Program of the 2001 International Worm Meeting

Talks will be 10 minutes with two minutes for questions; there are a few split presentations (you will be contacted by the organizers by June 1 if this applies to you).

Plenary 1
Royce Hall Auditorium, Friday 7:00-10:30 pm
Garth Patterson & Valerie Reinke, Chairs

1. MSP Signaling: Beyond the Sperm Cytoskeleton
   Michael A. Miller, David Greenstein

2. Promotion of germ cell fate by the multifunctional CCCH zinc finger protein PIE-1
   Christina Tenenhaus, Kuppuswamy Subramaniam, Melanie Dunn, Geraldine Seydoux

3. *IN VIVO* DYNAMICS OF POP-1 ASYMMETRY AND THE ROLE OF POP-1 IN THE INITIATION BUT NOT MAINTENANCE OF *end-3* REPRESSION
   Morris F. Maduro, Joel H. Rothman

4. Specification of Organ Identity by the C. elegans FOXA homologue PHA-4.
   Jeb Gaudet, Michael Horner, Susan E. Mango

5. Genetic Analysis of Endocytosis by the Coelomocytes of *Caenorhabditis elegans*
   Johnny Fares

6. *smu-1* and *smu-2* regulate the alternative splicing of *unc-52* pre-mRNA
   Angela K. Spartz, **Caroline A. Spike**, Jocelyn E. Shaw, Robert K. Herman

7. A Cycle of SMG-2 Phosphorylation Required for Nonsense-mediated mRNA decay in *C. elegans*
   Andrew Grimson, Kirk Anders, Phil Anderson

8. A Global Analysis of the *Caenorhabditis elegans* Operons
   Tom Blumenthal, Donald Evans, Chris Link, Alessandro Guffanti, Daniel Lawson, Danielle Thierry-Mieg, Jean Thierry-Mieg, Kyle Duke, Stuart Kim

9. Developmental expression map of C.elegans genome
   Yuji Kohara, Ikuko Sugiura, Tokie Oba, Masumi Obara, Takami Suzuki, Tadasu Shin-i, Yumiko Ueta, Mina Iwata, Keiko Hirono, Kyoko Nakata, Masahiro Ito, Yohei Minakuchi, Michiko Serizawa

10. A gene expression map for *C. elegans*
    Stuart K. Kim, Jim Lund, Stewart Scherer, Moni Kiraly, Kyle Duke, Min Jiang, Brian N. Wylie, George S. Davidson

11. 7,500 Genes and the Transition from Maternal to Zygotic Control of Development
    L. R. Baugh, A. A. Hill, E. L. Brown, Craig P. Hunter

12. Systematic gene inactivation with an RNAi feeding library
    Andrew Fraser, Ravi Kamath, Yan Dong, Gino Poulin, Peder Zipperlen, Maruxa Martinez-Campos, Marc Sohrmann, Julie Ahringer

13. Caenorhabditis Genetics Center
    Theresa Stiernagle, Sylvia Martinelli, Jonathan Hodgkin, Leon Avery, Robert Herman

14. The *C elegans* Knockout Consortium: Interim Report.
    Robert Barstead, Anil Dsouza, Mark Edgley, Keiko Gengyo-Ando, Erin Gilchrist, Jeff Holmes, Steven Jones, Yuji Kohara, Martin Lansdale, Sheldon McKay, Etsuko Machiyama, Shohei Mitani, Don Moerman, Gary Moulder, Sachiko Noguchi, Jamie Osborn, Bin Shen, Miwa Tamura, Malini Viswanathan

15. WormBase: A Web-accessible database for C. elegans biology
    Paul Sternberg, Dan Lawson, Erich M. Schwarz, Sylvia D. Martinelli, Wen J. Chen, Marco Mangone, Darin Blasiar, Ron Worthington, Raymond Y. Lee, Allan Day, Hans-Michael Mueller, Todd W. Harris, Danielle Thierry-Mieg, Jean Thierry-Mieg, John Spieth, R. Durbin, Lincoln D. Stein

Parallel 1A: RNAi
Royce Hall Auditorium, Saturday 9:00-11:00 am
Mary Montgomery, Chair

16. Characterization of RNAi-sensitive mutators and the *C. elegans Dicer* homolog
Sylvia E.J. Fischer, Rene F. Ketting, Pawel Pasierbek, Karen L. Thijssen, Ronald H.A. Plasterk

17. *ego-1*, a link between germline development and RNAi
Eleanor M. Maine, Valarie E. Vought, Jamie Wasilenko.

18. Using RNAi to identify new components of the RNAi machinery
Nathaniel R. Dudley, Jean-Claude Labbé, Bob Goldstein

19. Potential for Cross-Interference with RNAi
Jeff Norman, Erin Bishop, **Mary K. Montgomery**

20. Mutants of *C. elegans* defective in uptake of dsRNA in RNAi
Femke Simmer, Marcel Tijsterman, Kristy L. Okihara, Rene F, Ketting, Ronald H.A. Plasterk

21. RNAi analysis of 762 germline enriched genes.
Fabio Piano, Aaron Schetter, Diane Morton, Kris Gunsalus, Valerie Reinke, Stuart Kim, Kenneth Kemphues

22. Roles for embryonic lethal genes on *C. elegans* Chromosome I identified by RNA interference and 4-dimensional video-microscopy and identification of a new Par-like gene
Peder Zipperlen, Andrew G Fraser, Ravi S Kamath, Nathalie Le Bot, Monica Gotta, Maruxa Martinez-Campos, Julie Ahringer

23. Genome-wide functional analysis by RNAi-by-soaking with a non-redundant cDNA set
Ikuma Maeda, Atsuko Minamida, Masayuki Yamamoto, Yuji Kohara, **Asako Sugimoto**

24. Identification of *C. elegans* genes required for the DNA damage response using a combination of functional genomic approaches
Simon Boulton, Anton Gartner, Philippe Vaglio, Jérôme Reboul, David Hill, Marc Vidal

Parallel 1B: Signal transduction
Alex Hajnal, Chair

25. Two RGS proteins that inhibit Galphao and Galphaq signaling in *C. elegans* neurons require a Gbeta5-like subunit for function
Daniel L. Chase, Georgia A. Patikoglou, Michael R. Koelle

26. *eat-11* encodes GPB-2, a Gbeta5 ortholog that interacts with G_o alpha and G_q alpha to regulate *C. elegans* behavior
Merrilee Robatzek [*], Timothy Niacaris [*], Kate Steger, Leon Avery, James H. Thomas

27. An N-terminal region of *C. elegans* RGS proteins EGL-10 and EAT-16 directs inhibition of Go alpha versus Gq alpha signaling
Georgia A. Patikoglou, Michael R. Koelle

28. Novel downstream targets of GOA-1, EGL-30, GPA-12 and the *C. elegans* specific G-proteins
Alexander M. van der Linden, Edwin Cuppen, Femke Simmer, Celine Moorman, Ronald H.A. Plasterk

29. THE G-PROTEIN COUPLED RECEPTOR SML-1 ANTAGONISES RAS/MAP KINASE SIGNALLING DURING OLFACTION AND VULVAL DEVELOPMENT
Gopal Battu, Stefano Canevascini, Thomas Berset, Erika Fr„hli, Alex Hajnal

30. Modulation of inductive signaling during *C. elegans* vulval development
Nadeem Moghal, Chieh Chang, L. Rene Garcia, Paul W. Sternberg

31. *C. elegans* POP-1/TCF functions in a canonical Wnt pathway that controls cell migration and a noncanonical Wnt pathway that controls cell polarity
Michael A. Herman, Kelly Greene

32. AN AXIN-LIKE PROTEIN FUNCTIONS AS A NEGATIVE REGULATOR OF WNT SIGNALING IN THE Q NEUROBLAST LINEAGE
Hendrik C. Korswagen, Marco Betist, Damien Coudreuse, Hans C. Clevers

33. A genetic dissection of the minibrain kinase gene family and its role in Down syndrome
William B. Raich, Celine Moorman, Clay O. Lacefield, Oliver Hobert, Ronald H.A. Plasterk, Eric R. Kandel

34. Two Isoforms of the EGL-15 (FGFR) Serve Different Functions
Jay Goodman, Cathy Branda, Matthew Robinson, Michael Stern

Parallel 1C: Embryonic Polarity and Divisions
Pierre Gonczy, Chair

35. The Profilin PFN-1 is required for cytokinesis and cell polarity in the *C. elegans* embryo
Aaron F. Severson, Bruce Bowerman

36. The Anaphase-Promoting Complex is required to polarize the one-cell *C. elegans* embryo.
Chad Rappleye, Akiko Tagawa, Rebecca Lyczak, Bruce Bowerman, Raffi V. Aroian

37. *spn-4* encodes a putative RNA binding protein required for mitotic spindle orientation and cell fate patterning in the *C. elegans* embryo
José-Eduardo Gomes, Sandra E. Encalda, J. Clayton Carter, Kathryn A. Swan, Critopher A. Shelton, Bruce Bowerman

38. CDK-1 REGULATES SPINDLE ORIENTATION IN EARLY C. ELEGANS EMBRYOS
Martha C. Soto, Yanxia Bei, Craig C. Mello

39. Phosphotyrosine signaling acts in parallel with Wnt signaling to specify endoderm and to control cleavage orientation in early *C. elegans* embryos
Yanxia Bei, Jennifer Hogan, Martha Soto, Ka Ming Pang, John Collins, Craig C. Mello

40. Embryonic handedness choice in *C. elegans* involves a G_{alpha} protein encoded by the *spn-1* gene
William B. Wood, Barbara Robertson, Dominique Bergmann

41. *cdk-7* has independent roles in mRNA transcription and cell cycle progression in *C. elegans* embryos
Matthew R. Wallenfang, Geraldine Seydoux

42. A novel protein required for degradation of CCCH finger proteins in somatic lineages.
Kim Reese, Geraldine Seydoux

43. Wnt signaling and HAM-1 are required for asymmetric cell division of *C. elegans* neuroblasts.
Nancy Hawkins, C. Andrew Frank, Gregory Ellis, Bruce Bowerman, Gian Garriga

44. A POP-1 repressor complex restricts inappropriate cell type-specific gene transcription during *C. elegans* embryogenesis
Martin Victor, Dominica Calvo, Guangchao Sui, Margaret Po-Shan Luke, Gengyun Wen, Morris Maduro, Joel Rothman, Yang Shi

Parallel 1E: Synaptic Function
Ken Miller, Chair

45. Evidence that UNC-13 acts via syntaxin to promote vesicle priming
Janet E. Richmond, Robby M. Weimer, Erik M. Jorgensen

46. Analysis of *kin-13* PKC and *dgk-1* DAG kinase suggests that phorbol esters regulate a late stage of synaptic vesicle exocytosis.
Derek Sieburth, Jeremy Dittman, Stephen Nurrish, Joshua M. Kaplan

47. UNC-10 Rim, an active zone protein that regulates post-docking events at the synapse
Sandhya P. Koushika, Janet E. Richmond, **Richard Rolfe**, Gayla Hadwiger, Erik M. Jorgensen, Michael L. Nonet

48. SLO-1 Potassium Channel Regulates Duration of Neurotransmitter Release
Zhao-Wen Wang, Owais Saifee, Michael L. Nonet, Lawrence Salkoff

49. Electrophysiological measurement of the action of phorbol esters and serotonin on body wall neuromuscular physiology.
Jon Madison, Joshua Kaplan

50. Nidogen and Type XVIII Collagen Are Required During the Formation of Neuromuscular Junctions in C. elegans
Brian Ackley, Jennifer R. Crew, Seong Hoon Kang, Yishi Jin, James M. Kramer

51. *unc-122* and *unc-75*: Two genes that affect neuromuscular signaling
Paula Loria, Oliver Hobert

52. *ric-3*, A Novel Gene Which Enhances Acetylcholine Receptor Activity
Sarah Halevi, Margalit Eshel, Lina Yassin, Jim McKay, Millet Treinin

53. *sol-1* encodes a novel protein that is required for the function of *glr-1*.
Yi Zheng, Jerry E. Mellem, Andres V. Maricq

54. Analysis of Glutamatergic Neurotransmission by Knockout of Glutamate Transporter Genes.
Itzhak Mano, Tricia Wright, Monica Driscoll

Parallel 1F: Neural Development
Oliver Hobert, Chair

55. Neuronal fate specification by bHLH proteins in the *C. elegans* ray sublineage
Douglas S. Portman, Scott W. Emmons

56. *sem-4* Regulates Terminal Differentiation and Anteroposterior Patterning in the T Lineage
Anne Toker, Marty Chalfie

57. Inhibition of Touch Cell Fate by *egl-44* and *egl-46*
Ji Wu, Anne Duggan, Marty Chalfie

58. PAG-3 MAY COUPLE CELL LINEAGE CUES TO TERMINAL DIFFERENTIATION THROUGH HETERODIMERIZATION WITH UNC-3 IN DEVELOPING VA AND VB MOTOR NEURONS
Scott Cameron, Brinda Prasad, Joan B. McDermott, Eric Aamodt, Randall R. Reed, Bob Horvitz

59. *C. elegans* SEK-1 MAPK cascade regulates neuronal asymmetric development mediated by Ca2+ signalling
Miho Tanaka-Hino, Alvaro Sagasti, Naoki Hisamoto, Masato Kawasaki, Shunji Nakano, Jun Ninomiya-Tsuji, Cornelia I. Bargmann, Kunihiro Matsumoto

60. Regulation of olfactory receptor expression by a serine-threonine kinase.
Anne Lanjuin, Piali Sengupta

61. Synapse Formation between Neurons
Kang Shen, Cori Bargmann

62. *osm-5*, the *C. elegans* homologue of the murine cystic kidney disease gene *Tg737*, functions in a ciliogenic pathway
Courtney J. Haycraft, Peter Swoboda, Patrick D. Taulman, James H. Thomas, Bradley K. Yoder

63. Intraflagellar Transport particles complex A and B play different roles in *C. elegans cilia formation*
Hongmin Qin, Maureen M. Barr, Dennis R. Diener, Joel L. Rosenbaum

Parallel 1G: Meiosis & Chromosome Organization
Abby Dernberg, Chair

64. Multi-pathway Regulation of Meiotic Entry
Dave Hansen, E. Jane Albert Hubbard, Pawel Pasierbek, Josef Loidl, Tim Schedl

65. Roles and modes of action of *rad-51*
Cinzia Rinaldo, Paolo Bazzicalupo, Sara Ederle, Massimo Hilliard, **Adriana La Volpe**

66. Asymmetrically distributed oligonucleotide repeats in the *C. elegans* genome sequence that map to regions important for meiotic chromosome segregation
Marc D. Perry, Christopher Sanford

67. The *C.elegans* homolog of the human Bloom's syndrome gene is required for meiotic recombination and genome integrity
Chantal Wicky, Myriam Passannante, Ann Rose, Fritz Müller

68. Assembly and function of the synaptonemal complex in *C. elegans* meiosis.
Monica P. Colaiacovo, Amy MacQueen, Kirthi Reddy, Gillian Stanfield, Valerie Reinke, Stuart Kim, Anne M. Villeneuve

69. Germline and sex-limited loci distributed non-randomly among chromosomes and with respect to recombination rate
Asher D. Cutter, Sam Ward

70. MES-4, a protein required for germline viability, binds the autosomes but not the X chromosomes
Youyi Fong, **Laurel Bender**, Susan Strome

71. Specific functions of linker histone isoforms in C. elegans
Monika A. Jedrusik, Stefan Vogt, **Ekkehard Schulze**

72. Characterization of the *C. elegans* Heterochromatin protein 1 homologues.
Francesca Palladino, Florence Couteau, Frederic Guerry, Fritz Müller

73. The Role of Chromatin Organization in Germ Line Function and Maintenance
Bill Kelly

Parallel 1H: Sex Determination
Mario de Bono, Chair

74. The intracellular domain of the feminizing receptor TRA-2A interacts directly with the transcription factor TRA-1A.
David H. Lum, Patricia E. Kuwabara, David Zarkower, Andrew M. Spence

75. Export of the TRA-1/tra-2 mRNA Complex From the Nucleus Regulates C. elegans Sex Determination
Scott P. Segal, Laura Graves, Elizabeth B. Goodwin

76. Regulation of *fem-3* mRNA by the *mog* genes and MEP-1 for sex determination in the *C. elegans* hermaphrodite germ line.
Marco Belfiore, Judith Kimble, Gary Moulder, Robert Barstead, Alessandro Puoti

77. Rapid Evolution of the Sequence and Function of *fem-3*
Eric Haag*, Shanping Wang*, Sarah LaMartina, Judith Kimble

78. Hermaphrodite or female? Specification of germ cell fates during nematode evolution
Soochin Cho, Suk-Won Jin, Pei-Jiun Chen, Adam B. Cohen, Katherine A. Tucker, Meng Yao, Ronald E. Ellis

79. Role of sperm selection in the determination of paternity
Pavan Kadandale, Andrew Singson

80. Haldane's Rule in *Caenorhabditis* is implemented by sexual transformation.
Scott Baird

81. *mab-23*: a *doublesex/mab-3*-related gene required for male-specific differentiation and behavior in *C. elegans*
Robyn Lints, Scott W. Emmons

Parallel 1J: Dauers, Body Size & Cuticle
Frank Slack, Chair

82. A cyclic GMP-dependent protein kinase controls body size and life span in C. elegans
Yasumi Ohshima, Takashi Hirose, Yoshiya Nakano, Takashi Misumi, Osamu Ushijima

83. Neural regulation of body size through sensory signaling and a cGMP-dependent protein kinase
Manabi Fujiwara, Piali Sengupta, Nasrin Amiri, Hannah Volksman, Steven L. McIntire

84. IDENTIFICATION OF AMP KINASE AS A MODIFIER OF INSULIN RECEPTOR SIGNALING *IN VIVO* IN *C. elegans* and *Drosophila*
K.Ferguson, T. Kidd, R. Chatterjee, L.L'Archeveque, K. Shaw, C. Seidel-Dugan

85. *sma-11* encodes an EMK family serine threonine kinase that modulates TGF-beta signaling
Lisa L. Maduzia, Andrew F. Roberts, Huang Wang, Stephen Cohen, Richard W. Padgett

86. *daf-5*, a gene involved in dauer formation and adult longevity, is related to the Sno oncogene family
Mark L. Edgley, Kevin V. King, Patrice S. Albert, Donald L. Riddle

87. *daf-5* encodes a partner of the *daf-3* transcription factor in a TGF-beta signaling pathway
Li Sun, Garth I Patterson

88. *dda-1*: a Novel A-domain Containing Cuticulin-like Gene in *Caenorhabditis elegans*, Important for Dauer Body Shape.
Joaquin Muriel, Iain Johnstone, Gordon Lithgow, Danny Tuckwell

89. Alae formation and the role of CUT-1-like proteins
Maria Rosaria Sapio, Massimo A. Hilliard, Salvatore Arbucci, Michele Cermola, Reneé Favre, Paolo Bazzicalupo

90. END-2, an apparent nuclear hormone receptor, relocalizes from the cytoplasm to the plasma membrane in response to environmental conditions
Erin Newman-Smith, Gina Broitman-Maduro, Marina Len, Joel Rothman

Plenary II
Michael Herman & Michael Koelle, Chairs

91. Ventral nerve cord axons require the PVT neuron and zig genes to maintain their correct positioning in axonal tracts
Oscar Aurelio, Oliver Hobert

92. *sax-1* and *sax-2* act in parallel with *unc-34* to inhibit neurite outgrowth in the adult worm.
Maria E. Gallegos, Jennifer A. Zallen, Cori Bargmann

93. Dual roles of UNC-18 in synaptic transmission.
Robby M. Weimer, Janet E. Richmond, Warren S. Davis, Erik M. Jorgensen

94. Serotonin regulates repolarization of pharyngeal muscle
Timothy Niacaris, Leon Avery

95. Regulation of multiple male *C. elegans* spicule muscle behaviors during mating.
L. Rene Garcia, Paul W. Sternberg

96. "HOT" WORMS, COOL GENES: MUTANTS INVOLVED IN THERMOSENSATION
John S. Satterlee, Maura Berkeley, Hiroyuki Sasakura, Atsushi Kuhara, Ikue Mori, Piali Sengupta

97. A novel secretory protein, HEN-1, regulates integration of sensory signals and behavioral plasticity.
Takeshi Ishihara, Yuichi Iino, Akiko Mohri, Ikue Mori, Isao Katsura

98. Retrograde regulation of motor-neuron activities by body-wall muscle and intestine through the novel C2 domain protein AEX-1
Motomichi Doi, Kouichi Iwasaki

99. Early Sensory Deprivation Alters Behavior, Rate of Development and Neuroanatomy in C. elegans
Catharine Rankin, Susan Sangha, Jacqueline Rose, Ken Norman

100. The role of a cGMP dependent protein kinase in olfactory adaptation
Noelle L'Etoile, Cori Bargmann

101. Passive no longer: hermaphrodite-derived, mate-finding cue implicated in the nematode *Caenorhabditis elegans*
Jasper M. Simon, Paul W. Sternberg

102. Sex Drive in *C. elegans*
Jonathan Lipton, Scott W. Emmons

103. In Vivo Optical Imaging of Neuronal and Muscle Cell Activity
Rex Kerr, Hiroshi Suzuki, Christian Froekjaer-Jensen, Roger Y. Tsien, William R. Schafer

104. Generation of cell-type-specific RNA and gene expression profiles for Mechanosensation Neurons in *C. elegans*
Yun Zhang, Thomas Delohery, Charles Ma, Marty Chalfie

105. Creating transgenic lines in *C. elegans* by microparticle bombardment
Vida Praitis, Judith Austin

106. Identifying muscle genes that change transcription levels in response to varying cholinergic signals using full-genome microarrays.
Peter J. Roy, Stuart K. Kim

Parallel 2A: Behavior
Maureen Barr & Kouichi Iwasaki, Chairs

107. M5 controls terminal bulb contraction in *Panagrellus redivivus* but not in *C elegans*
Leon Avery, Mark Steciuk

108. *pbo-4* and *pbo-5* define a novel signaling pathway required to initiate the posterior body contraction
Paola Dal Santo, Erik M. Jorgensen

109. Altered Defecation Rhythm Produced by RNAi Suppression of Intestinal Potassium Channels, *kqt-2* and *kqt-3*.
Aguan Wei, Marina Kniazeva, Takayuki Teramoto, Min Han, Kouichi Iwasaki, Lawrence Salkoff

110. Control of the defecation motor program involves the G protein-coupled receptor AEX-2
Elaine K. Round, James H. Thomas

111. The family of genes encoding FMRFamide-related neuropeptides of *Caenorhabditis elegans*
Kyuhyung Kim, Chris Li

112. SUE-1: a novel *C. elegans* neuropeptide that regulates muscle contraction
Sarah Kniss, Judith Austin

113. Identification and characterization of *nic-1*, a gene involved in regulation of the nAChR.
Jinah Kim, Lisa Moore, Kari Dickinson, William Schafer

114. Genes that affect the behavioral response of *C. elegans* to ethanol
Andrew G. Davies, Tod R. Thiele, Steven L. McIntire

115. State-Dependency of Olfactory Adaptation
Jill C. Bettinger, Catharine L. Eastman, Steven L. McIntire

116. Activity of nociceptive neurons is required for social behaviour in *C. elegans*
Mario de Bono, David Tobin, Cori Bargmann

117. The *C. elegans* G-protein gamma subunit *gpc-1* is involved in adaptation to water-soluble attractants.
Gert Jansen, David Weinkove, Ronald H.A. Plasterk

118. EGL-47 is a putative G protein-coupled receptor that controls egg-laying behavior
James J. Moresco, Michael R. Koelle

119. UNC-58 IS AN UNUSUAL POTASSIUM CHANNEL OF THE TWIK FAMILY
Monika Tzoneva, James H. Thomas

120. *adc-1* MUTANTS FORAGE WHILE MOVING BACKWARDS
Melissa Hunter-Ensor, Bob Horvitz

Parallel 2B: Death & Neurodegeneration
Barbara Conradt & Nektarios Tavernarakis, Chairs

121. Analysis of the Sex-Specific Death of the Male-Specific CEM Neurons
Elizabeth Kimberly, Ning Pan, Beryl Hatton, Ute Meisel, and Ding Xue

122. The *cfi-1* gene inhibits expression of CEM cell fate in other *C. elegans* neurons.
Shai Shaham, Cori Bargmann

123. Dissection of DNA damage checkpoint regulation in C. elegans and cloning and initial characterisation of the novel rad-5/ clk-2 checkpoint gene
Anton Gartner, Arno Alpi, Björn Schumacher, Kai Hoffmann, Simon Boulton, Shawn. Ahmed

124. A Genetic Pathway Involved In Apoptotic DNA Degradation In Nematodes
Jay Z Parrish, Hye-Seong Park, Duncan A. Ledwich, Kristina Klotz, Helen E. Metters, Ding Xue

125. CED-12, A COMPONENT OF A RHO/RAC GTPASE SIGNALING PATHWAY, REGULATES CYTOSKELETAL REORGANIZATION

AND CONTROLS CELL-CORPSE ENGULFMENT AND CELL MIGRATION IN *C. elegans*
Zheng Zhou, Emmanuelle Caron, Angell Shieh, Erika Hartwieg, Alan Hall, Bob Horvitz

126. *mec-4(d)*-induced necrotic-like cell death in *C. elegans* requires calreticulin and regulators of ER-mediated Ca^{2+} release
Keli Xu, Nektarios Tavernarakis, Monica Driscoll

127. A Novel Pharmacogenetic Model for Parkinson's Disease: Dopamine Neurodegeneration in C. elegans
Richard Nass, David H. Hall, David M. Miller, Randy D. Blakely

128. MPTP-based test system for Parkinson´s Disease in *C. elegans*
Evelyn Braungart, Wolfgang Link, Manfred Gerlach, Karlheinz Tovar, **Marius C. Hoener**

129. The Gln-Ala repeat transcriptional activator CA150 interacts with huntingtin: neuropathologic and genetic evidence for a role in Huntington's disease pathogenesis
Sébastien Holbert, Isabelle Denghien, Tamara Kiechle, Adam Rosenblatt, Cheryll Wellington, Michael R. Hayden, Russell L. Margolis, Christopher A. Ross, Jean Dausset, Robert J. Ferrante, **Christian Néri**

130. HEAT SHOCK TRANSCRIPTION FACTOR (HSF) ACTIVITY REGULATES THE APPEARANCE OF POLYGLUTAMINE PROTEIN AGGREGATES
James F. Morley, Li-Jung Tai, Richard I. Morimoto

131. Identification of C. elegans proteins that directly interact with the human beta amyloid peptide.
Gin Fonte, Vadim Kapulkin, David Friedman, **Chris Link**

Parallel 2C: Outgrowth & Migration
Matt Buechner & Scott Clark, Chairs

132. *zag-1*, a Zn finger-homeodomain gene needed for axon guidance
Catherine Chiu, Katrina Sabater, Ray Squires, **Scott Clark**

133. The homeodomain protein CePHOX2/CEH-17 controls antero-posterior axonal outgrowth
Nathalie PUJOL, Pascal TORREGROSSA, Jean-François BRUNET

134. A possible repulsive role for FGF signaling in CAN migration
Tinya Fleming, Fred Wolf, Gian Garriga

135. Does UNC-119 mediate collagen-based axon guidance signal?
Wayne Materi, Kathy Bueble, Dave Pilgrim

136. Genetic Analysis of the Kallmann Syndrome Protein Ortholog *Cekal-1 in C. elegans*
Hannes E. Bülow, Katherine L. Berry, Oliver Hobert

137. A conserved interaction between integrins and a GCK kinase required for axonal navigation in *C elegans*
Patrice Poinat, Adèle De Arcangelis, Satis Sookhareea, **Michel Labouesse**, Elisabeth Georges-Labouesse

138. Interacting proteins with UNC-51, UNC-14 or UNC-33
Ken-ichi Ogura, Yoshio Goshima

139. VAB-8 AND UNC-51 PROTEIN INTERACTIONS MEDIATE AXON OUTGROWTH
Tina Lai, Gian Garriga

140. A genetic network involving unc-53, unc-5, unc-71, unc-51, unc-14, apr-1 and bg7 is controlling the dorsoventral outgrowth of the excretory canals.
Ingele Roelens, Dirk Inzé, Isabelle Maillet, Richard Feichtinger, Wim Van Criekinge, Titus Kaletta, Thierry Bogaert

141. MAX-1, a novel conserved protein, may function in UNC-5 mediated axon repulsion
Xun Huang, Hwai-Jong Cheng, Mei Zhen, Marc Tessier-Lavigne, Yishi Jin

142. A Constitutively Active UNC-40/DCC Reveals Downstream Signaling Components
Zemer Gitai, Erik Lundquist, Marc Tessier-Lavigne, Cori Bargmann

143. Roles of *unc-34* and *unc-40* in repellent guidance by *sax-3*
Tim Yu, Joe Hao, Wendell Lim, Marc Tessier-Lavigne, Cori Bargmann

144. Putative matrix proteins with thrombospondin type I repeats and serine protease inhibitor domains are involved in guidance of the distal tip cell
Takehiro Kawano, Hong Zheng, Joseph G. Culotti

145. Cellular mechanisms governing the specificity and timing of anchor cell invasion into the vulval epithelium
David R Sherwood, Paul W Sternberg

Parallel 2D: Mitosis & Asymmetry in the Early Embryo
Jill Schumacher & Bob Goldstein, Chairs

146. Maturation of the C. elegans centromere requires a CENP-C-like protein
Landon L. Moore, Mark B. Roth

147. Dissecting the molecular architecture and structure of the kinetochore by genetics and correlated light and electron microscopy
Kent L. McDonald, Mary Howe, Mike Albrecht, Patrick McDonel, Barbara J. Meyer

148. Analysis of chromosome segregation and kinetochore assembly in the one-cell stage *C. elegans*, embryo
Karen Oegema, Arshad Desai, Sonja Rybina, Matthew Kirkham, Anthony Hyman

149. Centrosome maturation in *C. elegans*: respective roles for gamma-tubulin and aurora–A kinase
Eva Hannak, Karen Oegema, Matthew Kirkham, Pierre Gönczy, Anthony A. Hyman

150. SPD-5 is a novel centrosomal protein required for mitotic spindle assembly
Danielle R. Hamill, J. Clayton Carter, Bruce Bowerman

151. *zyg-12* is required for the positioning of *C elegans* centrosomes
Christian J. Malone, John G. White

152. *zyg-8*, a gene required for spindle positioning in one cell stage embryos, encodes a Doublecortin-related kinase that promotes microtubule stability
Jean-Michel Bellanger, Matthew Kirkham, Andrei Pozniakowski, Tony Hyman, Pierre Gönczy

153. The mitosis-regulating kinase AIR-2 interacts with and phosphorylates the *C. elegans* kinesin-like motor protein BMK-1.
John D. Bishop, Jill M. Schumacher

154. Biochemical Identification of LIN-5 Associated Proteins with Mitotic Spindle Functions
Dayalan G. Srinivasan, Sander van den Heuvel

155. CDC-42, AGS-3 and heterotrimeric G proteins regulate spindle position and orientation
Monica Gotta, Julie Ahringer

156. *pom-1* is Required for Anterior/Posterior Polarity and Microtubule Dynamics in 1-Cell Embryos, and is Related to a Regulator of Cell Polarity in *S. pombe*
Jason Pellettieri, Valerie Reinke, Stuart Kim, Geraldine Seydoux

157. The NED-8 ubiquitin-like conjugation pathway regulates the microtubule and microfilament cytoskeleton in *C. elegans*.
Thimo Kurz, Danielle R. Hamill, Bruce Bowerman

158. The role of *C. elegans* Rho-binding kinase and myosin phosphatase in cytokinesis
Alisa J. Piekny, Paul E. Mains

159. OOC-5, required for polarity in the 2-cell embryo, encodes a AAA+ ATPase related to the human disease protein Torsin A
Stephen E. Basham, Lesilee S. Rose

Parallel 2E: Epithelial Morphogenesis & Polarity
Chris Rongo & Jim Waddle, Chairs

160. Analysis of gut epithelial polarity in the C.elegans embryo
Olaf Bossinger, Carin Theres, Daniela van Fürden, Christoph Segbert

161. Establishment of apical junctions in epidermal cells during *C elegans* embryogenesis: compaction by the LAP protein LET-413 and protein clustering by the MAGUK protein DLG-1.
Renaud LEGOUIS, Laura Mc MAHON, Jean Luc VONESCH, Michel LABOUESSE

162. Cooperative regulation of JAM-1 by Discs large and LET-413 controls junctional tightness of *C. elegans* epithelia
Mathias Köppen, Jeffrey Simske, Paul Sims, Christopher Rongo, David Hall, Christopher Lockwood, Gary Moulder, Anthony Radice, Robert Barstead, Jeffrey Hardin

163. Analysis of VAB-9, a Putative Integral Membrane, Cell Junction Protein
Jeffrey S. Simske, Jeff Hardin

164. The gap junction protein INX-3 is essential for embryonic morphogenesis.
Todd Starich, Jocelyn Shaw

165. A Role for Inositol 1, 4, 5-Triphosphate Receptor During the Process of Ventral Enclosure in *Caenorhabditis Elegans*
Christina L. Thomas, Jeff S. Simske, Jeff Hardin

166. The LAR-like Receptor Tyrosine Phosphatase PTP-3 and the VAB-1 Eph receptor tyrosine kinase have partly redundant functions in morphogenesis
Robert J. Harrington, Michael J. Gutch, Michael Hengartner, Nicholas Tonks, Andrew D. Chisholm

167. *kal-1*, the *C. elegans* homolog of the X-linked Kallmann syndrome gene, is involved in epidermal morphogenesis and neuronal growth.
Elia Di Schiavi, Elena I. Rugarli, Massimo A. Hilliard, Salvatore Arbucci, Anna Facciolli, Andrea Ballabio, Paolo Bazzicalupo

168. LAD-1, the *C. elegans* L1CAM homolog, participates in cell migration, and is a substrate for FGFR pathway-dependent phosphotyrosine-based signaling
Lihsia Chen, Bryan Ong, Vann Bennett

169. A tale of one gene encoding two proteins, one required to anchor muscles the other to maintain epidermal integrity
Julia M. Bosher, **Bum-Soo Hahn**, Renaud Legouis, Laura McMahon, Ann M. Rose, Michel Labouesse

170. *mua* genes function within the hypodermis, and may be regulated by the EKLF homolog MUA-1
John Plenefisch, Mark Bercher, Wurola Omotosho, Vera Hapiak, Suhant Khandekar, David Hall, Michelle Hresko

171. From a tetraspanin mutation, hopeful monsters
John Yochem

172. *tlp-1* is involved in the control of cell polarity and male tail tip morphogenesis during *C. elegans* development
Xiaojun Zhao, Ying Yang, David H. A. Fitch, Michael A. Herman

173. An innexin, *lep-1*, is required for male tail tip morphogenesis
Tania Del Rio*, Ying Yang*, David H.A. Fitch, Can Q. Nguyen, David H. Hall

Parallel: 2F: Vulval Development
Wendy Hanna-Rose & Joe Dent, Chairs

174. Regulation of Pn.p Cell Fusion in *C. elegans*
Scott Alper, Cynthia Kenyon

175. Functions of *lin-40* MTA, synMuv, and Ras in regulating the competence of the vulval precursor cells during vulval fate specification
Zhe Chen, Min Han

176. HOW HOX WORKS? *eff-1* is the name, effector of cell fusion is the game
Gidi Shemer, Benjamin Podbilewicz

177. Microevolution of vulval cell lineages within two nematode genera : *Caenorhabditis* and *Oscheius*.
Marie DELATTRE, Marie-Anne FÉLIX

178. ORDERING THE synMUV CLASS A PROTEINS: LIN-15A MAY BE IMPORTANT FOR THE NUCLEAR EXPRESSION OF LIN-56
Ewa M. Davison, Bob Horvitz

179. Activation of Wnt signaling bypasses the requirement for Ras signaling during vulval induction
Julie Gleason, Hendrick C. Korswagen, David M. Eisenmann

180. Docking sites on substrate proteins direct extracellular signal-regulated kinase (ERK) to phosphorylate specific residues.
Douglas A. Fantz, Dave Jacobs, Danielle Glossip, Kerry Kornfeld

181. Two *enhancers of raf, eor-1* and *eor-2*, function in parallel to *sur-2, lin-25*, and *lin-1* to positively regulate Ras signaling
Robyn M. Howard, Meera V. Sundaram

182. Protein Phosphatase 2A is a positive regulator of the Ras pathway.
Gautam Kao, Meera Sundaram

183. Mechanism and role of LIN-12 downregulation in response to Ras activation during VPC fate specification.
Daniel Shaye, Iva Greenwald

184. Regulation and function of *lin-11* in vulval development
Bhagwati P Gupta, Paul W Sternberg

185. Rac and candidate Rac-pathway genes control patterning and morphogenesis of secondary vulval cells
Ranjana Kishore, Meera Sundaram

186. Analysis of genes involved in late patterning of the vulva
Takao Inoue, Paul W. Sternberg

187. The *Caenorhabditis elegans evl-20* Gene Encodes a Developmentally Conserved ARF-like Protein Involved in Cytokinesis and Morphogenesis
Igor Antoshechkin, Min Han

188. A PROTEOGLYCAN BIOSYNTHETIC PATHWAY INVOLVED IN *C. elegans* EMBRYOGENESIS AND VULVAL MORPHOGENESIS AND IN HUMAN AGING DISORDERS
Ho-Yon Hwang, Bob Horvitz

Parallel 2G: Transcription & RNA Processing
David Eisenmann & Hitoshi Sawa, Chairs

189. A broad transcriptional requirement for CDK9/CyclinT *in vivo* involves overcoming elongation inhibition by SPT4/SPT5.
Eun Yong Shim, Amy Walker, Yang Shi, T. Keith Blackwell

190. Nuclear hormone receptor *nhr-23*(CHR3) is a critical regulator of all four larval molts of *Caenorhabditis elegans*
Marta Kostrouchova, Michael Krause, Zdenek Kostrouch, Joseph Edward Rall

191. Functional Analysis of C. elegans Nuclear Hormone Receptors
Marc R. Van Gilst, Keith R. Yamamoto

192. From Binding Sites to Target Genes: Molecular Characterization of the Intracellular Receptor Daf-12 Function
Yuriy Shostak, Keith R. Yamamoto

193. A pharyngeal muscle specific enhancer from *ceh-22* is targeted by PHA-4 and other factors
Tomas Vilimas, Craig Kuchenthal, Wei Chen, Alin Abraham, Peter Okkema

194. The novel factor PEB-1 functions during C. elegans pharyngeal organogenesis
Anthony P. Fernandez, Peter G. Okkema

195. The transcriptional Mediator complex functions as a switch of cell fates during vulval development
Akinori Yoda, Hiroko Kouike, Hideyuki Okano, **Hitoshi Sawa**

196. Functional analysis of splicing factors by RNAi using a nuclear retention assay
Peg MacMorris, Tom Blumenthal

197. A complex containing CstF and the SL2 snRNP connects 3' end formation and trans-splicing in *C. elegans* operons
Donald Evans, Ismael Perez, **Peg MacMorris**, Devin Leake, Carol Williams, Tom Blumenthal

198. Operon Resolution, Usage and Diversity of SL2-like spliced leaders in the phylum Nematoda
David B. Guiliano, Villius Pigaga, Mark .L. Blaxter

199. RNA editing is important for normal development and behavior in C. elegans
Brenda L. Bass, Leath A. Tonkin, Lisa Saccomanno, Daniel P. Morse, Mike Krause

200. GLD-2, a Putative Cytoplasmic Poly(A) Polymerase, is Required for Both Germline Development and Embryogenesis
Liaoteng Wang, Lisa Kadyk, Christian Eckmann, Marvin Wickens, Judith Kimble

201. *cdl-1*, whose mutation cause defects in programmed cell death and morphogenesis, encodes a homologue of stem-loop binding protein, and may contribute to proper core histone expression.
Yuki Kodama, Asako Sugimoto, Joel Rothman, Masayuki Yamamoto

202. The *lin-4* RNA is neither necessary nor sufficient for a post-initiation translational regulatory mechanism in the heterochronic pathway
Eric G. Moss, Kathy Seggerson, Lingjuan Tang

Parallel 3A: Sensory Transduction
Steve Nurrish, Chair

203. Touch Cell Proteins Needed for the Formation of Amiloride-sensitive Ion Channels
Miriam B. Goodman, Glen G. Ernstrom, Dattananda Chelur, Robert O'Hagan, Martin Chalfie

204. The complete *C. elegans* family of *osm-9*/capsaicin receptor related genes: a conserved family of putative ion channel subunits with roles in nociception and chemosensation.
David Tobin, David Madsen, Gary Moulder, Erin Peckol, Robert Barstead, A. Villu Maricq, Cori Bargmann

205. Neuropeptide modulation of touch sensitivity and locomotion
Tija Carey, Jamie Kass, Joshua Kaplan

206. Avoidance and bitter taste in *C. elegans* : evidences for a head-tail chemotopic sensory map
Massimo A. Hilliard, Ronald H.A. Plasterk, Cornelia I. Bargmann, Paolo Bazzicalupo

207. The novel conserved protein OCA-1 is required for chemosensory and osmosensory response by the ASH circuit.
Hidetoshi Komatsu, Shinya Matsumoto, Anne C. Hart

208. TAX-6 calcineurin acts as a negative regulator of sensory signal pathways to modulate excitability of sensory neurons
Atsushi Kuhara, Hitoshi Inada, Isao Katsura, Ikue Mori

209. Analysis of TTX-4 nPKC-epsilon required for sensory signaling
Yoshifumi Okochi, Kotaro Kimura, Masatoshi Okumura, Ikue Mori

210. Behavioral and genetic analysis of starvation-induced neural plasticity in thermotaxis
Akiko Mohri, Mizuho Koike, Eiji Kodama, Takafumi Mizuno, Kotaro Kimura, Ikue Mori

211. The Role of *srf-6* in Chemosensory Control of Surface Antigen Switching
David Phu, Douglas P. Olsen, Laura L. Miceli, **Samuel M. Politz**

Parallel 3B: Embryonic Cell Fates
Laura Berkowitz, Chair

212. Lamin-dependent localization of UNC-84, a protein required for nuclear migration in *C. elegans*
Kenneth K. Lee, Daniel Starr, **Merav Cohen**, Jun Liu, Min Han, Katherine L. Wilson, Yosef Gruenbaum

213. Translational control of maternal *glp-1* mRNA by POS-1 and its interacting protein PIP-1
Ken-ichi Ogura, Shohei Mitani, Keiko

Gengyo-Ando, Yuji Kohara

214. Mutations in *pos-1* dominantly suppress the Mex phenotype of *efl-1*, *dpl-1* and *mex-5* mutant embryos
Barbara Page, Jennifer Schisa, James Priess, Edwin Ferguson

215. MEX-3 Interacting Proteins Link Cell Polarity to Asymmetric Gene Expression
Nancy N. Huang, Darcy Mootz, Albertha J.M. Walhout, Marc Vidal, Craig P. Hunter

216. Early zygotic *pal-1* expression in the AB lineage depends on the LAG pathway
Lois Edgar, William B. Wood

217. The role of PLP-1 in mesendoderm specification and in other developmentally asymmetric cell divisions
E. Witze, E. Field, D. Hunt, J.H. Rothman

218. Control of hox gene expression might be more conserved between *Caenorhabditis elegans* and *Drosophila melanogaster* than previously thought.
Adrian Streit, Reto Kohler, Thomas Marty, Marco Belfiore, Krisztina Takacs-Vellai, Charles Stoyanov, José Fos, Francois Gautron, Christophe Folly, Ralf Schnabel, Markus Affolter, Fritz Müller

219. 4D-analysis of a collection of maternal effect mutants of *C. elegans*.
Juan Cabello, Richard Feichtinger, Heinke Schnabel, Ralf Schnabel

220. Variations in pattern formation and cell specification during nematode embryogenesis
Vera Lahl, Magdalena Laugsch, **Einhard Schierenberg**

Parallel 3C: Cell Cycle
Andy Golden, Chair

221. Metaphase-to-anaphase transition-defective mutants occur in Anaphase Promoting Complex genes and exhibit phenotypes in both oocyte and spermatocyte meiosis in *C. elegans*.
Edward S. Davis, Amanda Pletcher, Donald Fox, Barry Chestnut, Diane C. Shakes, Andy Golden

222. Making sperm without APC/C
P.L. Sadler, **D.C. Shakes**

223. Null mutations in wee1.3 are recessive lethal but rare dominant mutations specifically arrest the sperm cell cycle
Todd Lamitina, Steven W. L'Hernault, Ph.D.

224. *lin-9*, *lin-35* and *lin-36* negatively regulate G1 progression in *C. elegans*
Mike Boxem, Huihong Xu, Sander van den Heuvel

225. A novel synthetic approach to identify additional roles for lin-35/Rb in cell growth and proliferation.
David S. Fay, Sean Keenan, Min Han

226. CUL-4 functions to prevent DNA re-replication
Weiwei Zhong, Edward T Kipreos

227. C. elegans DNA damage checkpoint mutants mutate
Shawn Ahmed, Monika Tzoneva, James Thomas, Jonathan Hodgkin

228. Genes that ensure genome stability in *C. elegans*.
Joris Pothof, Marcel Tijsterman, Julie Ahringer, **Ronald Plasterk**

229. Heterochromatin binding protein 1 (HP1.1) in *Caenorhabditis elegans*: cell cycle analysis of chromatin structures and protein interactions
Masoud Bahrami, Ekkehard Schulze

Parallel 3D: Genomics
Marc Vidal, Chair

230. Open-reading-frame sequence tags (OSTs) support the existence of at least 17,300 genes in *C. elegans*
Jerome Reboul, Philippe Vaglio, Jean Francois Rual, Nicolas Thierry-Mieg, Troy Moore, Cindy Jackson, Tadasu Shin-i, Yuji Kohara, Danielle Thierry-Mieg, Jean Thierry-Mieg, Hongmei Lee, Joseph Hitti, Lynn Doucette-Stamm, James L. Hartley, Gary F. Temple, Michael A. Brasch, Jean Vandenhaute, Philippe E. Lamesch, David E. Hill, Marc Vidal

231. The worm transcriptome project
Jean Thierry-Mieg, **Danielle Thierry-Mieg**, Yutaka Suzuki, Sumio Sugano, Kazuko Oishi, Masako Sano, Hisayo Nomoto, Shinobu Haga, Satoko Nishizaka, Hiroko Hayashi, Fumiko Ohta, Sachiko Miura, Hiroko Uesugi, Michel Potdevin, Yann Thierry-Mieg, Vahan Simonyan, Adam Lowe, Tadasu Shin-I, Yuji Kohara

232. A snip-SNP map of the *C. elegans* genome: Applications to positional cloning.
Stephen R. Wicks, Raymond T. Yeh, Warren R. Gish, Robert H. Waterston, Ronald H. A. Plasterk

233. WorfDB: The *C. elegans* ORFeome database
Philippe Vaglio, Jerome Reboul, Philippe E. Lamesch, Jean-Francois Rual, David D. Hill, Marc Vidal

234. The BioKnowledge Library™: an Integrated Collection of Databases for Model Organism and Human Proteomes
Philip Olsen, Marek Skrzypek, Jodi Hirschman, Martha Arnaud, Maria Costanzo, Laura Robertson, Janice Kranz, James I. Garrels

235. Recently duplicated genes are less than half as likely to drive reporter gene expression: implications for genome annotation.
A.Mounsey, P.Bauer, D.McCarroll, I.A.Hope

236. Towards a physical and genetic map of *Pristionchus pacificus*
Jagan Srinivasan, Waltraud Sinz, Ralf J. Sommer

237. Phylogenomics: Analysis of EST datasets from species across the phylum Nematoda
Mark Blaxter, John Parkinson, Claire Whitton, David Guiliano, Jen Daub, Neil Hall, Mike Quail, Bart Barrell

238. Genes from Other Nematodes: A Progress Report on the GSC Parasitic Nematode EST Project
James P. McCarter, Sandra W. Clifton, Brandi Chiapelli, Deana Pape, John C. Martin, Todd N. Wylie, Michael Dante, Robert H. Waterston

239. Rates and Patterns of Mutation in the Nuclear and Mitochondrial Genomes of *Caenorhabditis elegans*
Dee R. Denver, Krystalynne Morris, Katherine Harris, Larrissa Vassilieva, Suzanne Randall Estes, Michael Lynch, W. Kelley Thomas

Parallel 3E: Aging & Stress
Pamela Larsen, Chair

240. Changes in Gene Expression Associated with Developmental arrest and Longevity
S.J.M. Jones, **D.L. Riddle**, A.T. Pouzyrev, V.E. Velculescu, L. Hillier, S.R. Eddy, S.L. Stricklin, D.L. Baillie, R. Waterston, M.A. Marra

241. Global profile of gene expression during aging
James Lund, Patricia Tedesco, Kyle Duke, Stuart K. Kim, Thomas E. Johnson

242. Global Analysis of Gene Expression Patterns in the Dauer Larvae of *Caenorhabditis elegans*
John Wang, Stuart K. Kim

243. Genome-wide analysis of transforming growth factor-ß-regulated genes in *Caenorhabditis elegans* by using microarrays
Tao Liu, Karen Zimmerman, Garth I. Patterson

244. Signals from the reproductive system that regulate lifespan and resistance to stress
Nuno Arantes-Oliveira, Javier Apfeld, Honor Hsin, Cynthia Kenyon

245. Effects of the gene disruption of two Mn-SOD isoforms on oxidative stress sensitivity and life span in *Caenorhabditis elegans*
Yoko Honda, Shuji Honda

246. *daf-2* Kinase Domain Alleles Prevent Hypoxic Death
C. Michael Crowder, Barbara A. Scott

247. *hif-1*, a homolog of mammalian hypoxia-inducible factor-1 alpha, is required for adaptation to low oxygen in *C elegans*
Huaqi Jiang, Rong Guo, Jo Anne Powell-Coffman

248. Signaling pathways in heat acclimation: A lesson from C. elegans mutants
Millet Treinin, Huaqi Jiang, Jo Anne Powell-Coffman, Judith Shleir, **Michal Horowitz**

Parallel 3F: Germline
Bill Kelly, Chair

249. P granules, nuclear pores, and RNA
JN Pitt, JA Schisa, JR Priess

250. The splicesomal Sm proteins play a role in P granule localization during early embryogenesis.
Scott A. Barbee, Alex L. Lublin, Thomas C. Evans

251. IFE-1, an isoform of eukaryotic initiation factor 4E, interacts with PGL-1 and is required for spermatogenesis in *C. elegans*.
Anahita Amiri, Brett Keiper, Ichiro Kawasaki, Yuan Fan, Yuji Kohara, Robert Rhoads, Susan Strome

252. GLHs associate with a LIM domain-containing protein and two other proteins necessary for fertility
Pliny A. Smith, W.M.A. Leung-Chiu, Lejla Mutapcic, April M. Orsborn, Ruth A. Montgomery, Karen L. Bennett

253. *cgh-1* , a conserved germline predicted RNA helicase required for gametogenesis and oocyte survival in *C. elegans*
Rosa E. Navarro, Eun Yong Shim, Yuji Kohara, Andrew Singson, T. Keith Blackwell

254. Control of germline cell fates by FBF, GLS-1, and GLD-2
Christian Eckmann, Brian Kraemer, Liaoteng Wang, Marvin Wickens, Judith Kimble

255. *puf-8*, a member of the puf family of RNA-binding proteins, promotes meiotic divisions and inhibits mitosis in primary spermatocytes.
Kuppuswamy Subramaniam, Geraldine Seydoux

256. Identification and characterization of multiple mRNA targets of GLD-1, an RNA binding protein required for germ cell development
Min-Ho Lee, Rueyling Lin, Tim Schedl

257. Two CCCH, zinc finger-containing proteins, OMA-1 and OMA-2, are redundantly required for oocyte maturation in *C. elegans*
Michelle Detwiler, Melanie Reuben, Xiumin Li, Eric Rogers, Rueyling Lin

258. Functional and molecular identification and physiological role of a ClC-2 Cl^- channel ortholog expressed in *C. elegans* oocytes
Eric Rutledge, Michael Christensen, Laura Bianchi, Rebecca Morrison, Adam Broslat, Andrew Beld, Alfred L. George, Jr., David Greenstein, Kevin Strange

Parallel 3G: Muscle Function & Development
Erik Lundquist, Chair

259. DYSTROPHIN AND ASSOCIATED PROTEINS IN C. elegans.
Gieseler, K, Grisoni, K, Mariol, M-C, **Segalat, L**

260. Functional analysis of ERM-1, a ryanodine receptor-interacting protein of *C. elegans*
Yasuji Sakube, Tomoyo Hamada, Hiroaki Kagawa

261. Functional analysis of the *C. elegans* Sarcoplasmic/Endoplasmic Reticulum Calcium transport ATPase
Richard Zwaal, Kurt Van Baelen, José Groenen, Anton van Geel, Veerle Rottiers, Titus Kaletta, Leonard Dode, Luc Raeymaekers, Frank Wuytack, Thierry Bogaert

262. Third and fourth tropomyosin isoforms of *Caenorhabditis elegans* are expressed in pharynx and intestines and are essential for development
Akwasi Anyanful, *Yasuji Sakube*, *Hiroaki Kagawa*

263. UNC-23 is a member of the BAG family of chaperone regulators and directly interacts with the non-inducible heat shock protein, HSP-1.
Poupak Rahmani, Donald G. Moerman

264. PAT-4 binds UNC-112 and Functions as an Adapter During Muscle Assembly
A. Craig Mackinnon, Hiroshi Qadota, Donald G. Moerman, Benjamin D. Williams

265. PAT-6, a Homlog of Actopaxin, Binds to PAT-4/Integrin-Linked Kinase
Xinyi Lin, Hiroshi Qadota, Don Moerman, Benjamin D. Williams

266. Functional analysis of the 5 LIM domain containing adaptor protein, UNC-97
Ken Norman, Shaun Cordes, Hiroshi Qadota, Poupak Rahmani, Don Moerman

267. *eat-1* Encodes Two Members of the ALP-Enigma Family of Proteins and is Required for Normal Muscle Function in *C. elegans*
Caroline R. McKeown, Mary C. Beckerle

268. Mutations in type XVIII collagen perturb fine patterns of matrix protein localization and elements of nervous system and muscle morphology
Jennifer R. Crew, Brian D. Ackley, Seong Hoon Kang, James M. Kramer

Parallel 3H: Somatic Gonad
Anna Newman, Chair

269. The *gnd* genes and *tra-1* control assembly and patterning of the gonad primordium
Laura Mathies, Sam Henderson, Robert Blelloch, Judith Kimble

270. POP-1, *sys* genes and sexual dimorphism during gonadogenesis
Kellee Siegfried, Trey Kidd, Judith Kimble

271. The presenilin SEL-12 is required during vulva muscle development for a normal egg laying behaviour in C. elegans.
Stefan Eimer, Roland Donhauser, Ralf Baumeister

272. The role of the SEL-12 presenilin in uterine pi cell induction and egg laying
Hediye Nese Cinar, Keri L. Sweet, Kim Hosemann, Karen Earley, Kavita Oommen, Anna P. Newman

273. Analysis of the presenilin complex
Diane Levitan, Lili Zhang, Caroline Goutte

274. Genes that suppress the Egl defect of *sel-12*, a *C. elegans* presenilin gene
Bernard Lakowski, Stefan Eimer, Christine Göbel, Roland Donhauser, Ralf Baumeister

275. Expression and function of the E/Daughterless protein HLH-2 during the AC/VU decision
Xantha Karp, Iva Greenwald

276. Mechanisms that establish anchor cell-specific expression of *lin-3*, *C. elegans* epidermal growth factor (EGF) homolog
Byung Joon Hwang, Paul W. Sternberg

277. Worms, Twist, and human Saethre-Chotzen syndrome
Ann Corsi, Tommy Brodigan, Erik Jorgensen, Mike Krause

278. Mesodermal cell fate specification in the postembryonic M lineage
Jun Liu, Andrew Fire

Parallel 3J: Membrane Biology
Raffi Aroian, Chair

279. Bt toxicity through a genetic lens: *bre-5* and carbohydrate modification
Joel S. Griffitts, Johanna L. Whitacre, Raffi V. Aroian

280. A putative GDP-GTP exchange factor is required for development of the excretory cell
Norio Suzuki, Matthew Buechner, Kiyoji Nishiwaki, David H. Hall, Naoki Hisamoto, Kunihiro Matsumoto

281. The ELAV Orthologue EXC-7 Binds *sma-1* mRNA to Regulate Excretory Canal Diameter
Masaki Fujita, Dana Hawkinson, Kevin V. King, David H. Hall, Hiroshi Sakamoto, **Matthew Buechner**

282. Beta-spectrin and synaptic vesicle release
Marc Hammarlund, Erik M. Jorgensen

283. UNC-16, A JNK signaling scaffold, regulates vesicle transport in *C. elegans*
Dana Byrd, Mercy Walcoff, Masato Kawasaki, Naoki Hisamoto, Kunihiro Matsumoto, Yishi Jin

284. RME-1, a novel EH domain protein required for endocytic recycling in *C. elegans* and mammalian cells
Barth D. Grant, Sharron X. Lin, Yinhua Zhang, Marie-Christine Paupard, David H. Hall, Frederick R. Maxfield, David Hirsh

285. Parallels between Mitochondrial Division and Endocytosis
Daniel Rubé, Ayako Hasegawa, Ashley Wright, Alexander van der Bliek

286. Targeting of rough endoplasmic reticulum membrane proteins and ribosomes to the cell body of *C. elegans* neurons
Melissa M. Rolls, David H. Hall, Martin Victor, Yang Shi, Ernst H. K. Stelzer, Tom A. Rapoport

287. FUSOMORPHOGENESIS: A TALE OF MONSTERS, ELF TAILS AND COLD FUSION
Benjamin Podbilewicz, Tamar Gattegno, Gidi Shemer, Clari Valansi, Irina Kolotuev

288. THE GENE *eff-1* IS REQUIRED FOR CELL FUSION IN EPITHELIAL SYNCYTIA
William A. Mohler, Gidi Shemer, Jacob del Campo, Victoria Scranton, John G. White, Benjamin Podbilewicz

Plenary III
Helen Chamberlin & Rueyling Lin, Chairs

289. Chromosome-Wide Regulation of Meiotic Crossing Over
Kenneth Hillers, Anne Villeneuve

290. AIR-2 and CeGLC-7 Function in Chromosome Cohesion in Meiosis
Eric Rogers, Xiumin Li, Rueyling Lin

291. Molecules underlying meiotic nuclear reorganization and the homolog pairing process.
Amy J. MacQueen, Monica P. Colaiacovo, Anne M. Villeneuve

292. Mitotic Chromosome Condensation and Segregation by a Conserved Protein Complex
Kirsten A. Hagstrom, Raymond Chan, Barbara J. Meyer

293. Chromosome Counting to Determine Sex: X and Autosomal Signals
Jennifer R. Powell, Catherine Pickle, John Gladden, Barbara J. Meyer

294. A novel Goloco motif protein is required for the asymmetric division of one cell stage embryos
Kelly Colombo, Stephan Grill, Tony Hyman, Pierre Gönczy

295. Forces responsible for asymmetric spindle positioning in single cell stage *C. elegans* embryos
Stephan W. Grill, Pierre Gönczy, Ernst H. K. Stelzer, Anthony A. Hyman

296. *C. elegans* MUTANTS IN THE *muc-1* GENE PROVIDE A MODEL FOR THE HUMAN LYSOSOMAL STORAGE DISEASE MUCOLIPIDOSIS TYPE IV
Brad Hersh, Erika Hartwieg, Bob Horvitz

297. Mutations in the *C elegans pqe-1* gene enhance polyglutamine-mediated ASH neurodegeneration
Cindy Voisine, Peter W. Faber, Daphne C. King, Anne C. Hart

298. UNC-83 and UNC-84 function at the nuclear envelope where they are required for proper nuclear migration
Daniel Starr, Chris Malone, Gerg Hermann, William Fixsen, James Priess, H. Robert Horvitz, Min Han

299. THE ACTIN-BINDING PROTEIN UNC-115 ACTS DOWNSTREAM OF RAC-2 IN NEURONAL MORPHOGENESIS
Eric Struckhoff, Jacob Eastman, **Erik Lundquist**

300. Cloning and characterization of *ced-12*/*elmo*, a novel member of the CrkII/Dock180/Rac1 pathway, which is required for phagocytosis and cell migrations
J.M. Kinchen, E. Brugnera, T.L. Gumienny, A. Tosello-Trampont, L.B. Haney, K. Nishiwaki, S. Walk, R. Francis, T. Schedl, Y. Qin, L. Van Aelst, K.S. Ravichandran, M.O. Hengartner

301. CED-12 functions in the CED-10 RAC-mediated pathway to control cell migration and cell-corpse engulfment
Yi-Chun Wu, Miao-Chi Tsai, Li-Chun Cheng,, Chung-Jung Chou, Nei-Yin Weng

302. Execution of necrotic-like cell death in *C. elegans* requires the activity of specific aspartyl proteases
Nektarios Tavernarakis, Keli Xu, Monica Driscoll

303. The *C. elegans* Autosomal Dominant Polycystic Kidney Disease Genes *lov-1* and *pkd-2* act in the same pathway
Maureen M. Barr, Paul W. Sternberg

304. A worm model for polycystic kidney disease
Kaletta, T, Van de Craen, M, Van Geel, A, Buechner, M, Burrow, C, Wilson, P, Bogaert, T

305. CATION DIFFUSION FACILITATOR PROTEINS AND ZINC IONS REGULATE RAS-MEDIATED SIGNALING
Janelle Jakubowski, Kerry Kornfeld

Plenary IV
Russell Hill & John Plenefisch, Chairs

306. Genetics and biochemistry of RNAi in *C. elegans*
Marcel Tijsterman, Rene F. Ketting, Kristy L. Okihara, Titia Sijen, Femke Simmer, Sylvia E. J. Fischer, Gregory Hannon, Ronald H. A. Plasterk

307. Target dependent accumulation of small RNAs during RNAi in *C.elegans*
Alla Grishok, Phillip Zamore, Craig C. Mello

308. A connection between RNAi and development in *C.elegans*
Alla Grishok, **Amy E. Pasquinelli**, Darryl Conte, Gary Ruvkun, Craig C. Mello

309. The maternal-effect gene *clk-2* affects developmental timing, is essential for embryonic development, encodes a protein homologous to yeast Tel2p, and is required for telomere length regulation in *C. elegans*.
Claire Bénard, Brent McCright, Yue Zhang, Stephanie Felkai, Bernard Lakowski, Siegfried Hekimi

310. The *C. elegans clk-2* gene encodes a novel regulator of telomere length and DNA damage response.
Chang-Su Lim, Abby Dernburg, Saira Mian, Kang-Ja Lee, Judith Campisi

311. DAF-9, a cytochrome P450 that influences diapause, reproductive development and longevity
Birgit Gerisch, *Cindy Weitzel*, *Corinna Kober-Eisermann*, *Veerle Rottiers*, *Adam Antebi*

312. Steroid hormone regulation of dauer formation and adult longevity
Kailiang Jia, **Patrice Albert**, Don Riddle

313. Two Pathways for the regulation of lifespan by metabolic genes in C. elegans
Andrew Dillin, Delia Garigan, Douglas Crawford, Aolin Hsu, Andrew G. Fraser, Julie Ahringer, Cynthia Kenyon

314. *C. elegans* life span is extended by a diet of *E. coli* lacking coenzyme Q
Pamela L. Larsen

315. INCREASED DOSAGE OF A *sir-2* GENE EXTENDS LIFE SPAN IN *C. elegans*
Heidi A. Tissenbaum, Leonard Guarente

316. Dramatic changes in chromatin composition associated with dauer formation, caloric restriction, and increased longevity.
Olga Ilkaeva, Chee-Kwee Ea, **James A. Waddle**

317. Aging at the cellular level is tissue specific: the nervous system does not degenerate while muscle and epithelia dramatically decline
Laura A. Herndon, Kristin M. Listner, Justyna M. Dudaronek, Paula Brown, **Peter J. Schmeissner**, David H. Hall, Monica Driscoll

318. Evolution of germ-line signals that regulate growth and ageing in nematodes
Mavji N. Patel, Christopher G. Knight, Constantina Karageorgi, Armand M. Leroi

319. Multiple genetic loci determine sensitivity of *C. elegans* to the deformation-causing pathogen *M. nematophilum*
Maria Gravato-Nobre, Hannah Nicholas, Delia O'Rourke, Debbie Whittington, **Jonathan Hodgkin**

Immunity & infection Workshop Man-Wah Tan, Chair

320. Identifying universal *Serratia marcescens* virulence factors using *C. elegans*.
C. Léopold Kurz, Sophie Chauvet, Emmanuel Andrès, Dominique Ferrandon, Mitchell Uh, Jean Celli, B. Brett Finlay, Jonathan Ewbank

321. Identification of inducible innate immune defences in *C. elegans.*
Gustavo Mallo, C. Léopold Kurz, Samuel Granjeaud, Yuji Kohara, **Jonathan Ewbank**

Microscopy workshop William Mohler, Chair

322. Automatic cell lineage acquisition system and analysis of early embryogenesis
Shuichi Onami, Shugo Hamahashi, Masao Nagasaki, Satoru Miyano, Hiroaki Kitano

323. New Methods for Imaging Endogenous Protein Structures in Live Cells and Tissues: High-Resolution Second Harmonic and Dual-Mode Non-Linear Microscopy
William A. Mohler, Paul J. Campagnola, Pamela E. Hoppe, Christian J. Malone

RNAi Workshop Geraldine Seydoux & Fabio Piano, Chairs

Teaching Workshop Jim Lissemore, Chair

Behavioral Quantitation Bill Schafer, Chair

Anatomy Workshop Zeynep Altun, Chair

WormBase Workshop Lincoln Stein, Chair

Union Biometrica Workshop Dwight Kirpatrick, Chair

Poster A

324. Intrinsically Thermotolerant Substrains Of *C. elegans* Maintain Their IT State, Show Increased Longevity, And Elevated Hsp16 Levels.
Joshua P. Ellwitz, Karen A. McFarland, Rebecca A. Buonpane, Nicole D. Smith, Karen E. Stine, Glenn E. White

325. Genetics of body fat and fat droplets in *C. elegans*
Kaveh Ashrafi, Francesca Chang, Gary Ruvkun

326. Age-related decline spares the nervous system: detailed characterization of cellular changes that accompany aging in wildtype and long-lived nematodes
Justyna M. Dudaronek, Laura A. Herndon, Paula Brown, David H. Hall, Monica Driscoll

327. Effect of Sterols on Life Span and Stress Resistance in *Caenorhabditis elegans*
Eun-Young Lee, Young-Ki Paik

328. Role of sensory cilia and sensory neurons in the regulation of worm lifespan
Joy Alcedo, Javier Apfeld, Bella Albinder, Jennifer Dorman, Honor Hsin, Bernadine Tsung, Cynthia Kenyon

329. *C. elegans* Sarcopenia: Characterization of Changing Muscle Protein Expression Patterns in Aging *C. elegans*
Peter J. Schmeissner, Justyna M. Dudaronek, Laura A. Herndon, Monica Driscoll

330. Molecular analysis of aging in individual nematodes
Tamara Golden, Simon Melov

331. GENE EXPRESSION ANALYSIS OF C. elegans LIFESPAN MUTANTS
Coleen T. Murphy, Cynthia Kenyon

332. Effects of ubiquinone metabolism on *clk-1* biology
Abdelmadjid K. Hihi, Siegfried Hekimi

333. Identification of two novel *clk* mutants
Chang-Su Lim, Kang-Ja Lee, Judith Campisi

334. Developmental control of coenzyme Q levels - the L2 arrest in *clk-1* mutants coincides with an increased reliance on respiratory energy metabolism
Tanya Jonassen, Pamela L. Larsen, Kym Faull, Catherine F. Clarke

335. No reduction of energy metabolism in Clk mutants.
Bart P. Braeckman, Koen Houthoofd, Annemie De Vreese, Kristel Brys, Isabelle Malcorps, Jacques Vanfleteren

336. Distinct mechanisms of Caenorhabditis elegans life extension by daf-2 mutation and by dietary restriction
John Thaden, Stuart Kim, Robert Shmookler Reis

337. Optimising dietary restriction-mediated lifespan extension
Michelle Keaney, David Gems

338. Caloric restriction activates stress resistance and confers life extension
Koen Houthoofd, Bart Braeckman, Annemie De Vreese, Isabelle Malcorps, Kristel Brys, Sylvie Van Eygen, Jacques R. Vanfleteren

339. Molecular mechanisms underlying the effects of caloric restriction on aging in *C. elegans*
Nektarios Tavernarakis, Monica Driscoll

340. DOES DAF-4 ALTERNATIVE POLYADENYLATION GENERATE A RECEPTOR ANTAGONIST?
Cathy V. Gunther, **Donald L. Riddle**

341. A *C. elegans* CREB PROTEIN MODULATES TGF-BETA SIGNALING
Mark Alkema, Bob Horvitz

342. DIN-1, a putative DAF-12 cofactor that regulates the *C.elegans* dauer diapause
Andreas H. Ludewig, Alexander Stein, Adam Antebi

343. A bin's-worth of bounty: SAGE data mining in dauer larvae and mixed-stage transcript profiles
Suzan J. Holt, Donald L. Riddle, Steven J. Jones

344. A Model of Human Niemann-Pick Type C disease in *C. elegans*
Jie Li, Michael Ailion, James H. Thomas

345. Pheromone Regulation of daf-7expression
Scott Kennedy, Gary Ruvkun

346. Searching for new proteins involved in dauer formation by two-hybrid protein interaction mapping.
Muneesh Tewari, Svetlana Busiguina, Patrick Hu, Gary Ruvkun, Marc Vidal

347. Natural variation in dauer formation genes
Michael Ailion, Travis Crump, Takao Inoue, James H. Thomas

348. Variation in dauer formation in *C. elegans*
M.P. Gardner, J.A. Jackson, M.E. Viney

349. DAF-7/ TGF-b expression required for larval development in *C. elegans* is controlled by presumed guanylyl cyclase DAF-11.
Mayumi Murakami, Makoto Koga, Yasumi Ohshima

350. A novel role for LAG-2/LIN-12 signaling in dauer recovery
Jimmy Ouellet, Richard Roy

351. IDENTIFICATION OF TWO GENES THAT CONTROL THE SURVIVAL OF THE MALE-SPECIFIC CEM NEURONS
Hillel Schwartz, Bob Horvitz

352. *sue-1* and *sue-2* are required for HSN death
Mona S. Spector, Dan Hoeppner, Michael O. Hengartner

353. Identification of Genes that Specify the Deaths of the Hemaphrodite-Specific Neurons
Ning Pan, Elizabeth Kimberly, Beryl Hatton, Manisha Shah Dudley, Ute Meisel, and Ding Xue

354. Specification of the Sexually Dimorphic Programmed Cell Death of the CEM Neurons
Phillip Grote, Claudia Huber, Barbara Conradt

355. Analysis of the Specification of the NSM Sister Cell Death: Is CES-1 a Transcriptional Regulator of *egl-1*?
Marion Thellmann, Julia Hatzold, Barbara Conradt

356. pvl-5 prevents Pn.p cell death during C. elegans vulval development.
Pradeep M. Joshi, David M. Eisenmann

357. Inactivation of *hus-1*, a conserved checkpoint gene in *Caenorhabditis elegans*, results in impaired cell cycle arrest and apoptosis induced by genotoxic stress.
E. Randal Hofmann, **Stuart Milstein**, Jennifer Ye, Anton Gartner, Michael O. Hengartner

358. Role of *C. elegans p53* in germ cell apoptosis and embryonic development.
W. Brent Derry, Joel H. Rothman

359. *abl-1* Regulates *C. elegans* Germ-cell Apoptosis
Xinzhu Deng, Alberto Villanueva, Xinhua Lin, Megan Dell, Oliver Hobert, Randy Hofmann, Zvi Fuks, Gian Garriga, Michael O. Hengartner, Richard N. Kolesnick

360. RNA interference, a way to check for inhibitors involved in germline cell death in *Caenorhabditis elegans*
Martin Jaeggi, Andrea Calixto, Stuart Milstein, Andy Fraser, Ravi Kamath, Peder Zipperlen, Maruxa Martinez-Campos, Julie Ahringer, Michael O. Hengartner

361. A screen for genes that control programmed cell death in the germ line
Stuart Milstein, Anton Gartner, Michael Hengartner

362. What factors regulate programmed cell death in the germ line of *C. elegans*?
Nicole Wittenburg, Barbara Conradt

363. Analysis of Torsin Protein Function in *C. elegans*: Towards a Nematode Model for Early-Onset Dystonia
Elaina G. Sexton, Songsong Cao, John Paul Bevel, Kim A. Caldwell, Guy A. Caldwell

364. Characterization of Human Early Onset Torsion Dystonia-Related Genes in *C. elegans*
Christina A. Doyle, Robyn Lints, Laurie Ozelius, Scott W. Emmons

365. A role of sarcoglycans in *C. elegans*
Sushant Khandekar, John Plenefisch

366. Use of C. elegans to study tumour suppressor genes
Laurent Molin, Jean Pierre Magaud

367. Characterization of a *C elegans* homolog of a human tumor biology target
Marsha Smith, Diane Levitan

368. RNAi screen of tumorigenesis-related genes: potential developmental roles
Aaron P. Putzke, Joel H. Rothman

369. NEURONAL SENSITIVITY TO POLYGLUTAMINE-PROTEIN AGGREGATES IN *CAENORHABDITIS ELEGANS* .
Heather Brignull, Richard Morimoto

370. MOLECULAR REGULATORS OF POLYGLUTAMINE AGGREGATE FORMATION IN *C. ELEGANS<i/>*
Susana Garcia, James Morley, Margarida Amaral, Richard Morimoto

371. The C. elegans Homologue of Human Huntingtin Interacting Protein 1 has Multiple Roles
J. Alex Parker, Ann M. Rose

372. Transgenic *C. elegans* overexpressing human alpha-synuclein as a model of Parkinson's disease.
Akihiko Koyama, Keiko Gengyo-Ando, Shohei Mitani, Takeshi Iwatsubo

373. Identification and characterization of the *C elegans* orthologue of the mammalian molecular adaptor Fe65, a cytosolic ligand of Alzheimer's beta-amyloid precursor protein
Marida Bimonte, Salvatore Arbucci, Davide Gianni, Tommaso Russo, Paolo Bazzicalupo, **Nicola Zambrano**

374. The Role of CaM Kinase II and GABA in preventing seizures in *C. elegans*
Allyson V. McCormick, Christine W. Miller, Elizabeth M. Newton, James H. Thomas

375. Genes which regulate social feeding behaviour in *C.elegans*
Nicola Tremain, Mario de Bono

376. FOOD-DEPRIVATION AND MODULATION OF BEHAVIOR: *mod-6* AND A SCREEN FOR NEW GENES
Daniel Omura, Rajesh Ranganathan, Bob

Horvitz

377. ACM-2, a neuronal muscarinic acetylcholine receptor, contributes to the regulation of feeding
Kate Steger, Leon Avery

378. Genetic analysis of the neuromodulatory control of search behavior in *C. elegans*
Thomas Hills, Fred Adler, Andres Villu Maricq

379. Isolation of mutants which exhibit social feeding behaviour
Gemma Brown, Mario de Bono

380. Genes regulating social feeding behaviour in *C elegans*.
Benny H. H. Cheung, Mario de Bono

381. Neurons regulating social feeding behaviour in *C. elegans*
Juliet C. Coates, Mario de Bono

382. Isolation and analysis of mutants abnormal in orientation to food.
Takashi Murayama, Makoto Koga, Yasumi Ohshima

383. A Genetic Screen for Components of the G Protein Signaling Pathways that Control Egg-Laying Behavior in *C. elegans*
I. Amy Bany, Michael Koelle

384. Mapping the site of action of G protein signaling components in *C. elegans* egg-laying system
Meng-Qiu Dong, Judy Pepper, Michael R. Koelle

385. Serotonin modulates locomotory behavior and coordinates egg-laying and movement in *C. elegans*
Laura Anne Hardaker, Emily Singer, Rex Kerr, William R. Schafer

386. Electrophysiologic characterization of cultured *C. elegans* mechanosensory neurons
Michael Christensen, Rebecca Morrison, Kevin Strange

387. Functional characterization of cultured *C. elegans* body muscle cells and cholinergic motor neurons
Ana Y. Estevez, Michael Christensen, Rebecca Fox, Rebecca Morrison, David Miller, Kevin Strange

388. Basic electrophysiological properties of body wall muscle from the Nematode *C elegans*.
Maelle Jospin, Laurent Segalat, Bruno Allard

389. Engineering pharyngeal pumping behaviors in *C. elegans*
Sarah Straud, Leon Avery

390. Ionic channels recorded from patches excised from the soma of the chemosensory neurons AWA and AWC
W T Nickell, S J Kleene

391. ELECTROPHYSIOLOGICAL ANALYSIS OF *RIC-3*
Mark T. Palfreyman, Erik M. Jorgensen

392. Olfactory Coding in *C. elegans* Requires Modulatory G_{alpha} proteins.
Paul D. Wes, Gert Jansen, Cori I. Bargmann

393. Analysis of octanol avoidance mutants and serotonin modulation of the ASH sensory circuit
Hana Sugimoto, Heather Dionne, Hidetoshi Komatsu, Tahira Sharmeen, Rhonda Hyde, Anne C. Hart

394. Mechanisms involved in regulating olfactory receptor expression
Katie Nolan, Trina Sarafi-Reinach, Jen Horne, Maura Berkeley, Anne Lanjuin, Piali Sengupta

395. Characterizing the Neural Circuitry of Chemotaxis to Volatile Odorants
Jesse Gray, Cori Bargmann

396. Sniffing out the mechanisms of ODR-7 function in AWA neurons
Marc E. Colosimo, Trina R. Sarafi-Reinach, Piali Sengupta

397. Characterization of the *E. coli*-induced suppression of olfactory habituation
William M. Nuttley, Karen Atkinson-Leadbeater, Derek van der Kooy

398. Isolation and analysis of chemotaxis mutants in *C. elegans*
Makoto Koga, Yasumi Ohshima

399. A screen for suppressors of the *let-60* chemotaxis defect
Yuichi Iino, Timothy Doyle, Takaaki Hirotsu

400. THE A, B, C s OF CHEMOSENSORY NEURON SPECIFICATION
Tali Melkman, Piali Sengupta

401. Identification and Characterization of Genes Required in a Cell-Cell Signaling Event that Results in Asymmetric Odorant Receptor Expression in *C. elegans*
Miri VanHoven, Alvaro Sagasti, Sarah Bauer, Stephanie Albin, Cori Bargmann

402. The regulation of sensory cilia specific genes in *C. elegans*
Peter Swoboda, James H. Thomas

403. The application of novel positional cloning strategies for forward genetics: the case of dyf-8.
Stephen R. Wicks, Erik Lyman, Kim Schuske, Erik Jorgensen, Ronald H.A. Plasterk

404. Olfactory G-protein signalling in *C. elegans*
Hannes Lans, Gert Jansen

405. A reverse genetic approach to neuropeptide function in the ASH sensory circuit
Jamie White, Anne Hart

406. Activity-dependent transcription in the AWA sensory neuron: the roles of *osm-9*, *ky440*, and other transduction molecules
Amanda Kahn, David Tobin, Cori Bargmann

407. Transient disruption of IP3 receptor function in *C. elegans*.
Denise S. Walker, **Nicholas J.D. Gower**, Gemma L. Bradley, Howard A. Baylis

408. Characterization of rcn-1, a calcipressin homologue in *C. elegans*
Jin Lee, Jaya Bandyopadhyay, Tami Kingsbury, Kyle Cunningham

409. Calcineurin Functions in C. elegans: growth and memory
Jiyeon Lee, Jaya Bandyopadhyay, Sunki Jung, Changhoon Jee, Joohong Ahnn

410. Characterization of synaptic defects in mau mutants
J-A Boudier, S. Hekimi, **M. Garcia**

411. Isolating redundant pathways that regulate synapse formation in *C. elegans*
Edward Liao, Vijhee Vijayaratnam, Mei zhen

412. *syd-5* may play a role in regulating synapse size in *C. elegans*
Renee Baran, Yishi Jin

413. *syd-2* and *rpm-1* function synergistically in synapse and muscle development
Mei Zhen, Xun Huang, Yishi Jin

414. Optical characterization of the vesicle pool size in ventral cord synapses
Jeremy Dittman, Joshua Kaplan

415. A screen to identify regulators of DGK-1
Cheryl Copelman, Stephen Nurrish

416. Identifying regulators of DGK-1.
Paul Morrison, Stephen Nurrish

417. A motoneuron-derived signal is required for differentiation of post-synaptic domains at GABAergic neuromuscular junctions in *C. elegans*
Christelle Gally, Jean-Louis Bessereau

418. Characterization of the *shn-1*, shank homologue in *C. elegans*
Changhoon Jee, Yoonsung Na, Joohong Ahnn

419. Characterization of Candidate Genes for Postsynaptic Proteins in *C. elegans* Nervous System.
Hidehito KUROYANAGI, Shigeo OKABE

420. Characterization of UNC-31 (CAPS) in C. elegans neurotransmission
Kim Schuske, Janet Richmond, Liakot Kahn, Hiroko Kouike, Erik Jorgensen, Kouichi Iwasaki

421. TOWARDS IDENTIFICATION OF *TRANS*-FACTORS REGULATING NEURON-SPECIFIC EXPRESSION OF THE *UNC-18* GENE
Shin-ichi Harada, Ryuji Hosono

422. The role of unc-86 in the development of serotonergic neurons
Jie Li, Ji Ying Sze

423. Identification of target genes of a transcription factor UNC-86.
Aki Yonezumi, Keiko Gengyo-Ando, Etsuko Machiyama, Sachiko Noguchi, **Shohei Mitani**

424. Cloning of a collagen which functions specifically during sensory organ morphogenesis in *C. elegans*.
Raymond Y.L. Yu, **King L. Chow**

425. Development of Left/Right Asymmetry in the Nervous System
Sarah Chang, Oliver Hobert

426. THE FAX-1 NUCLEAR HORMONE RECEPTOR FUNCTIONS DOWNSTREAM OF OTHER TRANSCRIPTIONAL REGULATORS TO SPECIFY NEURON IDENTITY
Tilak Sundaresan, Sheila Mathieson, Kristy Reinert, Elissa Murphy, Jessica Tanis, Nick Bianco, Ryan Martin, Yelena Vidgop, Bruce Wightman

427. Isolation of mutants defective in AFD thermosensory neuron development
Hiroyuki Sasakura, Hitoshi Inada, Ikue Mori

428. MEMBRANE LOCALIZATION OF THE ACTIN-BINDING PROTEIN UNC-115 CAUSES NEURONAL MORPHOGENESIS DEFECTS
Yie Yie Yang, Erik Lundquist

429. Two-hybrid Screen for UNC-44 AO13 Interacting Proteins
Ping Gong, Dina Darwis, Brent Bill, Jorge Munera, Jerry Perschall, Janna Lindemulder, Anthony J. Otsuka

430. A neuronal-specific classical cadherin encoded by the *hmr-1* locus is involved in axon patterning.
Ian D. Broadbent, J. Pettitt

431. *ast-1* and *ast-2*, novel genes important for fasciculation of axons in the ventral cord
Christina Schmid, Edward M. Hedgecock, Harald Hutter

432. RNAi MEDIATED DISRUPTION OF *VEM-1*, A NOVEL MEMBRANE-ASSOCIATED PROTEIN, PERTURBS THE PATTERNING OF A SUBSET OF AXONS IN THE VENTRAL NERVE CORD
Erik Runko, Zaven Kaprielian

433. Isolation and Characterization of Genes Involved in Motor Axon Sprouting
Nehal Mehta, Paula Loria, Oliver Hobert

434. GENETIC ANALYSIS OF AXON BRANCHING IN C. ELEGANS
Antonio Colavita, Marc Tessier-Lavigne

435. DNA arrays and mutations that reverse the direction of the ALM cell migrations
Nicole Toms, Jennifer Cooper, Brandi Patchen, **Eric Aamodt**

436. An expression pattern study of *mig-10*, a gene required for nervous system development in *C. elegans*
Maegan V. Rivard, Diana Rusiecki, James Manser, Elizabeth F. Ryder

437. *C. elegans* EMAP-like protein (ELP-1) is a microtubule-binding protein.
Kevin V. King, Jennifer L. Hueston, Kathy A. Suprenant

438. Molecular characterization of a novel microtubule binding protein
Jennifer Hueston, Kevin King, Eric Lundquist, Matthew Buechner, Kathy Suprenant

439. A *C.elegans* JSAP/JIP3 homolog, UNC-16, interacts with kinesin light chain, KLC-2
Rie Sakamoto, Masato Kawasaki, Dana Byrd, Yishi Jin, Naoki Hisamoto, Kunihiro Matsumoto

440. Microtubule based conventional kinesin activity is coupled to the actin-myosin system via an atypical kinesin
M. Yusuf Ali, Yoji Kohara, Shahid Siddiqui

441. Characterization of FERM-domain protein homologs in C. elegans
Peter Barrett, John Fleming, Olaf Bossinger, Verena Göbel

442. *vab-21* functions in epidermal morphogenesis and may encode intermediate filament protein IF-B1
Wei-Meng Woo, Andrew D. Chisholm

443. Functions of intermediate filaments and their regulatory proteins during embryonic tissue formation
Hiroshi Qadota, Katsuhisa Kasuya, Daisuke Tsuboi, Makiko Inoue, Mutsuki Amano, Kozo Kaibuchi

444. The cytoskeletal linker ERM is required for the integrity of epithelial luminal surfaces of internal organs in *C. elegans*
Verena Göbel, Peter Barrett, David H. Hall, Olaf Bossinger, John Fleming

445. *mir-1* and *mir-2* exhibit defects in pronuclear migration and spindle orientation in the P_0 stage *C. elegans* embryo
Jennifer B. Phillips, Rebecca Lyczak, and Bruce Bowerman

446. Spindle Dynamics and the Role of Gamma-tubulin in Early *C. elegans* Embryos
Susan Strome, James Powers, Melanie Dunn, Kimberly Reese, Christian J. Malone, John White, Geraldine Seydoux, William Saxton

447. Spindle rotation in the early *C. elegans* embryo
Amanda J. Wright, Craig P. Hunter

448. *spn-3*: A GENE INVOLVED IN SPINDLE POSITIONING
Leah R. DeBella, Lesilee S. Rose

449. *or358ts* is involved in mitotic spindle orientation and positioning in the one-cell stage *Caenorhabditis elegans* embryo
Sandra Encalada, Rebecca Lyczak, Bruce Bowerman

450. Genetic and Phenotypic Analysis of *spd-3* : A Mitotic Spindle Defective Mutant in *Caenorhabditis elegans*
Maria Vidal, Kevin O'Connell, John G. White

451. Three-dimensional reconstruction of the early C. elegans mitotic spindle
Thomas Müller-Reichert, Kent McDonald, Anthony Hyman

452. CeMCAK, a *C. elegans* kinesin with functions in meiosis and mitosis.
Debra Rose, Claire Walczak, Susan Strome, William Saxton

453. Cytoplasmic Dynein in the Early Embryo
Diane Schmidt, William Saxton, Susan Strome

454. The dynactin subunit, *dnc-1* (*or404ts*), is required for mitotic spindle orientation during early*C. elegans* embryogenesis
John Willis, Bruce Bowerman

455. Characterization of *rot-2*, a gene required for proper P_0 Spindle Orientation
Gregory C. Ellis, Rebecca Lyczak, Bruce Bowerman

456. Analysis of *spd-1*, a gene required for mitotic spindle integrity and cytokinesis in *C elegans*
Koen Verbrugghe, John G. White

457. A gain-of- function mutation in *cdc25.1* phosphatase causes intestinal-specific cell cycle aberrations.
Ivana Kostic, Richard Roy

458. GENETIC SCREEN FOR FZY-1 ALLELE
Lois Tang, Elaine Law, Risa Kitagawa, Ann M. Rose

459. Investigation of the function of Ran by RNAi
Peter Askjaer, Eva Hannak, Anthony A. Hyman, Iain W. Mattaj

460. Regulation of the exit from mitosis in C.elegans.
Ulrike Grüneberg, Anton Gartner, Erich Nigg

461. Screen for mutants defective in membrane deposit during cytokinesis
Agathe Authaler, Karsten Strauss, Florian Deyhle, Peter Heger, **Anne Spang**

462. Analysis of single-cell cytokinesis-defective mutants in *C. elegans*
Todd Heallen, Matt Wallenfang, Geraldine Seydoux, Jill M. Schumacher

463. Investigating the possibe role of calmodulin in cytokinesis
Ellen Batchelder, John White, Jeff Walker

464. Assessing the role of microtubules in the establishment of the first asymmetric cell division of the *C. elegans* embryo
Jean-Claude Labbé, Paul S. Maddox, E. D. Salmon, Bob Goldstein

465. Identification and characterization of *lin-14* genetic interactors
Diya Banerjee, Alvin Kwok, Gary Ruvkun, Frank J. Slack

466. A *C. elegans* Chromokinesin Required for Chromosome Segregation
Jim Powers, William Saxton, Susan Strome

467. Understanding the Role of a Cyclin B Homologue during Postembryonic Development in *Caenorhabditis elegans*
Shaolin Li, Richard Roy

468. The beta-tubulin gene, *tbb-2* is an activator of the *C. elegans* female meiosis specific genes, mei-1 and mei-2
Chenggang Lu, Paul E. Mains

469. Separase is required for the segregation of homologous chromosomes in meiosis I
Maria Siomos, Pawel Pasierbek, Michael Glotzer, Kim Nasmyth

470. A transgenic line produces post-prophase I chromosome segregation abnormalities in the female germline
Lynnette Kuervers, David Baillie

471. Control of M-Phase Entry during Oocyte Meiotic Maturation
Ikuko Yamamoto, David Greenstein

472. *daz-1* , a *C. elegans* homolog of DAZ (Deleted in Azoospermia), localizes to the cytoplasm of mitotic germ cells, and required at the entry phase of meiosis during oogenesis
Rika Maruyama, Takeshi Karashima, Asako Sugimoto, Masayuki Yamamoto

473. Hyper-resistance of meiotic cells to radiation due to a strong expression of a single *recA*-like gene in *Caenorhabditis elegans*
Takako Takanami, Hideyuki Takahashi, Atsushi Higashitani

474. him-8 and him-5
Philip M. Meneely, Navid Sadri, Anna Farago, Alex Ensminger, J. Robert Hogg, Sarah Johnson, Eirene Kontopoulos, Charles Phillips, Toni Amato, Bipin Subedi

475. Special handling for the X chromosome in meiosis
Kirthi Reddy, Linwah Yip, Monica Colaiacovo, Gillian Stanfield, Valerie Reinke, Stuart Kim, Anne Villeneuve

476. Genetic interplay between *rad-51* and other genes involved in meiosis
Cinzia Rinaldo, Paolo Bazzicalupo, Massimo Hilliard, Adriana La Volpe

477. Identifying new genes that function in meiotic DNA repair and double-strand break initiation
Gregory Chin, Anne Villeneuve

478. Regulation of gene expression, cellular localization, and in vivo function of *Caenorhabditis elegans* DNA topoisomerase I
Myon Hee Lee, Hyungki Park, Gaegal Shim, Junho Lee, Hyeon-Sook Koo

479. The yolk proteins and the vitellogenin receptor of *Rhabditis (Oscheius) pseudodolichura*, strain CEW1
C. Penha-Scarabotto, J.P. Moura, J.C. Serino Jr., R.B. Zingali, **C.E. Winter**

480. The ClC channel CLH-3 is activated during meiotic maturation in *C. elegans oocytes*
Eric Rutledge, Christoph Boehmer, Michael Miller, David Greenstein, Kevin Strange

481. Regulation of the ClC channel CLH-3 by serine/threonine dephosphorylation
Eric Rutledge, Kevin Strange

482. Control of Oocyte Meiotic Maturation and Gonadal Sheath Cell Contraction by MSP Signaling
Mary Kosinski, Michael A. Miller, David Greenstein

483. Reverse genetic analysis of a ubiquitin C-terminal hydrolase gene and an hnRNP homologue gene, which are important for gametogenesis in *C. elegans*.
Takeshi Karashima, Ikuma Maeda, Yuji Kohara, Asako Sugimoto, Masayuki Yamamoto

484. The *spe-5* Gene Encodes a Vacuolar (H+)-ATPase Beta Subunit that is Required for Spermatogenesis
Paul D. Hartley, Guang-dan Zhu, Steven W. L'Hernault

485. Progress towards the cloning of *fer-14*, a gene required for fertilization in *C. elegans* sperm
Todd Lamitina, Steven W. L'Hernault, Ph.D.

486. *spe-39* and Orthologs: A New Player in Intracellular Membrane Morphogenesis in Metazoans
Guang-dan Zhu, Steven W. L'Hernault

487. A *C elegans* Inositol 5- Phosphatase Homologue Involved In Inositol 1,4,5-triphosphate Signaling and Ovulation.
Yen Kim Bui, Paul W. Sternberg

488. The F-box protein FOG-2 binds the *C. elegans* multi-ubiquitin chain binding protein-1 (Mcb1) homolog to promote spermatogenesis in the hermaphrodite
Sudhir Nayak, Edward T Kipreos, Tim Schedl

489. The role of SPE-9 in C. elegans sperm-egg interactions.
Sonia Zannoni, Emily Putiri, Andrew Sinsgon

490. MAPPING spe-19; FURTHER PROGRESS, NEW APPROACHES
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491. Screening for sperm competition mutants
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Martin L. Hudson, David S. Karow, Kate H. Riviere, Michael A. Marletta, David B. Morton

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Kristin Simokat, Jeffrey S. Simske, Lois Edgar, William Wood, Jeff Hardin

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500. Molecular analysis of *pel-2* and *pel-3*, two genes required for embryonic pharynx differentiation and morphogenesis
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501. Comparative and functional analyses of the novel pharyngeal factor PEB-1
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502. Involvement of septins in development of the pharynx
Fern P. Finger, John G. White

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Jay D. Kormish, James D. McGhee

504. Analysis of *glo-1* which lacks autofluorescent and birefringent intestinal granules
Greg J. Hermann, Russell J. Hill, James

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Tetsunari Fukushige, Helen Tian, Barbara Goszczynski, Jim McGhee

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Keith Strohmaier, Joel H. Rothman

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509. The three *C. elegans* beta-catenin homologs make distinct protein interactions but retain functional redundancy *in vivo*
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511. Identification of discs large binding partners during epithelial development in C.elegans
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512. The role of erm-1 during zonula adherens formation in the gut epithelium of C. elegans
Daniela van Fürden, Carin Theres, Verena Gobel, **Olaf Bossinger**

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Bonnie L. Firestein, **Christopher Rongo**

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515. Identification of interacting molecules with GEI-4, a possible regulatory protein of intermediate filaments in *C. elegans*
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J.A.Smith, J.D.McGhee, J.S.Gilleard

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Mark E. Sheffield, Tim H. Lindblom, Jeff Hardin

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Anna P.Newman, Hediye Nese Cinar, Keri L. Sweet, Kavita Oommen

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Byung-Kwon Choi, Young-Ki Paik

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Nadeem Moghal, L. Rene Garcia, Paul W. Sternberg

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530. Genetic approaches to identify vulva-specific targets of LIN-39
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532. Genetic study of connectivity of ray sensory neurons in the *C. elegans* male
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534. The roles of a Semaphorin and an ephrin in patterning cell-cell contacts in the C. elegans male tail
Andrew C. Hahn, Scott W. Emmons

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Joanne C.N. Tam, K. Wah Chu, **Frankie C.F. Ko**, Raymond Y.L. Yu, King L. Chow

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S. Wa Tsang, **King L. Chow**

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Frankie C.F. Ko, King L. Chow

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Merav Cohen, Katherine L. Wilson, Yosef Gruenbaum

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Mike Shiffler, Guy A. Caldwell

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Shelli N. Williams, Kim A. Caldwell, Guy A. Caldwell

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Kim A. Caldwell, Jenny M. Whitworth, Shelli N. Williams, Guy A. Caldwell

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Kenneth K. Lee, Yosef Gruenbaum, Jun Liu, Katherine L. Wilson

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Merav Gazit, Orly Bar-Shalev, Avital Enosh, **Yonatan B. Tzur**, Kenneth K. Lee, Merav Cohen, Katherine L. Wilson, Susan Strome, Yosef Gruenbaum

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Jun Liu, Tom Rolef Ben-Shahar, Dieter Riemer, Millet Treinin, Perah Spann, Klaus Weber, Andrew Fire, Yosef Gruenbaum

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Ned Buttner, Bob Horvitz

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550. Modeling peroxisome biogenesis disorders and identification of novel peroxisomal proteins in *C. elegans*
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551. A *Caenorhabditis elegans* catalase with an unusual feature
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553. Emergence of an Ancient Intracellular Antiproteinase Defense System
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554. Activation of protein degradation in muscle: A non-transcriptional output of the Ras-MAP kinase signaling pathway
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Limor Broday, Thomas Buschmann, Stuart Milstein, Lila Pirkkaka, Michael Hengartner, Ze'ev Ronai

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Joanna Daulby, Nigel M. Hooper, Ian A. Hope, David Coates

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Keiko Hirono, Kyoko Nakata, Yumiko Ueta, Mina Iwata, Masahiro Ito, Yuji Kohara

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Joshua Snow, Claudia Morales, Rhonda Moore, Lynn Boyd

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Alan M. Zahler, A. Brock Roller, Jordan Benjamin, Tracy Farrer, W. James Kent

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Jeffrey R. Patton, Richard W. Padgett

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Cynthia DeRenzo, Kim Reese, Geraldine Seydoux

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Chun-Chieh Chen, Erbay Yigit, Hiroaki Tabara, Craig C. Mello

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Hiroaki Tabara, Erbay Yigit, Darryl Conte, Susan Parrish, Andrew Fire, Craig C. Mello

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Razia Ruksana, Hiromi Terami, Akwasi Anyanful, Yasuji Sakube, Hiroaki Kagawa

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M. Afaq Shakir, Bob Campbell

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Ralph Menzel, Thierry Bogaert, Rudolf Achazi

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584. Morphological Evolution of Rhabditidae
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Scott Everet Baird

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Krystalynne Morris, Dee R. Denver, Avinash Kewalramani, Katherine Harris, Suzanne Randall Estes, Michael Lynch, W. Kelley Thomas

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Waltraud Sinz, Jagan Srinivasan, Ralf.J.Sommer

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Michael Palopoli, William Soares, Dave Young

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Craig W. LaMunyon, Samuel Ward

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592. USING *C. ELEGANS* IN THE UNDERGRADUATE LABORATORY CLASSROOM: PROJECT LAB AND GENETICS
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593. A simple lab using single worm PCR and *dpy-5*
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594. An Upper-level Undergraduate Project-Based "Laboratory in Genetics" Course using *C. elegans*
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595. Using *C elegans* to teach genetics: SNP mapping in three weeks!
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596. Collaborative Research with Undergraduate Students: An Assembly Line Model
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597. Surfing the Genome: Using the *C. elegans* Genomic Sequence to Teach Molecular Genetics
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598. THERE'S A HUMP IN YOUR BACK!! RNA INTERFERENCE IN THE CLASSROOM
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599. Using PCR in an undergraduate lab course to detect deletions in the unc-93 gene
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600. *Caenorhabditis elegans* in the Undergraduate Physiology Curriculum
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601. A discovery oriented laboratory: from gene to mutant in the classroom
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602. Vesicle Transport and Fusion: An Organismal and Cell Biological Study for an Undergraduate Laboratory Exercise
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603. *Caenorhabditis elegans* as a Model Host for Human Bacterial Pathogens
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604. *Caenorhabditis* species as an infection model for the investigation of genes conserved between pathogens and their hosts
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605. A bummed pathogen meets with resistance: Towards the characterization and cloning of *bus-3* and *bus-5*.
Karen Yook, Tatiana Akimkina, Jonathan Hodgkin

606. Molecular cloning of *C. elegans bus* genes using Transposon Insertion Display.
Delia O'Rourke, Hannah Nicholas, Maria Gravato-Nobre, Jonathan Hodgkin

607. Developing genetic methods for a novel *C. elegans* pathogen, the bacterium *Microbacterium nematophilum*
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608. *C. elegans – M. nematophilum* interaction: Isolation and characterization of disease resistance mutants
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609. Multiple defects in a *C. elegans* mutant resistant to infection by *M. nematophilum*
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610. Understanding Bt-toxicity: Molecular and Cellular Analysis of Bt-resistance genes.
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611. Effects of *Stenotrophomonas maltophilia* on *Caenorhabditis elegans* and *Heterodera schachitii*.
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612. Insulin/IGF-like peptides of C. elegans
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613. *daf-2* signalling into the cell: looking for the second pathway
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616. Molecular Identification of Transcriptional Targets of the DAF-16 Winged Helix Transcription Factor
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619. Dauers: what makes them long-lived?
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621. Dissecting the Signaling Pathways Regulated by the PTEN Tumor Suppressor Homolog DAF-18 in *C. elegans*
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622. Nuclear Lamina Characterization in *C. elegans* during Apoptosis
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623. A *ced-4* SUPPRESSION SCREEN TO IDENTIFY NEW GENES INVOLVED IN PROGRAMMED CELL DEATH
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624. Identification of transcriptional regulators of the cell-death activator gene *egl-1*
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625. Identification and characterization of *cps-4* and *cps-5*
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626. TWO NEW CELL-DEATH GENES AND ENGULFMENT CONTRIBUTE TO CELL KILLING
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627. Phagocytosis of necrotic corpses—a new gene involved in NCD corpse disposal
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Xiaochen Wang, Duncan Ledwich, Valerie A. Fadok, Ding Xue

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Miwa Furuya, Asako Sugimoto, Joel H. Rothman, Masayuki Yamamoto

631. Analysis of the *C. elegans* homolog of the mammalian autophagy gene *beclin 1*
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632. Oxygen Deprivation in *Caenorhabditis elegans*
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Ellen Nollen, Frederic Gabriel, Jim Morley, Sanjeev Satyal, Kyle Duke, Stuart Kim, Richard I. Morimoto

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Jaegal Shim, Jae Young Kwon, Junho Lee

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Tadao Inoue, Youji Fukunaga, Yo Tabuse, Johji Miwa, Yasunori Yamaguchi

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Yvonne M. Hajdu-Cronin, Wen J. Chen, Paul W. Sternberg

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Joohong Ahnn, Byung-Jae Park, Sun-ki Jung, Duk-Gyu Lee, Jae-Ran Yu, Andrew Singson, Jaya Bandyopadhyay

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645. Characterization of the 13 *C. elegans* RGS Proteins: A reverse genetics approach
Heather Hess, Michael Koelle

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647. Genetic analysis of the Rap1-pathway in C. elegans
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Carol A. Bastiani, Wen J. Chen, Melvin I. Simon, Paul W. Sternberg

650. Type I and Type II phosphatidylinositol phosphate kinases have diverse functions in *C. elegans*
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651. A Novel Ser/Thr Protein Kinase is Involved in the Genesis of Alae
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653. Lov neurotransmission
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654. Characterization of the sperm transfer step of male mating behavior of C. elegans : Two sides to the story.
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Allyson Whittaker, Gary Schindelman, Shahla Gharib, Paul Sternberg

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Gunnar A. Kleemann, Alexandra L. Basolo

658. NOVEL ALLELES OF *SUP-9*, WHICH ENCODE A PUTATIVE K+ CHANNEL, REVEAL DEFECTS IN MALE MATING
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Dattananda Chelur, Marty Chalfie

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664. Developmental regulation of a novel outwardly rectifying mechanosensitive anion current, $I_{Cl,mec}$, in embryonic cells of *C. elegans*
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665. The ClC Chloride Channel CLH-5 Plays a Role in Mechanosensation
Laura Bianchi, Elizabeth M. Link, Alfred L. George, Jr.

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667. GENETIC AND PHENOTYPIC ANALYSES OF HABITUATION ABNORMAL MUTANTS
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668. IDENTIFICATION OF DIFFERENTIALLY EXPRESSED GENES ACCOMPANIED BY HABITUATION
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669. GENES SHOWING ALTERED EXPRESSION ACCOMPANIED BY HABITUATION
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670. Mechanosensory Signaling from the ASH Sensory Neurons: The Role of the GLR-2 Ionotropic Glutamate Receptor Subunit
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671. EVIDENCE FOR A *CHA-1*-SPECIFIC PROMOTER IN *C. ELEGANS*
Dennis Frisby, Tony Crowell, John McManus, Jim Rand

672. A *mod-5* SUPPRESSION SCREEN FOR GENES INVOLVED IN SEROTONERGIC NEUROTRANSMISSION
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673. Measuring neurotransmitter levels in isolated *C.elegans* vesicles by HPLC.
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674. Analysis of Fluoxetine Resistant Mutants of *C. elegans*
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675. Calcium imaging of vulval muscle activity in egg-laying behavior
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676. Suppressors that recover daf-7::gfp expression in tph-1(mg280)
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677. Characterization of Several G protein-coupled Receptors for the Secretin Family of Neuropeptides
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678. A screen for new Hic mutants
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679. Specificity of G-protein signaling in taste perception by the nematode *Caenorhabditis elegans*
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680. Neurotransmitter transporter genes in *C. elegans*.
Gregory P. Mullen, Gary Moulder, Robert Barstead, Jim Rand

681. A Genetic Interaction Between a Neurotransmitter Transporter and Synaptobrevin
GM Sandoval, G Ruvkun

682. Deletion of the dopamine plasma membrane transporter and its effect on behavior
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683. A Dopamine Receptor in *C. elegans*
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684. Identification and cloning of two putative novel excitatory GABA receptor subunits in *C. elegans*.
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685. Characterizing the *C. elegans* Ionotropic Glutamate Receptor GLR-5
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686. Characterization of Ionotropic Glutamate Receptor Subunits GLR-3 and GLR-6
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687. Identification of functional ion channel domains of *C. elegans* glutamate receptor subunits
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688. Alternative isoforms and mutants of the *glr-2* ionotropic glutamate receptor
Rachel Aronoff, Jerry Mellem, Andres Villu Maricq, Rolf Sprengel, Peter Seeburg

689. Identification of New Components in the *C. Elegans* PKD Pathway
Doug Braun, Maureen M. Barr

690. The physiological roles of AVR-14 in *C. elegans* and the parasite, *Haemonchus contortus*.
Nathalie Aptel, Alan Cook, Darrel Pemberton, Virginia Portillo, Candida Rogers, Lindy Holden-Dye, Adrian Wolstenholme

691. Expression of neuronal alpha-7-like nicotinic acetylcholine receptors in *C. elegans*
Karen K. Deal, **David M. Miller**

692. *eat-18* is required for nicotinic neurotransmission in the pharynx
Jim McKay, David Raizen, Leon Avery

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Denise S. Walker, Sung Ly, Katie Lockwood, **Nicholas J.D. Gower**, Howard A. Baylis

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E. Culetto, H.A. Baylis, J.E. Richmond, M.D. Squire, J.T. Fleming, J.A. Lewis, D.B. Sattelle

695. Towards understanding the cell biology of nicotine adaptation in Caenorhabditis elegans
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696. Identifying Modulators of Voltage-Gated Calcium Channels
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697. PHYSIOLOGICAL ROLE OF K-CL COTRANSPORTERS IN C. ELEGANS
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698. CHARACTERIZING THE NEURONAL FUNCTION OF THE SUP-9/SUP-10/UNC-93 TWO-PORE K+ CHANNEL COMPLEX
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699. CARGO RECOGNITION BY SYNAPTIC VESICLE KINESIN
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700. Movement of IDA-1::GFP-tagged vesicles in *C. elegans* nerve processes
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701. Cloning and characterization of a 'putative' transport defective mutant that mis-accumulates synaptic vesicle proteins in *C. elegans* neurons
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702. Direct Visualization of the Movement of Motor Proteins and their Cargo within Neurons of Living *C. elegans*
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704. Sensory axon guidance defects in *C. elegans*
Susan Kirch, Gage Crump, Cori Bargmann

705. *C. elegans* zfh-1, a zinc finger homeobox transcription factor involved in axon guidance
Irene Wacker, Irene Tham, Edward M. Hedgecock, Harald Hutter

706. RNAi screen to identify genes involved in axon guidance
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Douglas S. Portman, Oliver Hobert, Scott W. Emmons

708. *max-3(ju255)*, a new gene required for motoneuron axon guidance
Chris Suh, Jason McEwen, Yishi Jin

709. AHR-1 and netrins: Examining potential interactions during neuronal migration
Hongtao Qin, **Jo Anne Powell-Coffman**

710. THE IDENTIFICATION OF PROTEINS THAT INTERACT WITH UNC-73/TRIO IN *C. ELEGANS*
Robert M. Steven, Terry Kubiseski, Lijia Zhang, Joseph Culotti, Tony Pawson

711. A Semaphorin and a VEGF system controling morphogenesis in C.elegans.
Gratien Dalpe, Lijia W.Zhang, Enza Colonna, Marina Tarsitano, Paolo Bazzicalupo, Tetsunari Fukushige, Jim McGhee, Joe Culotti, Graziella Persico

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Myeongwoo Lee, Erin Cram, Bing Shen, Jean E Schwarzbauer

713. *mig-23* encodes an apyrase similar to the mammalian Golgi UDPases
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714. The role of the egg shell in cell migrations in the early *C. elegans* embryo
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715. A Screen for Suppressors of Distal Tip Cell Migration Defects Induced by Ectopic *unc-129* Expression
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716. ADM-1 AND ADM-2: DISINTEGRIN-METALLOPROTEASES IN CELL MIGRATION
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717. Roles of the ephrin EFN-4 and the semaphorin MAB-20 in embryonic morphogenesis
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718. A SCREEN FOR REGULATORS OF BAR-1 MEDIATED WNT SIGNALING IN THE Q NEUROBLAST LINEAGE
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719. How does *mig-13* guide anterior migrations?
QueeLim Ch'ng, Cynthia Kenyon

720. An *in vitro* System for Studying Cell Movements in Early Embryos
Jen-Yi Lee, Bob Goldstein

721. CHARACTERISATION OF MUTANT ALLELES INVOLVED IN C.ELEGANS CUTICLE FUNCTION
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722. The structural role and interactions of the DPY-7 cuticular collagen in the exoskeleton of Caenorhabditis elegans.
Martyn Quinn, Iain Johnstone

723. Localization of innexins
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724. Gap junction protein INX-13 is essential for excretory cell function.
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725. Characterization of *C. elegans* claudins
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726. Characterization of the leucine-rich repeat protein family in *Caenorhabditis elegans*
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Lars Nilsson, Simon Tuck

728. Keep Your Insides In and Your Outsides Out: VWFA Domain Proteins in the *daf-2* Cuticle and the Male Intestine
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729. Intexin (*itx*), a novel member of the Caspr family delineates the basolateral domain of intestinal cells and is found in ensheathing glia in the head
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730. Exploring the role of *zyg-11* in one cell stage embryos
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731. Unraveling the role of calcium signaling in the early *C elegans* embryo
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732. THE *C.ELEGANS* PROTEIN PHOSPHATASE 1 HOMOLOGUE(*CE*PP1) IS ESSENTIAL FOR EMBRYOGENESIS AND POST-EMBRYONIC DEVELOPMENT
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733. Evidence for cell-cycle regulation of the A/P polarity machinery in the 1-cell embryo
Adrian Cuenca, Geraldine Seydoux

734. Early embryogenesis differs even in close relatives of C. elegans
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735. Computer Simulation of Early Cleavage of *C. elegans* Embryo
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736. *pod-2*, along with *pod-1*, defines a new class of genes required for polarity in the early *Caenorhabditis elegans* embryo
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Dave Pilgrim, Wanyuan Ao, Serene Wohlgemuth, Tamara Checkland

738. Toward an understanding of *tbx-9*
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739. The *C. elegans* Homothorax/Meis homolog UNC-62 is required for embryonic patterning and viability
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740. Genes that Affect Asymmetric Cell Division and Cell Fate in the Nervous System
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741. *Cki-1* : a link between the timing of cell divisions and the acquisition of proper cell fates in the *C.elegans* somatic gonad
Ivana Kostic, Richard Roy

742. Genetic Analysis of Cell Cycle Control in the Germ Line
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743. A mutant which enters cell cycle during dauer
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744. Distinct developmental functions of the two *C. elegans* homologs of a cohesin subunit Rad21
Yoshiko Mito, Asako Sugimoto, Masayuki Yamamoto

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746. A GENETIC APPROACH FOR STUDYING THE FUNCTION OF THE *AIR-1* KINASE IN C. elegans
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Ananth Badrinath, John G. White

748. A novel synthetic approach to identify additional roles for lin-35/Rb in cell growth and proliferation.
David S. Fay, Sean Keenan, Min Han

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Vikas Dhingra, Chunling Jiang, Fernando Santiago, Edward T. Kipreos

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Michael Brauchle, Pierre Gönczy

751. The *C. elegans* homologues of eIF5A and the ATP synthase b subunit are required for germline development
Momoyo Hanazawa, Yuichi Iino

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Elyse Connors, Jodi Taszymowicz, Eleanor Maine

753. Genetic characterization of *fbf-1*, *fbf-2* and *puf-8*
Jen Bachorik, Beth Thompson, Liana Lamont, Peggy Kroll-Conner, Sarah Crittenden, Andrei Petcherski, David Rudel, Shanping Wang, Gary Moulder, Robert Barstead, Marvin Wickens, Judith Kimble

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Kuppuswamy Subramaniam, Geraldine Seydoux

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757. The *C. elegans ptc* and *ptr* genes: A cholesterol connection?
Patricia E. Kuwabara, Olivier Zugasti, Min-Ho Lee, Tim Schedl, Gregory Jefferis

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759. PGL-1, PGL-2, and PGL-3, a family of P-granule proteins, function redundantly to ensure fertility in both sexes of *C. elegans*
Ichiro Kawasaki, Anahita Amiri, Yuan Fan, Takeshi Karashima, Yuji Kohara, Susan Strome

760. The enigma of *(y2):* an XO-specific lethal mutation
Tammy F. Wu, John D. Plenefisch, Barbara J. Meyer

761. The nuclear receptor SEX-1 in *C. elegans*sex determination
Chin Yee Loh, Barbara J. Meyer

762. Systematic alteration in the sex determination system of *C. elegans*
Jonathan Hodgkin

763. Characterization of TRA-3 calpain, a protease involved in sex determination
Sharon B. Sokol, **Patricia E. Kuwabara**

764. TRA-1 is a Phosphoprotein and Interacts with FEM-2, a Protein Type 2C Phosphatase
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765. A genetic screen for regulators of sexual development in the male tail
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766. Secreted Signaling Ligand HER-1A Interacts with the TRA-2A Transmembrane Receptor to Regulate Sexual Cell Fates
A. Kristine Black, Nimerta Rajwans, Roxana Sultan, Marc D. Perry

767. *C. elegans* SMC complexes: from dosage compensation to chromosome segregation
Raymond C. Chan, Barbara J. Meyer

768. Molecular Characterization of the Dosage Compensation Gene, *dpy-21*
Stephanie Yonker, Barbara J. Meyer

769. The *let381* gene
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771. Characterization of CeTRAP240, a C. elegans Homolog of a Component in Multi-protein Transcriptional coactivator Complexes
Jen-Chywan Wang, Keith R. Yamamoto

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Jae Hyung An, T. Keith Blackwell

773. Analysis of PHA-4 activity on the *myo-2* promoter and identification of a new PHA-4 DNA binding site
John Kalb, Pete Okkema, Jim McGhee

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Adam S. Wenick, Oliver Hobert

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S.Aslam, A.Mounsey, P.Bauer, and I.A.Hope

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Sarah K. Lange, Linda S. Kaltenbach, Susan E. Mango

777. Sins and saps: investigating components of the *Caenorhabditis elegans* Sin3-histone deacetylase complex.
P.Bauer, A.Mounsey, and I.A. Hope

778. What is TLF doing during the *C. elegans* cell cycle?
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779. Evolution of homeotic gene function in nematodes
Arturo Gutierrez, Ralf J Sommer

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Tracee McMiller, Malini Jagdeo, Casonya M. Johnson

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Stephen T. Sewell, Ashwin Uttam, Daniel J. Grau, Andrew D. Johnson, Helen M. Chamberlin

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Eric J. Lambie, Diane L. Church, Rachel J. West, Ian A. White, Marc A. Ginsburg

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Krzysztof Drabikowski, Ruth Chiquet-Ehrismann

784. A Structure/Function Analysis of the *sys-1* Gene
Kami Montgomery, Katie Donlevy, Ron Ellis, Judith Kimble, **Jennifer Miskowski**

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786. Functional requirement for the histone deacetylase HDA-1 during gonadogenesis in *C. elegans*
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787. A cytochrome P450 involved in gonad development identified in RNAi screens
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788. Role of IP_3 signaling pathways in regulating contractile activity of gonadal sheath cells
Adam Broslat, Eric Rutledge, Christoph Boehmer, Kevin Strange

789. IDENTIFICATION AND CHARACTERIZATION OF *lin-35* Rb PATHWAY GENES THAT ANTAGONIZE RAS SIGNALING DURING VULVAL INDUCTION
Craig Ceol, Bob Horvitz

790. sur-7 positively regulates ras-mediated signalling during vulva development
John H. Yoder, Min Han

791. Subcellular Localization of MPK-1 Using GFP Fusion
Young-jai You, Melanie Cobb, Leon Avery

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Jessica Goldstein, Kerry Kornfeld

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Stefano Canevascini, Gopal Battu, Erika Froehli, Amit Dutt, Thomas Berset, Alex Hajnal

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Dennis Eastburn, Min Han

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Ginger Kelly, Kerry Kornfeld

796. GENETIC SCREENS FOR POSITIVE REGULATORS OF RAS SIGNALING
Christian Rocheleau, Alissa Goldman, Mandy Volk, Meera Sundaram

797. Characterization of a lin-12(d) suppressor, sel-7
Jiabin Chen, Xiajun Li, Iva Greenwald

798. spr GENES: SUPPRESSORS OF THE sel-12 PRESENILIN EGG-LAYING DEFECTIVE PHENOTYPE
Sophie Jarriault, Iva Greenwald

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Chieh Chang, Nadeem Moghal, Paul W. Sternberg

800. A second *ksr*-like gene in *C. elegans*: Is it *pex-1*?
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Eric Lambie

801. Genetics and genomics of heterochronic regulatory RNAs
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Lorenzo F. Sempere, Victor R. Ambros

803. Control of *ins-33* transcription by *lin-14* in the *C. elegans* hypodermis
Marta Hristova, Victor Ambros

804. Regulation of the *C. elegans* heterochronic gene *lin-41* by the small RNA *let-7*
Monica Vella, Te-Wen Lo, Eun-Young Choi, Amy Pasquinelli, Gary Ruvkun, Frank Slack

805. Characterization of the effect of early expression of the small RNA *let-7* on developmental timing in *C. elegans*.
Gabriel Hayes, Gary Ruvkun

806. Temporal Regulation of the *let-7* stRNA
Steven M. Johnson, Shin-Yi Lin, Frank J. Slack

807. *lin-64*, A NEW GENE INVOLVED IN DEVELOPMENTAL TIMING
Shin-Yi Lin, Mary Abraham, Mona Carlson, Amy Pasquinelli, Gary Ruvkun, Frank Slack

808. *dre-1*, a new heterochronic gene affecting gonadal and extragonadal developmental age
Nicole Fielenbach, *Adam Antebi*

809. The role of RNAi related genes in developmental timing in C. elegans
Amy E. Pasquinelli, Frank Slack, Alla Grishok, Craig Mello, Gary Ruvkun

810. The heterochronic gene *lin-57* encodes a hunchback-like protein and is temporally regulated via its 3' UTR
Juan E. Abrahante, John Cahoy, Ming Li, Eric A. Miller, Ann E. Rougvie

811. A MOLECULAR LINK BETWEEN DEVELOPMENTAL TIMING AND CIRCADIAN RHYTHMS
Heather Gardner, Mili Jeon, Ann Rougvie

812. Identification of genes regulating synaptic remodeling of DD motor neurons
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813. Role of *lin-41* in Developmental Timing of Vertebrates
Betsy R. Maller, Frank J. Slack

814. Characterization of the *mua* gene *vab-10* in C. elegans
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815. The Myosin Tailpiece is Essential for Thick Filament Function
Pamela E. Hoppe, **Rebecca C. Andrews**

816. sem-3 encodes a cis-prenyltransferase homolog, an enzyme required for production of an essential lipid.
Matthew K. Robinson, Michael J. Stern

817. C. elegans Plakin Protein: Another Molecular Similarity Between Fibrous Organelles and Vertebrate Hemidesmosomes
Michelle C. Hresko, Lawrence A. Schriefer, Robert H. Waterston

818. PARAMYOSIN-GFPs RESCUE MUTATIONS AND ARE USEFUL TO STUDY EMBRYONIC LETHALITY OF *CAENORHABDITIS ELEGANS*
Daisuke Fujii, Hiroaki Kagawa

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Hiromi Terami, Hiroaki Kagawa

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Tomoyo Hamada, Yasuji Sakube, Joohong Ahnn, Do Han Kim, Hiroaki Kagawa

821. Effect of the alpha 2 Ca^{2+} channel subunit UNC-36 on calcium transients in pharyngeal muscles.
Christian Frøkjær-Jensen, Rex Kerr, Roger Y. Tsien, William R. Schafer

822. Depletion of PKL (KIN-4) in Muscle Results in L1 Paralysis
Marianne Land, Charles S. Rubin

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824. Structural and Functional Disruption of Muscle Caused by Troponin I Mutation
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825. CAM-1/KIN-8 Receptor Tyrosine Kinase is Important for Coordinated Movement
Susan Poulson, David Madsen, Villu Maricq

826. PRODUCTION OF ANTIBODIES AGAINST *C elegans* PROTEINS
Ayanori Yamakawa, Youko Tokiwa, Syuugo Kubota, Yoshikuni Goto, Yumi Sasano, Ryuji Hosono

827. Biochemical Characterization of *C. elegans* Malate Dehydrogenase
Katherine M. Walstrom, Danny González

828. Loss of a dynamin related protein MGM-1 causes excessive mitochondrial fragmentation
Ashley Wright, Mauro Zappaterra, Alexander van der Bliek

829. Search for novel proteins affecting mitochondrial inner and outer membrane division.
Ayako Hasegawa, Dan Rube, Ashley Wright, Alexander van der Bliek

830. Nematodes with Mitochondrial Diseases
Bernard Lemire, William Tsang

831. RNAi-Mediated Embryonic Lethality by Three Genes Encoding Complex II Subunits of the Electron Transport Chain.
Harumi Ichimiya, Phil Hartman, Nao Ishii

832. Mitochondrial Complex I and II Defects Result in Excessive Superoxide Anion and Apoptosis.
Nanami Senoo-Matsuda, Phil Hartman, Nao Ishii

833. The Effects of Electron Transport Inhibitors on Survival of Wild-type *C. elegans*
Kayo Yasuda, Phil S. Hartman, Nao Ishii

834. Cool dauers: video capture + thermal stage microscopy = sequence + morphology
Paul De Ley, Irma T. De Ley

835. Cryofixation of *C. elegans* for ultrastructure, immunogold labeling and GFP fluorescence of the nervous system.
Steve Fields, Jim Rand

836. CAMBIO Initiative: Computational Algorithms for Multidimensional Biological Image Organization
Kevin W. Eliceiri, Charles Thomas, Curtis Rueden, Fong-Mei Lu, Narfi Stefansson, John G. White

837. Electron Tomography at an Organismal Level, a Unique Application of a Powerful Tool
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838. CAENORHABDITIS ELEGANS (NEMATODA) AS TEST SUBJECT FOR CRYOIMMOBILISATION
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839. The Low-Cost Worm Lab
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840. A High Resolution Digital 4-D-Microscopy
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841. A Computer-Assisted System For Reconstructing And Behaviorally Analyzing In 4D Every Cell And Nucleus During *C. elegans* Embryogenesis
Paul J. Heid, Edward Voss, Howard Butler, Jasper Humbert, Michael Clark, David R. Soll

842. New! Improved! Fixation protocols for TEM
David H. Hall, Gloria Stephney, Tylon Stephney, Frank Macaluso, Ya Chen

843. Cell lineage acquiring system for early embryogenesis
Shugo Hamahashi, Shuichi Onami, Hiroaki Kitano

844. NEXTDB: The nematode expression pattern database.
Tadasu Shin-i, Yuji Kohara

845. Correlation between transcriptome and interactome data in *S. cerevisiae*
Hui Ge, Zhihua Liu, Marc Vidal

846. Identifying promoter motifs and predicting gene expression patterns in *C. elegans* using data mining tools.
Frederick Tan, Brian Murphy, Dung Phu, Ian Pushee, Carolina Ruiz, Julia Krushkal, **Elizabeth Ryder**

847. Design of a Whole-Genome *C. elegans* Microarray for Investigating Protein-Genome Interactions
Jason D. Lieb, Todd Lowe, Patrick O. Brown

848. Integration of functional genomics approaches: protein interaction mapping using genes enriched in the C. elegans germline
Marian Walhout, Jerome Reboul, Olena Shtanko, Philippe Vaglio, Valerie Reinke, Stuart Kim, Marc Vidal

849. Mapping the genome(s) of the human nematode parasite *Brugia malayi*
Jen Daub, David Guiliano, Mark Blaxter, Jeremy Foster, Mehul Ganatra, Jenna Ware, Ibrahim Kamal, Barton Slatko, Neil Hall, Mike Quail, Bart Barrell, The Filarial Genome Project

850. NEMATODE.NET, a Tool for Navigating Sequences from Parasitic and Free-living Nematodes
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851. The worm is not enough - comparative genomics of *Ancylostoma ceylanicum*
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854. Characterization of a Short-Lived *C. elegans* Mutant
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856. Identification of genes involved in oxidative stress response and aging in *Caenorhabditis elegans*
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857. Longevity and gerontogenes in model organisms
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858. A genetic screen to identify novel players in *C. elegans* lifespan regulation
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859. A Screen for Mutations that Affect *Caenorhabditis elegans* Aging
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860. The knock-out of the peptide transporter gene *pep-2* results in delayed development and extended life-span in *Caenorhabditis elegans*
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861. Phosphorylation profiles of a quantitative trait loci (QTLs) on chromosome III, IV, V and X controlling longevity of *Caenorhabditis elegans*
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862. Enzymatic and *in vivo* functional characterization of a *C.elegans* RecQ DNA helcase
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863. Genetic analysis of the DAF-2 insulin-like signaling pathway of *C. elegans*
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864. A screen for mutations affecting the *daf-16*/FH-independent *daf-2*/IR signaling pathway
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865. GENETIC ANALYSIS OF THE NICTATION BEHAVIOR OF DAUER LARVAE
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866. The role of *egl-32<i/> in egg laying and its interaction with the TGFbeta pathway*
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867. Candidate downstream targets of TGF-beta signaling in dauer formation
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868. Defining new components of TGF-beta signaling pathways in *C. elegans*
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869. TGF-beta Signaling and Gene Expression
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870. The new progress about sma-3 in TGF-beta signaling in C. elegans
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871. *sma-9* and TGFbeta Signaling
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872. Regulation of endocytosis of albumin in the intestinal epithelial cells of *C.elegans* by TGFbeta-like signaling
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873. Characterization of Sma Mutants from a *lon-2* Suppressor Screen
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874. Genetic analyses of the adenylyl cyclase family in *C. elegans*
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875. *mig-14* functions in multiple developmental processes regulated by Wnt signaling
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876. Identification of EGL-27 molecular interactions
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877. Identification of *lin-44(sa490ts)* suppressors
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878. Neurodegeneration in *C. elegans*: analysis and cloning of a new *deg* gene
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879. A transgenic *C. elegans* model for Parkinson´s Disease
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880. Dissecting mechanisms of X-adrenoleukodystrophy neurotoxicity using *C. elegans*
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881. FUNCTION OF *C. ELEGANS apl-1*, A GENE RELATED TO HUMAN AMYLOID PRECURSOR PROTEIN.
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882. CHARACTERIZATION OF THE DEG-3 nAChR, A RECEPTOR INVOLVED IN NEURONAL DEGENRATION
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883. Expression analysis of C. elegans homologues of the human CLN3 gene involved in neuronal ceroid lipofuscinosis
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884. Exploring necrotic cell death in *C. elegans*
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885. *unc-79*: A Gene Involved in Anesthetic Responsiveness in *C. elegans*
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886. SNAP-25, a Rat General Anesthetic Target Implicated by *C. elegans* Genetics
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887. Defining the structural requirements for *unc-64(md130)*'s general anesthetic resistance
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888. Gain-of-function mutations in *egl-30* produce resistance to volatile anesthetics
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889. TOWARDS THE CLONING OF THE SUPPRESSORS OF *UNC-1*
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893. A Genetic Analysis of the Effects of Ethanol on Egg Laying
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894. Reproductive and Acute Toxicity of Platinum-based Chemotherapy Drugs in C. elegans
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895. Towards the Cloning of Gene That Can Mutate to Confer Lithium Resistance In *C. elegans*
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896. A genomic approach to understanding the actions of fluoxetine
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897. Steps Toward Solving the Drug Problem in Worms
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898. Functional roles of flavin-containing monooxygenase (FMO) genes in *C. elegans*: EMS-induced gene lesions screened using denaturing high-performance liquid chromatography (DHPLC)
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899. Neural integration of thermosensory and chemosensory information
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900. Reverse genetic approach to reveal sensory signal transduction pathway involving TTX-4 nPKC-epsilon
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909. DEG-3 LOCALIZATION REVEALS NEURONS WITH A COMPLEX MORPHOLOGY
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910. MUTATIONS THAT AFFECT SYNAPTIC LOCALIZATION OF GLR-1.
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916. Are input and output defects linked in *unc-4* mutants?
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918. Visualizing synapses in the motor neuron circuit
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919. In search of UNC-4 target genes, mediators of synaptic specificity.
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926. Guanylate Cyclase Beta 2 Homologues in *C. elegans*
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929. Neuronal Defects Elicited by Ectopic Expression of *C. elegans* Kallmann Protein
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931. *In Vivo* Imaging of HSN Growth Cones
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932. A GENETIC SCREEN FOR DA/DB AXON GUIDANCE MUTANTS IN C. ELEGANS
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933. Molecular characterization of the *seu-2* gene involved in axon guidance
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934. Identification of Genes Specifically Required for Ventralward Axon Guidance in *C. elegans*
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935. The *C. elegans* homologue of mammalian Hrs/Hrs-2 (C07G1.5) interacts with the *C. elegans* homologue of vertebrate EAST (C34G6.7), and the clathrin adaptor protein UNC-11A. Is there a link between clathrin mediated retrieval and synaptobrevin trafficking through the endocytic pathway? I. Characterization of Ce Hrs expression.
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939. Looking for the intracellular function of the sterol-sensing domain protein CHE-14 in ectodermal epithelial cells
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943. Mutations in *let-767*, a steroid processing gene, result in cholesterol hypersensitivity in *C. elegans*
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944. Isolation of Genes involved in the Regulation of Sterol Metabolism
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945. *C. elegans* genes coding for protein involved in lipid metabolism
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946. Role of sphingolipids in *C. elegans*
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947. Deletion of glucosamine 6-phosphate acetyltransferase causes sterility in homozygotes
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948. Characterisation of mammalian syndecan homologue in *C.elegans*
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949. Molecular and Genetic Characterisation of Cyclophilin Encoding Genes from *C. elegans*: a Focus on *cyp-1* and *cyp-5*.
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950. ISOLATION AND CHARACTERIZATION OF A NOVEL NADPH-DEPENDENT FLAVIN-REDUCTASE IN C. elegans AND HUMANS
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951. Examining the Role of Proprotein Convertases
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952. *CAENORHABDITIS ELEGANS* AS A MODEL ORGANISM IN THE STUDY OF NUCLEOSIDE TRANSPORTERS.
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954. Specification of the MS founder cell in *C. elegans*
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955. USING YEAST-TWO HYBRID AND RNA-MEDIATED INTERFERENCE TO IDENTIFY PROTEINS ESSENTIAL FOR PIE-1 ACTIVITY AND LOCALIZATION IN EARLY EMBRYOS
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956. Role of MEX-3 in Patterning PAL-1 Expression
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959. *C.elegans* RAB-5 is involved in asymmetric cell division in early embryogenesis
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960. A gain-of-function mutation in the *oma-1* gene results in C to EMS fate transformation
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961. The cell biology of Pod-1 and Pod-2 during polarity establishment in *C. elegans embryos*
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962. Embryonic development of the free-living marine nematode Pellioditis marina
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965. Analyzing genetic networks that regulate the development of the early *C. elegans* embryo
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967. Chromosome remodeling during meiosis
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968. t1171 is defective in chromosome segregation and cytokinesis
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969. Roles of the dosage compensation protein DPY-28 and the cohesin protein HIM-1 in mitosis and meiosis
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970. The HMGA proteins of *C. elegans*
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972. The ABI-complex is required for chromosome segregation but not for sister chromatid separation
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973. *In vivo* studies of the nuclear function of Barrier-to-Autointegration Factor (BAF)
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975. The role of *cdc-25.2* and *emb-29* in the cell cycle.
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976. *C. elegans* CKI-2 interacts with PCNA
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977. Investigation of CUL-2/RING finger complexes
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978. The regulation of cell cycle exit by cul-1 & lin-23 genes
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979. Securin and Separin: Roles in the C.elegans cell cycle?
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980. Characterization of mammalian homologs related to cell-cycle checkpoint control in *C. elegans*
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981. THE TIMELESS-LIKE GENE TIM-1 IS CSG-5 AND IS REQUIRED FOR CHROMOSOME SEGREGATION
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982. The *C.elegans* CIP/KIP-type cyclin-dependent kinase inhibitor CKI-1 is cell-autonomously required for postmitotic differentiation as well as cell cycle arrest.
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983. The *sys* genes and early gonadogenesis
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984. Characterisation of an XPF endonuclease-like gene in *Caenorhabditis elegans*
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985. Control of cell cycle withdrawal during *C. elegans* development
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986. *C. elegans* SR protein kinase, SPK-1, is required for germline proliferation and maturation.
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987. Genetic Analysis of Early Germline Proliferation
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988. Maintenance of embryonic stem cell identity in the germline blastomeres of *C. elegans*
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989. Identification of factors required for primordial germ cell development
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990. Suppressor analysis of FOG-1, a CPEB protein that regulates germ cell fate.
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991. RAS/MAP kinase signaling in meiotic prophase progression
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992. Forward and Reverse Genetic Approaches to Identify Genes Involved in Initial Meiotic Entry
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993. Defects in Timing and Position of the Initial Onset of Meiosis in *glp-1* Proximal Proliferation (Pro) Mutants
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994. MOG-6 and FEM-3 expression patterns are complementary in the hermaphrodite germ line
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995. *fem-3* Expression, Localization, and Interactions
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996. Identification and characterization of putative male-specific sexual regulators and TRA-1 target genes
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998. Disruption of *tra-2* translational control results in TRA-2 protein forming large nuclear structures
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999. Maternal-effect germline silencing of the sex-determining gene fem-1
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1000. A new transfomer mutant in *C. elegans*
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1001. Identification of a New X-Signal Element that Determines Sex in *Caenorhabditis elegans: ceh-39*
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1002. The HAT activity of CBP-1 is indispensable for its biological function during *C. elegans* embryogenesis
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1003. The Roles of the par Genes in Intracellular Motility Before the First Asymmetric Cell Division in *C. elegans*
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1004. Is PAR-2 an E3 Ubiquitin Ligase?
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1006. The role of Ce-BTF3 in endoderm development
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1007. Role of egl-5 in EGF/Wnt signal integration in the specification of P12 fate
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1008. Genetic Analysis of Coelomocyte Specification and Patterning
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1009. Coupling of cell type specification and cell division: the number of division rounds in the E lineage is regulated by the activity of the E-specifying *end* genes.
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1010. A screen for factors affecting the expression pattern of *lin-48*
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1011. DNA binding properties and potential downstream targets of the cell fate determining T-box gene *mab-9*.
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1012. Delineation of enhancer elements and trans-acting factors controlling *mab-21* activity in sensory ray differentiation.
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1013. Clustering analysis of gene expression patterns
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1014. Tissue-specific gene regulation by intronic elements in *C elegans*
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1015. Defining Regulatory Regions In Nematode Muscle Genes By Computational And Transgenic Studies.
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1016. The *lin-26* gene is regulated by tissue-specific cis-elements
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1017. The Roles of *cis*-elements in the Regulation of *C. elegans* Hox Gene *egl-5*
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1018. Identification of *Cis* -Regulatory Response Elements in Vulva Cell Fate Markers
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1019. Computer prediction of cis-acting elements from co-regulated genes.
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1020. Regulation of the *C. elegans* posterior *Hox* paralogs *nob-1* and *php-3*
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1022. Dissection of the upstream regulatory elements of the Inositol 1,4,5-trisphosphate receptor gene *itr-1*.
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1023. An investigation of the role of the *Exd/Pbx* orthologs *ceh-20* and *ceh-40* in the activation of *ceh-13*
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1024. Identification of genes involved in lateral inhibition during vulval cell fate specification
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1026. ISOLATION AND CHARACTERIZATION OF NEW SYNMUV GENES, INCLUDING *lin-61*, A CLASS B GENE WITH ROLES IN CELL DIVISION AND MULTIPLE POSTEMBRYONIC DEVELOPMENTAL PROCESSES
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1029. Organogenesis of the *C. elegans* vulva: *cog* mutants
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1030. Studies of mutations that disrupt vulval development in C. elegans
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1031. DOES *lin-15* STIMULATE CELL FUSION AND ANTAGONIZE VULVAL CELL FATES?
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1. MSP Signaling: Beyond the Sperm Cytoskeleton

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Sophisticated regulatory systems have evolved to coordinate fertilization and oocyte meiotic cell cycle progression. However, the molecular interactions that govern critical oocyte cell cycle transitions and their conservation in the metazoa are not clear. *C. elegans* oocytes, like those of most animals, arrest during meiotic prophase. Sperm promote the resumption of meiosis (maturation) and contraction of the smooth muscle-like gonadal sheath cells, which are necessary for ovulation. Using an *in vivo* bioassay, we have shown that the major sperm cytoskeletal protein (MSP) acts as a bipartite signal for both oocyte maturation and sheath contraction. During nematode sperm locomotion, MSP plays a role analogous to actin indicating that this 14 kDa sperm-specific protein has acquired extracellular signaling and intracellular cytoskeletal functions during evolution. Proteins with MSP-like domains have been found in plants, fungi, and other animals raising the possibility that MSP signaling functions may exist in other phyla.

We are particularly interested in understanding how MSP promotes the resumption of meiosis in oocytes. A receptor for a maturation-promoting factor has not been identified in any animal. Thus, identifying and characterizing the MSP receptor(s) is a major goal. We are taking a genomic approach to identify receptor candidates. Using an *in situ* MSP binding assay, candidates can be quickly screened for binding following RNAi. In this assay, MSP-flouroscein specifically binds oocytes and sheath cells of the proximal gonad arm. MSP-flouroscein is active in promoting both oocyte maturation and sheath contraction *in vivo*. Binding is not observed in distal female gonads, male gonads, or following incubation with BSA-flouroscein. Preincubation with a 20-fold excess of unlabeled MSP completely abrogates MSP-flouroscein binding. Further, binding is not observed in *emo-1(oz1)* oocytes, which lack a functional secretory system due to a mutation in a Sec61p γ homologue (Iwasaki et al., 1996). Using data from DNA microarrays (Reinke et al., 2000), we have identified three conserved transmembrane proteins whose transcripts are highly enriched in oocytes. MSP binding to oocytes is dramatically reduced in two loss of function backgrounds and enhanced in the other. Each gene is tightly linked to one of the two large MSP clusters in the genome--a finding of unknown significance. We currently have mutations in two genes and are doing detailed phenotypic analyses. We are also interested in examining protein localization *in situ* and evaluating MSP binding in a heterologous system. Our results will be discussed in the context of a new model for oocyte meiotic maturation.

Iwasaki et al. (1996). J Cell Biol 134:699-714

Reinke et al. (2000). Mol Cell 6:605-616

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2. Promotion of germ cell fate by the multifunctional CCCH zinc finger protein PIE-1

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The first cleavages of the zygote subdivide the embryo into five somatic lineages and the germ lineage. The embryonic germ lineage is distinguished from other lineages by three characteristics: 1) inhibition of mRNA transcription, 2) maintenance and expression of certain maternal RNAs associated with P granules, and 3) asymmetric cleavages. We have obtained evidence that the CCCH zinc finger protein, PIE-1, may regulate all three of these properties.

PIE-1 is a maternal protein that segregates with the embryonic germ lineage. In germline blastomeres, PIE-1 is both nuclear and cytoplasmic. Previously we have shown that one function of PIE-1 is to inhibit mRNA transcription . Using a transgenic rescue assay, we have obtained evidence that this function is linked to the nuclear pool of PIE-1, and that PIE-1 also performs a second independent function, possibly in the cytoplasm. This second function is required for efficient translation of *nos-2*, a maternally supplied, P granule-associated mRNA (see abstract by Subramaniam and Seydoux). A mutation in PIE-1s second CCCH finger reduces NOS-2 expression without affecting transcriptional repression and causes primordial germ cells to stray away from the somatic gonad, occasionally exiting the embryo entirely. Our results indicate that PIE-1 promotes germ cell fate by at least two independent mechanisms: 1) inhibition of transcription, which blocks zygotic programs that drive somatic development and 2) activation of protein expression from *nos-2*, and possibly other maternal RNAs, which promotes primordial germ cell development.

During mitosis, PIE-1 is also found around the centrosomes (Mello et al., 1996). We wondered whether this third pool of PIE-1 might perform yet another function in the germ lineage. Consistent with this possibility, *pie-1(-)* embryos show defects in the orientation of the P3 spindle, suggesting that PIE-1 may be required to help orient the cleavages of at least some germline blastomeres. We have found that the domain in PIE-1 required for targeting to the peri-centrosomal ring maps to the amino-terminus of the protein and interacts in a yeast-two hybrid screen with MDF-2, a component of the spindle-assembly check point (Kitagawa and Rose, 1999). Like PIE-1, MDF-2 associates with centrosomes and is required to orient the P3 spindle. We recently identified a mutation in PIE-1 that specifically disrupts its ability to associate with centrosomes, and are in the process of using this mutant to test the hypothesis that the peri-centrosomal pool of PIE-1 contributes to the asymmetric divisions of germline blastomeres.

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3. *IN VIVO* DYNAMICS OF POP-1 ASYMMETRY AND THE ROLE OF POP-1 IN THE INITIATION BUT NOT MAINTENANCE OF *end-3* REPRESSION

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The convergent action of Wnt and MAPK kinase signaling pathways results in unequal levels of the HMG box transcription factor POP-1 in sister cells that arise from anterior/posterior cell divisions: anti-POP-1 antibody stains less intensely in the nuclei of the posterior daughter cells as a result of these signals (Lin et al., 1995, 1998; Meneghini et al., 1999; Rocheleau et al., 1999). This 'POP-1 asymmetry' occurs reiteratively throughout embryogenesis. POP-1 asymmetry correlates with the generation of developmentally unequal daughters and POP-1 is required for these differences in many cases. The mechanisms by which this asymmetry is reiteratively generated are not well understood.

The *end-1* and *end-3* genes are candidate zygotic targets of maternal POP-1 in the EMS lineage. These genes, which specify the fate of the E cell, are bound directly by the zygotically expressed MED-1 and -2 GATA factors in both the E and MS lineages; POP-1 activity represses the *end* genes in MS, thereby restricting endoderm fate to E. To examine *in vivo* whether this repression involves the direct action of POP-1, we expressed a GFP::POP-1 fusion protein under control of the zygotic *med-1* promoter. This fusion expresses in all EMS descendants until the 8E stage, and rescues the maternal-effect lethal phenotype of a *pop-1(zu189)* mutation. We found that in MS, and other anterior, but not posterior, nuclei in the EMS lineage, GFP::POP-1 binds to an extrachromosomal array containing the *end-3* promoter (based on the "nuclear spot assay"). These observations support the model that Wnt/MAPK signaling blocks the repressive function of POP-1 in E, permitting MED-1,2 to activate *end-1,3* and specify endoderm fate. However, our findings also demonstrate that the observed *in vivo* interaction of POP-1 with *end-3* is neither necessary nor sufficient for repression: we observe interaction of POP-1 in Ea, in which *end-3* is apparently fully expressed, and detect no interaction in posterior daughters of the MS lineage, in which *end-3* expression is fully repressed. We propose that POP-1 can initiate a repressive state only at the time that transcription of a gene is first established (e.g., *end-3* in the E cell), and is not subsequently required to maintain this repressed state.

Examination of GFP::POP-1 also demonstrates the dynamic nature of POP-1 asymmetry *in vivo*. The GFP expression recapitulates the asymmetry seen with anti-POP-1 staining, demonstrating that this asymmetry reflects *bona fide* differences in nuclear POP-1 levels. During development, the GFP signal becomes cytoplasmic and uniformly distributed in mitotic cells. However, nuclear GFP::POP-1 asymmetry is re-established almost immediately after reformation of daughter interphase nuclei. Because GFP::POP-1 asymmetry is recursively generated even in cells in which we cannot detect message from the fusion gene, we suggest that this asymmetry does not depend on asymmetric synthesis of the protein, but involves other mechanisms such as differential degradation or nuclear localization.

Our observations also suggest that GFP::POP-1 differs qualitatively in anterior and posterior daughters. In anterior daughters only, we observe bright punctate fluorescence beginning immediately after mitosis; the punctate spots apparently coalesce into one or two large puncta by the end of interphase. We observe similar structures with myc-tagged POP-1, suggesting they may not simply be an artifact of the GFP fusion *per se*.

We have begun to examine the structural and genetic requirements for this dynamic behavior. The asymmetry in both POP-1 levels and presence of puncta requires WRM-1 and LIT-1, but not CBP-1. Neither the first 44 amino acids of POP-1, implicated in a canonical ß-catenin-Lef interaction with the ß-catenin BAR-1, nor BAR-1 itself, are required for either asymmetry. Furthermore, the conserved HMG box, and the carboxyl portion of the protein downstream of the HMG box, are dispensable for asymmetry, implicating the ~145 residues between these two domains. It will be of interest to identify the specific structural elements responsible for these asymmetries and to assess

further the biological relevance of the puncta observed in anterior nuclei specifically.

4. Specification of Organ Identity by the C. elegans FOXA homologue PHA-4.

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During development, cells acquire distinct cell fates in response to a host of regulators, many of which encode transcription factors. A longstanding issue has been the nature of the target genes controlled by these transcriptional regulators: do they activate a few genes at the top of a hierarchy, or control many genes that function at multiple stages throughout development? This question has been difficult to resolve because few direct targets have been identified.

To address how transcription factors influence cell fate decisions, we are studying the role of the fork head box protein PHA-4 during pharynx development. In embryos that lack pha-4 activity, cells that would normally become part of the pharynx develop as ectoderm instead. Conversely, ectopic expression of pha-4 produces excess pharyngeal cells at the expense of other cell types. Because pha-4 normally functions in pharyngeal cells irrespective of the cells' lineage or cell type, we propose that pha-4 endows cells with pharyngeal organ identity.

We have used a microarray chip approach to identify genes selectively expressed in the pharynx and have analyzed those genes to determine which are direct PHA-4 targets. We took advantage of maternal effect mutants that produce embryos with excess (par-1) or no (skn-1) pharyngeal cells to identify candidate pharyngeal genes. Included among our positive clones were most (>70%) genes known to be expressed in the pharynx, indicating that our approach worked well.

Three lines of evidence suggest that PHA-4 directly activates most or all pharyngeally-expressed genes. First, we examined the pharynx-specific expression of a random set of microarray positives and found that the expression of each depended on one or more predicted PHA-4 binding sites. Second,

PHA-4 is able to bind these sites in vitro. Third, temperature-shift studies indicate that PHA-4 is required throughout development, consistent with a model in which PHA-4 regulates both early and late acting genes. Our analysis also showed that the affinity of PHA-4 for different binding sites regulates the relative time of onset of the different target genes. When a PHA-4 binding site is converted by a single base-pair to a higher affinity site, expression initiates earlier. Conversely, a single base-pair mutation of a PHA-4 recognition sequence to one with lower affinity leads to a delay in the onset of expression. The affinity of PHA-4 binding in vitro matches the behavior of these reporter constructs in vivo. These findings suggest that modulating the affinity of PHA-4 binding sites within a promoter provides the embryo with a subtle mechanism to coordinate expression temporally throughout a developing organ. Since direct target genes of mammalian FOXA proteins include genes expressed early (e.g. endoderm) or late (e.g. liver) during vertebrate development, we suggest that global regulation of transcription within the foregut may be a common feature of this family of developmental regulators.

We are indebted to Stuart Kim, Rebecca Begley, Carrie van Doren, Kyle Duke, and Min Jiang, who performed the microarray hybridization experiments.

5. Genetic Analysis of Endocytosis by the Coelomocytes of *Caenorhabditis elegans*

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Endocytosis is a basic function of eukaryotic cells that leads to the internalization of fluid from the extracellular medium, nutrient uptake and the recycling of membrane components. In multicellular organisms, endocytosis has also been adapted by specialized cells for specific functions, including signaling down regulation, synaptic vesicle recycling, and antigen presentation. There are various routes by which endocytosis is accomplished, involving different cellular structures. The endocytic pathway utilized and the particular mechanisms used to regulate it depend on the ligand being internalized and the cell type examined. The multiplicity of options adds complexities that suggest that endocytosis should be studied using several different approaches and experimental systems.

The coelomocytes of *C. elegans* are scavenger cells that continuously and nonspecifically endocytose fluid from the pseudocoelom (body cavity). Green fluorescent protein (GFP) secreted into the pseudocoelom from body wall muscle cells is endocytosed and degraded by coelomocytes. We show that toxin-mediated ablation of coelomocytes results in viable animals that fail to endocytose pseudocoelomic GFP, indicating that endocytosis by coelomocytes is not essential for growth or survival of *C. elegans* under normal laboratory conditions. We examined known viable endocytosis mutants, and performed RNAi for other known endocytosis genes, for coelomocyte uptake defective (Cup) phenotypes. We also screened for new genes involved in endocytosis by isolating viable mutants with Cup defects; this screen identified 14 different genes, many with multiple alleles. A variety of Cup terminal phenotypes were observed, consistent with defects at various steps in the endocytic pathway. We describe here a more detailed analysis of *cup-5* one of the mutants identified from this screen.

We show that the *cup-5* loss of function mutation results in an enhanced rate of uptake of fluid-phase markers, decreased degradation of endocytosed protein, and accumulation of large vacuoles. Based on pulse chase experiments in coelomocytes marked with RME-8, a late endosomal marker, we identify these large vacuoles as late endosomes and/or lysosomes. Furthermore, CUP-5 itself localizes to compartments that are not labeled with EEA1, an early endosome marker, or RME-8, a late endosome marker.

The predicted CUP-5 protein is 611 amino acids with six predicted membrane spanning domains. CUP-5 is homologous to mucolipin-1 in humans. Loss of human mucolipin-1 underlies Mucolipidosis Type IV (MLIV), a lysosomal storage disease that results in severe developmental neuropathology. MLIV cells display an enhanced rate of uptake and accumulate large vesicles, suggesting that a defect in endocytosis may underlie the disease. This similarity in phenotypes between human and worm cells suggests that the *C. elegans cup-5* mutant may be a useful model for studying conserved aspects of mucolipin-1 structure and function and for analyzing its function in endocytosis.

Thanks to Iva Greenwald, in whose lab much of this work was done.

6. *smu-1* and *smu-2* regulate the alternative splicing of *unc-52* pre-mRNA

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Alternative splicing of pre-mRNA is a biologically important but poorly understand process. We are using a genetic approach to identify new genes that function *in vivo* to regulate alternative splicing. Mutations in *smu-1* and *smu-2* were isolated as extragenic suppressors of the synthetic lethal phenotype of *mec-8 unc-52(viable)* double mutants. *smu-1* and *smu-2* mutations also suppress other phenes of *mec-8* mutants, such as mechanosensory and chemosensory defects, apparently by a bypass mechanism; *mec-8* encodes a putative RNA binding protein that affects the accumulation of certain alternatively-spliced transcripts of *unc-52* and other genes (Lundquist *et al.* Development 1996 122: 1601). Finally, *smu-1* and *smu-2* mutations suppress the uncoordination conferred by nonsense mutations in exon 17 but not exon 18 of *unc-52*. We hypothesized that *smu-1* and *smu-2* encode factors that regulate the splicing of various target genes, at least some of which are also targets of splicing control by MEC-8. Indeed, our RT-PCR and RNase protection experiments indicate that mutation in *smu-1* or *smu-2* leads to enhanced skipping of exon 17 but not exon 18 of *unc-52* pre-mRNA. The phenotypes of *smu-1* and *smu-2* mutants seem to be identical and identical to the phenotype of *smu-1 smu-2* double mutants, suggesting that SMU-1 and SMU-2 work together.

smu-1 and *smu-2* both encode highly conserved proteins that are ubiquitously expressed and nuclearly localized. SMU-1 contains five WD motifs, which are implicated in protein-protein interactions, and is greater than 60% identical in amino acid sequence to a predicted human protein of unknown function; we suggest that this human protein regulates alternative splicing. We have molecularly characterized all six known *smu-1* mutations. Two appear to be null and confer a mildly deleterious but viable phenotype. *smu-2* encodes a protein that is 37%

identical to a mammalian nuclear protein called RED; homologues are also present in *Drosophila* and *Arabidopsis*. The similarities between SMU-2 and RED proteins occur throughout their full extents. SMU-2 is the only protein with significant similarity to RED in the *C. elegans* genomic sequence database. Neubauer *et al.* (Nat. Genet. 1998 20: 46) identified RED as a protein tightly associated with human spliceosomes. RED was named after the most distinctive feature of the protein, a domain consisting of alternating basic (arginine) and acidic residues (aspartic and glutamic acid); the function of this domain, which seems to be the least well conserved part of the protein, is not known. None of our three *smu-2* mutations is a molecular null, but *smu-2(RNAi)* mimics the *smu-2* phenotype: efficient suppression of *unc-52* and no inviability. GFP expression of a rescuing *smu-1::gfp* reporter disappears in a *smu-2* mutant background; we suggest that SMU-2 is required to stabilize SMU-1.

The first two authors contributed equally to this work.

7. A Cycle of SMG-2 Phosphorylation Required for Nonsense-mediated mRNA decay in *C. elegans*

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Messenger RNAs containing premature translation termination codons are rapidly degraded in all eukaryotes, a process termed nonsense-mediated mRNA decay (NMD). Functions of seven *smg* genes are required for NMD in *C. elegans*. SMG-2 appears to be the key regulator of NMD, with the remaining six *smg* genes regulating the state of SMG-2 phosphorylation. Activities of *smg-1*, *smg-3*, and *smg-4* are required to phosphorylate SMG-2, while activities of *smg-5*, *smg-6*, and *smg-7* are required to dephosphorylate SMG-2. We have identified SMG-1 as the likely direct kinase of SMG-2, identified SMG-5 as a component of a SMG-2 phosphatase, and characterized a complex of SMG and related proteins that is important for NMD.

Four lines of evidence suggest that SMG-1 is the direct kinase of SMG-2: (i) SMG-1 is predicted to be a protein kinase (S. O'Connor & P. Anderson). (ii) Activity of *smg-1* is required for phosphorylation of SMG-2 both *in vivo* and *in vitro*. (iii) Phosphorylation of SMG-2 *in vitro* is sensitive to wortmannin, a PI3-kinase inhibitor. (iv) SMG-1 and SMG-2 coimmunoprecipitate from crude extracts.

Three lines of evidence suggest that protein phosphatase 2A (PP2A) is the SMG-2 phosphatase: (i) SMG-5 interacts in a yeast two-hybrid screen with PR65/A, the scaffolding subunit of PP2A. (ii) SMG-5 co-immunoprecipitates with SMG-2, PR65/A, and PP2Ac, the catalytic subunit of PP2A. (iii) SMG-2 and PR65/A co-immunoprecipitate. We suggest that SMG-5 is a variable regulatory subunit of PP2A whose function is to target PP2A to its phosphorylated SMG-2 substrate.

We are presently characterizing additional proteins with which SMG-1, SMG-2, and SMG-5/PP2A interact using both yeast two-hybrid and co-immunoprecipitation assays. For example, SMG-3 interacts directly with

both SMG-2 and SMG-4 and bridges the interaction between SMG-2 and SMG-4. SMG-5 interacts directly with SMG-7. We believe that this complex of SMG and related proteins regulates the state of SMG-2 phosphorylation. In doing so, this complex communicates to downstream components of the mRNA degradation pathway whether the context of translation termination is normal or abnormal.

8. A Global Analysis of the *Caenorhabditis elegans* Operons

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Unlike genomes of other animals, the genomes of *Caenorhabditis elegans* and its close relatives contain numerous polycistronic transcription units similar to bacterial operons. The pre-mRNAs from these operons are processed into monocistronic mRNAs by 3' end formation and a specialized form of trans-splicing using the spliced leader, SL2. In order to determine how widespread operons are in the *C. elegans* genome, we have probed microarrays containing 17,817 predicted and known genes with a probe specific for mRNAs containing the SL2 sequence along with a control probe to polyA+ RNA. There are 1215 genes that form a separate group with high SL2/polyA+ ratios at the 99.9% level of confidence, and 86% of these appear to be downstream genes in operons, based on their genomic arrangements. We integrated this list with a list of ~400 genes in known operons and/or with confirmed SL2-containing cDNA clones. Of the genes previously confirmed to be SL2 trans-spliced, 91% are contained on the list of operons based on the microarray experiment, and almost all are in downstream positions. We estimate the genome contains ~850 operons, of which we provide experimental support for 807. On average, the operons contain 2.6 genes, and the longest contain seven genes. At least 13% of *C. elegans* genes are transcribed polycistronically. Operons are relatively common on chromosomes I and III, but much rarer on V and especially X. The average

spacing between the polyA site of the upstream gene and the trans-splice site of the downstream gene is 126 bp. Most of the operons uncovered in this analysis have not been reported previously, and we can cite numerous new examples of co-transcription of genes encoding apparently functionally related proteins. We suggest the possibility that close inspection of the operon list could reveal heretofore unknown protein relationships.

9. Developmental expression map of C.elegans genome

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We have been performing whole mount in situ hybridization using the cDNAs that have been classified in our EST project (See Thierry-Mieg et al.) as gene probes. Although there was a big delay in the project last year because we needed almost half a year to fix problems due to which our standard protocol suddenly didn't work, now we get the project back on the track and have finished some 7,600 genes. We have given minimal annotation to the in situ results; 10 stages for embryogenesis, 4 stages for larval-adult stage, on average 10 patterns (cell(s), tissue, region) per stage, 3 levels of relative intensity of signals per each pattern. Using the information, clustering analysis and extraction of consensus sequences in the upstream regulatory regions are in progress (see Ito et al.). Subsets of genes whose expression patterns are identical are being subjected to various analysis including RNAi, immunostaining and bioinformatic analysis (motif search, for example). This is a unique and powerful approach and, for example, we identified the function of proteasome in oocyte maturation and fertilization (see Hirono et al.). All the relevant data are integrated in NEXTDB and we are preparing to make them open by the worm meeting (See the demo by Shin-i et al.). In this report, we will show the content and the results of studies based on the database. Furthermore, we are planning "Annotation Relay" in which experts in various fields are invited to this lab by turns (a week or two per round and several people per round depending on the number of microscope) and look at the in situ slides on the microscope with the expert eyes to give more precise and deeper annotations to the in situ data than those we gave.

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10. A gene expression map for *C. elegans*

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The completion of the *C. elegans* genome sequence has identified nearly all of the genes in the genome (19,282 genes), but the function for most of these genes remains mysterious. A scant 6% of them have been studied using classical genetic or biochemical approaches (1135 genes), and only about 53% show homology to genes in other organisms (10,303 genes). The next challenge is to develop high-throughput, functional genomics procedures to study many genes in parallel in order to elucidate gene function on a global scale. One such approach is to use DNA microarrays to assay the relative expression of nearly every gene in the genome between two samples. Knowing when, where and under which conditions a particular gene is expressed can reveal the function of that gene.

A consortium of laboratories has used *C. elegans* DNA microarrays to profile gene expression changes in a wide variety of experiments. In each experiment, RNA from one sample was used to generate Cy3-labelled cDNA, and RNA from another sample was used to prepare Cy5-labelled cDNA. The two cDNA probes were simultaneously hybridized to a single DNA microarray and the ratio of the Cy3 to Cy5 hybridization intensities was measured, revealing which genes were relatively enriched in either RNA sample. Thirty different laboratories have collectively performed 553 experiments using these *C. elegans* DNA microarrays, including 179 experiments with microarrays containing 11,917 genes (63% of the genome) and 370 experiments using microarrays that have 17,817 genes (94% of the genome). The experiments compare RNA between mutant and wild-type strains, or between worms grown under different conditions. Many experiments have been done to date, including experiments on wild-type development, heat shock, Ras signaling, aging, the dauer stage, sex regulation and germ line gene expression. Individual microarray experiments reveal sets of genes that change in one mutant strain or growth condition. We combine the data from all of the experiments to assemble a gene expression database, and then used this database to group together co-regulated genes and visualize them using a three dimensional expression map. By matching the expression profile of an unknown gene to those of genes with known functions, the expression map can be used to ascribe functions to the large fraction of genes in the genome whose functions were previously unknown.

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11. 7,500 Genes and the Transition from Maternal to Zygotic Control of Development

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An immediate challenge of the post-genomic era is to quantitatively determine the precise temporal and spatial expression patterns of every gene in a model organism during its embryonic development. The relationship between genotype and phenotype can then be predictively modeled in terms of the regulation and consequences of these precise expression patterns. In addition to being quantitative, microarrays have the benefit of making measurements in parallel, thus enabling global observations of the transcriptome during development.

The *C. elegans* embryo is ideally suited for genomic analysis: it is experimentally accessible, well characterized genetically, has a compact genome, and the requirement for specific genes can be directly assessed using RNAi. To measure absolute abundance of transcripts during early development, we developed a sensitive and representative RNA amplification technique that enables quantitative analysis of mRNA levels from as few as 10 embryos. We then collected (in triplicate) pools of 10-15 embryos precisely staged (+/- ~3 min)by morphology at the 4-cell stage and allowed them to develop for proscribed amounts of time. mRNA from each of five time points spanning the 4 to ~102 cell stage was amplified and hybridized to oligonucleotide arrrays generating absolute measurements of transcript abundance for the entire genome. Approximately 7,500 genes are reproducibly detected in the time course, many of which are not represented in EST collections, and about 2,600 of them are significantly modulated over time (ANOVA $p<10^{-2}$). Transition from maternal to zygotic control of development is evident in the observed degradation of over 1,100 maternal transcripts along with the induction of approximately 1,500 zygotic transcripts. Among these, both simple and complex temporal expression patterns are detected. Intriguingly, reproducibility among replicates and similarity between adjacent timepoints increases towards the end of the time course. This result is consistent with the rate of molecular development decreasing and/or the stability of regulatory networks increasing as embryos approach the 100 cell stage. In either case, the observation suggests convergence onto a regulatory steady state. Statistical, computational and bioinformatic analysis of the data will be presented.

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12. Systematic gene inactivation with an RNAi feeding library

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We know the complete genome sequence of *C.elegans*, and can therefore predict the sequences of nearly all encoded genes. A major problem remains to understand what roles each gene plays in the development and function of the worm. The approach that we are taking to this problem is to use RNA-mediated interference (RNAi) to inhibit the function of each gene in the genome. To do this, we are constructing libraries of dsRNA-expressing bacteria; each strain targets a single predicted gene by RNAi when fed to worms. This feeding technique, pioneered by Timmons and Fire, allows for high throughput RNAi screening and, furthermore, the libraries can be used for an unlimited number of future screens (for example, to identify genes with subtle or conditional phenotypes).

We previously published the results of screening 87% of chromosome I genes (Fraser *et al*. 2000). We have now screened 88% of chromosome II genes and are currently screening an X chromosome library we have constructed. Data from chromosomes I and II are similar, with phenotypes detected for 14% of chr I and 12% of chr II genes. Also, as was seen on chromosome I, we find that fewer genes have an RNAi phenotype in the duplicated regions of chromosome II. We present an update of the project including a comparison of results obtained from chromosomes I, II, and X. In addition to identifying biological roles for many genes, we can also use our data to infer models of genome evolution and to discern relationships between the types of genes involved in different developmental processes. Finally, to increase our ability to identify RNAi phenotypes, we are screening for mutants with an increased sensitivity to RNAi; these should prove valuable for future RNAi-based screens.

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13. Caenorhabditis Genetics Center

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The Caenorhabditis Genetics Center (CGC), supported by NIH NCRR, supplies Caenorhabditis strains and information to researchers. The Minnesota team is responsible for acquiring, maintaining and distributing worm stocks, generating and maintaining a *C. elegans* bibliography, and publishing the Worm Breeder's Gazette (WBG). The English team acts as a clearing house for *C. elegans* genetic nomenclature and maintains the genetic map. The Texas team maintains the *C. elegans* web page.

The CGC now has over 4000 different strains. We strive to have at least one allele of every published gene and all chromosome rearrangements, duplications and deficiencies. In addition, we have several strains of species closely related to *C. elegans*. Strains are available upon written request, which should include a brief statement of the intended use of the strains. Email requests (to stier@biosci.cbs.umn.edu) are satisfactory. The CGC bibliography currently includes over 4300 research articles and book chapters. The WBG is published 3 times each year and currently has over 750 subscribers.

Various types of information from the CGC are available electronically. You can get current strain lists, the WBG subscriber directory, nomenclature guidelines, and the CGC bibliography at **http://biosci.cbs.umn.edu/CGC**. The English site contains a series of forms for submission of genetic map data, and this is the preferred method of data collection (**http://www.sanger.ac.uk/Projects/C_elegans/CGC**). Tables of data from the biennially produced map book are available for downloading or viewing. The Worm Breeder's Gazette and lots of other useful worm information are made available by Leon Avery at **http://elegans.swmed.edu/** .

We like to be acknowledged in papers for providing strains. We also like to receive reprints of worm papers.

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14. The *C elegans* Knockout Consortium: Interim Report.

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Our three laboratories comprise the *C. elegans* Gene Knockout Consortium, an international group of labs that provide gene knockouts by request to the worm community.

See web site - [http://elegans.bcgsc.bc.ca/knockout.shtml].

In the past two years, the consortium has received 882 requests for targeted gene disruptions. From this request list, consortium labs have eliminated the function of approximately 200 genes using a chemical mutagenesis approach [as of April, 2001]. All mutants and data provided by consortium laboratories are in the public domain and freely available to all researchers.

The *C. elegans* genome contains approximately 19,000 ORF's, of which only a fraction has been characterized through genetic mutational analysis. While our ultimate goal is to obtain knockouts in all the ORF's of this organism, for the immediate future we will focus on providing ko's requested by individual lab's and on nematode homologs of human genes.

We will report on our progress and on our evolving strategy for high throughput production of ko's.

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15. WormBase: A Web-accessible database for C. elegans biology

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The C. elegans genomic, genetic and biological database WormBase (http://www.wormbase.org) has two fundamental goals: to expand the information content of the database and to make it easier to use. As was its predecessor ACeDB, WormBase is a repository of physical map and genome sequence data (>99.6% complete) from The C. elegans Sequencing Consortium, and of genetic map data curated by the Caenorhabditis Genetics Center. WormBase continually updates gene structures and WormPep (a compendium of the best guess of C. elegans proteins) based on ESTs, cDNA sequence, C. briggsae genomic sequence, protein homology, and other relevant data.

A central element of WormBase is professional curation of the literature and of large-scale datasets. We are systematically extracting information from the C. elegans literature (about 4400 papers in April 2001). New data sets added in the past year include integrated transgenes, RNAi experiments from the large-scale screens and from the literature; gene expression data from the literature, SNP location and map data; and diagrams of cells from John White. Revisions of data models are underway to accommodate additional types of data on gene expression, regulation and function, cell function, as well as a more refined view of sequence features. As part of the curation effort, we are working with C. elegans researchers to develop broadly usable structured vocabulary for intensely studied aspects of C. elegans biology such as stages of embryonic development. To increase the sharing of data among model organism databases, WormBase has joined the Gene Ontology Consortium to help modify their shared, structured vocabulary to accomodate C. elegans biology.

WormBase is now updated weekly. To increase speed of access through the Internet , mirrors of the website will be available by the time of this meeting. On the website, the classic graphical displays of ACeDB have been supplemented with new viewers: A SimpleMap viewer gives the look of an old style hand-drawngenetic map, but with clickable links. Genome Hunter allows browsing of the genome in a continuous fashion, displaying genes, cDNAs, C. briggsae homologies, and other features such as the location of sequences used in RNAi experiments; it also allows easy downloading of sequence. A new Pedigree Browserdisplays the cell lineage highlighting cells of interest. In addition to Simple Search from the home page, searches for expression patterns, RNAi data, cells, BLAST searches, and complex queries are available. A User guide has been added. We rely on users to inform us of errors in information or software performance, as well as your desires for content and presentation. Users can forward data to curators via web-based forms, email, or via file transfers. WormBase exists to serve the C. elegans and broader biomedical community, and the WormBase Consortium thanks our many data contributors and collaborators, especially those providing their large-scale datasets, and those providing feedback.

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16. Characterization of RNAi-sensitive mutators and the *C. elegans Dicer* homolog

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The transposon Tc1 is active in somatic cells of all *C. elegans* isolates. However, Tc1 activity in the germ line is restricted to certain strains: for example Tc1 is active in the germ line of Bergerac BO, but silenced in Bristol N2. Mori *et al.* showed that multiple genetic loci are involved in regulation of germ-line transposition (Mori *et al.*, 1988).

In an EMS screen for activation of Tc1 in the germ line of Bristol N2, 43 mutants were isolated that fall into two classes: mutants that are resistant to *pos-1*RNAi, such as *mut-7(pk204)*(Ketting *et al.* 1999) and *mut-14(pk738)* (see abstract Tijsterman *et al.*), and mutants that are wild type for *pos-1* RNAi. The latter class consists of 20 mutants. These mutants are sensitive to RNAi directed against germline-expressed genes (*pos-1*, *rba-2*), as well as somatically-expressed genes (*unc-22*). Furthermore, one of the mutants, *mut-11 (pk724)*, was tested for *gld-1* cosuppression, and found to be sensitive. Similarly, two previously isolated Tc1 mutators, *mut-4(st700)* and *mut-6(st702)* are completely sensitive to both RNAi (Tabara *et al.*, 1999; our data) and cosuppression (Ketting and Plasterk, 2000).

RNAi-sensitive mutators do share some additional phenotypes with *mut-7(pk204)*. Both RNAi-resistant and RNAi-sensitive mutators are ts-sterile. DAPI-staining of the gonads several mutants shows a reduction of gonad size and the presence of univalents in diakinesis. Like *mut-7*, most RNAi-sensitive mutators are Him. It is possible that the different mutations are directly causing the Him-phenotype and the ts-sterility; on the other hand, these phenotypes might result from the activation of transposons in the germ line, reminiscent of the hybrid dysgenesis syndrome in *Drosophila*. Initial mapping of the ts-sterility phenotype of fifteen of the RNAi-sensitive mutants indicates this class of mutators consists of at least seven genes. Further progress will be reported.

In vitro experiments using *Drosophila* have shown that in the initiation step of RNAi, dsRNA is cleaved into guide RNAs. Next, these guide RNAs can target a nuclease complex to the mRNA, which is then degraded. We now show that also in *C. elegans* extracts, exogenous dsRNA is cleaved into 23 bp guide RNAs. So far, one gene has been shown to be involved in the formation of guide RNAs: *Drosophila Dicer*, encoding a RNase III enzyme. The *Dicer* gene has one clear homolog in *C. elegans*: K12H4.8. In order to analyze the role of *Dicer* in RNAi in *C. elegans*, adeletion mutant of K12H4.8 was isolated from a chemical deletion library. A 1.7 kb deletion removes the helicase domain and part of the PAZ domain, and is likely to be a complete null allele. The mutant, after outcrossing, is sterile and shows a weak *unc-22* phenotype after feeding of *unc-22* dsRNA. Rescue experiments and phenotypes will be discussed.

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17. *ego-1*, a link between germline development and RNAi

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The adult *Caenorhabditis elegans* gonad contains proliferating and differentiating germ cells arrayed in a linear fashion from mitotic germ cells at the distal end to mature gametes at the proximal end. Germline development requires the coordinate regulation of diverse events such as cell proliferation, sex determination, meiotic progression, and gamete formation.

ego-1 was originally identified in genetic screens for enhancers of *glp-1* (Qiao et al., 1995). GLP-1 is a Notch-type receptor that mediates cell-cell interactions during somatic and germline development; in the germ line, it mediates a proliferative signal from the somatic distal tip cells to the distal germ cells. Based on genetic studies, ego-1 gene activity promotes GLP-1-mediated signaling as well as later germline events such as meiotic progression and gametogenesis. *ego-1* mutants are sterile with defects at various developmental stages. In ego-1 mutants, germ cells enter meiosis prematurely, consistent with a decrease in GLP-1-mediated signaling; some mitotic germ cells arrest, suggesting a mitotic progression defect; early meiotic prophase occurs slowly and oocytes contain desynapsed chromosome pairs, as if chromosome pairing/ recombination is abnormal; and oocytes are small and fail to take up yolk (Qiao et al., 1995; Smardon et al., 2000; E.M. and V.V., unpublished).

ego-1 encodes a member of the RdRP (RNA-directed RNA polymerase) family (Smardon et al., 2000) that includes *Neurospora* QDE-1 and *Arabidopsis* SDE-1/SGS-2. In addition to their developmental defects, *ego-1* mutants are defective in RNAi for a large proportion of germline-expressed genes (Smardon et al., 2000; E.M. and V. V., unpublished). Similarly, mutations in *qde-1* and *sde-1/sgs-2* disrupt some forms of RNA silencing, suggesting that RdRP-like proteins play a role in RNA silencing. We have found that expression of an integrated *pie-1:gfp:histone* transgene (a kind gift of G. Seydoux and J. Austin) is resistant to RNAi in the *ego-1(-)* background. Normally, expression of the *pie-1:gfp:histone* transgene produces high GFP levels in the proximal germ line. When fed *gfp* dsRNA (using the Timmons and Fire L4440 vector/HT115 E. coli system), N2 and *ego-1(-/+)* animals lose the GFP fluorescence within 24 hr whereas *ego-1(-)* animals retain strong GFP fluorescence after three days. We used this construct to assay the RNAi response in *ego-1(-)* progeny of *ego-1(+/-)* mothers, and find that they express GFP at low levels. Therefore, the RNAi defect seems to be partially rescued by expression of maternal *ego-1(+)*. We obtained evidence of weak maternal rescue using other germline-expressed genes, as well. These results suggest that the dose of functional EGO-1 protein is critical for a robust RNAi response.

We are investigating the biological and biochemical role of EGO-1 using various approaches, as will be discussed. To study EGO-1 distribution in the germ line and the effects of EGO-1 overexpression, we have generated *ego-1:gfp* fusion constructs and are collaborating with J. Austin (U Chicago) to generate integrated transgenes (Praitis et al., 2001). Intriguingly, based on anti-GLD-1 (Jones et al., 1996) and anti-PGL-1 (Kawasaki et al., 1998) staining, we find that P granule composition and distribution are altered in the ego-1 germ line. To date, all known P granule-associated proteins are RNA binding proteins. We are collaborating with C. Cameron (Penn State) to test whether EGO-1 has RdRP activity and/or binds RNA *in vitro*. We have also begun using the yeast two-hybrid system to screen for EGO-1 interacting proteins. One possibility consistent with our current data is that EGO-1 activity promotes diverse processes in the germ line to ensure the formation of functional gametes

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18. Using RNAi to identify new components of the RNAi machinery

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RNAi is an effective tool to phenocopy the loss of function of many genes in a variety of organisms, including *C. elegans* (1). During an RNAi-based screen using germline-enriched RNAs (2), we found that a pool of eight dsRNAs that included *glp-1* dsRNA produced viable embryos (0-2% lethality), yet injecting the *glp-1* dsRNA alone produced a high degree of embryonic lethality (87-97%). These results might either be explained by non-specific suppression of *glp-1*(RNAi) by multiple dsRNAs, or by specific suppression by a single dsRNA. To distinguish between these possibilities, *glp-1* dsRNA was co-injected with specific members of the pool. We found that only one dsRNA from the pool could suppress *glp-1* dsRNA-mediated lethality. Additionally, removing this suppressing dsRNA from the pool of eight restored a highly penetrant *glp-1* phenotype. We were surprised to find that the suppression effect was not a suppression of *glp-1* loss of function, since two *glp-1* alleles (*q224* and *q231*) could not be suppressed; rather it appeared to be a suppression of the RNAi process itself, since co-injection could also suppress the lethality produced by other dsRNAs (*mom-2*, *par-2*, or *hmp-2*). Since these results suggest that components of RNAi can themselves be targets of the RNAi machinery, we next asked whether known components of the RNAi machinery (3) could behave similarly. RNAi of either *mut-7* or *rde-1* could also suppress the lethality produced by RNAi of essential genes. The effect appears specific since unrelated dsRNAs, even at 10-fold concentrations, could not suppress this lethality. These results suggest it is possible to identify genes with roles in the RNAi pathway using RNAi.

We will discuss our results and present data on a second dsRNA that also can suppress RNAi. We are currently testing candidate components of the RNAi machinery by this method, and we are developing tools for a large-scale screen to find additional components.

1. Fire et al. (1998) Nature 391:806-11

2. Reinke et al. (2001) Proc Natl Acad Sci USA 98:218-23

3. Ketting et al (1999) Cell 99:133-41; Tabara et al (1999) Cell 99:123-32

19. Potential for Cross-Interference with RNAi

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RNAi is a useful reverse genetic tool for analyzing gene function in many, although not all, eukaryotic species. Given the large number of genes that have arisen due to gene duplication and that share highly conserved domains with other family members, we were concerned with the following question: What is the potential for cross-interference between the introduced dsRNA and endogenous sequences other than those specifically targeted? As part of our experimental approach to address this question, we cloned homologous sequences from *C. elegans* and the closely related *C. briggsae* and tested these sequences for the ability to cause interference in both species. This experimental approach allowed us to accomplish three goals: (1) determination of the effectiveness of short sequences to generate reliable RNAi phenotypes; (2) potential for cross-interference between the introduced dsRNA and mRNAs other than those specifically targeted; and (3) preliminary characterization of the functions of conserved genes involved in embryonic patterning in *C. briggsae.* We have found that the ability of dsRNA to cause effective interference relies on a combination of length, longest 100% stretch of nucleotide identity and overall sequence identity with the target mRNA. Our results are consistent with current models of the mechanism governing RNAi. But our results also suggest that although an incredibly diverse set of organisms appear to respond to the presence of dsRNA in a similar manner, i.e., by silencing the activity of cognate sequences presumably through a conserved pathway, even closely related species can differ in their sensitivity to dsRNA and the specificity of their responses.

20. Mutants of *C. elegans* defective in uptake of dsRNA in RNAi

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RNA interference is the experimental gene silencing by double stranded RNA. One of the most striking aspects of RNAi in *C. elegans* is that it works not only when the dsRNA is injected into the target tissue, but also when the dsRNA is added to the food of the animal or when the animals are soaked in a solution of dsRNA (Timmons and Fire 1998; Tabara *et. al.*,1998) indicating that RNAi can spread through the entire organism and can even exert its effect via the gonads on the next generation of animals.

We tested several natural isolates of *C. elegans* for their response to feeding on dsRNA, and found that most natural isolates are sensitive. One strain, CB4856 from Hawaii, is not sensitive to dsRNA encountered in the environment. It is, however, sensitive to injected or transgene encoded dsRNA. This suggests that a factor involved in uptake of the dsRNA is not functional in this Hawaiian strain. We are currently mapping the *rsd* (RNA Spreading Defective) genes that make CB4856 resistant to environmental dsRNA (we mapped one close to the center of LGI).

In a mutant screen for RNAi deficient mutants, using a RNAi sensitive, spreading-proficient strain and *E. coli* expressing dsRNA for an essential maternal gene, we found mutants that respond to injected dsRNA, indicating that the core RNAi machinery is intact, but that fail to respond to dsRNA when introduced by feeding. So far we have identified six loci. The identity of one of the genes, *rsd-3*, suggests a role in vesicle transport. We will report on genetic mapping and identification of this and other genes.

21. RNAi analysis of 762 germline enriched genes.

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We are interested in identifying genes that play essential roles in early embryogenesis. Toward this goal, we have been studying the function of ovary expressed genes using RNAi. Here we report on the RNAi analysis of 762 genes selected by microarray studies to have high expression in the ovary. We inject dsRNA representing each gene into 9 worms and follow each worm and its progeny separately. We score for both embryonic and post-embryonic defects. For each gene giving embryonic lethality above 80%, we obtain time-lapse movies of the first 50 minutes of embryogenesis from embryos from three separate affected worms. Our protocol and results differ slightly from other large scale RNAi studies and we will present a comparison of the results.

We have found that 31% of the germline enriched genes give rise to embryonic lethal phenotypes. Interestingly, although there is a strong correlation between a high degree of sequence conservation and embryonic lethality, the major lethal class is composed of proteins that are not annotated, thus these RNAi data provide the first functional data for these genes from any genome. Using the microarray data we are exploring the connection between expression patterns and phenotypes. Our analysis thus far allows us to extend a previous observation that genes required for embryogeneis are less likely to map on the X-chromosome.

We are categorizing genes using defects seen in early embryogenesis. We expect that genes giving similar embryonic defects will identify components of biochemical pathways and protein complexes allowing us to predict functions for previously uncharacterized genes.

22. Roles for embryonic lethal genes on *C. elegans* Chromosome I identified by RNA interference and 4-dimensional video-microscopy and identification of a new Par-like gene

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Early embryonic development involves complex events such as the regulation of cell and nuclear divisions and the establishment of embryonic polarity. To identify genes involved in such events, we collected 4-dimensional time-lapse video recordings of the first three cell divisions and analysed terminal phenotypes after RNA interference (RNAi) of 147 embryonic lethal genes previously identified in a systematic screen of *C. elegans* chromosome I. Over half gave defects in early processes such as meiosis, the assembly or position of the first mitotic spindle, cytokinesis, and proper nuclear positioning.

One striking finding from our studies is that some phenotypic classes primarily contain genes involved in a shared biochemical process. For example, many genes whose RNAi resulted in 1-cell arrest encode proteasome or APC components, those that cause altered pronuclear envelope morphology are often involved in nuclear transport or are components of the nuclear envelope, and those resulting in abnormal nuclear morphology are often involved in the packaging or synthesis of DNA. Such correlation between phenotype and cellular function can be used to inform analyses of genes whose biochemical function is unknown. Genes of unknown biochemical function indeed occur in the phenotypic classes mentioned above suggesting that these genes might encode new factors involved in the identified processes.

In addition to looking for defects during early cleavages, we also characterised genes with no early RNAi phenotype (nearly half of embryonic lethal genes) by examining terminal arrest phenotypes. This analysis identified a common arrest phenotype for genes involved in

basal transcription and further identified candidate genes for late embryonic processes such as embryonic elongation.

When applied to the whole genome, this approach should identify the vast majority of genes required for early cell processes, paving the way for a greatly improved understanding of these processes and their regulation at the molecular level.

We will also present initial characterisation of a gene we identified on chromosome I with a Par-like RNAi phenotype. The first cleavage is symmetric, the two resulting daughters cleave nearly synchronously, and P-granules are mis-segretated at the two-cell stage. Furthermore, PAR-2 protein is localised all around the cortex instead of being only at the posterior, suggesting an early role in embryonic polarity.

23. Genome-wide functional analysis by RNAi-by-soaking with a non-redundant cDNA set

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The genome of *C. elegans* consists of approximately 19,000 genes, and the function(s) of many of them are still unknown. We have been performing a systematic functional analysis of the expressed genes by the RNAi-by-soaking method [1]. In this method, L4 worms are soaked in dsRNA solutions for RNA delivery, and their F1 phenotypes as well as P0 germline phenotypes are examined. As templates for *in vitro* dsRNA synthesis, a set of tag-sequenced non-redundant cDNAs corresponding to ~10,000 genes (representing half of the predicted genes) has been used. Because the size of the cDNA set is comparable to the number of detectable genes by a whole-genome DNA microarray[2], this cDNA set is likely to contain most genes expressed in the development of *C. elegans* .

We examined the RNAi phenotypes for ~2,800 genes (15% of the predicted genes) to date, and 27% of them showed detectable developmental phenotypes under a dissection microscope. The ratio of the cDNA clones that caused phenotypes was apparently higher than that of the predicted ORFs (13~14%)[3,4], consistent with the finding by Fraser et al.[3] that ORFs having EST are more likely to give RNAi phenotypes. Thus, the RNAi screen using the non-redundant cDNA set is an efficient way to comprehensively identifying essential genes for development. The general RNAi phenotypes observed were; F1 embryonic lethality (14% of the examined clones), F1 post-embryonic lethality (4.5%), P0 sterility (7%), F1 sterility (1%), and morphological abnormality (0.7%).

Of these, we analyzed the phenotypes of F1 sterility in detail with DIC microscopy and DAPI staining, and have identified 31 genes that might play important roles in germline development. Each cDNA clone affected a distinct process, such as proliferation of germ cells, germline sex determination, gonadogenesis, gametogenesis, and fertilization.

The genes corresponding to the cDNA clones we used distribute almost evenly to all chromosomes. The incidence of the essential genes was comparable among the autosomes (26% ~ 34%), however, that on the X chromosome was significantly lower (16%). Because a mutation in the essential genes on the X chromosome would directly lead to the lethality/sterility of males, they appear to be removed from the X chromosome in the process of evolution.

We aim to complete the RNAi screen of the whole cDNA set. The functional information obtained in this work will provide a starting point for further analysis of each gene.

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24. Identification of *C. elegans* genes required for the DNA damage response using a combination of functional genomic approaches

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To address novel questions about metazoan DNA damage checkpoints and DNA repair (referred to as the DNA damage response or "DDR"), we are using functional genomic approaches in *C. elegans*. Our first goal was to identify novel genes involved in DDR by generating a protein-protein interaction map. Database searches using known DDR proteins from other organisms identified 75 *C. elegans* ORFs that encode putative orthologs (dORFs). These dORFs were cloned using the Gateway recombinational cloning technology and transferred to both RNAi-feeding and two-hybrid vectors. High-throughput RNAi was used to establish that many dORFs are indeed required for DDR in the worm. A DDR protein interaction map was generated by screening each of the 75 dORFs for two-hybrid interactors. This map contains 165 potential interactions. Finally RNAi was used for many of the novel potential interactors. In each case we scored for defects in DNA damage-induced cell cycle arrest and apoptosis to ascertain checkpoint integrity. Using this combination of large-scale two-hybrid and RNAi approaches we have identified novel DDR worm genes and many of those have potential orthologs in mammals. These findings suggest that protein interaction mapping and RNAi can be complementary and can be used in combination to uncover novel genes involved in other biological processes.

25. Two RGS proteins that inhibit Galphao and Galphaq signaling in *C. elegans* neurons require a Gbeta5-like subunit for function

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Gβ proteins have traditionally been thought to complex with Gγ proteins to function as subunits of G protein heterotrimers. The divergent $G\beta_5$ protein, however, can bind either Gγ proteins or regulator of G protein signaling (RGS) proteins that contain a G-gamma-like (GGL) domain. RGS proteins are inhibitors of G protein signaling that act as Gα GTPase activators. While $G\beta_5$ appears to bind RGS proteins *in vivo*, its association with Gγ proteins *in vivo* has not been clearly demonstrated. It is unclear how $G\beta_5$ might influence RGS activity. In *C. elegans* there are exactly two GGL-containing RGS proteins, EGL-10 and EAT-16, and they inhibit $G\alpha_o$ and $G\alpha_q$ signaling, respectively.

We knocked out the gene encoding the *C. elegans* $G\beta_5$ ortholog, GPB-2, to determine its physiological roles in G protein signaling. The *gpb-2* mutation reduces the functions of EGL-10 and EAT-16 to levels comparable to those found in *egl-10* and *eat-16* null mutants. *gpb-2* knockout animals are viable and exhibit no obvious defects beyond those that can be attributed to a reduction of EGL-10 or EAT-16 function. GPB-2 protein is nearly absent in *eat-16; egl-10* double mutants, and EGL-10 protein is severely diminished in *gpb-2* mutants.

These results indicate that $G\beta_5$ functions *in vivo* complexed with GGL-containing RGS proteins. In the absence of $G\beta_5$, these RGS proteins have little or no function. The formation of RGS-$G\beta_5$ complexes is required for the expression or stability of both the RGS and $G\beta_5$ proteins. Appropriate RGS-$G\beta_5$ complexes regulate both $G\alpha_o$ and $G\alpha_q$ proteins *in vivo*.

26. *eat-11* encodes GPB-2, a Gbeta5 ortholog that interacts with $G_o$alpha and $G_q$alpha to regulate *C. elegans* behavior

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In *C. elegans*, the GOA-1 $G_o\alpha$/EGL-30 $G_q\alpha$ signaling network regulates locomotion and egg laying. Genetic analysis shows that the locomotion and egg-laying defects of activated Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) are suppressed by perturbations of this network, which include loss of the GOA-1 $G_o\alpha$, DGK-1 diacylglycerol kinase, EAT-16 G-protein γ subunit-like (GGL)-containing RGS, or the unidentified protein encoded by the gene *eat-11* [1].

To define the role of *eat-11* in G_o/G_q signaling, we cloned *eat-11* and report that it encodes GPB-2, an ortholog of mammalian $G\beta_5$. Unlike other Gβ proteins, $G\beta_5$ has been shown to bind specifically to GGL-containing RGS proteins, and the $G\beta_5$/RGS complex can promote the GTP-hydrolyzing activity of Gα subunits. In addition to EAT-16, the GGL-containing RGS protein EGL-10 also participates in the GOA-1/EGL-30 signaling network. We find that several *gpb-2*(*lf*) alleles, including a putative null allele, confer locomotory and egg-laying phenotypes intermediate between *eat-16*(*lf*) and *egl-10*(*lf*). This indicates that GPB-2 may regulate the opposing activities of $G_o\alpha$ and $G_q\alpha$ via an interaction with both the EAT-16 and EGL-10 RGS proteins. Similar models have been proposed based on independent studies of *gpb-2* deletion mutants [2, 3]. Two other *gpb-2*(*lf*) mutations, C266Y and D307N, confer different behavioral phenotypes which suggest that these mutations specifically disrupt the activity of the EAT-16 RGS without affecting EGL-10.

We also analyzed the role of *gpb-2* in defecation and feeding. *gpb-2* and *eat-16* regulate enteric muscle activity similarly, and this function appears to be independent of G_o signaling. In this behavior, the C266Y and D307N mutants behave like the other *gpb-2*(*lf*) mutants, including the putative null, indicating that GPB-2 normally interacts with only the EAT-16 RGS in the enteric muscle. For feeding behavior, we find that loss of GPB-2 or EAT-16 function decreases the activity of the M3 pharyngeal motor neuron. M3 is important for generating coordinated pharyngeal motions that allow efficient feeding. Thus, the misregulation of M3 in *gpb-2*(*lf*) mutants may explain their starved appearance, which was first described by L. Avery. We also show that some aspects of GPB-2 function may occur downstream of a pharyngeal muscarinic receptor, and that these functions are required for normal feeding and growth.

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27. An N-terminal region of *C. elegans* RGS proteins EGL-10 and EAT-16 directs inhibition of Go alpha versus Gq alpha signaling

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Regulators of G protein signaling (RGS proteins) inhibit G protein signaling. While all RGS proteins contain an RGS domain that contacts G alpha subunits and activates their GTPase activity, a subset also contains a G-gamma-like (GGL) domain, as well as a conserved N-terminal region of unknown function. In *C. elegans* two members of this subset, EGL-10 and EAT-16, selectively inhibit GOA-1 (Go alpha) and EGL-30 (Gq alpha) signaling, respectively. To assess the role of the subregions of these RGS proteins in G alpha target selectivity, we generated transgenes in *C. elegans* expressing EGL-10, EAT-16, their conserved subregions, or chimeras between the two proteins. We determined which transgenes could rescue the defects seen in mutants lacking endogenous EGL-10 or EAT-16. EGL-10 and EAT-16 retained the ability to specifically inhibit GOA-1 or EGL-30, respectively, even when expressed from the same heterologous promoter, demonstrating that distinct G alpha specificity is a property of the RGS proteins themselves, and not of their expression patterns. We found that coexpression of N-terminal and GGL-RGS fragments of EGL-10 gave full EGL-10 activity. In contrast, expression of either fragment alone gave little activity. Thus, both regions of EGL-10 cooperate to inhibit G protein signaling, yet need not be covalently attached to each other. Finally, using EGL-10/EAT-16 chimeras we found that the GGL-RGS region of either protein is capable of acting on either GOA-1 or EGL-30. These data suggest that a key factor determining G alpha target selectivity of the chimeras is the manner in which the GGL-RGS region is linked to the N-terminal region.

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28. Novel downstream targets of GOA-1, EGL-30, GPA-12 and the *C. elegans* specific G-proteins

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In a recent study, we have shown a functional role for the second G-protein β subunit, GPB-2, in GOA-1 (mammalian homologue of Goα) and EGL-30 (mammalian homologue of Gqα) signaling. GPB-2 is most related to the divergent mammalian Gβ5 subunit that, unlike the other mammalian Gβ subunits (Gβ1-4), has the ability to interact with the Gγ subunit-like (GGL) domain of a subset of RGS proteins. Using both genetic and yeast two-hybrid analysis, we found that GPB-2 interacts with the regulator of G-protein signaling (RGS) proteins EAT-16 and EGL-10 in *C. elegans*, and that GPB-2 regulates the functions of these proteins in the Goα / Gqα signaling network (1).

Two interesting outcomes of this study were that: first, animals lacking both *gpb-2* and *goa-1* function have a synthetic larval lethal phenotype, which is rescued when EGL-30 activity is reduced. While this result suggests that Gqα activity causes the *gpb-2 goa-1* double mutant synthetic lethality, no suppression is observed when EGL-8 (a potential downstream effector of EGL-30) activity is reduced. Thus, we expected to find as yet unidentified mediators of EGL-30 signaling in a screen for suppressors of the synthetic lethal phenotype of *gpb-2 goa-1* double mutants. To date, we have isolated 28 suppressors of this synthetic lethality, and found that 4 of these are alleles of *egl-30*. Thus, some of the remaining are likely to disrupt novel downstream effectors of EGL-30.

Secondly, we have shown that members of a G-protein signaling pathway can be identified using yeast two-hybrid analysis. Thus, we have performed two-hybrid interaction screens using all 20 *C. elegans* Gα subunits. We have identified 11 different proteins that interact with 4 different Gα subunits. Some of these proteins suggest novel signaling pathways downstream of G-proteins. Currently, we are investigating the biological significance of these interactions.

Furthermore, we have screened for novel downstream targets of GPA-12 (mammalian homologue of G12). Overexpression of dominant active GPA-12 under control of a heat-shock promoter results in larvae that arrest at the L1 stage but eventually recover. We have found 42 mutations that suppress this phenotype, and are in the progress of characterizing these suppressors. Thus, we will present novel targets of several different G-proteins identified using diverse techniques.

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29. THE G-PROTEIN COUPLED RECEPTOR SML-1 ANTAGONISES RAS/MAP KINASE SIGNALLING DURING OLFACTION AND VULVAL DEVELOPMENT

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During vulval development, three out of six vulval precursor cells (VPCs) are induced by a signal from the gonadal anchor cell (AC) to adopt vulval cell fates. The AC triggers vulval differentiation by activating a highly conserved RTK/RAS/MAP kinase pathway in the VPCs. *C. elegans* is attracted to certain volatile compounds such as isoamyalcohol, diacetyl or benzaldehyde. When animals are exposed to a volatile attractant, the RAS/MAP kinase pathway is activated in two pairs of chemosensory neurons (AWA and AWC) in the head (Hirotsu et al. (2000) *Nature* 404, 289-293). Furthermore, chemotaxis is compromised by mutations that perturb the activity of the RAS/MAP kinase pathway.

We have performed genetic screens to identify negative regulators of RAS/MAP kinase signalling during vulval induction. While cloning one of these inhibitory genes (*meg-1*), we have identified the *sml-1* gene (for *sm*ell; ORF F49E12.5) because overexpression of *sml-1(+)* efficiently rescues the *meg-1* multivulva (Muv) phenotype. *sml-1* encodes a seven-pass transmembrane protein similar to the *sra* family of chemosensory receptors. A SML-1::GFP fusion is expressed in the AWA and AWC chemosensory neurons, but no expression can be observed in the VPCs. We have isolated a *sml-1* deletion allele *(zh13)* that removes most of the coding region. *sml-1(zh13)* single mutants exhibit slightly increased chemotaxis to isoamylalcohol or diacety but no obvious vulval defects. However, loss of *sml-1* function partially suppresses the chemotaxis defect of *let-60(n2021)* or *lin-45(sy96)* mutants and the vulvaless (Vul) phenotype of *let-60(n2021), let-60(n2031^{dn})/+* or *sem-5(n2019)* mutants. Thus, SML-1 acts antagonistically to the Ras/MAP kinase signalling pathway both during vulval induction and chemotaxis. Overexpression of *sml-1* under control of its own promoter (*sml-1(XS)*) causes several phenotypes, some of which can also be observed in starved animals. *sml-1(XS)* animals are defective in chemotaxis to volatile attractants, they are weakly Vul, Unc, Egl and 14% of the animals enter the dauer stage at 25°C. SML-1 function is in part mediated by the GPA-5 G protein-alpha subunit. A *gpa-5* loss-of-function mutation (Jansen et al. (1999), *Nature Genetics* 21:414-419) partially rescues the chemotaxis defect of *sml-1(XS)* animals and it suppresses the *let-60(n2021)* chemotaxis and vulval defects. Interestingly, we observed that under limiting food conditions the Muv phenotype of *let-60(n1046gf)* decreases, while *sml-1(zh13); let-60 (1046gf)* double mutants appear to be insensitive to food conditions. Thus, SML-1 may act as a sensor in the AWA and AWC neurons to regulate vulval induction in response to the external conditions.

30. Modulation of inductive signaling during *C. elegans* vulval development

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In *C. elegans* hermaphrodites, the vulval precursor cells (VPCs) P3.p-P8.p can adopt vulval or hypodermal fates. In wild-type animals, the anchor cell secretes LIN-3, which activates its receptor, LET-23, and causes P5.p-P7.p to adopt vulval fates. Although P3.p, P4.p, and P8.p also express LET-23, it is thought that their distance from the anchor cell prevents levels of activated LET-23 from accumulating necessary to overcome default hypodermal fates. We have used two approaches to study modulation of responses to this inductive signal. In one approach, we tested candidate loci for interaction with this pathway. We find that a loss of function mutation in the *egl-15* negative regulator, *clr-1*, a receptor tyrosine phosphatase, increases responsiveness of all 6 VPCs to the inductive signal. In contrast, an activated allele of *egl-30* Gq alpha facilitates responsiveness of P5.p-P7.p, but not P3.p, P4.p, and P8.p. In a second approach, we identified EMS-induced mutations that enhance the choice of vulval fates by P3.p, P4.p, and P8.p in the presence of a gain-of-function allele of *let-23*. Some of these mutations, such as *sy622*, strongly enhance the responsiveness of P3.p, P4.p, and P8.p, while only weakly affecting P5.p-P7.p. These results indicate that there are both pan-Pn.p, as well as Pn.p-specific mechanisms operating to modulate vulval induction. Pan-Pn.p mechanisms might function as buffers to lower the overall noise inappropriately contributing to vulval induction. In conjunction with pathways defined by *egl-30(gf)* and *sy622* which increase responsiveness of P5.p-P7.p and decrease responsiveness of P3.p, P4.p, and P8.p, respectively, a robust response by only a subset of cells might be guaranteed during inductive signaling.

31. *C. elegans* POP-1/TCF functions in a canonical Wnt pathway that controls cell migration and a noncanonical Wnt pathway that controls cell polarity

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In *C. elegans*, Wnt signaling pathways are important in controlling cell polarity and cell migrations. In the embryo, a novel Wnt pathway functions through a ß-catenin homolog, WRM-1, to down-regulate the levels of POP-1/Tcf in the posterior daughter of the EMS blastomere. The level of POP-1 is also lower in the posterior daughters of many anteroposterior asymmetric cell divisions during development. I found that this is the case for of a pair of postembryonic blast cells in the tail. In wild-type animals, the level of POP-1 is lower in the posterior daughters of the two T cells, TL and TR. Furthermore, in *lin-44/Wnt* mutants, in which the polarities of the T cell divisions are frequently reversed, the level of POP-1 is frequently lower in the anterior daughters of the T cells. I used a novel RNA mediated interference technique to interfere specifically with *pop-1* zygotic function and determined that *pop-1*is required for wild-type T cell polarity. Surprisingly, none of the three *C. elegans* ß-catenin homologs appeared to function with POP-1 to control T cell polarity. Wnt signaling by EGL-20/Wnt controls the migration of the descendants of the QL neuroblast by regulating the expression the Hox gene *mab-5*. Interfering with *pop-1* zygotic function caused defects in the migration of the QL descendants that mimicked the defects in *egl-20/Wnt* mutants and blocked the expression of *mab-5*. This suggests that POP-1 functions in the canonical Wnt pathway to control QL descendant migration and in novel Wnt pathways to control EMS and T cell polarities. We are currently using available mutations and zygotic RNAi to determine what role other known Wnt pathway components play in the control of T cell polarity.

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32. AN AXIN-LIKE PROTEIN FUNCTIONS AS A NEGATIVE REGULATOR OF WNT SIGNALING IN THE Q NEUROBLAST LINEAGE

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The Wnt signaling pathway plays a key role in many developmental processes by regulating the expression of specific Wnt-responsive target genes. Binding of Wnt to its receptor Frizzled leads to the stabilization of the effector beta-catenin, which in turn migrates to the nucleus to activate Wnt target gene expression. The level of beta-catenin in the cell is tightly controlled by a complex consisting of the tumor suppressor protein APC, Axin and the kinase GSK3, which marks beta-catenin for degradation by the proteasome. This ensures that, in the absence of Wnt signaling, target gene expression is turned off. In *C. elegans*, Wnt signaling is mediated by the beta-catenin BAR-1, which interacts with the Tcf-like transcription factor POP-1 to activate the expression of Wnt target genes such as the Hox gene *mab-5* in the Q neuroblast lineage (1).

To learn more about the mechanism and regulation of BAR-1/beta-catenin signaling, we have performed yeast-two-hybrid screens to isolate BAR-1 interacting proteins. One of these interactors, C37A5.9, was isolated multiple times and was shown to be specific for BAR-1. Thus, no binding was observed with the adhesion specific beta-catenin HMP-2 or POP-1/Tcf. C37A5.9 encodes a protein which is similar to Axin. C37A5.9 is 18-21% identical to vertebrate and *Drosophila* Axin and contains the expected conserved RGS and DIX domains. In flies and vertebrates, Axin functions as a scaffold to assemble the complex of APC, GSK3 and beta-catenin. This function is essential for beta-catenin downregulation. We find that in addition to BAR-1, C37A5.9 also binds GSK-3 and we are currently testing if it interacts with the APC-like protein APR-1 as well.

Is C37A5.9 a functional Axin homologue and a negative regulator of Wnt signaling? To investigate this, we studied the RNAi phenotype of C37A5.9. Loss-of C37A5.9 function does not result in obvious visible phenotypes, but does induce a striking defect in Q neuroblast migration. The direction of Q neuroblast migration is specified by the Wnt target gene *mab-5*. This Hox gene is only expressed in the Q cell lineage on the left side of the animal and induces posterior migration. On the right side, *mab-5* is not expressed and the QR daughter cells migrate in the opposite, anterior direction. In C37A5.9(*RNAi*) animals, the direction of the QR daughter cell migration is reversed. This phenotype suggests that loss of C37A5.9 function activates *mab-5* expression in the QR lineage, which in turn induces posterior migration. A similar phenotype is also observed in the known negative regulator *pry-1* (2). The extrapolated position of C37A5.9 on the genetic map is close to that of *pry-1*. Therefore, we sequenced C37A5.9 in a *pry-1*(*mu38*) mutant background. We find that *mu38* creates a stop codon (W268Stop) between the RGS and DIX domains in the predicted C37A5.9 protein sequence. These results strongly suggest that C37A5.9 is *pry-1*. The Pry-1 mutant phenotype is in agreement with a function of C37A5.9/PRY-1 as an Axin; loss-of *pry-1* function results in a BAR-1/beta-catenin dependent activation of the Wnt pathway (2). We propose that C37A5.9/PRY-1 is a functional Axin homologue and that a conserved Axin/APC/GSK3-like complex regulates Wnt target gene expression in *C. elegans*.

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33. A genetic dissection of the minibrain kinase gene family and its role in Down syndrome

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Increased gene dosage of minibrain kinase (MnbK) has been implicated in the cognitive defects of Down syndrome, the most common cause of mental retardation. MnbK belongs to the DYRK subfamily of protein kinases, which are capable of phosphorylating both serine/threonines and tyrosines. Despite their potential importance, little is known about the normal function of these genes, and it is unclear how increased expression of MnbK produces cognitive defects. Towards these ends, we are investigating the *C. elegans* members of the DYRK kinase subfamily.

Two members of the DYRK kinase subfamily were identified in the database, which we call *mbk-1* and *mbk-2* (minibrain kinase), as well as a related kinase called *hpk-1* (homeodomain interacting protein kinase). Based on sequence similarity of the kinase domains and overall gene structure, *mbk-1* is the DYRK1A/MnbK homolog, and *mbk-2* is equally similar to mammalian DYRK2 and DYRK3. To determine the expression and subcellular localization of these genes, we generated translational fusions with GFP. *mbk-1::gfp* is ubiquitously expressed, and the gene product primarily localizes to the nucleus. *mbk-2* is alternately spliced into 3 forms; all 3 forms are cytoplasmic and are expressed in overlapping patterns that include subsets of neurons in the head, ventral nerve cord, and tail. *hpk-1::gfp* localizes to nuclear puncta and is expressed strongly in embryos and L1 larvae, and weakly in older larvae and adults.

In an effort to model the consequences of increased MnbK gene dosage in Down syndrome, MBK-1 was overexpressed using its endogenous promoter. In addition, we overexpressed putative "kinase dead" and "kinase active" mutants of MBK-1. We found that overexpression of *mbk-1(gf)* produces strong defects in AWC-mediated olfaction. AWC sensed odors fail to be sensed; odors sensed by both AWA and AWC elicit only a partial response, while AWA-sensed odors elicit an intact response. The defects are dosage sensitive, since heterozygotes show partial odortaxis defects. Using a heat shock promoter, we determined that the defects result from acute MBK-1 activity, since a single heat shock one hour prior to the assay was sufficient to produce the defect, while heat shocking 24 hours prior to the assay had little effect. The odortaxis defect is at least partially cell autonomous, since *mbk-1* overexpression from the *gcy-10* promoter was sufficient to produce weak defects. Additional phenotypes for *mbk-1(gf)* include synergistic enhancement of partial loss-of-function mutations in *let-60* and *mpk-1* during vulval development, as well as a strong dauer constitutive phenotype with *daf-7* at 15° C.

To determine the normal function of the minibrain genes, we have identified deletion mutants in *mbk-1* and *hpk-1*, and are screening for an *mbk-2* deletion. Both *mbk-1* and *hpk-1* deletion mutants are viable, and are currently being outcrossed prior to characterization.

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34. Two Isoforms of the EGL-15 (FGFR) Serve Different Functions

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The *C. elegans* fibroblast growth factor receptor (FGFR) EGL-15 is required for two processes: (1) an essential function during early larval development and (2) sex myoblast (SM) chemoattraction to the developing gonad. Alternative splicing of exon 5 generates EGL-15 isoforms that differ in the extracellular domain, just N-terminal to the ligand-binding region. The EGL-15(5A) isoform is required for chemattraction, whereas the EGL-15(5B) isoform is required for viability.

The *C. elegans* genome also encodes two FGF ligands that appear to function in separate processes: *egl-17* in SM chemoattraction, and *let-756* in an essential function. One simple model to account for these data is that the alternative isoforms specify the FGF they interact with and that the ligand thus establishes the type of response. However, we have shown that LET-756, when expressed from the *egl-17* promoter, can act as an SM chemoattractant. Conversely, EGL-17, when expressed from the *let-756* promoter, can rescue the essential function of *let-756*. Furthermore, when LET-756 acts as a chemoattractant it requires the chemoattraction EGL-15 isoform and when EGL-17 acts in the essential function it does not. Thus, each EGL-15 isoform is responsible for determining the particular downstream response.

Interestingly, both EGL-15 isoforms function in SM migration, but in different capacities. In the absence of the chemoattraction mediated by EGL-15(5A), the SMs are repelled from the gonad. Surprisingly, mosaic animals in which both isoforms are lost in the SMs show neither the attraction nor the repulsion. Thus, EGL-15(5B) is required for the repulsion. Furthermore, the mosaic results show that both isoforms act in the migrating SMs, one for attraction and one for repulsion. Preliminary results using isoform-specific antibodies support the presence of both isoforms within the migrating SMs. Transcript analysis indicates that the intracellular regions of EGL-15(5A) and 5B are identical, setting up an intriguing paradox as to how the different isoforms stimulate apparently opposite effects within the same cell.

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35. The Profilin PFN-1 is required for cytokinesis and cell polarity in the *C. elegans* embryo

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Proper regulation of the actin cytoskeleton is crucial for diverse developmental asymmetries in the first cell cycle of the *C. elegans* embryo. In wild type embryos, the oocyte pronucleus migrates to meet the stationary sperm pronucleus in the posterior of the embryo. At the same time, polarized cytoplasmic flows can be detected in the posterior half of the embryo, and P-granules become asymmetrically distributed along the A/P axis. Finally, the first mitotic spindle becomes displaced toward the posterior of the embryo, resulting the production of daughter blastomeres with different sizes in the ensuing cytokinesis.

We have identified three *C. elegans* homologs of the actin binding protein profilin based on sequence homology. Inactivation of one of these profilin genes, called *pfn-1*, by RNAi results in defects in all of the actin-dependent processes described above. In *pfn-1(RNAi)* embryos, both pronuclei migrate to meet in the center of the embryo, and the first mitotic spindle is centrally positioned. Cytoplasmic flows are severely reduced, and P-granules persist in the middle of the embryo. Finally, *pfn-1* mutant embryos fail in the related processes of polar body extrusion during meiosis, pseudocleavage, and cleavage furrow ingression during cytokinesis. Thus, PFN-1 is required for the formation or function of the contractile ring, as well as for the establishment of embryonic asymmetries.

Because the phenotypes described above resemble those resulting from disruption of the actin cytoskeleton following treatment with the actin-depolymerizing drug cytochalasin D, we examined the effect of *pfn-1* interference on the actin cytoskeleton by staining *pfn-1* embryos with antibodies that recognize actin and nonmuscle myosin II (NMY-2). While both proteins accumulate at high levels around the entire cortex of wild-type embryos, we find actin and myosin only in dispersed patches in *pfn-1* embryos. Additionally, the localization of the Formin Homology protein CYK-1, which is required for cytokinesis, and PAR-2 and PAR-3, which are essential for normal embryonic polarity, are severely disrupted in *pfn-1* embryos. Thus, PFN-1 appears to function at a high level in the hierarchy of proteins regulating the assembly of the actomyosin cytoskeleton.

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36. The Anaphase-Promoting Complex is required to polarize the one-cell *C. elegans* embryo.

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To better understand the process by which cell polarity is established, we are analyzing genes required for the characteristic asymmetries of the one-cell C. elegans embryo. We recently identified a new class of genes which we designate *pod* loci as mutation of these genes results in loss of anterior-posterior polarity and in osmosensitive embryos ('*pod*' = polarity and osmotic defective). We have shown that this osmosensitivity likely results from improper formation of the extracellular eggshell. This mutant phenotype suggests that early embryonic polarity is established, in part, by a process that is also shared by the process of eggshell formation.

To identify additional components, we have initiated a study of mutants that produce osmotically sensitive embryos and also exhibit loss of polarity phenotypes. Through two different genetic screens, one of which directly identifies osmosensitive embryos, we have isolated a number of other mutations that represent new loci of the *pod* class and comprise 10 complementation groups. The genetic behavior of these mutants subdivides the *pod* class into two groups. Class I pod mutants show roughly 50% penetrant loss of one-cell polarity and gene function is required only maternally. This class consists of 5 complementation groups including *pod-1*, *pod-2*, and *emb-8*. Class II pod mutants (5 complementation groups) are both paternally and maternally required for viability. In contrast to class I, mutation of four of the class II loci cause nearly 100% penetrant polarity defects.

Mapping and complementation analyses indicate the Class II *pod* alleles mutate genes whose products are core members of the anaphase-promoting complex (APC/cyclosome). These genes were previously identified in unrelated screens as loci required for the completion of meiosis (*emb-27, emb-30, mat-1. mat-2, and mat-3*). However, unlike the previously identified *mat*alleles, our *pod* alleles complete meiosis and progress through the cell cycle but divide symmetrically and fail to polarize PAR-3 and germline granules. Thus, these *pod* alleles reveal that the anaphase-promoting complex functions in additional cellular roles and is the first example of this protein complex outside of cell cycle control. How is APC connected to the establishment of polarity? We hypothesize that regulated protein degradation is the process that properly positions molecular markers required to polarize the early embryo. We are currently testing predictions of this model.

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37. *spn-4* encodes a putative RNA binding protein required for mitotic spindle orientation and cell fate patterning in the *C. elegans* embryo

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The orientation of the mitotic spindle is thought to be a crucial during development for proper segregation of cytoplasmic determinants upon asymmetric cell division. Nonetheless, the mechanisms by which cells control the orientation of the spindle remain to be understood. One example of asymmetric cell division with an oriented mitotic spindle occurs in P_1, the posterior blastomere of the 2-cell stage *C. elegans* embryo. In P_1 the centrosomes, after duplication and migration, are positioned transversely to anterior-posterior (a-p) axis of the embryo, subsequently the centrosome-nucleus complex rotates roughly 90 degrees, orienting the P_1 mitotic spindle along the a-p axis of the embryo. It has been suggested that this rotation involves interactions between astral microtubules and a remnant site from Cytokinesis, however the underlying mechanism remains unknown.

We have identified maternal effect embryonic lethal mutations in a gene, we call *spn-4*, required for orientation of mitotic spindle in the P_1 blastomere, in these mutants the P_1 spindle stays transverse to the a-p axis. Moreover the ectopic longitudinal orientation of the spindles observed in *par-3* mutants at the 2-cell stage requires the function of the *spn-4* gene, and the distribution of PAR-2 protein is normal in *spn-4* mutant embryos. Thus *spn-4* probably acts downstream of, or in parallel with, the *par* genes to positively regulate rotation of the spindle in P_1. Although spindle orientation is abnormal in P_1, this cell division remains remarkably asymmetric in *spn-4* embryos: the daughter blastomeres are unequal in size with the bigger daughter dividing before the smaller one, and the P granules and PIE-1 protein are still segregated to the smaller daughter cell, like in wild type. In addition, and apparently independently of the spindle orientation phenotype, *spn-4* mutant embryos also exhibit defects in cell fate patterning. *spn-4* embryos fail to produce endoderm and pharyngeal mesoderm, like *skn-1* mutants, and also produce ectopic anterior body wall muscle, as in *mex-3* mutants. Futhermore in *spn-4* embryos the germline-specific P granules are still segregated to a single P_4-like blastomere, but its daughters proliferate abnormally during embryogenesis, instead of staying quiescent like in wild type. Interestingly PIE-1 protein levels decline prematurely in *spn-4*, as it is not detectable in most of these P_4-like blastomeres or in its daughters, while in wild type it is present in P_4 and transiently in its daughters Z_2 and Z_3. This premature loss of PIE-1 might explain loss of germ cell quiescence in *spn-4* mutants. We determined the molecular identity of *spn-4*, and it encodes a putative RNA binding protein containing a single RNA Recognition Motif (RRM). SPN-4 is closely related to another C. elegans RRM protein, FOX-1, a component of the dosage compensation and sex determination machinery and it's known to act post-transcriptionally. We therefore suspect that SPN-4 also acts post-transcriptionally, by regulating the stability or translation of mRNAs encoding components more specifically implicated in the multiple developmental pathways that are defective in *spn-4* mutants. Thus SPN-4 seems to be mediating a subset of asymmetries downstream of the polarity initiated by the PAR proteins.

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38. CDK-1 REGULATES SPINDLE ORIENTATION IN EARLY C. ELEGANS EMBRYOS

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The 4-cell stage blastomere EMS divides asymmetrically along the anterior-posterior (A/P) axis of the embryo and produces two daughters with distinct cell fates. The fates of the EMS daughters and the A/P orientation of the EMS division axis are specified by signaling from the neighboring blastomere P2. P2/EMS signaling involves at least two partially redundant pathways, a WNT signaling pathway and a phoshpotyrosine signaling pathway (see abstract by Bei et al.,). A complex composed of WRM-1/ß-catenin and the SER/THR kinase LIT-1 controls cell fate downstream of P2/EMS signaling. However, neither of these factors are required for the A/P oreintation of the EMS division. While mutations in some components of WNT signaling have been shown in blastomere isolation experiments to have defects in EMS spindle orientation, intact embryos do not reveal this role. Here we describe a recessive maternal effect mutation, *ne236*, that completely converts the normally A/P orientation of the EMS spindle to left/right in intact embryos. Despite the change in EMS, cell divisions previous to the six cell stage are normal, and the germ line cells continue divide properly and to segregate P granules properly until the end of embryogenesis. All embryos from homozygous *ne236* mothers arrest development without body enclosure and with cell fate changes including changes consistent with defects in P2/EMS signaling. Surprisingly, we found that *ne236* is an allele of *cdk-1* (previously known as *ncc-1*), the C. elegans homolog of the yeast cyclin dependent kinases CDC-2 and CDC28. Null alleles of *cdk-1* exhibit mid-larval mitotic arrest and lethality that appears to coincide with exhaustion of maternally provided CDK-1. Depletion of maternal CDK-1 by RNAi causes a one cell meiotic arrest. In contrast, *cdk-1(ne236)* homozygotes have no obvious zygotic phenotype, and produce mutant embryos that have normal cell divisions and the normal numbers of well differentiated cells. Furthermore *ne236* in trans to a deficiency appears identical to *ne236* homozygotes suggesting that the *ne236* allele is not significantly impaired for meiotic or mitotic functions of *cdk-1*. Thus *ne236* appears to cause a recessive loss of one specific function of CDK-1 leaving most or perhaps all of its other cell cycle regulatory activities intact. Interestingly, genetic tests suggest that the spindle orientation defect of *cdk-1(ne236)* is suppressible by removing certain P2/EMS signaling components, including *wrm-1*, the ß-catenin homolog. Since loss of *wrm-1* has a strong EMS cell fate defect but gives the wild type A/P spindle orientation, this finding suggests a model for division axis control in EMS. The default spindle orientation for EMS may in fact be A/P. WRM-1 in concert with other factors may mask this default polarity cue when, for example, the function of CDK-1 is altered, or, as further experiments have revealed, when other components of the P2 to EMS signal are perturbed (see abstract by Bei et al.,). We therefore propose that CDK-1 may directly or indirectly regulate target(s) at a key step in the cell cycle to unmask an asymmetry site that directs EMS spindle orientation.

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39. Phosphotyrosine signaling acts in parallel with Wnt signaling to specify endoderm and to control cleavage orientation in early *C. elegans* embryos

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At the 4-cell stage of embryogenesis in *C. elegans* a cell contact mediated induction specifies both the endoderm fate and the anterior-posterior (A/P) orientation of the endoderm precursor called EMS. In vitro manipulations of the 4-cell stage blastomeres indicate that the signaling cell in this induction is the posterior-most cell called P2 and we refer to this signaling process as P2/EMS signaling. Wnt signaling components have been implicated in P2/EMS signaling for cell fate determination. Some of the components in the pathway are also important for the A/P orientation of the EMS division. However, with the exception of an intriguing allele of cdk-1 (See abstract by Soto et al.), no mutants identified to date prevent the A/P cleavage orientation of EMS cell in the intact embryo. Here we show that SRC-1, a *C elegans* homolog of $pp60^{c\text{-}src}$, and MES-1, a protein distantly related to receptor tyrosine kinases, are responsible for the more intense accumulation of phosphotyrosine at the junction between P2 and EMS. The accumulation of the intense phosphotyrosine at this junction correlates precisely with the localization of the MES-1 protein at the P2/EMS contact site. The src-1 and mes-1 mutants synergize with mutants in mom-2/wnt, mom-3, mom-5/FZ, and sgk-1/GSK-3ß to cause a complete loss of the endoderm fate and a left-right (L/R) rather than A/P division of EMS. These results suggest that phosphotyrosine and Wnt signaling act in parallel in the intact embryo to specify endoderm and to orient the division axis of the endoderm precursor cell EMS.

In mammalian cells cell adhesion components are substrates for the SRC tyrosine kinase and results from *Drosophila* have shown that cell adhesion plays a role in cell polarity. Previous work in *C.elegans* has shown that classic components of cell adhesion junctions including E-cadherin (HMR-1), α-catenin (HMP-1) and ß-catenin (HMP-2) are cortically localized at all cell-cell contact sites in the early embryo, but are not required for P2/EMS signaling. Genetic analysis of L/R EMS divisions in *cdk-1(ne236)* have suggested a model that EMS has a groundstate potential to divide A/P and that misregulation of another ß-catenin (WRM-1) in the early embryo may lead to masking of this groundstate potential (see abstract by Soto et al.). Consistent with this model, we find that inhibition of WRM-1 or of HMR-1 by RNAi can restore the EMS A/P division orientation (but not endoderm) in our double mutants deficient in P2/EMS signaling. These results suggest that SRC-1 and MES-1 function in parallel with Wnt pathway components to unmask the potential of EMS to orient its division axis. Although the in-vivo localization of WRM-1 is still not known it is tempting to speculate that as in other systems this ß-catenin homolog is localized at cell-contact sites and that local signaling at the EMS/P2 junction leads to the release of WRM-1. This release may accomplish two things, unmasking of a cortical site that directs the A/P division of EMS and activation of WRM-1 for signaling downstream to specify the endoderm fate. We are currently testing predictions of this model by looking for changes in the localization of adhesion components such as HMR-1, HMP-1, and HMP-2 in cells undergoing P2/EMS signaling. We are also examining the genetic requirements for EMS spindle orientation in blastomere isolation assays where cell contacts are broken and then restored. Our preliminary results have shown that the intense phosphotyrosine at the EMS-P2 boundary can only rarely be restored in such experiments. Similar experiments have shown that SRC-1 appears to function in both cells for the phosphotyrosine accumulation at cell-cell contact sites.

40. Embryonic handedness choice in *C. elegans* involves a G_{alpha} protein encoded by the *spn-1* gene

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The apparent left/right (L/R) symmetry of the early *C. elegans* embryo is broken between the 4- and 6-cell stages of cleavage when the L/R-oriented spindles in the anterior blastomeres ABa and ABp skew in a clockwise direction (viewed dorsally). The resulting cleavage produces an asymmetric 6-cell embryo and establishes the handedness of all subsequent L/R asymmetries in development (Wood, Nature 349:536, 1991). Handedness choice is essentially invariant, so that animals with reversed situs are not seen in wild-type populations ($<10^{-4}$). The *ts* maternal-effect mutation *spn-1(it143)* causes misorientation of spindles in the second and third cleavages at 25º, affecting ABa and ABp most severely. The result is 70% embryonic lethality, with 40% of the surviving progeny showing reversed situs (Bergmann & Wood, Int. *C. elegans* Meeting Abstr. 187, 1999). We mapped *spn-1* to a region of LGI including the predicted Gα protein gene *gpa-16* (Jansen et al., Nature Genet 21:414-419, 1999). The *spn-1* mutation failed to complement *gpa-16(pk481)*, a deletion mutation (kindly provided by G. Jansen), which in a *gpa-16(pk481);dpy-20(e1282)* strain also causes incompletely penetrant maternal-effect embryonic lethality. Sequencing of *gpa-16* cDNA from *spn-1(it43)* revealed a single base change, causing the substitution G202D in a conserved region of the predicted Gα protein product. Together with the recent demonstration that this protein is involved in control of a Gβγ complex regulating centrosomal migration and hence spindle orientation in early embryos (Gotta & Ahringer, Nature Cell Biol 3:297-300, 2001), our findings begin to explain how the *spn-1(it143)* mutation affects handedness choice and suggest that the initial symmetry-breaking event may act through heterotrimeric G protein signaling.

41. *cdk-7* has independent roles in mRNA transcription and cell cycle progression in *C. elegans* embryos

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In a screen for embryonic lethal mutants with aberrant expression of a *pes-10*:GFP transgene, we isolated a number of mutants that fail to activate mRNA transcription in early embryos. Most of these mutants arrest with a terminal phenotype similar to that observed when RNA polymerase II function is removed - 100-cell stage arrest with no apparent differentiation (Powell-Coffman et al., 1996). In contrast, one mutant, *ax224*, showed a more severe terminal phenotype - mutant embryos arrest at about the 50 cell-stage, and DAPI staining reveals defects in chromatin segregation and DNA replication. We have mapped and cloned this mutant, and have shown that it is an allele of the *C. elegans* homologue of Cdk7 (Thanks to Amy Walker for first pointing out the map position of *C. elegans cdk-7*).

Cdk7 is the catalytic subunit of TFIIH and is thought to phosphorylate the carboxy-terminal domain (CTD) of RNA polymerase II during transcription. Consistent with this role, we find that *ax224* embryos have reduced levels of phosphorylated CTD. Cdk7 has also been proposed to be the Cdk activating kinase (CAK) that activates cell cycle Cdks. CAK activity of Cdk7 has been observed in vitro in several systems, but *in vivo* evidence for an essential role in cell cycle progression has been difficult to obtain due to Cdk7's essential role in transcription. Evidence from yeast actually argues against Cdk7 acting as a CAK since the budding yeast homologue of Cdk7, Kin28, does not possess CAK activity *in vivo* or *in vitro*. Instead a distantly related gene Cak1 functions as the sole essential CAK in *S. cerevisiae*. Cak1 is not conserved in higher eukaryotes (Liu and Kipreos, 2000), leaving open the question as to which Cdk functions as the CAK outside of yeast. Consistent with *cdk-7* acting as the CAK in *C. elegans*, we find that *ax224* embryos have cell cycle defects beyond what is expected from a block in transcription. These defects include

lengthened interphase and M phase, and inappropriate DNA replication. In addition, we find that phosphorylation of NCC-1 (*C. elegans* cdc2, Boxem et al., 1999) is reduced in *ax224* mutants (Thanks to Andy Golden for the NCC-1 antibody). Finally, the *cdk-7(RNAi); cdk-7(ax224)* "double mutant", which presumably lacks most *cdk-7* activity, arrests in the one-cell stage with a phenotype similar to that observed in *ncc-1(RNAi)* embryos. Taken together, our data demonstrates that *cdk-7* has independent roles in transcriptional activation and cell cycle control, and suggests that *cdk-7* may be the sole essential CAK in *C. elegans*.

42. A novel protein required for degradation of CCCH finger proteins in somatic lineages.

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The first cleavages of the embryo are asymmetric and give rise to 5 somatic lineages and the embryonic germ lineage. Several maternal proteins are asymmetrically segregated during these cleavages: in particular three proteins, PIE-1, POS-1 and MEX-1, segregate preferentially with the germ lineage and are excluded from somatic lineages. These proteins have in common a pair of CCCH zinc fingers (ZF1 and ZF2). We have found that, when fused to GFP, ZF1s from PIE-1, MEX-1 and POS-1 cause GFP to be degraded specifically in somatic blastomeres. Consistent with a role in protein degradation, mutations in PIE-1 ZF1 cause abnormal stabilization of PIE-1 in somatic blastomeres. These observations suggest that the ZF1 in PIE-1, MEX-1 and POS-1 targets these proteins for degradation specifically in somatic blastomeres.

To identify factors that function in this process, we performed a yeast-two hybrid screen for proteins that interact with PIE-1 ZF1 and identified a novel protein, F59B2.6 (thanks to Zheng Zhou and Bob Horvitz for their excellent library). F59B2.6 does not appear to have any recognizable motifs. GST pull down experiments have confirmed that F59B2.6 interacts directly and specifically with ZF1 and does not interact with ZF2. RNA-mediated interference of F59B2.6 causes abnormal accumulation of PIE-1,MEX-1 and POS-1 in somatic blastomeres, and results in embryonic lethality. F59B2.6(RNAi) embryos show no defects in PAR-2, PAR-6 and GLP-1 localization, indicating that this gene is not generally required for embryonic polarity. These observations suggest that F59B2.6 recognizes ZF1-containing proteins and targets them for degradation in somatic blastomeres. Although CCCH fingers in other proteins (e.g. mammalian TIS11) have been implicated in

binding to RNA, our findings raise the possibility that CCCH fingers can also function as determinants of protein stability.

43. Wnt signaling and HAM-1 are required for asymmetric cell division of *C. elegans* neuroblasts.

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Both intrinsic and extrinsic mechanisms can polarize an asymmetrically dividing cell. Many asymmetrically distributed intracellular molecules have been described that segregate fate to daughter cells during mitosis. In *C. elegans*, Wnt signaling has also been shown to polarize asymmetrically dividing cells. We propose that in *C. elegans*, Wnt signaling may distribute intracellular molecules required for asymmetric cell division.

We are focusing on asymmetric divisions in the lineage that generates the HSN and PHB neurons. In this lineage, an HSN/PHB neuroblast divides asymmetrically to generate an anterior daughter cell that dies and a posterior daughter cell, the HSN/PHB precursor. This precursor then divides to produce the HSN and PHB neurons. Asymmetric division of the HSN/PHB neuroblast requires the *ham-1* gene. Mutations in *ham-1* disrupt the asymmetry of the division and the protein is asymmetrically localized to one side the cell.

We have shown that HAM-1 functions with members of the Wnt signaling pathway to polarize the HSN/PHB neuroblast. Both a *dsh-2* deletion mutant, as well as RNAi experiments with *mom-5*, produced a Ham-1 phenotype in the HSN/PHB lineage. *mom-5* encodes a Frizzled (Fz) homolog and putative Wnt receptor, while *dishevelled (dsh)* proteins function downstream of Fz. The phenotypes of *dsh-2; ham-1* double mutants support the hypothesis that *ham-1* and *dsh-2* function in the same pathway. We raised antibodies against DSH-2 and the protein is detected from the 4-cell stage of embryogenesis until the 1 1/2 to 2 fold stage, and is primarily membrane associated. In cells also expressing HAM-1, the two proteins co-localize. Cells with asymmetrically localized HAM-1 also localize

DSH-2 asymmetrically. In *mom-5* mutant embryos, DSH-2 is largely delocalized to the cytoplasm. Finally, mutations in *egl-27* exhibit weakly penetrant Ham-1 phenotypes and show genetic interactions with *ham-1*. *egl-27* encodes a MTA1 homolog, a member of the NURD complex (nucleosome remodeling and histone deacetylase). *egl-27* and Wnt pathway mutants have overlapping phenotypes suggesting that EGL-27 may play a role in transducing a Wnt signal.

Our results are consistent with a model where Wnt signaling asymmetrically localizes molecules like DSH-2 and possibly HAM-1 to polarize dividing cells. We are currently trying to identify the Wnt required for polarizing the HSN/PHB neuroblast.

44. A POP-1 repressor complex restricts inappropriate cell type-specific gene transcription during *C. elegans* embryogenesis

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During animal development, cell fate determination is a critical decision process that sets the stage for future differentiation of specific cell types. In *C. elegans*, previous studies have shown that histone acetyltransferase CBP-1 counteracts the repressive activity of the histone deacetylase HDA-1 to allow endoderm differentiation, which is specified by the E cell. In the sister MS cell, the endoderm fate is prevented by the action of an HMG box-containing protein POP-1 through an unknown mechanism. In this study, we show that the actions of CBP-1, HDA-1 and POP-1 converge on *end-1*, an initial endoderm-determining gene. In the E lineage, CBP-1 is required to overcome inhibition by both HDA-1 and POP-1 in order to activate *end-1*. We further identify a molecular mechanism for the endoderm-suppressive effect of POP-1 by demonstrating that POP-1 functions as a transcriptional repressor that inhibits inappropriate *end-1* transcription. We provide biochemical and functional evidence that POP-1 represses transcription via the recruitment of HDA-1 and UNC-37, the *C. elegans* homolog of the co-repressor Groucho. These findings demonstrate the importance of the interplay between acetyltransferases and deacetylases in the regulation of a critical cell fate-determining gene during development. Furthermore, these results identify a strategy by which concerted actions of histone deacetylases and other co-repressors ensure maximal repression of inappropriate cell type-specific gene transcription. This combinatorial scheme appears to be conserved in multiple organisms.

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45. Evidence that UNC-13 acts via syntaxin to promote vesicle priming

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Release of neurotransmitters from synaptic vesicles requires the fusion of the synaptic vesicle membrane with the plasma membrane. Membrane fusion at nerve terminals is extremely rapid and requires the vesicle to be in a readied or primed state. Priming is thought to require the formation of the SNARE complex, comprised of the vesicle protein synaptobrevin and the plasma membrane proteins SNAP-25 and syntaxin. In solution syntaxin adopts a default-closed conformation that is incompatible with SNARE complex assembly. In this closed state, the amino terminus of syntaxin occludes its SNARE motif preventing interactions with the other SNARE proteins.

UNC-13 is another protein that is required for vesicle priming. UNC-13 binds the amino terminus of syntaxin in a region not involved in interactions with the other SNARE proteins. One possible model for UNC-13 function is that it promotes the open configuration of syntaxin by binding to the syntaxin amino terminus and thus facilitates interactions with the other SNARE proteins. If the function of UNC-13 is to open syntaxin then one would predict that a constitutively open form of syntaxin should bypass the requirement for UNC-13 in vesicle priming.

Dulubova et al. (EMBO J. 1999 Aug 16;18(16):4372-82.) identified two amino acid substitutions which cause syntaxin to constitutively adopt the open state. We engineered mutations into *C. elegans* syntaxin at these conserved residues (UNC-64 L166A/E167A). Expression of the open form of syntaxin in *unc-13(s69)* mutants significantly improved the behavioral deficits of *unc-13(s69)* animals. Furthermore, primed vesicles and evoked release were restored to wild-type levels in *unc-13(s69)* mutants expressing the open form of syntaxin. In contrast, overexpression of wild-type syntaxin failed to rescue the behavioral or priming defects of *unc-13(s69)* mutants. The ability of open syntaxin to rescue evoked release was specific to *unc-13* mutants, since open syntaxin failed to rescue evoked release in the synaptobrevin null mutant *snb-1(js124)* or the neuronal calcium channel mutant *unc-2(e55)*. These data support a model in which UNC-13 primes synaptic vesicles for fusion by promoting the open configuration of syntaxin.

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46. Analysis of *kin-13* PKC and *dgk-1* DAG kinase suggests that phorbol esters regulate a late stage of synaptic vesicle exocytosis.

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Phorbol esters enhance release of neurotransmitter at many synapses and in some cases appear to regulate the exo/endocytic machinery directly. To understand the mechanisms by which phorbol esters and diacylglycerol (DAG) regulate synaptic transmission, we have studied G protein modulation of the *C. elegans* neuromuscular junction (NMJ). Our prior results (and those from other labs), showed that phorbol ester treatment induces hypersensitivity of animals to the paralytic effects of aldicarb (the Hic phenotype), and we showed that phorbol ester binding to the C1 domain of UNC-13 is required for this effect. Here we report that a protein kinase C (PKC), encoded by the *kin-13* gene, also contributes to phorbol ester-induced stimulation of acetylcholine (ACh) release at NMJs. We isolated the allele *nu448* in a screen for mutations that suppress the Hic phenotype of *dgk-1* mutants. The *dgk-1* gene encodes a diacylglycerol kinase (DAG kinase); therefore, *dgk-1* DAG kinase mutant neurons are predicted to have elevated levels of DAG. We positionally cloned the *nu448* gene and found that it corresponds to an allele in the *kin-13* PKC gene. The predicted *kin-13* PKC protein is most similar to the PKC-epsilon isoform and contains two C1 domains (which bind DAG), a pseudosubstrate domain, and a kinase domain. The *nu448* lesion is a nonsense mutation the kinase domain and behaves like a null in trans to a deficiency.

Homozygous *nu448* mutants are resistant to aldicarb (Ric), have sluggish locomotion, and shallow body bends. The Ric phenotype of *nu448* mutants is likely to reflect a defect in ACh release from motor neurons, since these animals have normal sensitivity to the paralytic effects of the ACh agonist levamisole. Over-expression of wild type *kin-13* PKC in transgenic animals causes a phenotype that is opposite of the *nu448* phenotype. Transgenic animals are Hic and have exaggerated body bends. Because the absence of *kin-13* PKC function does not completely paralyze animals, it is likely that *kin-13* PKC plays a modulatory rather than an essential role in acetylcholine release.

The *kin-13(nu448)* PKC mutation partially suppresses the Hic phenotype of *dgk-1* DAG kinase null mutants, but it completely suppresses the Hic phenotype of *goa-1* $G\alpha_o$ mutants, suggesting that *kin-13* PKC acts downstream of $G\alpha_o$ in a pathway modulating neurotransmitter release. Loss of *kin-13* PKC function also suppresses the Hic phenotype of animals expressing a putative gain-of-function *unc-64* syntaxin mutation. Because syntaxin is a component of the core vesicle release machinery, this result suggests that *kin-13* PKC is required for a late stage of regulated vesicle exocytosis. *kin-13* PKC mutants are partially resistant to phorbol ester-induced hypersensitivity to aldicarb, suggesting that *kin-13* PKC is an important presynaptic target for phorbol esters. We have previously shown that a mutation in the C1 domain of UNC-13 also confers partial resistance to phorbol esters. In double mutants that lack both *kin-13* PKC and that carry the UNC-13 C1 domain mutation, there is a further reduction in phorbol ester sensitivity, suggesting that KIN-13 PKC is a phorbol target that acts in parallel with UNC-13 to facilitate release. Fluorescence imaging using VAMP-GFP expressed in the motorneurons shows that in *kin-13* mutants the average VAMP fluorescence at each NMJ is increased by ~20%, reflecting an accumulation of synaptic vesicles at release sites. Conversely, we find that that VAMP fluorescence is decreased at the NMJs in *dgk-1* DAG kinase mutants by a similar amount. These data implicate KIN-13 PKC in facilitating neurotransmitter release by regulating a late step in the fusion of primed synaptic vesicles. In addition, they suggest that the size of the synaptic vesicle pool at NMJs is regulated by DAG levels.

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47. UNC-10 Rim, an active zone protein that regulates post-docking events at the synapse

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Rim1 was previously identified as a Rab3 effector localized to the presynaptic active zone in vertebrates. We cloned a *C. elegans* homolog of Rim and demonstrate that it is encoded by the gene *unc-10*. Null mutants lacking the entire Rim coding sequence are viable, but exhibit a variety of behavioral defects consistent with synaptic transmission deficits. These defects are more severe than those of *rab-3* mutants suggesting Rim may play roles beyond rab3 signaling. Electrophysiological analysis of the null mutant revealed a 3-fold decrease in the amplitude of evoked release events and a five-fold decrease in the frequency of spontaneous miniature events at the neuromuscular junction. Despite the physiological defects, neuromuscular junctions of Rim null mutants exhibited normal presynaptic densities and normal levels of docked vesicles. These data suggest that Rim acts after docking, perhaps in regulating priming of synaptic vesicles for exocytosis.

Vertebrate Rim interacts with several proteins: UNC-13, cAMP-GEFII and rab3. To further our understanding of the molecular mechanism of Rim function, we are also determining if these interactions are conserved using two hybrid and *in vitro* biochemical techniques.

Like the vertebrate protein, *C. elegans* Rim is localized to a discrete sub-domain of the synapse. Antibodies against the N-terminal domain exhibit bright punctate staining in wild type animals. The staining pattern is more restricted than that obtained using antibodies directed against synaptic vesicle proteins. Specifically, individual punctum can be resolved in both the ventral and dorsal nerve cord. We are currently assessing which domains of Rim are required for function and synaptic localization. In another approach to identify other potential Rim interacting proteins, we created a functional Rim-GFP fusion that localizes similarly, though slightly less discretely than the antibody staining. Using this fusion expressed under the Rim promoter, we screened for mutants which disrupts normal localization. *js569*, a mutant with sub-lethal and uncoordinated phenotypes was isolated in this screen. Characterization of this interesting mutation is in progress.

48. SLO-1 Potassium Channel Regulates Duration of Neurotransmitter Release

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In order to identify negative regulators of synaptic transmission, we screened the entire genome of *C. elegans* for suppressors of *unc-64(e246)*, a hypomorphic syntaxin allele that greatly depresses synaptic function. Among 10 suppressors examined, six were alleles of the Ca^{2+}-activated K^{+} channel (SLO-1), notably, the only K^{+} channel gene identified. *unc-64* animals were lethargic and resistant to treatment with the cholinesterase inhibitor aldicarb. *slo-1* mutations partially suppressed the lethargy and aldicarb-resistance of *unc-64*. Expression of wild-type SLO-1 in neurons, but not muscle, reversed the suppression. To investigate the physiological basis of suppression, we examined evoked excitatory postsynaptic currents (EPSCs) and spontaneous miniature postsynaptic currents (MPSCs) at the neuromuscular junction under voltage-clamp (-60 mV) conditions. The EPSC was induced by stimulating the ventral nerve cord with a 0.5-ms DC pulse. Evoked EPSCs at the *unc-64* neuromuscular junction were small, with an amplitude of ~15% and quantal content of ~5% of wild-type. Addition of a *slo-1* mutation prolonged EPSC duration, resulting in a 4-fold increase in quantal content. *slo-1* mutations also prolonged EPSCs and increased their amplitudes in the absence of *unc-64*. In contrast, MPSC frequency and amplitude were not altered by *slo-1* mutations, suggesting that the effects of the mutations were presynaptic. These results suggest that BK channels plays a key role in synaptic transmission, possibly by terminating Ca^{2+} influx into the presynaptic nerve terminal.

49. Electrophysiological measurement of the action of phorbol esters and serotonin on body wall neuromuscular physiology.

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We are interested in understanding how endogenous neuromodulators regulate synapse function and behavioral responses. In *C. elegans*, 5-HT and diacylglycerol (DAG) modulate the rate of locomotion. Recent genetic and pharmacological analysis has shown that 5-HT and DAG, or phorbol esters, act presynaptically on motor neurons to control acetylcholine (ACh) release at body wall neuromuscular junctions (NMJs) [1,2]. 5-HT acts through a G-protein Galphao subunit, GOA-1, to reduce ACh release at body wall neuromuscular junctions (NMJs) [1]. In contrast, DAG or phorbol esters act through presynaptic phorbol ester receptors, such as UNC-13, to potentiate ACh release [2]. To further understand the mechanisms that underlie these changes in synaptic function, we have carried out whole cell voltage clamp recordings from body wall muscle and have recorded the pre-and post-synaptic effects of 5-HT and phorbol esters on adult body wall NMJ physiology.

We have recorded from body wall muscle both excitatory post-synaptic currents (EPSCs) and muscle responses to ACh application in the presence of 5-HT and phorbol esters. At concentrations of 5-HT greater than 50 microM the amplitude of ACh-activated currents and EPSC amplitudes are both reduced by 40% in the presence of 5-HT, suggesting that 5-HT regulates the activity of muscle ACh receptors. By contrast, 25 microM 5-HT does not alter muscle responsiveness to ACh; however, this concentration of 5-HT now significantly reduced the rate of EPSCs. In addition, we have also analyzed the action of phorbol esters on synaptic transmission at body wall NMJs. At concentrations of 10 nM or greater we observe a 2-3 fold increase in the rate of EPSCs while no post-synaptic changes in ACh activated currents are observed. We have analyzed putative targets of phorbol ester potentiation of synaptic transmission. The protein kinase C mutant,

kin-13 (Sieburth and Kaplan, IWM, 01) is resistant to phorbol ester induced aldicarb hypersensitivity. Voltage clamp recordings from *kin-13* mutants show that stimulation of spontaneous EPSC rate by phorbol esters is reduced. These results suggest PKC mediates phorbol ester induced changes in presynaptic release probability at the body wall NMJ.

These changes in synaptic physiology caused by 5-HT and phorbol esters are consistent with the genetic and pharmacological analysis observed previously. These experiments will serve as the basis to determine how changes in calcium sensitivity or vesicle pool size contribute to changes in synaptic function and what molecular targets contribute to the action of these endogenous neuromodulators.

1. Nurrish et al., Neuron 1999 24: 231.
2. Lackner et al., Neuron 1999 24: 335.

50. Nidogen and Type XVIII Collagen Are Required During the Formation of Neuromuscular Junctions in C. elegans

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Using a combination of behavioral assays and synaptic markers we have identified that nidogen, nid-1, and type XVIII collagen, cle-1, are required for the proper organization of GABAergic and cholinergic neuromuscular junctions in the nematode Caenorhabditis elegans. Both molecules have previously been shown to regulate cell migration and axon guidance during neural development and are the first components of the extracellular matrix to have been shown to localize to the basement membrane surrounding the nervous system. Both proteins localize along axons in distinctly punctate pattern that partially codistributes with a synaptic marker for cholinergic synapses, UNC-17. NID-1 null animals have severe synapse formation defects including an increased number of apparent puncta that have a decreased size. A deletion of the nidogen G2 domain results in more subtle NMJ defects, providing the first evidence for an in vivo function of this domain. Two different alleles of cle-1 result in a truncation of the NC1 domain from the type XVIII collagen. Both alleles result in viable mutants that display a reduced number of puncta that appear enlarged. NID-1 and CLE-1 mutants display subtle uncoordinated locomotory defects that are only apparent using a thrashing assay. These data corroborate a role for the basement membrane in synapse formation, but reveal previously unknown functions for these specific molecules.

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51. *unc-122* and *unc-75*: Two genes that affect neuromuscular signaling

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We have been interested in uncovering the molecular events underlying the neurite "sprouting" defects observed in the Lim homeodomain mutant *lim-6* (Hobert et al. *Development 126*: 1547-1562). We have focused our attention on identifying mutants that lead to similar sprouting defects in DVB, a GABAergic motorneuron whose development and function is crucial for execution of the defecation motor program in *C. elegans.* We found that mutations that affect communication of DVB with its target muscle cause DVB motor axon sprouting (see abstract by Mehta, Loria and Hobert). Moreover, we have discovered that two previously identified but mainly uncharacterized genes, *unc-122* and *unc-75*, cause low penetrant DVB sprouting defects. Both mutants are especially interesting in that they enhance the DVB sprouting defect seen in *unc-25* null animals and thus appear to act in a pathway parallel to GABAergic neurotransmission in DVB. Similar enhancement of *unc-25* sprouting is observed in *unc-31* mutants where peptidergic neurotransmission is presumably disabled. Based on this analysis, we hypothesize that DVB interacts with its target through GABA-mediated fast neurotransmission and a parallel aminergic/peptidergic pathway and that *unc-122* and *unc-75* may act in the latter.

To test this hypothesis, we set out to clone *unc-122* and *unc-75.* Since *unc-122* and *unc-75* mutants are also coilers, for cloning purposes we relied predominantly on their more obvious unc/coiling phenotypes. 3-factor and deficiency mapping, transformation rescue, and sequencing revealed that F11C3.2 corresponds to *unc-122*. UNC-122 is predicted to be a type II transmembrane protein, containing a 19 amino acid N-terminal intracellular domain. The extracellular portion of the protein contains a collagen-like repeat followed by a highly conserved olfactomedin (OLF) domain. Two independently isolated alleles of *unc-122* show premature stops within the OLF domain suggesting a crucial role for this region of the protein. The *unc-75* locus maps very close to *spe-9* on LGI and we have obtained transgenic rescue of *unc-75* using a YAC within this region (gift of Andrew Singson). SNP mapping has narrowed our focus and we expect cloning of *unc-75* in the very near future.

The coiling phenotypes of *unc-122* and *unc-75* suggest defects in motorneurons or the muscles they innervate. Our mosaic analyses have clearly established that *unc-122* function is not required neuronally, but is in fact crucial within muscle lineages. In an effort to pinpoint the intracellular localization of UNC-122, antibody staining is currently underway using transgenic lines containing a rescuing epitope-tagged version of the protein.

To delineate the pathway in which *unc-122* and *unc-75* act in regard to their coiling phenotype, we tested interaction with GABA- and cholinergic-neurotransmission. Double mutants of *unc-122* or *unc-75* with *unc-25* both coil and shrink. Thus, GABAergic neurotransmission is not involved in the coiling observed. We next investigated whether defects in cholinergic neurotransmission could account for the coiling phenotypes of each mutant. Drug studies show that *unc-122* is hypersensitive to both aldicarb and levamisole, indeed suggesting a defect in cholinergic synaptic transmission. But, *unc-29unc-122* double mutants still coil strongly indicating that levamisole-sensitive acetylcholine receptors are not involved. Similar drug studies are underway for *unc-75* and preliminary results suggest that *unc-75* is also slightly hypersensitive to acute doses of aldicarb and is thus likely involved in neurotransmission. Our current hypothesis is that as in DVB, *unc-122* and *unc-75* may also act in an aminergic/peptidergic pathway that interacts with cholinergic neurotransmission, an interaction that has been observed in other systems as well (Weiss et al. *J. Physiology* 87: 141-151). We anticipate that further analysis of *unc-122* and determination of the molecular identity of *unc-75* will soon provide us a much clearer understanding of both coiling and DVB neurite sprouting defects.

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52. *ric-3*, A Novel Gene Which Enhances Acetylcholine Receptor Activity

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Mutations in *ric-3* were isolated as suppressors of the neurodegeneration caused by the activating *u662* mutation in the nictotinic acetylcholine receptor (nAChR) DEG-3[1] and in a screen for mutants resistant to the acetylcholinesterase inhibitor aldicarb[2]. *ric-3* mutants are resistant to levamisole, an agonist of the body wall muscle nAChR. They also show a reduction in the rate of pharyngeal pumping that correlates with a reduced MC spike, indicating impaired cholinergic transmission. Thus, *ric-3* affects cholinergic transmission mediated by three distinct nAChRs with no apparent defects in transmission by other neurotransmitters. Immunocytochemistry showing DEG-3 accumulation in the cell bodies of *ric-3* worms suggests that RIC-3 is a postsynaptic component of the machinery that assembles or transports nAChRs.

We have cloned ric-3 and identified it as T14A8.1. *ric-3* is a novel gene coding for a highly charged protein predicted to contain 2 TM domains and an extensive coiled-coil region. Characterization of four cDNAs kindly received from Y. Kohara has shown the existence of two transcripts, coding for proteins of 363 and 378 amino acids. While no strong homology is found with known proteins, *ric-3* homologues are present in the *Drosophila* genome and in *Xenopus* and human EST databases. A rescuing RIC-3:GFP fusion construct shows RIC-3 expression in muscle and neurons. RIC-3: GFP fluorescence indicates apparent ER localization, and some presence in neuronal processes. The ER localization is consistent with a role in receptor assembly, although presence at the plasma membrane cannot be ruled out.

RIC-3 coexpressed with the DEG-3/DES-2 receptor in *Xenopus* oocytes significantly enhances receptor activity, while having no effect on native oocyte receptors or on coexpressed glutamate receptors. A similar enhancement is observed when RIC-3 is coexpressed with the homomeric rat alpha 7 nAChR. This result is consistent with the possibility that RIC-3 confers cholinergic specificity on the machinery which assembles and transports membrane receptors.

We will also present work using biochemistry in the *Xenopus* oocyte system to further elucidate the mechanism by which RIC-3 enhances nicotinic receptor activity.

[1] Treinin, M. and M. Chalfie (1995). A mutated acetylcholine receptor subunit causes neuronal degeneration in *C. elegans*. Neuron 14:871-877.

[2] Miller, K.G., A.Alfonso, M. Nguyen, J.A. Crowell, C.D Johnson, and J.B. Rand, (1996). A genetic selection for *Caenorhabditis elegans* synaptic transmission mutants. Proc. Natl. Acad. Sci. USA 93:12593-12598.

53. *sol-1* encodes a novel protein that is required for the function of *glr-1*.

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In the vertebrate central nervous system the majority of excitatory signaling is mediated by glutamate and its receptors. In order to function, glutamate receptors must be localized to specific sites where they are then inserted into the membrane. To gain insight into the molecular mechanisms that regulate this process, we designed a genetic screen to identify genes required for localization and insertion of the *C. elegans* glutamate receptor GLR-1.

We have previously reported that transgenic worms expressing a constitutively active form of GLR-1 [GLR-1 (A/T)] show a "lurching" phenotype -- they rapidly alternate between forward and backward movement. The hyper-reversal phenotype provides a sensitized background to uncover genes essential for glutamate receptor function. By screening for mutations that suppress the hyper-reversal behavior, we have identified a gene, *s*uppressor *o*f *l*urcher (*sol-1)*, which encodes a novel protein that is expressed in the nervous system.

By characterizing the expression of a GLR-1::GFP fusion in *sol-1* mutants, we have shown that SOL-1 is not required for GLR-1 expression. Interestingly, like *glr-1* mutants, *sol-1* worms are nose touch defective, suggesting that SOL-1 is required for GLR-1 function. Furthermore, glutamate-gated currents mediated by GLR-1 are completely abolished in the command interneuron AVA in *sol-1* mutant worms. In contrast, we can still record normal glutamate-gated currents mediated by the NMDA receptor, NMR-1, in AVA. We are currently investigating how SOL-1 affects the function of GLR-1.

54. Analysis of Glutamatergic Neurotransmission by Knockout of Glutamate Transporter Genes.

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The amino acid L-Glutamate (Glu) is a neurotransmitter that mediates most of the excitatory neurotransmission in the human brain and is thus central to development, basic physiology and higher brain functions. Exaggerated activation of glutamatergic transmission leads to neuronal cell death (termed excitotoxicity) and is believed to be a cause or a contributing factor to many acute and chronic neurological disorders including stroke and ALS. In order to ensure accurate response to rapid Glu signals and to avoid buildup of toxic levels of this transmitter, synaptic Glu is rigorously pumped out of the synapse by specialized synaptic Glu Transporters (GluTs). Malfunction of GluTs has been specifically identified as a key event in the initiation and/or progression of the neuronal damage seen in stroke and in ALS. Further emphasis on the importance of the role of GluTs in normal and pathological Glu neurotransmission is suggested by the particularly high degree of conservation of these genes from nematodes to humans. We therefore suggest that a detailed description of the molecules and the processes involved in normal and pathological functions of GluTs in the nematode might be used to gain similar insight to analogous processes in higher organisms. We aim at creating synaptic Glu buildup akin to that seen in disease conditions by systematic knockout of *C. elegans* GluT genes. We have so far knocked out 4 of the 6 GluT genes in the worm, and we are producing fold-back RNAi constructs to knockout the expression of the remaining two GluT genes. Analysis of the phenotypes of these deletion mutants by themselves and in combination with other Glu-related mutants suggests that GluTs are key regulators of synapses that control pharyngeal pumping, thermotaxis and the responses to a range of chemical, osmotic and mechanical stimuli. GluTs appear to cooperate with other components of the synapse to maintain a functional balance of Glu

neurotransmission. Initial observations suggest a possible increase in sensitivity of a knockout strain to chemical insults. Together with other studies of Glu neurotransmission in the nematode, these observations might serve as the foundation for a detailed molecular and cellular description of key processes of neuronal function. Furthermore, the phenotypes of these GluT knockout mutants might serve as the basis for genetic screens aimed at the identification of additional participants in normal and pathological aspects of Glu neurotransmission.

55. Neuronal fate specification by bHLH proteins in the *C. elegans* ray sublineage

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The use of repeated sublineages is likely to be a common theme in development. In the nervous system, sublineages provide an ideal opportunity to examine the integration of mechanisms that specify general neuronal properties with those that specify the more restricted characteristics of subtype identity. We have been studying the sensory rays of the *C. elegans* male tail to characterize the regulatory network that controls selection of the ray precursor cells and establishes the four distinct fates of their progeny.

During the male L3 stage, nine bilateral pairs of posterior seam cells are specified to become the ray precursor cells, or Rn cells. Through the execution of the ray sublineage, each Rn cell clonally gives rise to the three cell types of each ray (the ray structural cell Rnst and the ray neurons RnA and RnB) as well as a cell that undergoes programmed cell death. Superimposed on the ray sublineage are patterning mechanisms that generate differences between corresponding cells of different rays, including structural cell morphology and ray neuron neurotransmitter identity. The *atonal*-family bHLH transcription factor LIN-32 is expressed in all of the ray precursor cells, and is required to specify their fates as neuronal precursors. Because strong *lin-32*alleles cause nearly complete ray loss, and ectopic expression of *lin-32* can specify ectopic ray sublineages, *lin-32* can be thought of as a ray proneural gene, acting early to implement commitment to the ray sublineage.

However, *lin-32* has later functions in ray development as well. By using weak *lin-32*alleles along with ray-neuron-specific GFP markers, we have found that loss of *lin-32* function can disrupt multiple steps in the ray sublineage, uncoupling cell fate specification in one branch from that of another. *lin-32*function is also required for the terminally-differentiated characteristics of ray neurons and ray structural

cells. These findings suggest that *lin-32* has additional independent functions after the specification of the Rn cell, and are supported by recent results in*Drosophila* demonstrating that *atonal* functions in multiple steps of neuronal development as well.

To better understand these multiple functions of *lin-32*, we screened for suppressors and enhancers of the ray loss phenotype of a weak *lin-32* allele and recovered two alleles of the gene *hlh-2*. *hlh-2* encodes CeE/Da, the worm member of the E/*daughterless* family of general bHLH heterodimerization partners. Our *hlh-2*mutations enhance the multiple ray defects caused by weak *lin-32* alleles, and disrupt the formation of DNA-binding LIN-32:HLH-2 complexes *in vitro*. Together, these results indicate that *lin-32* and *hlh-2* are generally required throughout the ray sublineage for multiple fate-specification events, and imply that the LIN-32:HLH-2 complex has multiple, independent targets for different steps in ray development.

To identify targets of the LIN-32:HLH-2 complex that implement ray cell fates, we are taking a microarray-based approach in collaboration with Stuart Kim (Stanford University). Using the hermaphrodite-lethal mutation *dpy-28(y1ts)*, we have compared whole-genome mRNA expression patterns in adult male populations lacking rays (*hlh-2; lin-32* mutants) to those with excess rays (*lin-22* mutants). This approach has thus far identified two new genes expressed in rays, the Onecut-homeodomain transcription factor *ceh-39*and the G-protein coupled receptor C03A7.3. We are generating additional data in order to identify a more complete set of ray genes, and are using methods to identify sequence motifs enriched in the promoters of ray-specific genes. We plan to expand this analysis by profiling the gene expression patterns of wild-type animals from mid-L3 to adult, in order to be able to visualize changes in transcription during the ray sublineage.

56. *sem-4* Regulates Terminal Differentiation and Anteroposterior Patterning in the T Lineage

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The *C. elegans* gene *sem-4* is a member of the *spalt* gene family. *spalt* genes encode Zn finger proteins expressed in neural tissue in worms, frogs, mice, fish, and human beings. In flies, *spalt* is involved in head and tail patterning; in worms, *sem-4* is expressed strongly in the head and tail. *sem-4* animals have many neuronal defects, including the production of ectopic touch-neuron-like cells in the tail. These ectopic cells arise inappropriately from the T lineage (where *sem-4* is expressed) and express several touch cell markers, including *mec-3*. Mitani *et al*. suggested that *sem-4* might negatively regulate *mec-3* in differentiating cells (Mitani S, Du H, Hall DH, Driscoll M, and Chalfie M, *Development* **119**, 773-783 (1993)). Basson and Horvitz proposed that mutations in *sem-4* transform the T.pp sublineage into a touch cell (PLM) sublineage (Basson M and Horvitz HR, *Genes & Dev*. **10**, 1953-1965 (1996)). We find that *sem-4* determines the cell fate of both terminally differentiating cells and precursors in the T lineage, through regulation of *mec-3* and interaction with the anteroposterior patterning genes *egl-5*, *mab-5*, and *lin-39*.

We have shown that *sem-4* directly represses *mec-3* expression and touch cell function when expressed ectopically in the six touch neurons. Zn fingers 2 and 3 are critical for this repression. Moreover, SEM-4 binds to at least two specific sites in the *mec-3* promoter and likely represses *mec-3* transcription directly through this interaction. Because ectopically expressed *sem-4* can prevent differentiation of the touch cells through direct repression of *mec-3*, we propose that *sem-4* normally represses *mec-3* expression and touch cell fate in the T lineage. These results delineate a direct role for *sem-4* in the process of terminal differentiation of T lineage neurons.

We have also examined regulation by *sem-4* of precursor cell fate in the T lineage. In *sem-4* animals, the fate of the T.pp precursor is variable, as is the number of cells ectopically

expressing *mec*-3; T.pp gives rise to three distinct lineages which differ from the wild-type lineage and which produce different numbers of *mec-3* positive cells. One of these lineages resembles the wild-type T.pp lineage but produces one ectopic *mec-3* expressing cell. The second abnormal lineage resembles the PLM touch neuron lineage, as first suggested by Basson and Horvitz, and produces one ectopic *mec-3* expressing cell (Basson and Horvitz, 1996). The third abnormal lineage resembles the Q lineage (which generates the AVM and PVM touch neurons) and produces at least two ectopic *mec-3* expressing cells.

We find that mutations in the HOM-C genes affect the production of ectopic touch neurons in *sem-4* animals. Mutations in *egl-5*, *mab-5*, and *lin-39* decrease the production of ectopic touch neurons; mutation of *egl-5* produces the largest decrease. Mutation of each of the HOM-C genes affects the development of different touch cells: PVM migration is abnormal in *mab-5* mutants (Chalfie M, Thomson NJ, and Sulston J, *Science* **221**, 61-63, (1983)), AVM migration is abnormal in *lin-39* mutants (Clark SG, Chisholm AD, and Horvitz HR, *Cell* **74**, 43-55 (1993), Wang BB, Miller-Immergluck MM, Austin J, Robinson NT, Chisholm A, and Kenyon C, *Cell* **74**, 29-42 (1993)), and we find that *mec-3* expression in PLM neurons is significantly reduced in an *egl-5* mutant. These data suggest that the ectopic touch neurons in *sem-4* animals have characteristics of AVM, PVM, and PLM touch neurons. Therefore, the lineage transformations in *sem-4* animals may be homeotic transformations in which the T.pp lineage is transformed either to a different tail lineage (PLM) or to more anterior (AVM and PVM) touch neuron lineages. We propose that *sem-4* negatively regulates the HOM-C genes in the T lineage. This idea is consistent with evidence that mutations in *spalt* cause ectopic expression of *sex combs reduced*, the *Drosophila* homologue of *lin-39* (Casanova J, *Roux's Arch Dev Biol*, **198**, 137-140 (1989)). In other lineages, *sem-4* may positively regulate the HOM-C genes: Grant *et al*. found that *sem-4* interacts with *lin-39* and possibly with *mab-5* during vulval development (Grant K, Hanna-Rose W, and Han M, *Dev*. *Biol*. **224**, 496-506 (2000)); we have observed that *sem-4* animals exhibit an AVM migration defect very similar to that present in *lin-39* animals.

57. Inhibition of Touch Cell Fate by *egl-44* and *egl-46*

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In wild-type *C.elegans* six cells develop as receptors for gentle touch. In *egl-44* and *egl-46* mutants (which were first identified because of defects in the HSN neurons; Desai *et al*., 1988, Nature 336: 638), however, the FLP neurons also possess touch receptor-like features and express touch receptor genes (Mitani *et al*., 1993, Development 119: 773). Because these genes appear to repress the expression of touch cell fate, we have cloned them and characterized their action on touch cell-specific genes. *egl-44* encodes a TEF(transcrition enhancer factor)-like protein, and like TEF proteins, EGL-44 contains a TEA/ATTS DNA-binding domain and a putative transcriptional regulation domain. A *gfp::egl-44* rescuing fusion is expressed in nuclei of neurons (including the FLP and HSN cells), hypodermis and interstine, but not in touch cells. *egl-46* encodes a zinc-finger protein that with two Drosophila proteins and two proteins from humans and mice defines a novel zinc-finger subfamily. A *gfp::egl-46* rescuing fusion is expressed predominantly and transiently (except for in a few cells) in nuclei of neurons, including FLP, HSN, and the touch cells. The late and transient expression of *gfp::egl-46* in the Q lineages and the previously identified lineage defects in *egl-46* mutants, suggest that the gene may control the production and differentiation of terminal cells in this lineage. In contrast to its expression in many other cells, *egl-46* is continually expressed in the FLP neurons; this expression is dependent on *egl-44*.

To test whether coexpression of *egl-44* and *egl-46* prevented the expression of touch cell characteristics, we ectopically expressed *egl-44* and *egl-46* in the touch cells. This expression resulted in touch insensitivity and the loss of *mec-7*, *mec-4*, and *mec-18* expression. In

addition, as with the FLP cells, coexpression of *egl-44* and *gfp::egl-46* led to the continued expression of the flourescent protein. These defects were not seen when *egl-44* was expressed in the touch cells of *egl-46* mutant animals. These results suggest that expression of both genes are needed to repress touch cell fate in the FLP cells.

EGL-44 and EGL-46 are likely to repress the expression of touch cell function genes by binding to their promoters and preventing the binding of the MEC-3::UNC-86 complex. EGL-44 and EGL-46 bind to each other in *in vitro* S-Tag pull down experiments. In addition, the binding of EGL-44 to specific sites in the *mec-4* and *mec-7* promoters is enhanced by the presence of EGL-46. These binding sites are near those of UNC-86 and MEC-3, and the binding of these latter proteins is preventd by the presence of EGL-44 and EGL-46. We are currently examining the roles of different domains of EGL-44 and EGL-46 in this binding to understand further the action of these genes in the combinatorial control of touch cell fate.

58. PAG-3 MAY COUPLE CELL LINEAGE CUES TO TERMINAL DIFFERENTIATION THROUGH HETERODIMERIZATION WITH UNC-3 IN DEVELOPING VA AND VB MOTOR NEURONS

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During the development of multicellular animals the potentials of blast cells are progressively restricted until differentiated cell types are formed. During this process, cells decide between survival and programmed cell death. We are interested in understanding the mechanisms that control this decision and have chosen to focus on how the pattern of programmed cell deaths in the ventral cord is determined. In the midbody of wild-type animals the six Pn.aap cells survive and differentiate to form VC motor neurons, while the Pn.aap cells in the anterior and posterior die. From two genetic screens seeking mutants abnormal in the numbers of VC motor neurons or programmed cell deaths, we found that *unc-3* and *pag-3* mutants have both extra cell corpses and extra VC motor neurons. In *pag-3* mutants these abnormalities are a consequence of a defect in neuroblast fate determination wherein the Pn.aaa neuroblasts reiterate the fate of their mothers, the Pn.aa cells, and generate extra Pn.aap-like cells. The extra Pn.aap-like cells can become VC-like motor neurons or undergo programmed cell death. Mutations in *unc-3* do not affect P cell divisions but instead affect the

fates established in differentiating cells generated by those lineages. Evidence derived from analyses of the phenotype of *unc-3 pag-3* double mutants, laser ablation studies, and expression patterns of cell type-specific markers in mutants suggests that loss of *unc-3* function may result in extra cell corpses and VC motor neurons by affecting VB motor neuron differentiation.

pag-3 and *unc-3* both encode transcription factors. In developing wild-type animals antiserum recognizing PAG-3 first detected PAG-3 in the Pn.aa neuroblasts, the anterior daughters of which are abnormal in *pag-3* mutants. Expression was not detected in the Pn.ap cells, which are also neuroblasts, indicating that PAG-3 expression is activated by cell lineage cues specific to the Pn.aa neuroblasts. PAG-3 was also present in all cells generated by Pn.aa, including the VA, VB and VC motor neurons, suggesting that PAG-3 may function during differentiation of these cells. An antiserum recognizing UNC-3 first detected protein in differentiating motor neurons. More specifically, UNC-3 and PAG-3 were coexpressed in the VA and VB but not in the VC motor neurons. We found that UNC-3 and PAG-3 can heterodimerize *in vitro*. We propose that PAG-3 expression is activated by Pn.aa-lineage-specific cues to determine neuroblast fate and that PAG-3 may then serve a second function in a complex with UNC-3 in the differentiating VA and VB motor neurons generated by Pn.aa. A *Drosophila* counterpart of PAG-3, Senseless, is required for the development of peripheral nervous system neurons. Expression of Senseless and the proneural proteins, which, like UNC-3, are HLH transcription factors, are interdependent. Vertebrate counterparts of UNC-3 are widely expressed in the developing nervous system and are coexpressed with vertebrate counterparts of PAG-3 in some tissues. We suggest that we have discovered a universal mechanism whereby PAG-3 counterparts determine blast cell fates in specific lineages and then act during terminal differentiation in cells derived from those blast cells in part through heterodimerization with HLH transcription factors.

59. *C. elegans* SEK-1 MAPK cascade regulates neuronal asymmetric development mediated by Ca2+ signalling

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Lateral asymmetric patterns of gene expression are key to the development in many systems. Within the bilaterally symmetric pair of olfactory AWC neurons in *Caenorhabditis elegans*, the candidate odorant receptor STR-2 is asymmetrically expressed in either the left or the right neuron, but never both. The asymmetric AWC cell fate is regulated by axon contact during development and by Ca2+ signalling. The mitogen-activated kinase (MAPK) pathway is a highly conserved signalling cascade that converts extracellular signals into various outputs. We identified a *C. elegans* MAPK kinase (MAPKK), SEK-1, that can activate p38 MAPK. Here we show that SEK-1 is required for asymmetric expression in AWC neurons. Genetic and biochemical analyses reveal that SEK-1 functions within the same pathway downstream of UNC-43, the type II Ca2+- and calmodulin-dependent protein kinase (CaMKII), and NSY-1 MAPKK kinase (MAPKKK). We conclude that the NSY-1-SEK-1 MAPK pathway establishes asymmetric cell fate decision during neuronal development. This MAPK cascade is activated by Ca2+ signalling through CaMKII.

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60. Regulation of olfactory receptor expression by a serine-threonine kinase.

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Regulation of olfactory receptor expression by a serine-threonine kinase.

The ability to sense chemical cues in the environment is crucial for *C. elegans* to find food, avoid harm, and choose an appropriate developmental program. Initial contact with these cues occurs via G-protein coupled odorant receptors localized in the cilia of sensory neurons. Distinct sensory neurons play highly specialized sensory roles. Therefore, specific receptors must be expressed within the correct neuron and at the correct level to enable the worm to interpret the cue correctly and thus initiate the proper behavioral response. This is a remarkable feat given the complex and constantly changing environment. Very little is known in any organism about how olfactory receptor expression is regulated, and how sensory information is integrated to modulate behavior and development. We describe the gene *sns-8*, a kinase required to regulate expression of a subset of odorant receptors.

sns-8 was isolated from genetic screens to find factors that affect expression of the odorant receptor, *str-1*. *str-1* expression is dramatically reduced in *sns-8* mutants. We investigated the extent of olfactory receptor expression defects, and found that a subset of receptors expressed in the AWB olfactory neurons and a receptor expressed in the ASH polymodal neurons require SNS-8 function. In addition to having defects in the regulation of olfactory receptor expression, *sns-8* mutants are small (see abstract by Meduzia et al.) and are developmentally delayed.

sns-8 encodes a serine-threonine kinase closely related to PAR-1. It is broadly expressed throughout the nervous system and is cytoplasmically localized. SNS-8 is required neuronally to rescue its body size defects - adding *sns-8* to an ever-increasing group of genes that implicate neuronal, and perhaps sensory, activity in the regulation of body size (see abstract by Fujiwara et al). We have found that expression of SNS-8 specifically in the AWB neurons restores *str-1* expression - indicating that SNS-8 acts cell autonomously to regulate receptor expression.

We have evidence indicating *sns-8* mutants have compromised sensory signaling. *sns-8* worms show a weakened ability to assess correct dauer inducing conditions. *sns-8* mutants show a synthetic dauer constitutive phenotype (Syn-Daf) with other genes known to regulate sensory inputs into the dauer pathway. These genes include *egl-4* and *tph-1*. *egl-4* mutants have numerous characterized sensory defects and has been shown to function in the Group II TGF-ß signaling pathway for dauer formation (Daniels et al, 2000). *egl-4* encodes a cGMP-dependant kinase (see abstract by Fujiwara et al). While neither *sns-8* nor *egl-4* mutants alone form dauers at 20ûC, we have shown that *sns-8; egl-4* double mutants inappropriately form dauers at this temperature. Surprisingly, we have found that *egl-4* mutants also show defective *str-1* expression, perhaps indicating a previously undescribed role for *egl-4* in the regulation of expression of a subset of receptors. In addition, we find that when *sns-8* is combined with a mutant that knocks out serotonin synthesis, *tph-1*(Sze et al. 2000), these doubles also show a Syn-Daf interaction at 20ûC. We therefore propose a model in which *sns-8* regulates sensory inputs into the TGF- ß dauer regulatory pathway by regulating the expression of a subset of olfactory receptors.

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61. Synapse Formation between Neurons

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Synaptogenesis between neurons is a key mechanism underlying the generation, maintenance, and plasticity of neuronal circuits. The molecular events that govern the development of synapses between neurons are poorly understood. In C. elegans, neurons form stereotypical synapses with each other, providing an ideal model system to study this question.

We are studying the synapses formed between the HSNs and VC4,5 neurons that regulate egg-laying behavior. Presynaptic vesicles in the HSN motor neurons were labeled with SNB-1-YFP (Nonet, 1999). Postsynaptic sites in VC4,5 neurons were labeled with LIN-10-RFP (Rongo et al. 1998). Using these markers, two clusters of synapses can typically be visualized in the vulva region of adult animals.

We used these markers to examine the formation of synapses during development. SNB-1-YFP and LIN-10-RFP are expressed by the L2 larval stage, but the HSN to VC synapses are not mature until the adult. The clustering of presynaptic vesicles in HSN is first apparent in early L4 larva. When the vesicles first cluster, postsynaptic LIN-10 in the VC4,5 neurons has a homogenous staining pattern. Later in the L4 stage, LIN-10 forms puncta along the VC processes, which resolve to locations next to presynaptic vesicles in early adult. These results suggest that presynaptic specializations precede postsynaptic specializations, at least those marked by LIN-10.

We then asked what determines the location of the synapses. We found that the vulva may induce synaptogenesis between these neurons. In dig-1 animals whose vulvae are anteriorly displaced, the synapses between HSN and VC4,5 were anteriorly displaced accordingly. In unc-40 and unc-6 mutants, HSN axons do not contact the vulva because of axon guidence defects. HSN presynaptic vesicles showed abnormal clustering in these mutants. Vulvaless animals resulting from ablation of vulval precursor cells also showed abnormal HSN presynaptic clustering. These results suggest that the developing vulva induces synapse formation between HSN and VC4,5, consistent with its known role in inducing HSN and VC branching.

Presynaptic vesicle clustering and synaptic transmission are not essential for the assembly of the postsynaptic apparatus. In unc-104 mutants, presynaptic vesicles are trapped in the HSN cell body, but postsynaptic clustering of LIN-10 was normal. In unc-13 mutants, synaptic transmission is blocked, but both LIN-10 localization in VC4,5 and SNB-1 localization in HSN was normal.

We are using these markers to identify and characterize mutants with defects in synapse formation, and have isolated a mutant that shows ectopic HSN synapses in the vulval area.

Reference:

Nonet, M.L. (1999). Visualization of synaptic specializations in live C. elegans with synaptic vesicle protein-GFP fusions. J. Neurosci.Methods 89, 33-40

Rongo C, Whitfield CW, Rodal A, Kim SK, Kaplan JM. (1998) LIN-10 is a shared component of the polarized protein localization pathways in neurons and epithelia. Cell. 94:751-9.

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62. *osm-5*, the *C. elegans* homologue of the murine cystic kidney disease gene *Tg737*, functions in a ciliogenic pathway

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Cilia and flagella are important organelles involved in diverse functions such as fluid and cell movement, sensory perception, and embryonic patterning. They are devoid of protein synthesis, thus their formation and maintenance requires the movement of protein complexes from the cytoplasm into the cilium and flagellum axoneme by intraflagellar transport (IFT), a conserved process common to ciliated or flagellated eukaryotic cells. Mutations in murine Polaris (*Tg737*) result in cystic kidney disease and left-right axis patterning defects associated with aberrant cilia formation or maintenance. *Tg737* is expressed in ciliated epithelial cells, and the encoded protein, Polaris, localizes to the base of cilia and within the cilium axoneme. Here we characterize *osm-5*, the *C. elegans* homologue of the murine cystic kidney disease gene *Tg737*. *osm-5* is expressed in ciliated sensory neurons and its expression is regulated by DAF-19, an RFX-type transcription factor, that governs the expression of several other genes involved in cilia formation in the worm. OSM-5 protein is predicted to contain ten tetratricopeptide repeats (TPRs), a motif involved in protein-protein interactions. Similar to murine Polaris, OSM-5 protein was found to concentrate at the cilium base and within the cilium axoneme by immunofluorescence and an OSM-5::GFP translational fusion. Furthermore, time-lapse imaging of OSM-5::GFP fusion protein shows fluorescent particle migration within the cilia. Analysis of three mutant *osm-5* alleles indicates that *osm-5* is required for cilia formation and that the TPRs are essential for proper function of the protein. Overall, our data support a conserved role for OSM-5 in ciliogenesis, most likely as a component of the IFT process.

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63. Intraflagellar Transport particles complex A and B play different roles in *C. elegans cilia formation*

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Intraflagellar Transport (IFT) is a microtubule-dependent movement of particles along the eukaryotic flagellum and cilium beneath the membrane. IFT has been shown to be a fundamental process for assembly and maintenance of eukaryotic cilia and flagella, including *C. elegans* sensory and mammalian primary cilia. In mammals, defects in IFT may result in Polycystic Kidney Disease, situs inversus, and retinal degeneration. IFT particles were first isolated from the green alga *Chlamydomonas* as two complexes, A and B, composed of about 16 individual polypeptides. The functions of complex A and B are unknown.

We and others have found that several *C. elegans* cilium structure genes encode IFT polypeptides and motors. By analogy to *Chlamydomonas*, it is assumed *C. elegans* IFT particles also form two complexes, although direct evidence for this hypothesis has been lacking. We are exploring the biochemical, cellular, and molecular roles of the IFT particles in *C. elegans*. Density gradient centrifugation of *C. elegans* extracts shows OSM-6, an IFT particle complex B polypeptide, of MW 52KD, sediments at about 16S, suggesting an association with an IFT complex similar to that found in *Chlamydomonas*.

In *C. elegans*, cilia are sensory organelles required for a broad range of sensory behaviors. Mutations affecting IFT proteins result in defective ciliogenesis and corresponding abnormal sensory perception. Based on gene sequence homology and mutant phenotypes, we categorized previously identified *C. elegans* IFT sensory mutants into complex A and complex B mutants. For example, in the complex A mutant che-11, cilia can still form, OSM-6::GFP accumulates in cilia, yet no obvious motility was detected in IFT assays. In the complex B mutant osm-5, cilia are highly stunted, OSM-6::GFP localizes to the stumpy cilia, yet bi-directional movement looks normal inside the stumpy cilia. These results suggest that the similar sensory phenotypic defects in *C elegans* IFT complex A and B mutants may have different causes. IFT complex A and B may play different roles in formation and function of cilia. We are currently exploring the roles of complex A and B in both ciliogenesis and sensory signal transduction.

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64. Multi-pathway Regulation of Meiotic Entry

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At the distal end of the late-larval and adult *C. elegans* gonad, a population of proliferating germ cells serve as stem cells for potentially hundreds of gametes. As cells move proximally, away from the influence of the Distal Tip Cell (DTC), they leave the mitotic cell cycle and enter meiotic prophase. GLP-1/Notch receptors in distal germ cells are activated by a signal from the DTC, promoting the proliferative state. Gain-of-function (gf) consitutively active alleles of *glp-1* cause germ cells to remain proliferative, thereby forming a germline tumor. *gld-1* and *gld-2* function redundantly to inhibit proliferation and/or promote entry into meiosis and are negatively regulated by the *glp-1* pathway (Kadyk and Kimble, 1998; Francis et al. 1995). Animals lacking *gld-1* and *gld-2* form a germline tumor similar to that seen with a *glp-1(gf)* allele.

We have employed two genetic screens to identify other genes that are involved in regulating the switch from mitosis to meiosis. One screen identified mutations that enhance a weak *glp-1(gf)* allele, (*oz112 oz120)*. This screen identified *teg-1* (tumorous enhancer of *glp-1(gf)*), which encodes a 353 amino acid protein with similarity to a human protein that interacts with the T-lymphocyte receptor, CD2. The other screen identified mutations that are synthetic tumorous with *gld-2*. From this, we obtained multiple alleles of *syt-1* (synthetic tumorous), which we cloned and identified as the *nos-3* gene (Kraemer et al 1999). This screen also identified another allele of *teg-1*, demonstrating that *teg-1* is synthetic tumorous with *gld-2*. Neither *teg-1* nor *nos-3* mutants are tumorous on their own. Thus, *teg-1*, *nos-3* and *gld-1* appear to each function redundantly with *gld-2* to inhibit proliferation and/or to promote entry into meiosis.

Close examination of the germline tumors in *gld-2; teg-1*, *gld-2; nos-3* and *gld-2 gld-1* animals, however, revealed that none are equivalent to the *glp-1(oz112gf)* tumor, even though the mutations in these four loci appear to be null. Using two markers (anti-REC-8 that stains chromosomes of proliferative nuclei when specific staining conditions are used and anti-HIM-3 (Zetka et al 1999) that stains chromosomes of meiotic nuclei), we have shown that each of these synthetic tumors contain meiotic nuclei while the *glp-1(oz112gf)* tumors do not. These results suggest that these four genes (*gld-1*, *gld-2*, *teg-1* and *nos-3*) could be involved in regulating the progression of meiotic prophase rather than entry into meiotic prophase. Thus the germline tumor would result from germ cells returning to mitosis after failing to progress through meiotic prophase, analogous to the *gld-1* single in female germ cells (Francis *et al* 1995). Conversely, if these genes do act to promote meiotic entry, the presence of meiotic nuclei in the tumors suggests that a loss of *gld-2* and *gld-1* (or *gld-2* and *teg-1* or *nos-3*) is not equivalent to constitutive activation of GLP-1, indicating that there is additional genetic complexity in this process. To distinguish between these two models, we used the weak *glp-1* gf allele *ar202*. At 15C the germ lines of *glp-1(ar202)* animals are essentially wild type, while at higher temperatures animals display a 'Pro' phenotype (proliferative germ cells in the proximal end of the gonad; see abstract by Pepper, Lo and Hubbard) and a late onset tumorous phenotype (the size of the distal mitotic zone is expanded). We tested *gld-1*, *gld-2* and *teg-1* for their ability to enhance the late onset tumorous phenotype of *glp-1(ar202)* at 15C. We reasoned that if a gene were normally required to promote entry into meiosis, loss of its activity could enhance the late-onset tumorous phenotype of *glp-1(ar202)*.
Conversely, if no enhancement of *glp-1(ar202)* is seen, the gene might not be involved in regulating meiotic entry, but rather meiotic progression.

Mutations in *gld-1*, *gld-2* and *teg-1* each enhance the late onset tumorous phenotype of *glp-1(ar202)* at 15C, supporting the hypothesis that these genes regulate entry into meiosis. Furthermore, there are far more meiotic nuclei in the *gld-2 gld-1* and *gld-2; teg-1* doubles than in the *gld-2 gld-1; teg-1* triple mutant. This suggests that each gene functions to promote entry into meiosis, perhaps through three

separate pathways. Since some evidence of meiotic nuclei still exists in the triple mutant, a possible fourth pathway could be involved.

65. Roles and modes of action of *rad-51*

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Our understanding of genetic control of meiotic recombination comes from studies in the unicellular eukaryote *Saccharomyces cerevisiae*. Most gene involved in control of recombination and meiosis are conserved throughout evolution. However, metazoa have developed a germ cell line distinct from the somatic cell line requiring a different kind of regulation compared to unicellular eukaryotes. Furthermore, in distant species, in spite of evolutionary conservation, similar genes may be subject to different regulation and interactions. Accordingly, elimination of a conserved molecule or interference with a conserved pathway may lead to quite different results in different organisms. For this reason, genetic and biochemical studies in different organisms are likely to contribute to an understanding of the crucial features and fundamental components of recombination pathways and their evolution.

Among eukaryotes, *C. elegans* is the only organism known so far provided with a single *rec*A like gene, the homolog of *RAD51*. Surprisingly, the meiosis specific *DMC1* gene present in fungi, plants and mammals, is absent in C. elegans. RNA interference of the *rad-51* gene in *C. elegans* leads to a number of visible phenotypes such as i) high levels of embryonic lethality, ii) increase in the frequency of males, iii) reduced fertility, and iv) hypersensitivity to ionizing radiation *in soma*. We have analysed in details the mechanisms leading to the above described phenotypes, in order to understand the different functions and modes of action of *rad-51* in somatic and germ line cells.

We demonstrated that this gene is required at several steps of gametogenesis: gene

inactivation, in fact, affects pre-meiotic repair, sister chromatid exchange and homologous recombination and triggers a meiotic checkpoint. Unlike what has been described in mammals, *rad-51* expression is not required during embryogenesis, but is required and enhanced in soma in response to DNA damage induced by ionizing radiation.

66. Asymmetrically distributed oligonucleotide repeats in the *C. elegans* genome sequence that map to regions important for meiotic chromosome segregation

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Some feature of the *C. elegans* X chromosome distinguishes it from the five autosomes allowing X-linked genes to be dosage compensated. This biological regulatory mechanism is mediated by a conserved multiprotein complex found bound along both X chromosomes in XX animals. We used a bioinformatics approach to ask if the worm genome sequence contains any obvious identifiable elements that are also distributed along the X and could play a role in this process. Our analysis revealed several short candidate sequences that were highly enriched on X. As a control for this study we asked if any of the other five chromosomes contained their own chromosome-specific sequences. In each case we identified short repetitive DNA sequence elements that are enriched on one of the six chromosomes. Strikingly, each sequence is asymmetrically distributed such that the elements are clustered towards one end of that chromosome. We realized not only that our initial assumptions about dosage compensation were likely to be incorrect, but also that these repetitive sequence patterns were reminiscent of another feature of worm chromosomes.

The termini of *C. elegans* chromosomes have been proposed to play an important role in meiotic prophase either when the homologs may be participating in a genome-wide search for their proper partners, or in the initiation of synapsis. For each one of the six chromosomes one end appears to stimulate crossing-over with the correct homolog; while the other end of the chromosome seems to lack this property. The six repetitive sequence elements described above have a distribution that closely parallels these putative meiotic pairing centers (MPC) or homolog recognition regions (HRR). We propose that these six DNA sequence elements, which are largely chromosome-specific, may correspond to the genetically defined

HRR/MPC elements. To test this hypothesis we have begun a yeast one-hybrid screen to ask if any worm proteins can bind specifically to these short sequences.

67. The *C.elegans* homolog of the human Bloom's syndrome gene is required for meiotic recombination and genome integrity

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The *him-6* gene is required for meiotic recombination and genome stability during proliferation of premeiotic cells in *Caenorhabditis elegans*. *him-6* encodes a putative recQ type of DNA helicase which is homologous to the human BLM (Bloom's Syndrome) gene product. It is also related to the *E. coli* RecQ helicase, the *S. pombe* rqh1+ gene and the *S. cervisiae* Sgs1 gene. Blooms syndrome (BS) is a rare autosomal recessive disorder characterized by genomic instability. The BLM protein is thought to function as antirecombinase in induced recombination resulting, e.g., from blocked replication.

The *him-6* gene is predominantly expressed in the germ line. Mutations in *him-6* result in a decrease in meiotic recombination. Oocyte chromosomes of both *him-6(e1423)* and *him-6(e1104)* homozygotes lack chiasmata, suggesting that the chromosomes fail to undergo crossing-over or pairing. However, at pachytene stage, where they are fully paired in wild type animals, no visible defects were observed in *him-6* mutants. Unlike *BLM*$^{-}$, however, *him-6* mutations cause no apparent somatic phenotypes.

Double mutants for *him-6* and *top-3* (the gene encoding topoiosomerase IIIα) exhibits severe defects in the proliferation of the germ line nuclei, a phenotype which is not observed in *top-3* single mutants. The number of nuclei in the gonad is greatly reduced and there are some evidences of chromosomal damage. These nuclei do not appear to progress through meiosis and the chromosomal defects are not suppressed by a mutation in the *spo-11* gene, which is essential for the initiation of meiotic

recombination. Altogether, this suggests a profound defect in the ability of *him-6 top-3* double mutants to maintain genome integrity during the stage of germ line proliferation. We propose that HIM-6 has at least two distinct functions. First, it promotes normal levels of meiotic recombination, a novel function for eukaryotic BLM proteins, and second, together with topoisomerase IIIα, it has DNA repair activity during the proliferation of premeiotic cells. We are currently investigating the possibility that HIM-6 participates in a large repair complex, together with other factors such as, e.g., MRE-11.

68. Assembly and function of the synaptonemal complex in *C. elegans* meiosis.

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Meiosis is the specialized cell division process by which diploid organisms generate haploid gametes. In preparation for the meiosis I division, homologous chromosomes recognize each other, pair, and undergo recombination. During this process a proteinaceous structure known as the synaptonemal complex (SC) forms at the interface between side-by-side aligned homologs. Crossing over is completed in the context of the SC, leading to the formation of chiasmata that connect the homologs and allow them to orient toward opposite poles of the meiosis I spindle.

Despite the ubiquitous presence of the SC structure from yeast to mammals, its functions in meiosis are poorly understood and a matter of much debate. In order to investigate proposed roles for the SC during meiotic prophase and to understand how its components interact in a macromolecular assembly we set out to identify genes involved in synapsis.

Our genetic screens and functional genomic approaches have synergized to identify three *syp* (synapsis protein) genes important for achieving successful synapsis between homologous chromosomes. The consequences of loss or reduction of function of the *syp* genes are exemplified by the phenotype of *syp-2* mutants. As *syp-2* mutants enter meiotic prophase, nuclei undergo a normal spatial reorganization of chromosomes, load on meiosis-specific chromosome axis protein HIM-3, and homologs do indeed initially pair. However, *syp-2* mutants fail to stabilize pairing, and homologs dissociate prematurely. Further, the polarized nuclear organization characteristic of entry into prophase (normally lost upon completion of synapsis) persists for a prolonged period. *syp-2* mutants lack chiasmata at the end of meiotic prophase, presumably reflecting failure in

crossing over. *syp-2* mutants also exhibit elevated levels of germ cell apoptosis, suggesting either that they trigger a DNA damage checkpoint by initiating but failing to complete recombination, or that absence of synapsis *per se* triggers a checkpoint response.

SYP-2 and the other SYP proteins contain extended coiled-coil domains and are likely structural components of the central region of the SC. SYP-2 localizes at the interface between paired aligned homologs, and is lost from chromosomes by late diakinesis (in contrast to components of the chromosomal axes, which persist until the meiotic divisions). SYP-1 shows similar localization to SYP-2, and SYP-1 fails to become localized in *syp-2* mutants. We are currently testing whether SYP-2 localization is dependent on chromosome axis protein HIM-3 or meiosis-specific cohesin REC-8.

Our analysis of SYP protein function and localization suggests that: 1) the SC is important for stabilizing intimate chromosomal associations subsequent to initial pairing; 2) the SC is required for formation of crossover recombination; 3) the synapsis process itself may drive redistribution of chromosomes out of a polarized nuclear organization.

69. Germline and sex-limited loci distributed non-randomly among chromosomes and with respect to recombination rate

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Genes that are involved in reproduction or that have sex-limited expression clearly interest evolutionists due to their potential role in fitness. Theory suggests that such gene classes may also represent prime candidates for rapid evolution by sexual selection, and that loci involved in processes of rapid antagonistic coevolution should accumulate non-randomly on chromosomes. We apply a functional genomics perspective to the evolutionary issue of gene spatial arrangement using data comprising >90% of the genes predicted to be present in the Caenorhabditis elegans genome. This study demonstrates an approach by which microarray technology may be used to distinguish classes of genes for use in the genomic-scale testing of predictions generated by evolutionary theory-and illustrates some limitations of current genomic evolutionary theory. Randomization tests that take into account the physical positions of loci show that germline-related genes are distributed in an aggregated fashion along chromosomes. Furthermore, our findings indicate that germline-related and gender-related loci are represented disproportionately in regions of low recombination and that germline-related and gender-related loci are underrepresented on the X chromosome. Although definitive conclusions regarding the causes of these genomic patterns are premature, we hypothesize that processes ranging from mating system byproducts to biochemical constraints during meiosis and stabilizing selection may account for different components of our observations.

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70. MES-4, a protein required for germline viability, binds the autosomes but not the X chromosomes

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We report the first example of a protein, MES-4, that binds specifically to the autosomes, and not to the X chromosomes. *mes-4* was discovered, along with *mes-2*, *mes-3*, and *mes-6*, in a screen for maternal-effect sterile mutants. All four genes give a similar mutant phenotype: death of the germline in the progeny of *mes/mes* mothers. *mes-4* encodes an 898 amino acid protein which has a predicted homolog in *Drosophila* and is related to a human and a mouse gene. All four MES-4-related proteins share 3 PHD fingers, a CXC domain, a SET domain, and a PKF (pinky finger) domain. PHD fingers are thought to mediate protein-protein interactions. SET domains are shared by many chromatin-binding proteins. Intriguingly, MES-2 and MES-6 are the *C. elegans* homologs of *Drosophila* Enhancer of zeste and Extra sex combs, both members of the Polycomb group of chromatin-associated transcriptional repressors. Indeed, studies of germline expression of transgenes[1] suggest that all four MES proteins participate in repressing expression of at least some genes in the germline.

By immunofluorescence staining, MES-4 appears associated with chromosomes in the germline and in embryos. MES-4 is expressed robustly in the distal, mitotic region of the germline, then becomes barely detectable in the meiotic region, including the oocytes. MES-4 levels increase dramatically after fertilization. The level of MES-4 later declines in somatic cells, but persists in the primordial germ cells. A striking feature of MES-4 staining in germ cells and early embryos is that it localizes exclusively to the autosomes and is not detectable on the X chromosomes. Insight into the differential binding behavior of MES-4 comes from results of immunostaining worms bearing "repetitive arrays" (composed of plasmid DNA) vs. worms bearing "complex arrays" (composed primarily of high-complexity worm genomic DNA): MES-4 binds to the complex extrachromosomal arrays, but not to the repetitive arrays. The previous observation that transgenes in repetitive arrays are generally silenced in the germline, but transgenes in complex arrays can be expressed, is intriguing in light of our observations that MES-4 binds to autosomes and complex arrays, but not to X chromosomes and repetitive arrays: perhaps the X's adopt a different chromosome state than the autosomes and are transcriptionally repressed in the germline, like repetitive arrays. This hypothesis is supported by the recent finding[2] that, of genes whose expression is enriched in the germline, very few are located on the X. Two possible models for the role of MES-4 binding to the autosomes are: 1) MES-4 directly activates expression of autosomal genes, some of which may participate in repression of X-chromosome gene expression, and 2) MES-4 protects the autosomes from binding by transcriptional repressors that function in the germline.

1. Kelly and Fire, 1998, Development 125, 2451.

2. Reinke *et al.*, 2000, Mol. Cell 6, 605.

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71. Specific functions of linker histone isoforms in C. elegans

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Single gene linker histone knockouts have been produced in a number of metazoan organisms ranging from Tetrahymena to mouse. None of these knockouts produced a significant phenotype. Therefore it has been proposed that linker histones could be dispensable for eukaryotic live. The presence of a linker histone multigene family, however, is a typical feature of the genomes of multicellular organisms. Therefore it may be speculated that although the linker histone could be dispensable for the live of individual eukaryotic cells, it may serve specific and essential functions in the development of multicellular organisms. We used RNAi to analyze the complete linker histone gene family of C. elegans, which comprises eight genes (H1.1 - H1.6, H1.X, and H1.Q). Specific and significant phenotypes were obtained for three of these genes.

Histone H1.1 is essential for the chromatin silencing in the male and female germ line of C. elegans. Moreover, the depletion of histone H1.1 leads to a lack of germ cell proliferation and differentiation in the hermaphrodite, but not in the male. This type of hermaphrodite sterility is similar to the phenotypical appearance of the mes mutants, which is ascribed to be caused by a loss of chromatin silencing. No further linker histone of C. elegans is involved in germ line development or gametogenesis.

RNA interference with H1.4 expression in him-8 created adult males with spike tails. These males were not attracted to hermaphrodites and did not mate. H1.4 RNAi resulted also in variable numbers of SPD neurons in the affected mail tail structures. We conclude that H1.4 is needed for the completion of male tail morphogenesis.

The primary structure of H1.X defines a novel type of linker histone. H1.X is a nuclear as well as a cytoplasmic protein, and it is prominently associated with the tonofilaments of the seven marginal cells of the pharynx. The tonofilaments are a cytoskeletal structure created by the intermediate filament system. H1.X is also expressed in body and vulva muscles. H1.X RNAi created uncoordinated animals with an elongated pharynx muscle, which were occasionally egg laying defective.

We conclude that linker histone isoforms are very specifically involved in various aspects of the development of the multicellular organism C. elegans.

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72. Characterization of the *C. elegans* Heterochromatin protein 1 homologues.

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The highly conserved Heterochromatin protein 1 (HP1) class of chromobox genes has been implicated in gene regulation, DNA replication, chromosome segregation and nuclear architecture via the assembly of macromolecular complexes in chromatin. In order to understand how higher order chromatin structure can regulate these different processes throughout development, we have been studying the *C. elegans* HP1 homologues. *C. elegans* contains two HP1 homologues, which we have named HPL-1 and HPL-2 (HP1-like 1 and 2). To determine the pattern of expression of HPL-1 and HPL-2, we have analyzed HPL-1 : :GFP and HPL-2 : :GFP reporter lines. Both reporters are strongly expressed in most, if not all somatic nuclei of larvae and adult animals. In embryos, strong expression is first apparent starting at the 50 cell stage. We are also able to detect a weaker HPL-2 : :GFP fluorescence in germline nuclei, oocytes and early embryos. Strikingly, in both oocytes and nuclei from embryos, we observe the presence of a limited number of fluorescent foci over a more diffuse background fluorescence, suggesting that the fusion protein may associate with particular chromosomal regions. To gain insight into the function of HPL-1 and HPL-2 we have performed RNA interference experiments. While *hpl-1* (RNAi) results in no detectable phenotype, *hpl-2* (RNAi) at 25° results in 50-80% sterility amongst the F1 progeny of injected animals. This sterility appears to be due to a defect in oocyte maturation. Associated with this sterility, we also see a less penetrant evl phenotype and the occasional induction of pseudovulvae anterior or posterior to the normal vulva. The correct specification of vulval cell fates requires both the activation of RTK/Ras/Map kinase members as well as negative regulation by a set of genes known as the synMuvs. synMuv genes comprise two functionally redundant sets of genes that appear to antagonize Ras pathway signaling. Several synMuvB genes have been shown to encode chromatin associated factors. We will present data showing that at 25° *hpl-2* (RNAi) results in a highly penetrant synMuv phenotype. Our interpretation of these phenotypes, together with the GFP localization studies, is that HPL-2 may be involved in the organization of repressive chromosomal subdomains, and that its absence results in the expression of genes which need to be silenced for proper oocyte maturation and vulval development. The *C. elegans* germline contains a chromatin-dependent mechanism for silencing transgene arrays. To ask whether *hpl-2* plays a role in this mechanism we are testing whether *hpl-2* (RNAi) relieves the silencing of a ubiquitously expressed *let-858 : :gfp* reporter transgene in the germline. Preliminary experiments suggest that this is indeed the case. These studies should shed light on the role of HP1 proteins throughout development in general, and in particular on the formation of heterochromatin-like complexes as a conserved mechanism of gene regulation.

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73. The Role of Chromatin Organization in Germ Line Function and Maintenance

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The germ line in *C. elegans* is maintained by a variety of suppressive mechanisms throughout development and during adulthood. In the adult germ line both transcriptional and post-transcriptional mechanisms play roles in maintaining germ cell viability and function. Transcriptional suppression involves chromatin-based mechanisms that are products of a compromise reached by the competing goals of meiotic DNA: the chromosomes must be compacted along their lengths for synapsis and recombination, yet must also be transcriptionally active for maternal product synthesis. One might thus hypothesize that genes essential for germ line function have evolved mechanisms to escape aspects of chromatin silencing that likely accompany the compaction of meiotic DNA.

Non-integrated transgenes are efficiently targeted for silencing in the germ line by chromatin-based mechanisms, since mutations that are defective in this silencing exhibit defects in chromatin organization. In addition, Valerie Reinke along with Stuart Kim and Anne Villeneuve, as well as my lab, have observed that the entire X chromosome appears to be silenced. Markers for transcriptionally active chromatin (e.g. histone acetylation) are uniquely excluded from the X chromosome in immature germ cells in both males and hermaphrodites. As germ cells mature past pachytene stage in hermaphrodites, however, the X chromosome becomes increasingly labeled by probes that correlate with transcriptional activation. This "activation" is not observed in male germ cells. Interestingly this correlates well with microarray data from Reinke et al. that indicates a paucity of sperm-specific or germ cell-intrinsic genes on the X, but a normal distribution of putative oocyte-specific genes (Mol. Cell. 6:605).

Silenced transgenes strongly mimic the observed underacetylation of X chromosome histones in the early stages of meiotic prophase I, but fail to become "activated" in oocytes. Conversely, a transgene (for a normally autosomal gene) integrated onto the X exhibits a germ line expression that is always strongest in oocytes. This suggests the presence of cis-acting elements peculiar to the X that may be involved in post-pachytene activation of the X chromosome in oocytes. The nature of germ line X-regulation is unknown, but is unlikely to involve components of dosage compensation (which do not assemble onto the X until early embryogenesis). Indeed, an absence of X-linked genes essential for both male and female germ cells would appear to obviate a need for dosage compensation in germ cells, as well as permit X silencing in the immature germ cells of both sexes. This may in turn have played a role in the frequent appearance of hermaphroditism in nematode evolution.

The effects of silencing mutants on transgene and X silencing, an analysis of X silencing in other hermaphroditic and gonochoristic nematode species, and the role of chromatin organization in germ line function will be presented and discussed.

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74. The intracellular domain of the feminizing receptor TRA-2A interacts directly with the transcription factor TRA-1A.

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In *XX* animals the *tra-2* gene negatively regulates the activity of the three *fem* genes. The absence of *fem* activity in *XX* animals frees *tra-1*, the terminal regulator of somatic sex determination, to promote female development. The *tra-2* gene encodes two proteins TRA-2A and TRA-2B. The larger of the two proteins, TRA-2A, is a predicted transmembrane protein with a large C-terminal intracellular domain. TRA-2B is expressed in the hermaphrodite germline and is predicted to be a soluble protein consisting of just the C-terminal domain of TRA-2A.

The intracellular domain of TRA-2A negatively regulates the FEMs through a direct interaction with FEM-3. Overexpression of the C-terminal domain of TRA-2A (TRA-2c) is sufficient for the negative regulation of FEM activity and the transformation of *XO* animals into females. To investigate the mechanism of TRA-2c feminizing activity we overexpressed various TRA-2c fragments. Surprisingly, a C-terminal fragment of TRA-2c (TRA-2c$_{delta}$5), incapable of interacting with FEM-3 in the yeast 2-hybrid system, maintained weak feminizing activity. Both yeast 2-hybrid and biochemical data demonstrate that TRA-2c$_{delta}$5 physically interacts with TRA-1A. *tra-2(mx)* mutations affect *tra-2* activity in both the soma and germline. Three *mx* mutations all disrupt the TRA-2c/TRA-1A interaction *in vitro* and reduce or eliminate the feminizing activity of TRA-2c$_{delta}$5 in overexpression assays *in vivo*.

We predicted that mutations in *tra-1* that disrupt the TRA-1/TRA-2 interaction should have a phenotype similar to *tra-2(mx)* alleles. Several alleles of *tra-1* have a *smg*-sensitive germline phenotype similar to that of the *tra-2(mx)* alleles. We found that mutations corresponding to three of the *smg*-sensitive *tra-1* alleles lie within the TRA-2 binding domain. Two of the *tra-1* alleles carry missense mutations of which one disrupts the TRA-2/TRA-1 interaction *in vitro*. A third *tra-1* allele possesses a nonsense mutation that truncates TRA-1 leaving ten amino acids of the minimal TRA-2 binding domain. Like the *tra-2(mx)* mutations the *tra-1 smg*-sensitive mutations are predicted to disrupt the TRA-2/TRA-1 interaction *in vivo*.

Our surprising results suggest that in both the germline and soma TRA-2 plays a role in regulating TRA-1A through a direct interaction. TRA-1A has been demonstrated to directly regulate transcription of several genes and is predicted to be nuclear localized. We observe strong nuclear localization of both GFP::TRA-1A and Myc::TRA-1A in transgenic animals. Consistent with a nuclear role for TRA-2 regulation of TRA-1A, we observe nuclear localization of a highly active GFP::TRA-2c fusion protein. Our data raise the intriguing possibility that in the soma TRA-2A is cleaved to allow the intracellular domain to enter the nucleus and interact with TRA-1A.

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75. Export of the TRA-1/tra-2 mRNA Complex From the Nucleus Regulates C. elegans Sex Determination

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Regulation of the sex determination gene *tra-1* is necessary for proper sexual development in *C. elegans*. *tra-1* is the terminal regulator in the somatic sex determination pathway, and is also necessary for germline sex determination. *tra-1* produces two proteins, TRA-1A and TRA-1B. TRA-1A is a member of the GLI family of transcription factors, and contains five zinc-fingers. TRA-1B is co-linear with the amino terminus of TRA-1A, but only contains the first two zinc fingers. (Unless otherwise stated, both proteins will be referred to as TRA-1). We find that the nuclear levels of TRA-1 correlate with sexual cell identity. Immunofluorescence analysis indicates that the levels of nuclear TRA-1, in both adult intestines and germlines, is higher in hermaphrodites than in males. By western analysis, the amounts of TRA-1 are similar in both males and hermaphrodites. Thus, sub-cellular localization, not protein levels, is responsible for the differences in nuclear levels of TRA-1 between the sexes. The importance of TRA-1 localization patterns in sexual development is further supported by the finding that XX *tra-2(lf)* mutants, which are masculinized, show less nuclear TRA-1 than XX wild-type animals. In addition, XX *tra-2(lf);fem-1(lf)* double mutants, which are feminized, show similar nuclear levels of TRA-1 as wild-type XX animals.

The sex-specific sub-cellular localization of TRA-1 is also dependent upon the interaction of TRA-1 with the *tra-2* mRNA. Previously, we found that nuclear export of the *tra-2* mRNA is regulated by TRA-1 binding to the *tra-2* 3' untranslated region (3'UTR). TRA-1 binds the *tra-2* 3'UTR, and promotes *tra-2* mRNA export to the cytoplasm (Graves et. al., 1999). Here we show that the nuclear levels of TRA-1 and sexual cell identity are regulated by nuclear export, and that this is mediated by TRA-1 binding the *tra-2* 3'UTR. Treatment of XO animals with Leptomycin B, (an inhibitor of CRM1 mediated nuclear export), increases nuclear TRA-1. Leptomycin B treatment also results in aberrant activation of the vitellogenin-2 promoter in wild-type XO animals but not in *tra-1(null)* animals. Therefore, inappropriate female development occurs when nuclear export is inhibited. This indicates that nuclear export of TRA-1 inhibits its activity, and consequently, female development. Export of TRA-1 requires binding to the *tra-2* 3'UTR: a genetic mutation in the *tra-2* 3'UTR that deletes the TRA-1 binding site results in an overabundance of nuclear TRA-1 and inappropriate female development in both XX and XO animals. Both increased nuclear TRA-1 and female development are suppressed by over-expression of RNA containing the TRA-1 binding site, emphasizing the importance of RNA binding for TRA-1 nuclear export and sexual development. The mutual dependence of TRA-1 and *tra-2* mRNA on one another for nuclear export suggests they export the nucleus as a TRA-1/*tra-2* mRNA complex. This work identifies a novel RNA based mechanism for controlling PolII transcriptional regulatory activity and cell fate determination.

Graves. et al. (1999) *Nature*. (399), 802-805.

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76. Regulation of *fem-3* mRNA by the *mog* genes and MEP-1 for sex determination in the *C. elegans* hermaphrodite germ line.

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The hermaphroditic nematode *Caenorhabditis elegans* produces both sperm and oocytes from a single pool of germ cell precursors. While the first germ cells differentiate into sperm in the larva, adult hermaphrodites produce only oocytes. This fundamental switch of germ cell fate is dependent on the repression of the *fem-3* mRNA through a regulatory element in its 3'untranslated region (UTR). Several genes that are required for the *in vivo* repression of *fem-3* expression have been identified. Previous studies have shown that *fbf* (***f****em-3* **b**inding **f**actor) encodes a protein which represses *fem-3* by binding its 3'UTR, and that *mog-1*, *-4* and *-5*, which represent the *C. elegans* orthologs of the yeast splicing factors PRP16, PRP2 and PRP22 respectively, are also required for the sperm/oocyte switch. We have shown that MOG-1, MOG-4 and MOG-5 do not bind to each other, nor to other *fem-3* regulators such as FBF and NOS-3. In addition, the three MOG proteins do not bind to the *fem-3* 3'UTR. Using yeast two-hybrid screens, we have identified MEP-1 (**M**OG interacting and **E**ctopic **P**-granules), an RNA-binding zinc finger protein that binds to each of the three MOG proteins. A *mep-1* deletion allele causes a phenotype that is very similar to that obtained by RNA interference: the strongest Mep-1 mutant phenotype is early larval arrest; however *mep-1* mutants from heterozygous mothers do reach adulthood and are sterile in that they are defective in oogenesis and in somatic gonadal arm elongation. We also noticed that a *mep-1* mutant produces less germ cells than a wild type animal. A *mep-1::GFP* reporter transgene and MEP-1 antibodies indicate that, similarly to the three MOG proteins, MEP-1 is localized to the nucleus. In addition, like the *mog* genes, *mep-1* is required *in vivo* to negatively regulate the expression of a reporter transgene that carries the *fem-3* 3'UTR. These data suggest that MEP-1 might correspond to a new factor involved in the sperm/oocyte switch and that it might work along with the MOG proteins in a nuclear complex that post-transcriptionally regulates *fem-3* via its 3'UTR.

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77. Rapid Evolution of the Sequence and Function of *fem-3*

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fem-3 plays a central role in both somatic and germline sex determination in *C. elegans*, and its careful translational regulation is thought to be a key feature of hermaphrodite development (reviewed by Puoti et al., 1997). FEM-3 functions via a direct antagonistic physical interaction with a cytoplasmic domain of TRA-2 (Mehra et al., 1999), but little else is known about this novel protein. We are studying sex determination genes in non-*elegans* species of *Caenorhabditis* for both structure-function and regulatory purposes, and also to gain insight into the mechanisms by which hermaphrodites evolved from gonochoristic ancestors. Using a synteny approach, we have cloned *fem-3* homologues from both *C. briggsae* and *C. remanei*, which together form a clade that is the sister taxon to *C. elegans* (Fitch et al., 1995; Rudel and Kimble, 2001).

The FEM-3-binding domain of TRA-2 is hypervariable in evolution relative to the rest of the TRA-2 (Mehra et al., 1999; Haag and Kimble, 2000), and we find that FEM-3 itself is also extremely divergent. Cr-FEM-3 consists of 439 aa and Cb-FEM-3 412 aa, compared with 388 aa for Ce-FEM-3, the differences due primarily to variation in the start codon location. The pairwise identity across the domain common to all three is 33% (Ce-FEM-3 vs. Cr-FEM-3), and 39% (Ce-FEM-3 vs. Cb-FEM-3), making FEM-3 the most rapidly evolving sex determination gene yet discovered, and therefore one of the most quickly evolving genes in the entire genome. Such rapid evolution of FEM-3 and the TRA-2 domain with which it interacts suggests three possibilities. First, many sequences may be able accomplish the same function (lack of constraint). Second, high mutation rates in the sequence of one binding partner may have been offset by selection for compensatory changes in the other to produce a constant affinity (compensatory coevolution). Third, there may have been selection for a distinct phenotype that led to a net change in the affinity of interaction (directional selection). We are attempting to distinguish between these scenarios by yeast two-hybrid and *in vitro* assays.

Unexpectedly, the role of *fem-3* in sex determination appears to have diverged among these species, as tested by *fem-3(RNAi)*. Although *fem-3* promotes male development in somatic tissues in *C. elegans* and *C. remanei*, it is apparently not required for spermatogenesis in *C. remanei*. RNAi analysis of *Cb-fem-3* is underway, as are experiments to test for conservation of the translational control of *fem-3* by FBF in both *briggsae* and *remanei*. Taken together, our results lead us to two major conclusions. First, FEM-3 and the FEM-3/TRA-2 interaction are "hotspots" of especially rapid sequence change during the evolution of sex determination, for reasons that are not yet clear. Second, the essential role in germline sex determination played by *fem-3* in *C. elegans* is not conserved in its sister species. Whether control of germline sex by *fem-3* is an ancestral or derived feature remains to be determined.

*These authors made equal contributions to this work.

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78. Hermaphrodite or female? Specification of germ cell fates during nematode evolution

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Most animal species consist of two different sexes, whose body plans and behaviors are specialized to help them find and mate with each other. Despite the importance of sexual reproduction, all aspects of this process evolve rapidly. To elucidate how these rapid change occur, we are studying the control of germ cell fate in the genus *Caenorhabditis*, which contains nematodes with two different types of reproductive systems. *C. elegans* and *C. briggsae* have male and hermaphrodite sexes, whereas *C. remanei* has male and female sexes. In particular, we want to understand why *XX* animals become self-fertile hermaphrodites in some species, but become females in closely related species.

In, *C. elegans*, both FOG-1, a Cytoplasmic Polyadenylation Element Binding (CPEB) Protein, and FOG-3 are required for germ cells to become sperm instead of oocytes. *C. elegans* also produces three other CPEB proteins, CPB-1, which is required for spermatogenesis, and CPB-2 and CPB-3, which have no known function (Luitjens *et al*. 2000). To learn how the control of sexual fate in germ cells has changed during evolution of the Caenorhabditids, we cloned the homologs of these genes from *C. briggsae* and *C. remanei*.

We find that each species has four CPEB genes, just like *C. elegans*. In each species, RNA-mediated interference (RNAi) directed against these genes caused the same germline phenotypes previously reported in *C. elegans*, with one exception. In *C. briggsae*, *cpb-1(RNAi)* also resulted in a second phenotype, in the male tail. These data suggest that the divergence and specialization of these CPEB proteins pre-dates the origin of the genus *Caenorhabditis*, but this process is still continuing.

We also find that each species has a single *fog-3* gene, and that the structures and sequences of these genes are conserved. RNAi experiments show that FOG-3 is required in each species for germ cells to become sperm rather than oocytes, just like in *C. elegans*. Furthermore, in each species, the level of *fog-3* transcripts is correlated with spermatogenesis. This observation suggests that the control of *fog-3* expression might be responsible for determining if *XX* animals become females or hermaphrodites. How might this work? Transgenic rescue experiments using chimeric constructs show that the *C. elegans* and *C. briggsae* FOG-3 proteins are functionally interchangeable, as are the promoters from all three species. Furthermore, these promoters each contain multiple TRA-1A binding sites. Thus, our results suggest that the underlying mechanisms for controlling germ cell fates are similar in each species, but that the regulation of *fog-3* expression has changed during recent evolution. Since we have shown that the *fog-1* and *fog-3* promoters are interchangeable in *C. elegans*, we propose that changes in the transcriptional regulation of this pair of genes might be responsible for the rapid evolution of germ cell fates. These changes could be caused by the modification of one of several upstream regulatory factors.

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79. Role of sperm selection in the determination of paternity

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Fertilization has always been considered a male-dominated arena, in the sense that very little importance was given to the role of the oocyte in determining which sperm would fertilize it. This was probably due to the view that the oocyte's role in this process was minimal. However, an increasing body of evidence suggests that this is not the case. That, in fact, the oocyte has a considerable impact in determining which sperm will successfully fertilize it. Despite this increased awareness of the oocyte's role in the process of paternity-determination, there has been no direct evidence for sperm selection by the oocyte. We believe that we have provided the first direct observation for sperm selection, based on our studies in the worm.

A number of mutant strains have been generated that are defective in fertilization. These mutants make sperm that are incapable of fertilizing the oocytes, despite being normal in every other respect (i.e., morphology, activation, locomotion, localization to the spermatheca and sperm-competition). Some alleles of these mutants are slightly "leaky," with much-reduced brood sizes as compared to wild type animals. Thus, observing the pattern in which fertilized and unfertilized eggs are laid in such semi-fertile mutants gives us a direct indication of the pattern in which the sperm are being utilized. By such experiments, we have confirmed that one class of the fertilization-defective mutants that we study show a pattern of sperm utilization that is consistent with the hypothesis that the oocyte can discriminate between, and selectively utilize one population of sperm in preference to another. The other class of mutants show no such sperm-selection, and exhibit a random pattern of sperm utilization. To further confirm the phenomenon of sperm selection by the oocyte, we have introduced two distinct and controlled populations of sperm into mutant hermaphrodites that make no sperm of their own. Under such conditions, we show that fertilization-capable sperm are preferentially utilized by the oocyte over fertilization-defective sperm. We will also report the results of ongoing work that seeks to understand the evolutionary advantages, if any, of evolving a mechanism for sperm selection. Finally, based on our molecular characterization of the mutants used in this study, we propose a model by which such a mechanism of sperm selection could be operating. This is the first direct observation of sperm selection at the post-copulatory, pre-zygotic stage and has important implications for our understanding of micro evolutionary mechanisms. Our observations, along with the powerful molecular and genetic tools available, lead us to believe that the mutants used in this study will prove to be valuable reagents in furthering our knowledge of the molecular players and mechanisms involved in sexual selection.

80. Haldane's Rule in *Caenorhabditis* is implemented by sexual transformation.

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Crosses between *C. briggsae* strain AF16 males and *C. remanei* strain EM464 females resulted in gender-biased broods in which all F1 adults were sterile females. This was consistent with Haldane's Rule: 'When in the F1 offspring of two different animals races one sex is absent, rare, or sterile, that sex is the heterozygous [heterogametic] sex' (Haldane, 1922). The observed gender bias in AF16::EM464 adults resulted from sexual transformation, not male-specific lethality. This was determined using a single-worm PCR assay to detect the C. briggsae homolog of the X-linked *unc-18* gene (*Cb_unc-18*) in F1 adults. *Cb_unc-18* was detected in approximately half of F1 adult hybrids consistent with a karyotypic XX:XO ratio of 1:1. *C. briggsae* and *C. remanei* variants have been identified that suppress this hybrid sexual transformation phenotype. In crosses between *C. briggsae* strain HK104 males and *C. remanei* strain EM464 females adult male and female hybrids were obtained. In these hybrids, a strong correlation was observed between karyotype and gender. Similar results were obtained for crosses between *C. briggsae* strain AF16 males and *C. remanei* strain PB228 females and for crosses between *C. briggsae* strain HK104 males and *C. remanei* strain PB228 females. The effects of the HK104 and PB228 variants on the suppression of sexual transformation of XO hybrids was cumulative. In hybrid males obtained from crosses employing HK104 or PB228, the copulatory bursa and sensory rays were absent or greatly attenuated and the spicules were short and/or crumpled. In hybrid males obtained from crosses employing HK104 and PB228, the fan and rays were slightly attenuated and the spicules usually were well formed. These results implicate defects in sex determination as a reproductive isolating mechanism in Caenorhabditis. Genetic studies of the HK104 and PB228 variants will be used to identify the genes that implement this reproductive barrier.

81. *mab-23*: a *doublesex/mab-3*-related gene required for male-specific differentiation and behavior in *C. elegans*

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The sex determination pathway is comprised of global regulators required for the differentiation of all sexually dimorphic features (such as *tra-1*) and downstream transcription factors that regulate sex-specific differentiation of specific tissues. Examples of this second class of genes are *doublesex, fruitless* and *dissatisfaction* of *Drosophila* and *mab-3* in *C. elegans*. Our interest is in the genetic pathways that regulate the differentiation of male rays, sex-specific sensory organs used to sense the hermaphrodite during mating. In a forward screen for genes that control specification of neurotransmitter phenotype in ray neurons we have identified a second downstream transcription factor gene of the sex determination pathway, *mab-23*.

mab-23 is required to ensure the appropriate restriction of dopaminergic fate to three of the nine ray pairs. Dopamine is made by the A-type neurons of rays 5, 7, and 9, and serotonin by the B-type neurons of rays 1, 3 and 9. This stereotyped assignment of neurotransmitter identities among the rays is dependent on a TGF-beta (DBL-1) signal and, for rays 3 to 5, on the activity of the Abdominal B-like HOX transcription factor EGL-5. In *mab-23* mutants, the A-type neurons of rays 1, 2, 3 and 6 ectopically express dopaminergic fate. This ectopic expression does not depend on DBL-1, but for rays 3 to 5, does depend on EGL-5. Our data suggest that in wild type, *mab-23* functions to repress expression of dopaminergic fate in rays 1 to 6, thereby rendering them dependent on DBL-1 for dopaminergic fate induction. As a result of prepatterned differences in competence between the rays and their differential exposure to DBL-1 ligand, the DBL-1 pathway acts preferentially in ray 5 to alleviate this repression, allowing dopaminergic fate to be expressed.

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mab-23 mutations have several additional effects in the male, but no effect in the hermaphrodite. *mab-23*mutant males exhibit variable swelling of the dorsal tail hypodermis (Hodgkin & Doniach (1997) Genetics 146: 149-164). They are copulation defective, showing defects in contact response, a behavior mediated in part by the rays (which are morphologically wild type in *mab-23* males). They are also defective in turning and serotonin-induced tail curling, both of which are mediated by male sex muscles. Consistent with these defects, we find that *mab-23* reporters are expressed in a variety of male-specific tissues including the male rays, sex muscles, neurons of the ventral chord and preanal ganglia. In contrast, hermaphrodite expression is confined to a few non- sex-specific tissues that also express in the male, namely ventral body wall muscle and a few neurons of the head and tail.

We have cloned *mab-23* and found that it encodes a putative DM domain transcription factor related to MAB-3, *Drosophila* Doublesex and vertebrate testis-specific factor DMRT1, three phylogenetically conserved regulators of sexual differentiation. DM domain transcription factors constitute a novel class of Zn-chelating, DNA binding proteins. The two *mab-23* mutant alleles contain sequence changes predicted to disrupt DM domain function.

The structural and functional similarity of MAB-23 and MAB-3 raises the question of their functional relationship to one another and to other determinants of male differentiation. *mab-23* and *mab-3* mutant phenotypes affect partially overlapping sets of tissues. The primary defects in *mab-3* males are the presence of a tail spike, the production of yolk protein in the gut and missing rays. By contrast, *mab-23* males display none of these phenotypes but have behavioral defects that suggest aberrant patterning of multiple tissues of the male posterior. Specification of rays requires both *mab-3* and *mab-23*. Both genes are expressed in the ray lineages from the Rn cell stage through into the adult. *mab-3* is required primarily for the generation of ray cells while *mab-23* effects neurotransmitter patterning and axon path-finding in ray neurons. We find that expression of *mab-23* in the rays is not dependent on *mab-3*, and thus we suggest that these two genes act in parallel and are coordinately regulated to promote male-specific differentiation. In hermaphrodite tissues, *mab-3* expression is repressed directly by TRA-1A and we have found potential TRA-1A binding sites in the *mab-23* promoter that are conserved in *C. briggsae*. Our current efforts are directed at testing the functional relevance of these sites.

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82. A cyclic GMP-dependent protein kinase controls body size and life span in C. elegans

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Body size is an important characteristic of an organism. However, mechanisms of body size determination are mostly unknown. As a basis to study the mechanisms in C. elegans, we developed a system to measure body length and diameters, and to estimate volume, of a worm by an automatic image analysis. Also, we isolated more than 20 independent mutants with a larger body volume, including one which had been isolated previously by Ikue Mori. Four of them, which have up to twice as large volume as the wild type, were analyzed. They are grossly normal in morphology and growth, except for weak egg-laying defects(Egl), dark intestine(Din) and abnormal male tail structures. They continue to grow in adults to be approximately twice as big four days after becoming adults. The number of intestinal nuclei in the mutants is close to that of the wild type, suggesting that intestinal cells are bigger. The four mutants showed elongated life span by about 50%.

One of the mutations(ks16) was mapped using SNPs to a 50kb region on chromosome IV, and near to egl-4. Complementation tests showed that the four mutations are allelic with one another and to egl-4(n477). The region to which ks16 mapped contains a single candidate gene F55A8.2 that is predicted to encode a cGMP dependent-protein kinase(PKG) of 780 amino acids. The phenotypes of ks16 was rescued by a YAC Y39C2 carrying F55A8.2 and by a cDNA construct for the PKG. We found nonsense mutations in the PKG coding sequences of three mutants among the four. The PKG carries a predicted cyclic nucleotide binding and kinase domains with(PKGa) or without(PKGb) an N-terminal glycine-rich region. These results indicate that the PKG encoded by egl-4 controls body size and life span, in addition to sensory behaviors, dauer formation and egg-laying reported by Daniels et al., Genetics 156, 123-141, 2000). Genes are found in other animals that are highly homologous in the kinase domains. Knock out mice for such a gene(cGKII) were reported to be dwarfs(Pfeifer etal., 1996). GFP is expressed in head neurons and hypodermis under the control of PKGa promoter, and in body wall muscles under the control of PKGb promoter. We have produced polyclonal antibodies that specifically detect an 80kDa protein in a C. elegans extract to characterize the PKG protein. We are trying to examine the shape and volume of major organs and cellular DNA contents to determine their changes in the mutants. We also plan to analyze the other big mutants. One of our goals is to make the worm up to 10 times as big by two or more mutations and/or transgenes.

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83. Neural regulation of body size through sensory signaling and a cGMP-dependent protein kinase

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In *C. elegans*, body size may be regulated by the nervous system. Lewis and Hodgkin initially reported that cilium-defective mutants such as *che-2* and *che-3* are smaller than wild-type animals. A smaller body size is also observed in other cilium-defective mutants and in the *tax-4* mutant (*tax-4* encodes a cGMP-gated channel that is necessary for chemosensation). These observations suggest that if an animal cannot sense an environmental cue such as food, body size may be reduced through altered neural activity. Such regulation may be useful if a smaller body is economical.

In order to analyze the putative neural regulation of body size, we isolated suppressor mutants of the *che-2* small body size phenotype (*chb*). 15,000 haploid genomes were screened, yielding 28 candidates. None of these mutants suppresses the dye-filling defect of *che-2*. Some of these suppressors show the same body size with or without the *che-2* mutation in the background, suggesting that they have possible defects downstream of sensory signals. In this class (9 of 28), *chb-1*, *chb-2*, *chb-3* and *chb-4* have been mapped to chromosomes IV, II, I and V respectively, using the snipSNP method (thanks to Wicks and Plasterk).

In the course of mapping of *chb-1*, we found that *chb-1* is allelic with *odr-9/egl-4* by complementation testing. The phenotype of *odr-9/egl-4* is well characterized and reported (S. Daniels *et al.* 2000). *chb-1* shares with *odr-9/egl-4* all of these phenotypes including a chemotaxis defect, an egg-laying defect, dauer formation at 27 C, and aldicarb-resistance. We identified the *chb-1* gene by rescuing with cosmids and cDNA as well as by mutation site analysis. This gene encodes a cGMP-dependent protein kinase.

There are at least 2 different promoter regions for this gene (a and b). We have found that the a. promoter region can direct expression in 10 pairs of head neurons, and the b. promoter region can direct the expression in body wall muscle. We found that the cDNA driven by the a. promoter is sufficient to rescue the suppressor phenotype of *che-2* small body size and all other known phenotypes, and that the cDNA driven by the b. promoter has no rescuing ability. The fact that a cDNA driven by a panneuronal promoter, H20 (thanks to Ishihara and Katsura), can also rescue all phenotypes supports a model in which this gene acts in the nervous system. Interestingly, the a. promoter does not direct expression in chemosensory neurons such as AWA, AWC, and ASE. Although we cannot exclude the possibility of a low level of expression in these neurons, this may suggest that the role of this gene is in the interneurons downstream of the sensory neurons required for chemotaxis

In conclusion, we propose a model in which the body size of worms is regulated through sensory signaling, and a cGMP-dependent protein kinase is involved in the downstream processing of this sensory information.

84. IDENTIFICATION OF AMP KINASE AS A MODIFIER OF INSULIN RECEPTOR SIGNALING *IN VIVO* IN *C. elegans* and *Drosophila*

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We are using model organism genetics to identify new genes in the Insulin Receptor (InR) signaling pathway. Such genes might encode targets for the development of novel therapeutics for the treatment of Type 2 Diabetes. Both *C. elegans* and *Drosophila* have homologs of the human insulin receptor, and genetic analyses in these organisms have demonstrated that the regulation of downstream signaling components also is conserved. We have taken several genetic approaches to identify new components of the InR signaling pathway, using InR mutants in *C. elegans* and *Drosophila* as entry points. In *C. elegans* , we performed large-scale RNAi of selected gene classes, including kinases, phosphatases, and proteases, and screened for suppressors of the dauer constitutive phenotype of *daf-2* (InR) mutants. To facilitate detection of weak modifiers of *daf-2* signaling, we screened for suppression in *daf-2* backgrounds that display more moderate mutant phenotypes than the canonical *e1370* allele used in the majority of previous screens. In parallel, a reverse genetics screen was done in *Drosophila* , using transposon insertion lines to look for modifiers of InR mutant phenotypes. Strikingly, subunits of the metabolic sensor AMP kinase were identified as one of the modifiers of InR signaling in both screens. AMP kinase is a key modulator of metabolic flux in mammalian cells, regulating pathways involved in fatty acid and carbohydrate metabolism. Furthermore, AMP kinase activity stimulates glucose transport in an insulin-independent manner. The identification of AMP kinase as a modifier of insulin signaling in *C. elegans* and *Drosophila* indicates that this interaction is conserved, and will allow genetic dissection of the mechanism of this interaction.

85. *sma-11* encodes an EMK family serine threonine kinase that modulates TGF-beta signaling

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Members of the Transforming Growth Factor beta (TGF-beta) superfamily affect many processes including cellular proliferation, differentiation, development and death. Mutations in several pathway components in humans result in various cancers including colon, pancreatic and breast carcinomas, as well as cause defects in bone and heart formation. In order to better understand the effects of TGF-beta on these processes, we are studying the signaling mechanisms of the Sma/Mab TGF-beta-like pathway in *C. elegans*. The Sma/Mab pathway is comprised of the ligand, *dbl-1*, two receptor serine/threonine kinases, *sma-6* and *daf-4*, and the downstream effector Smads, *sma-2*, *-3*, and *-4*. Mutations in any of these components result in animals that are small in body size (Sma) with defects in male tail (Mab) morphogenesis. We have performed several screens based on body size to isolate novel Sma/Mab pathway components. Of the many small mutants recovered from our screens, we have focused on *sma-11* (see abstract by Sengupta lab on *sns-8,* which is allelic to *sma-11*).

Although much of the general TGF-beta signaling cascade has been revealed, many issues still remain unresolved. We are particularly interested in understanding how Smad activity is regulated. We have found that *sma-11* encodes a serine threonine kinase with homology to the EMK (ELKL motif kinase) family of kinases. These kinases have been shown to phosphorylate microtubule associated proteins, thereby affecting microtubule (MT) stability, and are essential for cell and embryonic polarity (Drewes 1997). Recently, it has been demonstrated that mammalian Smads are able to physically interact with beta tubulin (Dong 2000), suggesting that microtubules might play a role in retaining Smads in the cytoplasm. The molecules involved in

regulating the interaction of Smads with MTs are unknown, and no genetic evidence currently exists to gauge the importance of this interaction to TGF-beta signaling. We have performed immunoprecipitation experiments in *Drosophila* S2 cells and find that SMA-2 binds beta tubulin. Our data suggest that this interaction is lost when the TGF-beta pathway is stimulated. We hypothesize that the SMA-11 kinase may be the trigger that allows the Smads to dissociate from MTs thereby allowing them to transmit a robust TGF-beta signal. We are currently testing this model to determine whether SMA-11 stimulates the release of SMA-2 from the microtubules. Given the strong mutant phenotype of *sma-11*, it is likely that association and release of Smads from MTs may be a fundamental requirement for TGF-beta signaling. Therefore, we are further investigating the role *sma-11* plays in regulating Smad activity and TGF-beta signaling.

86. *daf-5*, a gene involved in dauer formation and adult longevity, is related to the Sno oncogene family

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We have identified *daf-5* as W01G7.1, a predicted gene related to the Sno family of oncogenes. Loss-of-function mutations in *daf-5* result in a dauer-defective (Daf-d) phenotype. They have been shown to suppress dauer-constitutive (Daf-c) mutations in the TGF-beta signaling branch of the dauer pathway, and to enhance the life span extension of *daf-2* (insulin receptor) mutants. Genetic epistasis tests have placed *daf-5* downstream of the *daf-1* type I and *daf-4* type II TGF-beta receptors and at the same step as the Daf-d gene, *daf-3*, which encodes a SMAD transcription factor (Patterson et al., Genes & Development 11: 2679-2690, 1997). This extends the parallel between dauer signaling and mammalian TGF-beta signalling, in which the SnoN oncoprotein has been shown to interact directly with SMAD transcription factors (Stroschein et al, Science 286: 771-774, 1999).

daf-5 was physically mapped using DNA dimorphisms between the strains N2 and RC301 to refine its position prior to DNA transformation experiments. The cosmid W01G7 was found to rescue *daf-5*, using the elimination of suppression of a *daf-7* Daf-c mutation as the assay for *daf-5(+)* activity. W01G7.1 RNAi treatment of *daf-7* resulted in strong suppression of the Daf-c phenotype, indicating that this candidate gene corresponds to *daf-5*. We then sequenced several alleles of *daf-5*, finding alterations that result in premature stops or amino acid substitutions. Southern analysis of a putative Tc1-insertion allele (*m512*) using a cDNA probe demonstrated that the entire locus is deleted, and that *m512* is a null. It exhibits the same phenotype as the other Daf-d alleles. The mutation resulted from excision of a resident Tc1 element adjacent to *daf-5* in the parent mutator strain. PCR analysis

of mutant genomic DNA showed that the genes flanking *daf-5* are intact. The cDNA was sequenced in its entirety, confirming all predicted splice sites, and RT-PCR experiments indicate that *daf-5* is trans-spliced to SL1. GFP reporter studies showed that the *daf-5* promoter is active in neurons in the head, consistent with the expression patterns of the *daf-1* and *daf-4* receptors and the *daf-3* SMAD.

daf-3 and *daf-5* mutants have no longevity phenotype on their own, but they enhance the life span of *daf-2* mutants, showing that neural TGF-beta signaling plays a minor role in determining adult life span that is only revealed in a *daf-2* background.

87. *daf-5* encodes a partner of the *daf-3* transcription factor in a TGF-beta signaling pathway

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The reproductive/dauer developmental decision is controlled by environmental cues, such as food, pheromone and temperature. A TGF-beta-related pathway promotes reproductive over dauer development. Mutations in *daf-7* (a TGF-beta-like ligand), *daf-1* and *daf-4* (type I and type II receptor kinases), and *daf-8* and *daf-14* (Smad transcription factors) induce dauer at restrictive temperature regardless of environmental cues. The Daf-c phenotype of these mutants is suppressed by mutations in *daf-3* (a Smad) or *daf-5*. Epistasis analysis suggests that *daf-5* and *daf-3* are either antagonized by this TGF-beta-related pathway or acting in a parallel pathway.

Our cloning of *daf-5* showed that *daf-5* encodes a novel protein with weak similarity to chromatin remodeling proteins such as Sno/Ski and ACF-1. Vertebrate Sno has been recently shown to be capable of acting as an inhibitor of TGF-beta pathways by interacting with Smad proteins. However, because of the low similarity, the functional relationship of DAF-5 to Sno/Ski is uncertain. We searched for a better Sno/Ski homolog in *C. elegans* and found nothing; however, we found convincing Sno/Ski homologs in the nematodes *Brugia malayi* and *Globodera rostochiensis*, and in *Drosophila*. Thus, either one group of nematodes has lost Sno/Ski, or *daf-5* is a Sno/Ski that is so highly diverged as to be almost unrecognizable. The genetic function of *daf-5* in dauer formation is different from that suggested for Sno/Ski by the vertebrate tissue culture studies in that *daf-5* is not simply acting to block signaling by the receptors.

We sequenced twenty *daf-5* alleles and identified four missense mutations, one inframe deletion, seven nonsense mutations, one frame-shift, and two splice site mutations. Three alleles had no mutation in the coding region. Five out of fifteen alleles affected amino acid residues 158-173 (of a 627 amino acid residue

protein). This hotspot is in a region that shows no significant homology to other proteins.

We examined *daf-5* expression with *daf-5*::*GFP* constructs. One contains 6.5Kb of upstream sequence and an intact *daf-5* coding region, which has GFP inserted in the first exon. This construct rescued *daf-5*. The other construct has GFP fused to the C-terminal end of the first exon of *daf-5* with a longer (13Kb) upstream region. The rescuing construct shows a relatively strong expression in ganglia in the head and tail and in the anterior pharynx. A small number of animals show weak expressions in the hypodermis, muscles, intestine, and distal tip cells. The non-rescuing GFP construct is more strongly expressed, and shows more consistent expression in the hypodermis, muscles, intestine, and distal tip cells; still, its expression is strongest in the head and tail ganglia. This observation is consistent with expression and functional data from the Riddle and Thomas labs that indicate that the TGF-beta receptors function in the nervous system. We are testing whether *daf*-5 functions in the nervous system by expressing*daf-5* with nervous-system-specific and other promoters to see where *daf-5* must be expressed to rescue a *daf-5* mutant.

daf-5 and *daf-3* have been shown to interact with each other in the yeast-two-hybrid system (see abstract by Hu, Tewari, Ruvkun and Vidal). We are going to perform immuno-precipitation from extracts of *C. elegans* to see if *daf-5* and *daf-3* physically interact during different developmental stages and to determine if the interaction is controlled by TGF-beta signaling.

88. *dda-1*: a Novel A-domain Containing Cuticulin-like Gene in *Caenorhabditis elegans*, Important for Dauer Body Shape.

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A-domains are 200-amino acids modules identified in a range of mammalian extracellular matrix proteins. Our previous studies on A-domains of human integrin a subunit show that they are responsible for collagen binding, and also for the interaction of these integrins with other ECM proteins such as laminin. Studies of a number of other human A-domain-containing proteins indicate a collagen or ECM-binding function.

The cuticle of *C. elegans* is an extracellular matrix that covers the whole worm forming the exoskeleton. This determines and maintains the shape of the animal, is important for motility, and acts as a protective barrier between the animal and its environment. The cuticle consists predominantly of small collagen-like proteins extensively cross-linked. At least 6 different layers can be clearly distinguished using electron microscopy. The outermost face remains insoluble when treated with reducing agents, due to the presence of non-collagenous proteins called cuticulins.

The high degree of organisation within the cuticle may require the presence of ECM organisers. Equivalent roles in mammals are played by A-domain proteins, and the *C. elegans* A-domain proteins are potentially responsible for organising cuticle components.

We report the characterisation of *dda-1*; a modular gene containing an A-domain, a cuticulin domain and a transmembrane region. Using promoter fusion to a GFP reporter gene we have detected stage specific expression restricted to three folded embryo and L2d

predauer. When this gene was knocked out by RNA interference, we observed a strong dumpy phenotype in dauers, accompanied by partial or total absence of the alae. Two ribbons all along and underneath the lateral edges of the alae at L1 and dauer stages, were detected by immunofluorescence, using a polyclonal anti DDA-1 antibody.

We establish in our work that *dda-1* (*d*umpy *d*auer 1) is not only the first gene which, when disrupted causes a dumpy phenotype in dauers, but is also the first candidate gene for a cuticle organiser in *C. elegans*.

89. Alae formation and the role of CUT-1-like proteins

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The most visible specialized structures of *C.elegans* are the alae, lateral simmetric longitudinal extroflessions of the cuticle that run the entire length of the animal. They are present only in L1s, dauers and adults and their morphology is different and characteristic for each stage. We would like to understand how alae are generated, how their form is attained and which genes are responsible for their stage specific forms. We began by studying the role in this process of *cut-1* (C47G2.1). *cut-1* is expressed during dauer larvae cuticle formation and the protein is present in the dauer cuticle in a highly cross-linked, insoluble form. Ultrastructural immuno-detection indicates that CUT-1 is localized in a narrow ribbon underneath the dauer alae. We have used RNA interference to reduce the expression of *cut-1* and study what happens to the alae. The main defect we have observed under the stereoscope, is that dauer larvae appear, somewhat dumpish. Under higher magnification they show a larger diameter, completely lack the alae and appear to not undergo radial shrinking. EM confirms that the alae are missing and indicates that the only sections of the circumference that are enlarged are those corresponding to where the alae usually form.

Analysis of the *C.elegans* genomic sequence has revealed a family of 30-32 genes encoding for proteins that share homology with CUT-1. They contain entirely or in part a 260 residues domain which we had named CUT-H (for CUT-1 Homology) and which appears to be related to the ZP domain present in a number of vertebrate proteins. Two members of this family, F22B5.3 and R07E3.3, present a higher degree of homology to *cut-1* . We have named them *cut-3* and *cut-5* respectively and have investigated their role in an attempt to identify the genes that might serve, in the formation of the alae of L1s and adults, the function that *cut-1* exerts in the formation of the alae of dauers. *cut-3* is

expressed only in late embryonic stages and RNA interference of this gene results in dumpish L1s which completely lack alae. No effect on the shape of the body or of the alae of dauers and of adults was observed. *cut-5* is expressed in late embryogenesis and during dauer formation. Unlike CUT-1 and CUT-3, CUT-5 participates to the formation of alae in both L1 and dauers. RNA interference with *cut-5* results in L1s and dauers with abnormal and discontinous alae. In *cut-5(RNAi)* worms the correlation between presence of the alae and body diameter (shrinking) is particularly evident because can be observed in different sections along the length of the same animal. Again no effect on adult alae was observed suggesting that these may be formed by a different mechanism. Expression of CUT-3 under the *cut-1* promoter shows that CUT-3 can partially rescue the lack of alae and of radial shrinking in *cut-1(RNAi)* worms. The resulting alae have a dauer-like rather than an L1-like shape indicating that the stage specific morphology of the alae depends on the developmental stage rather than on the properties of single stage specific cuticlin proteins. At present our working hypothesis is that in L1 and dauers alae are formed by a mechanism in which the internal layers of the dorsal and ventral halves of the cuticle are pulled together along the lateral lines. The alae would result from a sort of wrinkling of the more external layers of the cuticle on the more internal ones. CUT-1 CUT-3 and CUT-5 would participate in this mechanism by acting as substrates for a cross-linking activity that generates the pulling force.

90. END-2, an apparent nuclear hormone receptor, relocalizes from the cytoplasm to the plasma membrane in response to environmental conditions

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end-2 encodes a putative nuclear hormone receptor (NHR) that, based on gene reporter studies, is expressed in the endoderm throughout the life of the worm. NHR family members are transcriptional regulators that are activated when bound to their small lipophilic ligands such as steroids. While some NHRs are localized to the nucleus, others are cytoplasmic in the absence of ligand and translocate to the nucleus upon ligand binding. Once in the nucleus, they bind target sequences and regulate gene expression.

We have found that while END-2 is present in the nucleus during early development, unexpectedly, it appears to localize to the plasma membrane under certain conditions later in development. END-2 antibody reveals nuclear expression of the protein in posterior cells of early embryos (pre-bean stage), consistent with RNA *in situ* analysis. However, by the bean stage, END-2 antigen relocalizes to the plasma membranes of developing gut cells. This membrane association continues through to hatching. Our preliminary biochemical results further indicate that END-2 partially associates with the membrane fraction obtained from both early and late embryos based on western blot analysis of embryonic extracts. We are biochemically investigating how END-2 associates with this membrane fraction.

We found that in larvae, END-2 antigen is either primarily cytoplasmic (and nuclear-excluded) or is localized to the membrane, depending on the preparation examined. The variable localization of END-2 to the cytoplasm or membrane in larvae is attributable to variations in environmental conditions. When worms are grown in uncrowded conditions, END-2 is present throughout the cytoplasm of gut cells.

However, when they are cultured at high density, the protein appears to partition to the membrane. The effect of crowding on END-2 localization may be a response to dauer pheromone, a hypothesis that we are currently testing.

This intriguing localization pattern of END-2 is atypical for NHRs and suggests a possible novel mode of action. One postulate is that membrane-associated END-2 transduces a signal from the membrane. Such a possibility is consistent with the evidence that a number of steroids can transduce a signal that is independent of transcription, and in some cases the ligands can act without entering the cell. Alternatively, the non-membrane localized form of END-2 may function like a typical NHR and localization to the membrane may be a mechanism for sequestering END-2, rendering it inactive. We are performing genetic and biochemical studies to investigate these alternatives.

91. Ventral nerve cord axons require the PVT neuron and zig genes to maintain their correct positioning in axonal tracts

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While navigation of an axon to its target destination has been intensively studied in several different organisms, it has never been addressed how it is ensured that axons physically remain within defined axonal tracts throughout the life of the animal. One would intuitively assume that the same molecules and environmental cues that guide an axon to its destination will continue to assure the correct positioning of an axon throughout its life and/or that there are simple physical constraints that prevent axons from altering their position within an axonal tract. Quite unexpectedly, we have uncovered that axons are kept in their correct position through a novel maintenance mechanism. We describe here the cellular and genetic basis for this maintenance mechanism.

Our genome-sequence based expression pattern analysis of Ig-domain containing proteins revealed a novel family of eight secreted 2-Ig domain proteins, encoded by the zig genes that are expressed in several domains within the nervous system and other tissues (Aurelio et al., IWM 1999). We describe here that all of the six neuronally expressed zig genes are expressed in the PVT guidepost neuron that sends an axon from its cell body in the tail to the nerve ring. PVT is a source of UNC-6 and has been shown to be required embryonically for attraction of tail axons into the ventral nerve cord (VNC)(Wadsworth et al., 1996; Ren et al., 1999). Surprisingly, we found that the expression of three of the six PVT-expressed zig genes (zig-2, zig-4 and zig-8) is turned on only after the animals have hatched and thus long after the VNC axons have established their tracts in mid-embryogenesis. This observation prompted us to laser-ablate PVT postembryonically in L1 larvae. Using a sra-6::gfp marker to visualize the left and right VNC , we found that adult animals that had PVT killed at the L1 stage fail to maintain a correctly separated left and right VNC; instead axons cross from the left to the right tract and

vice versa. Since axons are affected that have already established their tracts from the tail ganglia to the nerve ring during embryogenesis, this observation suggests that PVT is required postembryonically to maintain the correct separation of axonal tracts. It is also notable that by removing PVT, we have not removed a purely architectural barrier that physically separate the left from the right VNC (this barrier is constituted by a hypodermal ridge), but merely a cell that is located at the end of the VNC which sends an axon along the right VNC.

This effect of PVT is presumably mediated by the postembryonically expressed zig genes. A zig-4 null mutant, kindly provided by the knock-out consortium, displays a similar, yet less penetrant axon crossover phenotype. This defect can be rescued by driving zig-4 behind its own promoter, which is within the VNC exclusively expressed in PVT . The lower penetrance of the defect as compared to the PVT laser ablation suggests that the other zig genes participate in the PVT-mediated maintenance mechanisms. We will describe several models that could explain how secreted ZIG-4 protein could ensure the correct maintenance of left and right VNC structure.

Given the importance of postembryonic zig gene expression, we asked how their onset of expression is regulated. We found that the expression of the postembryonically expressed zig genes zig-2, zig-4 and zig-8 (but not the embryonically expressed zig genes zig-1, zig-3 and zig-5) depends on the combinatorial activity of the lim-6 and ceh-14 LIM homeobox genes in the PVT neuron.

Our findings illustrate two novel and unexpected aspects of axonal patterning: 1) The existence of an active maintenance mechanisms for axon positioning, mediated by the PVT neuron. 2) The dedication of a new gene family for this maintenance mechanism.

References: Wadsworth et al. , 1996, Neuron 16(1):35-46; Ren et al., 1999, J Neurobiol. 39(1):107-18.

92. *sax-1* and *sax-2* act in parallel with *unc-34* to inhibit neurite outgrowth in the adult worm.

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Neurite outgrowth is a precisely regulated process. Furthermore, evidence suggests that the growth potential of neurons decreases with age. Both cell extrinsic and cell intrinsic factors have been implicated in this decline. We are interested in factors that prevent aberrant neurite outgrowth in the adult. These negative-acting factors could play a role in the potential of axons to regenerate following injury in humans.

The initial outgrowth and guidance of axons are normal in *sax-1* and *sax-2* mutants, but at later larval stage and in the adult, *sax-1* and *sax-2* mutants have neurons with enlarged, irregular shaped cell bodies and ectopic neurites. Similar phenotypes have been seen in mutants that disrupt neuronal activity and function, but neuronal function is apparently normal in *sax-1* and *sax-2* mutants. This observation suggests that *sax-1* and *sax-2* may act more directly with the actin cytoskeleton to regulate cell shape and inhibit ectopic neurite outgrowth.

To ask if repulsive axon guidance factors also play a role in regulating neurite outgrowth in the adult, we have begun to make double mutants of *sax-1* and *sax-2* with various axon guidance mutants including *unc-34*, the worm homolog of Enabled. Evidence suggests that Enabled mediates repulsive guidance through the *sax-3*/*robo* guidance receptor. Indeed, *unc-34; sax-1* and *unc-34; sax-2* double mutants have a strongly enhanced ectopic neurite outgrowth defect in late larval stages and the adult. This result suggests that *sax-1* and *sax-2* may act in redundant parallel pathways with *unc-34* to inhibit ectopic neurite outgrowth at late larval stages. Alternatively, *sax-1* and *sax-2* may create a permissive environment for axon guidance pathways to respond to cues present in the adult animal.

sax-1 encodes a serine/threonine kinase related to the Ndr protein kinase family in humans (62% id.) and flies (60 % id.). The function of Ndr kinase in neurons is unknown, but the Dm-NDR kinase and other closely related kinases have been shown to affect cell shape and polarity in nonneuronal cells. Since double mutant analysis places *sax-1* and *sax-2* in the same genetic pathway, I am in the process of cloning *sax-2* in order to identify other components of this kinase pathway. I now have rescue with a small pool of cosmids. Progress in cloning and additional phenotypic characterization will be presented.

93. Dual roles of UNC-18 in synaptic transmission.

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Neurotransmitter is released from presynaptic nerve terminals by the fusion of synaptic vesicles with the plasma membrane. Synaptic vesicle exocytosis requires the formation of SNARE complexes, comprised of synaptobrevin, SNAP-25 and syntaxin. In solution, syntaxin adopts a closed configuration that is incompatible with SNARE complex formation. UNC-18, a neuronal protein implicated in vesicle exocytosis, binds to syntaxin in the closed configuration. Loss-of-function mutations in *unc-18* nearly eliminate vesicle exocytosis. A model based on these data proposed that UNC-18 bound closed syntaxin, promoted the opening of syntaxin, and thereby promoted the formation of the SNARE complex and hence exocytosis. A prediction of this model is that if one could eliminate the syntaxin - UNC-18 interaction then neurotransmission would be blocked. To test this model we engineered mutations into syntaxin that reduce UNC-18 binding, and mutations into UNC-18 that reduce syntaxin binding. We have tested the consequence of these mutations on synaptic vesicle exocytosis.

Scanning alanine replacement experiments of the mammalian syntaxin homolog identified two residues, L165/E166, that when mutated to alanine render syntaxin incapable of binding UNC-18. We made the corresponding mutations into *C. elegans* syntaxin, L166A/E167A. Pulldown experiments demonstrate that this mutant form of syntaxin does not bind UNC-18 from worm lysates. We expressed this mutant form of syntaxin in a null background, *unc-64(js115)*. Mutant syntaxin that does not bind UNC-18 is capable of rescuing the lethality and locomotory phenotypes associated with the syntaxin null, suggesting that the mutant protein functions *in vivo* and that UNC-18 function is not via syntaxin. In fact, such worms are hypersensitive to Aldicarb, suggesting that UNC-18 interactions with syntaxin inhibit

release.

Studies of the UNC-18 homolog in *Drosophila*, ROP, identified a mutation, R50C, that decreases the affinity of ROP for syntaxin without affecting other known UNC-18 interactions. We made the corresponding mutation in UNC-18, R39C, and expressed the protein in *unc-18(e234)* animals. Expression of R39C UNC-18 rescues the paralysis associated with *e234* suggesting the mutant protein functions *in vivo* and that the interaction of UNC-18 with syntaxin is not relevant to the UNC-18 role in facilitation of exocytosis. However, animals expressing the mutant UNC-18 are hypersensitive to the effects of Aldicarb, suggesting that more acetylcholine is released in these animals. Thus, UNC-18 binding to syntaxin appears to inhibit rather than facilitate vesicle exocytosis.

Finally, if UNC-18 is required to promote the open configuration of syntaxin for SNARE complex formation then a constitutively open form of syntaxin should bypass the requirement for UNC-18. The L165/E166 mutations which reduce UNC-18 binding also renders syntaxin in a constitutively open configuration. To test if the open form of syntaxin can bypass the requirement of UNC-18 we made doubles with *unc-18(e234)*. Expression of open syntaxin in the *unc-18* mutant fails to suppress the Unc-18 phenotype. This suggests that the facilitory role of UNC-18 is not to promote the open configuration of syntaxin for SNARE complex formation. Consistent with this result our preliminary ultrastructural analysis of *unc-18* mutant synapses suggests that the facilitory role of UNC-18 is in synaptic vesicle docking, which precedes SNARE complex formation.

Together, these data suggest that UNC-18 has dual roles in synaptic transmission: a facilitory role in vesicle docking that precedes SNARE complex formation, and an inhibitory role via the SNARE protein syntaxin. We are currently testing *in vivo* whether the inhibitory role of UNC-18 is via inhibition of SNARE complex formation.

94. Serotonin regulates repolarization of pharyngeal muscle

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Serotonin stimulates the pharynx to promote rapid pumping. We have used an electrophysiological assay to show that serotonin decreases pharyngeal action potential duration and enhances activity of the pharyngeal motor neuron M3. Gramine, a competitive serotonin antagonist, and octopamine have effects opposite those of serotonin. Gramine and octopamine decrease pharyngeal pump rate, increase action potential duration and decrease activity of the M3 motor neuron. In the presence of gramine or octopamine, action potentials can exceed one second in duration, which would limit pharyngeal pumping rates to 60 pumps per minute. In the presence of serotonin, however, the average action potential duration is shortened to 250 ms. Pumps of this length are compatible with serotonin-stimulated pumping rates we observe, which are often in excess of 200 pumps per minute. Thus, serotonergic regulation of action potential duration may be required for rapid pharyngeal pumping.

We have identified two genes, *eat-18* and *avr-15*, that are important for regulation of the action potential by serotonin, gramine and octopamine. Mutations in *avr-15* specifically disrupt M3 function[1]. Since M3 promotes repolarization of the pharynx, enhancement of M3 activity is one likely mechanism for serotonin's effect on action potential duration. EAT-18 is known to be required for function of the pharyngeal motor neuron MC, however it is unclear if this is the only pharyngeal defect in *eat-18* mutants. Serotonin, gramine and octopamine have no effect on action potential duration in *eat-18*; *avr-15* double mutants. However, *eat-18* and *avr-15* single mutants can respond to these drugs, suggesting that serotonin and octopamine regulate action potential duration by *eat-18* and M3-dependent mechanisms.

[1] Dent *et al*. (1997) EMBO J. 16:5867-79

95. Regulation of multiple male *C. elegans* spicule muscle behaviors during mating.

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During mating, *C. elegans* males monitor the activities of hermaphrodites and react appropriately to impregnate their mates. To address how males interpret and integrate mating cues into appropriate responses, we are dissecting the spicule insertion step of mating behavior since spicule movements provide a readout for experimental manipulation. Observations of mating reveal that the spicule protractor muscles display at least three contractile behaviors. As the male tries to penetrate the vulval lips, the spicule muscles twitch at a frequency of 7 Hz. Partial spicule penetration through the vulva triggers the spicule muscles to switch from oscillating contractions to a 30 sec sustained contraction. Sustained muscle contraction can be increased for an additional 30 sec by the transfer of seminal fluid. By observing the effects of ablating male tail cells, we found that the PCA, PCB, PCC, HOA, and HOB sensory neurons initiate oscillating spicule muscle contractions, whereas SPC motor neurons trigger sustained muscle contraction. Also, modulation of sustained contraction requires the connection between the somatic gonad and the cloacal opening.

We have identified components that regulate spicule insertion by analyzing chemical agents and identifying novel genetic mutations that induce spicule muscle contraction. The acetylcholine (ACh) agonists levamisole, arecoline, nicotine, and oxotremorine induce the spicules to protract. This is consistent with our observations that the PCB, PCC, and SPC neurons express the ACh vesicular transporter UNC-17, suggesting that these cells use ACh to excite the spicule muscles. Multiple ACh receptors, defined by their agonist sensitivities, utilize different calcium channel genes to induce protraction. Levamisole receptors require *unc-68*(ryanodine receptor calcium channel): arecoline receptors require *egl-19* (voltage gated calcium channel subunit); nicotine and

oxotremorine receptors require both *unc-68,* and *egl-19* to cause spicule protraction. Similar to agonist exposure, males mutant in *prc-1,-2,-3,* and *-4,* protract their spicules constitutively. These mutations require wild-type *egl-19* but not *unc-68* to induce the Prc phenotype. These *prc* genes might be involved in the regulation of extra-cellular calcium mobilization that is utilized by the arecoline-induced protraction pathway. During mating, *egl-19(lf)* and *unc-68(null)* males show distinct defects during spicule insertion. *egl-19(lf)* males produce normal oscillating spicule muscle contractions, but fail to trigger sustained contractions; whereas, *unc-68(null)* males display the opposite defect. Taken together, these observations suggest that multiple cholinergic pathways differentially regulate intra- and extra-cellular calcium to produce distinct muscle contraction behaviors during mating.

96. "HOT" WORMS, COOL GENES: MUTANTS INVOLVED IN THERMOSENSATION

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Although ambient temperature is a critical environmental cue that must be sensed by an organism, little is known about thermosensory neuron development or thermosensory signal transduction. Temperature changes can elicit dramatic behavioral and metabolic changes. In *C. elegans*, the primary thermosensory neurons are the AFD neural pair, which have specialized microvillar sensory endings that are believed to mediate thermosensation. We conducted a genetic screen designed to isolate mutants with defects in AFD-specific gene expression, and identified mutants in four complementation groups.

We have isolated two alleles of the previously identified thermotaxis mutant *ttx-1*. Mutants in *ttx-1* are defective in expression of all AFD specific genes examined, have morphological defects in the AFD sensory endings, and are cryophilic. In *ttx-1* mutants, the AFD neurons adopt an olfactory neuron-like fate.
Misexpression of *ttx-1* in other sensory cells is sufficient to convert many other sensory cells to an AFD-like fate, including expression of AFD-specific genes and elaboration of AFD-like sensory endings. In addition, our work suggests that the AFD temperature input is important in regulating dauer recovery, perhaps via temperature regulation of insulin production. (Work on *ttx-1* was done in collaboration with Hiroyuki Sasakura, Atsushi Kuhara, and Ikue Mori).

ttx-1 encodes a homeodomain protein of the *Otx/Crx* family, making *ttx-1* the first identified transcription factor that regulates thermosensory neuron differentiation specifically, in any organism. Members of the *Otx/Crx* family have previously been shown to function in the specification of visual structures in *Drosophila* and vertebrates, and *Crx* in particular, has been shown to be required for maintenance of vertebrate photoreceptor cell fate. Interestingly, an orthologous gene pair, *Chx10* and *ceh-10*, have also been shown to specify the fates of the postsynaptic partners of the photosensory (bipolar cells) and thermosensory (AIY) neurons respectively. This suggests, as proposed by Svendsen and McGhee (1995), that the photosensory and thermosensory circuits may share a common evolutionary origin.

We have identified three other genes that function to regulate AFD-specific gene expression. Two of these genes, *tax-2* and *tax-4*, encode subunits of a cyclic nucleotide-gated channel which has been shown by other researchers to function in AFD, as well as other sensory cells. The fourth class of mutants have molecular lesions in the *cmk-1* gene which encodes the worm ortholog of CaMKI, a calcium/calmodulin-dependent protein kinase. There are no published accounts of a CaMKI mutant in any multicellular eukaryote, so the characterization of *cmk-1* mutants will reveal a great deal about the specific neuronal function of CaMKI. *cmk-1* mutants are thermophilic, and have reduced expression of some, but not all, AFD-specific genes. *cmk-1* is expressed in many neurons including AFD, although the function of this gene in other cells is still unknown. These results suggest that gene expression in the AFD neurons may be regulated by an activity-dependent mechanism.

Why are *tax-2*, *tax-4* and *cmk-1* required for proper expression of AFD specific genes? Do these molecules participate directly in thermosensory signaling? Are they part of a pathway allowing the thermosensory neurons to habituate to, or learn and remember, a food-associated cultivation temperature? Do defects in these molecules cause the worm to perceive the ambient temperature as higher or lower than in fact it is? We will present our progress in testing these hypotheses, with an emphasis on elucidating the pathway by which four cool genes make worms "feel the heat".

97. A novel secretory protein, HEN-1, regulates integration of sensory signals and behavioral plasticity.

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Integration of sensory signals and neuronal plasticity are essential steps for informational processing in the nervousl system. Animals sense many environmental stimuli simultaneously, and these sensory signals are modulated depending on their experiences and environment to generate a proper response. To elucidate its molecular mechanisms, we study a *hen-1(ut236)* mutant, which was originally identified as a mutant defective in interaction of two sensory signals. *C. elegans* move toward attractive odorants and avoid aversive Cu^{2+} ion. In our paradigm, worms must cross aversive Cu^{2+} ion barrier to reach attractive odorants, and their behavior varies with the concentration of both stimuli. In this assay, *hen-1* prefers to avoid Cu^{2+} ions rather than to move toward the attractive odorants, although the dose responses to odorants and to Cu^{2+} are indistinguishable from those of wild type. This phenotype suggests that *hen-1* has defects in the integration of two sensory signals but not in the chemosensation. Positional cloning reveals that the *hen-1* gene encodes a novel secretory protein with an LDL-ligand binding motif and is expressed in ASE and AIY neurons. Immunohistochemical studies showed that HEN-1 is localized in their processes at the nerve ring and their cell bodies in wild type animals, but it is not transported to the processes and mislocalized to cell bodies in the *unc-104* mutant in which synaptic vesicles are not transported. By using cell type specific promoters, we found that the HEN-1 functions cell nonautonomously in the nervous system. In addition, the expression of the HEN-1 in embryonic stage is not sufficient for the rescue,

but expression in the late larval or adult stage is. This result suggests that it is necessary for functions in the mature neuronal circuit, but not for development of the nervous system.

The *hen-1* mutant has also defects in two types of behavioral plasticity induced by paired sensory stimuli. Wild type animals conditioned with NaCl and starvation change their behavior to avoid NaCl, but *hen-1* shows weak behavioral change after conditioning. In another paradigm, wild type animals cultivated with food migrate to the cultivated temperature, but, after starvation, they change their behavior to avoid the temperature (see abstract by Mohri et al.). The *hen-1* mutant shows normal thermotaxis in well-fed condition, but, unlike wild type animals, *hen-1* conditioned with starvation at a cultivated temperature does not change their behavior to avoid the temperature (Aho phenotype). These defects in learning can be rescued by the wild type *hen-1* transgene. Although, in these paradigms, worms are conditioned with starvation and other stimuli, we cannot find any abnormality in behavioral change induced by sole starvation. These results suggest that the HEN-1 controls neuromodulation of sensory signals and, as a result, it affects integration of these signals and learning induced by paired stimuli.

98. Retrograde regulation of motor-neuron activities by body-wall muscle and intestine through the novel C2 domain protein AEX-1

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While motor neurons regulate activities of muscles and internal organs by releasing neurotransmitters and neuropeptides, reciprocal retrograde signaling from postsynaptic cells is essential for temporal correlation between pre- and postsynaptic activities during synaptic development and synaptic-efficacy regulation. For example, the synaptic-efficacy changes at neuromuscular junctions are tightly coupled with muscle-size changes through communication between muscle and neurons. The retrograde signaling is also implicated in playing an essential role for memory formation in the central nervous system. An activity-dependent retrograde signal in *C. elegans* is also implicated in proper synaptic connectivity between SAB motor neurons and head muscle. However, the mechanism of retrograde signaling is largely unknown. Here we report that muscle and intestine in *C. elegans* regulate neural activities through the novel C2 domain protein AEX-1.

aex-1 mutants were previously isolated based on defects in defecation, a GABAergic-neuron controlled behavior. By further characterizing *aex-1* phenotypes, we found that *aex-1* mutants were resistant to the acetylcholinesterase inhibitor aldicarb: aldicarb resistance well correlates with defects in synaptic vesicle release. These phenotypes in *aex-1* mutants are similar to those identified previously in mutants of presynaptic genes, such as *aex-3* (Rab3 GDP/GTP exchange factor), *cab-1* (AEX-3 binding protein) and *snt-1* (synaptotagmin). We also observed that *aex-1* mutations do not cause dramatic morphological changes in the nervous system. All of these findings suggest that AEX-1 might be another regulatory component for synaptic vesicle release.

aex-1 encodes a 1009 amino acid protein with one potential calcium-binding C2 domain. AEX-1 has a similarity to the rat Munc13-4 and human BAP3 proteins: function of these

proteins is unknown. Using GFP fusion constructs, we found that *aex-1* is predominantly expressed in muscles and intestine. Furthermore, we revealed that muscle-specific AEX-1 expression rescues aldicarb-resistance and intestine-specific expression rescues defecation defects, but neuron-specific expression rescues neither. Therefore, AEX-1 regulates neural activities from muscles and intestine.

To understand a presynaptic response by this retrograde regulation, we investigated a well-characterized G-protein pathway at presynaptic terminals: both EGL-30 G_q alpha and GOA-1 G_o alpha proteins regulate a pathway that leads to translocation of UNC-13 to active zones for synaptic vesicle release. UNC-13 in the *aex-1* mutant was diffusely localized through axons of the ventral nerve code while that in the wild-type accumulates at presynaptic terminals. This diffuse pattern was also rescued by muscle-specific AEX-1 expression. Our genetic analysis also revealed that neural *egl-30(gf)* is epistatic to muscular *aex-1* for the UNC-13 localization. These data suggest that an AEX-1-dependent signal feeds into this G-protein pathway to regulate the UNC-13 localization for synaptic efficacy. These findings indicate the presence of a general mechanism in non-neural tissues to modulate neural activities through AEX-1.

99. Early Sensory Deprivation Alters Behavior, Rate of Development and Neuroanatomy in C. elegans

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A basic feature of mammalian nervous systems is that during development activity dependent processes sculpt the final patterns and strengths of synaptic connections. Changes in synapses have been reported as a result of altered early experience. Rats raised in isolation in wire cages show fewer synapses in the visual cortex than rats raised in groups in an enriched environment (Sirevaag & Greenough, 1987). Recent evidence of this same phenomenon in invertebrates suggests that this may be a basic feature of nervous systems that is conserved across phylogeny. In these experiments we investigate the effects of early experience on the nervous system and behavior of the nematode *C. elegans*.

Our procedure involved preparing NGM plates with 1 drop of *E. coli* (making a circle with approximately 1 cm. diameter) and letting 4-day old adults lay either a single fertilized egg (for isolate groups) or 20-30 eggs (for colony groups). Adult worms and excess eggs were removed from the plates and all of the plates were placed on cushioning in a 20 degree incubator for four days. For behavioral experiments 4-day old isolate and colony worms were transferred to blank NGM plates and given a single tap. For egg counting experiments plates were removed from the incubator at the designated hour and the number of eggs on the plate counted (for colony plates the number of adult worms was counted and the # of eggs/worm was determined). For imaging studies 4-day old worms were mounted and photographed using a confocal microscope.

We found that depriving *C. elegans* of sensory stimulation by raising them in isolation leads to 4-day old adults that show: 1) decreased responsiveness to a mechanical stimulus (a tap),

2) slower rates of physical development (worms raised in isolation are smaller and slower to begin laying eggs than age-matched worms raised in groups), and 3) altered morphology of synapses between the mechanosensory neurons and the interneurons of the tap withdrawal circuit. A mutant strain of worms without functional chemoreceptors (*osm-6*) showed the effect of isolation on the adult response to tap suggesting that chemical cues did not play a role in this effect. Other behaviors such as spontaneous reversals and response to a heat probe were not altered by isolation.

The anatomical differences between worms raised in isolation and worms raised in groups were seen using two different transgenic strains of worms. One strain, developed by Michael Nonet of Washington University was *pmec-7*::VAMP::GFP (this allowed assessment of the effects of isolation on the pre-synaptic mechanosensory neurons). The other strain was a *glr-1*::GFP strain (KP1477) developed by Josh Kaplan of UC Berkeley (the *glr-1* receptors are on the interneurons and allow assessment of post-synaptic changes). Using these strains we have seen clear differences in the synapses of worms raised in isolation compared to worms raised in groups.

These studies add to the demonstrations of plasticity in the nervous system of *C. elegans*. Experience during development plays a role in determining the strength of connectivity in the adult. Thus *C. elegans* can serve as a model system in which to investigate the molecular mechanisms of this phenomenon.

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100. The role of a cGMP dependent protein kinase in olfactory adaptation

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C. elegans can accurately discriminate among a wide variety of volatile chemosensory cues. At least five classes of odors are detected by the two AWC sensory neurons. The signaling cascade in the AWC neurons is thought to be initiated by odorant binding to a G-protein coupled receptor, which activates one or more G alphas, this is postulated to lead to increased cGMP levels either by stimulating a guanylyl cyclase or inhibiting a phosphodiesterase. cGMP gates a cation channel comprised of the two subunits, TAX-4 and TAX-2. Though each AWC neuron responds to at least two volatile compounds, adaptation to each odorant occurs independently. We discovered that *ky95*, an allele of a cGMP dependent protein kinase, is required for adaptation to the AWC-sensed odorants benzaldehyde, butanone and isoamyl alcohol. This gene is allelic with *egl-4/odr-9/chb-1* (Fujiwara and McIntire pers. comm). We have rescued the adaptation defects of the null *egl-4(n479)* with a cGMP dependent protein kinase cDNA expressed solely within the sensory neurons AWC, AWA, AWB, ADF and ASH. Although some of the other neurons could participate in adaptation, we suggest that olfactory adaptation occurs within the AWC sensory neuron itself. Expression of the kinase under a heat-shock promoter was used to show that the kinase is required in the adult for adaptation and not during development. We have tentatively identified the beta subunit of the cGMP-gated cation channel, TAX-2, as a target of phosphorylation: at short time points animals expressing an unphosphorylatable TAX-2 channel exhibit impaired adaptation. This raises the question of how adaptation can be both odorant specific yet result from regulation of the TAX-2/TAX-4 channel that is responsive to all three odorants. We are testing models that may explain this paradox. Another potential target for phosphorylation may be the Smad transcription factor DAF-3. A *daf-3* mutation is epistatic to the null *egl-4(n479)* mutation for adaptation at long time points. This result suggests that the signaling cascade at the cilia eventually alters gene expression in the nucleus consistent with other suggestions

(Miyahara et al. 1999 International Worm Meeting abstract 593). We propose that the cGMP dependent protein kinase is an integrator of olfactory signaling. Once a threshold of cGMP concentration or duration is met, a cohort of proteins within the cell alters its odor-responsiveness in an odorant specific manner.

101. Passive no longer: hermaphrodite-derived, mate-finding cue implicated in the nematode *Caenorhabditis elegans*

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Hermaphrodites have long been considered passive mates, exhibiting no behavior involved in mating. Our findings correct this misconception and demonstrate that hermaphrodites do contribute. We report that a mate finding cue exists: males reverse more frequently and stay longer in regions of agar conditioned with hermaphrodites as compared to unconditioned agar. This cue is sexually dimorphic, given off only by the hermaphrodite and eliciting a response only in male. From our studies we suggest a form of kinesis that works by attracting males to their mating partners from a distance and functions, once males arrive, in holding attracted males in close proximity. Males from three feral isolates of *C. elegans* also respond in a similar manner to this cue (CB4555 from Pasadena, CA; CB4932 from Taunton, England; and CB4856 from Hawaii, USA), and thus this cue is robust and not correlated with NPR-1-related social behavior.

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102. Sex Drive in *C. elegans*

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C. elegans males cultured alone on a bacterial lawn will not remain indefinitely on the food source, a behavior we refer to as leaving. We have analyzed leaving and found that it is a regulated behavior with characteristics consistent with the view that it is a mate-searching behavior. Sexual behaviors such as mate-searching and copulation are considered motivated behaviors governed by ill-defined states of the nervous system known as drive states. Using a quantitative leaving assay, we have determined physiological and environmental variables that influence the probability of leaving and have isolated both constitutive and defective leaving mutants. Thus, we are able to functionally and genetically dissect an apparent nematode counterpart of sex drive.

We have found that leaving can be described as a stochastic process defined by a probability that differs for males, hermaphrodites, and juveniles. In a leaving assay, a single animal is placed in the center of a small bacterial lawn on a 9cm plate and the subsequent time interval during which it leaves, defined as wandering beyond a circle of 3.5cm radius, is determined. Twenty or more animals are assayed per experiment and the fraction of non-leavers as a function of time is plotted. Leaving can be described by a simple first-order rate equation characterized by a single probability of leaving per hour (P_L). In our assay, P_L for adult males is 0.17+/-0.008. Leaving is both stage- and sex-specific: P_L for adult hermaphrodites is 0.002 and sexually immature (L4) worms of either sex do not express appreciable leaving (P_L<0.001).

The presence of other nematodes affects leaving. Male leaving was completely and reversibly abrogated by presence of hermaphrodites (P_L<0.001); in contrast, males placed with other males still expressed leaving behavior (P_L=0.103). We have obtained evidence that the hermaphrodite signal required to detain males is short-range or may be contact-dependent. We isolated a mutation, *cod-16(bx72)*, in which male leaving was not inhibited by the presence of hermaphrodites. Both *cod-16* males and hermaphrodites tested alone exhibited wild type leaving behavior. Thus, the *cod-16* locus may define a gene specifically required for male detection of hermaphrodites.

In *C. remanei*, a gonochoristic species for which mating is obligate, leaving was expressed by both sexes (P_L male=0.21; P_L female=0.07). Strikingly, females mated prior to testing had a greater tendency to stay on food (P_L=0.03), suggesting that the drive state governing leaving behavior might be regulated by signals from the reproductive system. Thus, leaving is not male- or species-specific, but a behavior that correlates with the requirement to find a mate.

Different drive states may vie to direct behavior toward specific goals (e.g. food or sex). Thus, it is expected that sex drive would be regulated by reproductive and nutritional status. Indeed, we found that leaving probability in *C. elegans* is regulated by signals from the gonad in both sexes and by food-deprivation. A role for gonad signaling was demonstrated by ablation studies and by examining the behavior of mutants with abnormal development of the gametes. Overnight food-deprivation completely and reversibly blocked male leaving. Moreover, hermaphrodites efficiently detain males only in the presence of food, consistent with a prioritization of feeding over sex.

In a small pilot screen, the leaving assay enabled us to readily isolate several leaving defective mutants that moved well but did not express normal leaving kinetics. *bx117*, *bx121*, and *bx122* express greatly reduced P_L (<0.025) and likely represent three genes. *bx122* is an allele of *unc-77*, a loopy Unc mutant in which egg laying is hypersensitive to serotonin (Schafer and Kenyon, 1996). Interestingly, *bx121* hermaphrodites were also strongly hypersensitive to serotonin in a paralysis assay. A role for serotonin in the regulation of leaving was further supported by the finding that serotonin-deficient *tph-1* mutant males exhibited markedly reduced P_L (=0.04), whereas males lacking dopamine were unaffected.

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103. In Vivo Optical Imaging of Neuronal and Muscle Cell Activity

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Genetically-encoded optical sensors, such as the FRET-based, ratiometric calcium-sensitive protein cameleon, have many potential advantages for cell-specific non-invasive neural imaging. The use of optical indicators is particularly attractive in *C. elegans* due to the animal's transparency, the ease with which transgenic animals can be generated, and the difficulty of electrophysiological methods. However, because of their relatively slow kinetics and small signal size, it has been difficult to use genetically-encoded sensors like cameleon in excitable cells. We have recently overcome these hurdles and developed imaging methods that have allowed us to detect and measure in vivo calcium transients in neurons and muscle cells.

While developing the technique, we initially focused on detecting and measuring the calcium influx accompanying contraction of the pharyngeal muscle. We expressed various cameleons under control of the pharyngeal-specific promoter *myo-2*, and imaged the fluorescence ratio emitted by the pharyngeal muscle cells. We observed prominent peaks in these ratiometric traces characteristic of calcium transients, which precisely accompanied muscular contractions and which were accompanied by an increase in FRET. Using this approach, we have made the surprising discovery that mutations eliminating the activity of UNC-36, the conserved alpha-2 subunit of the calcium channel, cause a significant increase in the magnitude of the pharyngeal calcium transient. This suggests either that the alpha-2 subunit functions in muscle channels to negatively regulate calcium influx or that it acts indirectly on the muscle by altering synaptic transmission in pharyngeal neurons. Experiments are in progress to distinguish between these hypotheses.

We have also used cameleon to detect calcium transients in neurons. Direct electrical stimulation using an extracellular electrode produced reliable responses in cameleon-expressing neurons. Preliminary experiments indicate that the neurons of *unc-36* animals may be less easily excited than in wild-type, suggesting that the alpha-2 subunit may promote calcium influx in neurons. We have recorded from the mechanosensory PLM neurons using cameleon driven by the *mec-7* promoter and have observed responses to a train of regularly spaced mechanical stimuli. In collaboration with the Driscoll lab, we are currently analyzing the effects of mutations in the putative mechanotransduction channel MEC4/MEC10 to determine their effects on mechanically-activated neural activity. We also plan to use these imaging methods to investigate the activity patterns of interneurons receiving synaptic input from PLM. By simultaneously imaging the activity of sensory neurons and their post-synaptic partner, we hope gain insight into the mechanisms underlying the integration and processing of sensory information in these simple sensory circuits.

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104. Generation of cell-type-specific RNA and gene expression profiles for Mechanosensation Neurons in *C. elegans*

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One of the great advantages of *C. elegans* as an experimental system is the ability to characterize mutant phenotypes at the level of single type of cells. With the sequencing of the genome, the use of DNA microarrays offers the opportunity to look at the genome-wide transcriptional activity under different experimental conditions. Unfortunately, array experiments using whole worm RNA do not afford the resolution needed to look, for example, at the differences in expression between specific wild type and mutant nerve cells. We have developed a technique to generate cell-type-specific RNA to use with cDNA microarrays to study gene expression profiles for a single type of *C. elegans* cells and have applied it to study the differentiation and function of the embryonic touch receptor neurons (ALM and PLM). While saturation mutagenesis for touch insensitive mutants have identified several genes needed for touch cell development and function, those screens could not have identified redundant genes, pleiotropic genes or genes that mutate to a touch super-sensitive phenotype. DNA array data should reveal such genes as well as provide candidates for as yet uncloned *mec* genes.

To identify *mec-3*-dependent genes expressed in the touch cells, we collected late-stage embryos from strains with integrated GFP arrays: either $P_{mec\text{-}18}$ *gfp* in a wild-type background for differentiated touch cells or $P_{mec\text{-}3}$ *gfp* in a *mec-3 (e1338)* background for *mec-3* mutant cells that should have become touch cells. Cells from dissociated embryos were cultured overnight (many extended processes in culture) and the GFP-positive cells were enriched 100-fold by fluorescence-activated cell sorting. Typical post-sort values from cell sorting are $4x10^6$ cells with 40-50% being GFP positive. mRNAs from these cells were linearly amplified 10^6-fold using a modified Eberwine's method, labeled and applied to cDNA microarrays (in Stuart Kim's lab) representing virtually all of the *C. elegans* genes.

The reliability and sensitivity of the expression profiles is suggested by the finding that 6 of the 10 known *mec-3*-dependent genes were among the top 15 genes and 8 out of 10 known *mec-3*-dependent genes (including *mec-3* itself) had ratios above 4.0 (the two other genes had ratios higher than 2.0). In addition, the profile also provides several groups of interesting candidates besides unknown function genes with ratios equal to or higher than ratios of known *mec-3*-dependent genes: 1) the gene with the highest ratio maps to the same position as the previously uncloned *mec-17* gene, which is needed for the maintenance of touch cell fate (we have confirmed that this gene is expressed exclusively in the touch cells and have identified two missense mutations in *mec-17 (u265)*), 2) two other genes map to the area of *mec-15* and a single mutation on X that disrupts touch cell microtubules, 3) several genes that contribute to the T-complex chaperonins, which are needed for the formation of tubulins, 4) several channel genes in addition to *mec-4* and *mec-10* and 5) genes for G-protein coupled receptors and transcription factors. We are also using RNAs from sorted and unsorted cells to identify touch-cell-enriched genes that are not *mec-3*-dependent.

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105. Creating transgenic lines in *C. elegans* by microparticle bombardment

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C. elegans transgenic lines are typically created by injecting DNA into the hermaphrodite germline to form multicopy extrachromosomal DNA arrays. We have developed an alternative method for *C. elegans* transformation, using microparticle bombardment, that produces single- and low-copy chromosomal insertions and can be used to express transgenes in the germline as well as in somatic cells. In this approach, gold beads are coated with transgene DNA and accelerated to high speeds allowing them to penetrate the *C. elegans* cuticle. After bombardment, we find gold beads located in germline nuclei as well as throughout the animal. Previous work demonstrated that integrated lines could be created by injecting *sup-7*-containing plasmids into oocyte nuclei (1). We hypothesize that introduction of transgenic DNA into a germline nucleus is an essential step in integrative transformation.

Because integrative transformation by microparticle bombardment is a relatively rare event, we use a selection to identify transformed animals after bombardment. *unc-119* mutants are unable to form dauers. By bombarding *unc-119* mutants and including an *unc-119* rescuing fragment in our bombardment plasmids, we can use starvation to select against untransformed *unc-119* worms. Using this approach, we find that approximately 1/4 of our bombardments produce homozygous or heterozygous integrated lines. Although *unc-119* provides a very effective selection for identification of integrated lines, the morphological phenotype of *unc-119* mutants and the expression of UNC-119 throughout the nervous system limit its usefulness. To address this issue, we are presently testing the suitability of temperature-sensitive and drug resistance genes as additional co-transformation markers for integrative transformation.

(1) Fire, A. EMBO Journal 5:2673-2680.

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106. Identifying muscle genes that change transcription levels in response to varying cholinergic signals using full-genome microarrays.

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The excitatory neurotransmitter acetylcholine (ACh) not only controls muscle cell contraction, but can also lead to a change in muscle cell physiology. For example, in mammals, chronic exposure to the ACh agonist nicotine can desensitize cells, leading to addiction. To better understand how a post-synaptic cell responds to ACh, we are examining the genome-wide transcriptional changes in C. elegans striated muscle cells in response to varying cholinergic signal.

To do this, we first needed to develop a method to measure relative gene expression levels in single tissues in C. elegans. Previous DNA microarray experiments have compared gene expression levels from entire worms rather than from specific tissues. A major limitation to this approach is that gene expression changes in one tissue, such as muscle, could be obscured by expression in other tissues. To isolate muscle mRNAs, we use the myo-3 promoter (a gift from A. Fire) to drive expression of an epitope-tagged poly(A)-mRNA-binding protein in striated muscle. The mRNA/tagged-protein complex is co-immunoprecipitated from whole-animal lysates using antibodies against the epitope tag. mRNAs significantly enriched in muscle are identified by comparing the immunoprecipitated mRNA to the mRNA present in the initial worm extract using DNA microarrays. We call this technique "mRNA-tagging". L1-muscle mRNA-tagging (6 repeats) revealed 1453 genes that were significantly enriched, including most characterized muscle-specific genes (positive controls) and excluding most non-muscle genes (negative controls). We repeated the entire experiment using L2 larvae (6 repeats) and found that there were few significant differences between L1 and L2 muscle. Together, these results indicate that mRNA-tagging successfully identifies genes expressed in striated muscle.

To profile transcriptional changes in striated muscle in response to ACh, we either excited muscle cells using the nicotinic ACh agonist levamisole or prevented normal muscle contraction using an ACh receptor mutant. We then used mRNA tagging to identify genes expressed in excited and mutant muscle, and compared those genes to each other and to those expressed in "normal" muscle. 304 genes show increased expression and 851 genes show decreased expression in levamisole-excited muscle, relative to normal or mutant muscle. The gene expression data indicates that ACh excitation decreases the relative expression of ACh receptors and other ligand-gated ion channels, which may represent new ACh receptor candidates. Specifically, the expression levels of seven ligand-gated receptors, two 2nd messenger-gated receptors, and two characterized ACh receptor genes (lev-1 and unc-29), were significantly lower in the levamisole-treated or normal worms than they were in the mutant. The overall effect may be to desensitize cells to acetylcholine following strong activation, and to sensitize cells in ACh receptor mutants.

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107. M5 controls terminal bulb contraction in *Panagrellus redivivus* but not in *C elegans*

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In the *C elegans* pharynx contractions of the corpus (first bulb) and terminal bulb (TB) are synchronized because excitation begins in the corpus and spreads through gap junctions to the TB. In *Panagrellus redivivus* the TB pumps three times faster than the corpus, suggesting that the *Panagrellus* TB must have another source of excitation. Albertson and Thomson's EM reconstructions of the *C elegans* pharynx showed that a single motor neuron, M5, synapses onto TB muscles. However, M5 has little or no detectable function in *C elegans*. When we killed M5 in *Panagrellus*, TB contractions were at the same rate as and synchronized with corpus contractions, as in *C elegans*.

Why might it be advantageous for the *Panagrellus* TB to pump faster than the corpus? The innexin EAT-5 couples the *C elegans* corpus and TB. In an *eat-5* null mutant the TB is unsynchronized with the corpus and pumps more slowly. *eat-5* mutants grow almost normally on *E. coli* HB101 (easier to eat than most *E. coli* strains) but can't grow on DA837 (a derivative of OP50). By selecting for suppressors of *eat-5* that allowed growth on DA837, we isolated one strain, *sef(ad1614) eat-5*, in which corpus and TB are still unsynchronized, but the TB pumps about twice as fast as in the *eat-5* single mutant. This result suggests that the rate of TB pumping determines growth on DA837. We hypothesize that the faster TB pumping in *Panagrellus* may allow it to use low-quality food sources. Each of several different nematode species that we have tested grows faster on HB101 than DA837, but the difference varies widely from species to species. *Panagrellus* is less affected by the food source than any other species tested.

To find other differences between pharyngeal nervous system function in the two species we killed each of the 14 pharyngeal neuron types in each of 5-10 worms of each species, then measured the time required to grow to adulthood on DA837. By this crude test two *C elegans* pharyngeal neuron types are important: M4, essential for contraction of posterior isthmus muscles, and MC, which controls the rate of pumping. In *Panagrellus* 2/12 neuron types tested affected growth rate: M2 and M5. In *C elegans* M2 innervates isthmus muscles but has no detectable effect on pharyngeal function. We have not yet determined the feeding defect in M2-minus *Panagrellus*. M4 had no detectable effect on *Panagrellus* growth. (For technical reasons, MC and I2 have not yet been tested.) Thus, in both species only a small subset of pharyngeal neuron types is important for grossly normal feeding, but the important subsets are different.

Nematodes have anatomically similar nervous systems despite wide variations in behavior. For instance, the locomotory nervous systems of adult *C elegans* and *Ascaris lumbricoides* are cell-for-cell identical, despite the great difference in size and locomotory behavior (Stretton et al). How can the same nervous system produce different behavior? Based on our results we propose the following ROGUT (Ridiculously Oversimplified Grand Unified Theory): The 118 neuron types in the adult female nematode represent a menu of possible behaviors. In any given species, only a subset of these types actually functions; which subset determines behavior. While this idea cannot explain all variation in nematode behavior, we believe it may be part of the explanation.

The most controversial implication of this hypothesis is that a neuron type may persist through evolution in the absence of selection for its function. It is possible that M5 (for instance) has an important function in *C elegans* in the wild that we cannot detect in the lab. If so, that function must be different from its function in *Panagrellus*, since M5 function in *Panagrellus* is easily detected in the lab. But if M5 has a function in *C elegans* different from its function in *Panagrellus*, it is surprising that its function in *Panagrellus* is predicted by its anatomy in *C elegans*. In any case, the substantial differences in pharyngeal nervous system function we have discovered are difficult to reconcile with the hypothesis that nematode nervous system

constancy is maintained by selection for function. Instead we favor the hypothesis that nematodes have similar nervous systems because of developmental constraints.

108. *pbo-4* and *pbo-5* define a novel signaling pathway required to initiate the posterior body contraction

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The *C. elegans* defecation cycle is characterized by the activation of three distinct muscle contractions. Every 50 seconds, a cycle begins with the posterior body contraction, followed by the anterior body contraction, and finally, by the enteric muscle contraction. Laser ablation studies have shown that two GABAergic motor neurons, AVL and DVB, are required the anterior body contraction and the enteric muscle contraction. Neuronal ablations did not affect cycle timing or the execution of the posterior body contraction. In addition, mutations that disrupt neurotransmission and secretion also have no effect on the posterior body contraction. Together, these results suggest that a non-neuronal signal is required for proper timing of the cycle and initiation of the posterior body contraction.

We demonstrated that the defecation cycle is regulated by an endogenous clock that resides in the intestine. Specifically, mutations in the inositol trisphosphate (IP_3) receptor, an intracellular calcium channel, eliminate clock function. Moreover, we observe calcium spikes in the intestine that correlate with the timing of the cycle and immediately precede a posterior body contraction. These data suggest that a calcium spike may stimulate the release of a signal from the intestine that could then be received by the body muscles.

How does a calcium signal in the intestine trigger a muscle contraction without a neuronal intermediate? To identify the nature of the signal and its target, we screened for mutations that specifically eliminate the posterior body contraction. From this screen we identified the *pbo-4* and *pbo-5* genes. We cloned *pbo-4* and demonstrated that it encodes a protein homologous to Na^+/H^+ exchangers (NHEs). Plasma membrane NHEs mediate the exchange of one Na^+ ion into the cell for one H^+ ion out, and thus acidify the extracellular environment. In mammalian cells, some isoforms are activated by calcium via calmodulin binding.

Similarly, PBO-4 contains a potential Ca^{2+}/calmodulin binding domain on the C-terminal, cytosolic portion of the protein that could mediate calcium signaling. Interestingly, *pbo-4* expression is restricted to the posterior intestine. This suggests that PBO-4 activity is part of the signal that initiates the posterior body contraction. What is the target? *pbo-5* encodes a novel, ligand-gated ion channel that is distantly related to acetylcholine receptors. *pbo-5* expression is restricted to the posterior muscles that lie adjacent to the PBO-4 expressing cells. These data predict a model in which a calcium spike activates PBO-4 to secrete protons from the intestine. PBO-5 might then recognize this signal, depolarize the body muscles, and initiate the posterior body contraction. We are currently characterizing the electrophysiological properties of PBO-5 to determine whether it forms a homomeric channel and to test whether changes in pH affect receptor activation.

109. Altered Defecation Rhythm Produced by RNAi Suppression of Intestinal Potassium Channels, *kqt-2* and *kqt-3*.

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KCNQ potassium channel genes encode M-type potassium channels, and are notable because nearly all known human orthologs are associated with hereditary diseases, affecting epithelial cells, cardiac muscle and neurons. *C. elegans* possess three distinct KCNQ orthologs, *kqt-1* (C25B8.1), *kqt-2* (M60.5) and *kqt-3* (Y54G9a.3), among a total of 65 potassium channel genes. We examined the *in vivo* role for these channel genes through a combination of electrophysiological and genetic techniques. Our results revealed an unexpected role for intestinal KQT potassium channels in the control of defecation rhythms.

kqt-1 and *kqt-3* cRNAs expressed functional potassium currents in *Xenopus* oocytes, and were analyzed by voltage-clamp recordings. KQT-1 and KQT-3 currents exhibited particularly slow activation kinetics, depolarized voltage-dependence and pharmacological blocking profiles characteristic of KCNQ channels. Though *kqt-2* failed to express functional channels alone, co-injections with *kqt-1* or *kqt-3* suggest that KQT-2 is capable of forming heteromeric channels with either KQT-1 or KQT-3. This close resemblance of functional properties between KQT and KCNQ channel subunits, suggests that these two sets of genes may mediate similar conserved cellular functions *in vivo*.

Consistent with a role for KCNQ channels in epithelial cells, *kqt* genes were found to express in worm intestine. Full-length translational GFP fusions were made for all three *kqt* genes and used to transform lines of worms. Intestinal cells were prominently labeled by *kqt-2*::GFP and *kqt-3*::GFP, with less consistent labeling by *kqt-1*::GFP. Expression of *kqt-2*::GFP was exclusive to intestinal cells, and appeared to label all cells uniformly. In contrast, *kqt-3*::GFP expression was enhanced in anterior- and posterior-most intestinal segments.

To examine intestinal *kqt* function, RNAi was performed for all three *kqt* genes and treated animals assayed for defecation behavior. Efficacy of RNAi-mediated suppression was independently confirmed for *kqt-2* and *kqt-3*, by the loss of intestinal GFP fluorescence reported by *kqt-2*::GFP and *kqt-3*::GFP transgenes. Suppression of either *kqt-2* or *kqt-3* resulted in prolonged defection cycle intervals (pBoc-to-pBoc), relative to N2 controls, with unaltered defecation motor steps. No effect was observed with *kqt-1* RNAi, consistent with weaker *kqt-1*::GFP intestinal expression. Average cycle intervals were prolonged ~56% with *kqt-3* RNAi (75 ± 6 sec) and ~28% with *kqt-2* RNAi (60 ± 2 sec), relative to N2 (48 ± 2 sec) and *kqt-1* RNAi (50 ± 2 sec). Cycle interval histograms revealed bimodal normal distributions of intervals produced by *kqt-2* and *kqt-3* RNAi treated animals, with similar means (62 and 110 sec, *kqt-2* RNAi; 59 and 103 sec, *kqt-3* RNAi). This shared time structure suggests that KQT-2 and KQT-3 may act on a common process, perhaps as a heteromeric channel. This suggestion was further supported by double *kqt-2/kqt-3* RNAi experiments, showing prolonged cycle intervals (79 ± 5 sec) which were non-additive relative to *kqt-3* and *kqt-2* RNAi treated animals.

Because defecation cycles are controlled by intestinal cytoplasmic Ca^{+2} oscillations mediated by ITR-1 inositol triphosphate receptors, one intriguing possibility is that KQT channels may interact with this process. Thus, in addition to hypothesized roles in shaping prolonged action potentials and mediating ionic transport, our results suggest that KQT/KCNQ-like channels may serve an unexpected role in regulating intracellular calcium oscillations.

110. Control of the defecation motor program involves the G protein-coupled receptor AEX-2

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Worms defecate by executing three sets of muscle contractions every 45-50 seconds when they are feeding. A calcium spike in the posterior intestine immediately precedes and probably initiates the first of these muscle contractions (1), called the posterior body-wall muscle contraction (pBoc). Approximately 3 seconds after the pBoc, an anterior body-wall muscle contraction (aBoc) pushes the posterior pharynx backward into the anterior intestine. Immediately following the aBoc, a contraction of the enteric muscles in the region of the anus expels intestinal contents (Exp). We have been working to characterize *aex-2*, mutations in which cause defects in both the aBoc and Exp (Aex) steps of the defecation motor program.

We have cloned *aex-2* and shown that it encodes a 7-transmembrane domain protein with homology to the G protein-coupled receptor family. However, the *aex-2* sequence does not fall into any specific small molecule receptor subfamily, suggesting that it mediates a novel signaling pathway in *C. elegans*. This signaling pathway may be peptidergic, since mutations in two enzymes known to process small signaling peptides also cause an Aex phenotype. *aex-5* encodes a proprotein convertase, which cleave proproteins into small signaling peptides (2), and *egl-21* encodes a carboxypeptidase E (3), which process peptides once they have been cleaved. *aex-2* may encode a receptor for one or more peptides processed by these two gene products.

An *aex-2::gfp* fusion expresses GFP in the enteric muscles, NSM, AWB, and several other cells in the head of the worm, including possibly AVL, a neuron responsible for stimulating the aBoc. AEX-2 may be receiving a signal in AVL that in turn activates the anterior body-wall muscles to execute the aBoc. AEX-2 may also be receiving a signal in the enteric muscles, inducing them to execute the expulsion. An

unknown signal or signals from the intestine initiates the process of defecation, but how that signal is transmitted to activate the three motor steps is unknown.

In an effort to identify other proteins functioning in this pathway, we are screening for suppressors of the *aex-2* expulsion defect. We have screened over 10,000 haploid genomes and have identified more than ten alleles that suppress *aex-2(sa3)*, a hypomorphic allele that has a missense mutation in the sixth transmembrane domain. While no mutation confers complete suppression, many have interesting phenotypes, including three mutants of a novel class that occasionally execute more than one expulsion per cycle. None of these mutations suppresses the aBoc phenoytype of *aex-2*, suggesting that while the aBoc and Exp phenotypes are caused by one mutation, they are mechanistically separable. We are currently mapping and further characterizing these new alleles.

1. Dal Santo, P, et al., 1999. Cell 98: 757-67.

2. Thacker, C. and A.M. Rose, 2000. Bioessays 22: 1-9.

3. Carey, T. and J.M. Kaplan West Coast Worm Meeting Abstract, 2000.

111. The family of genes encoding FMRFamide-related neuropeptides of *Caenorhabditis elegans*

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FMRFamide (Phe-Met-Arg-Phe-NH_2) and related peptides (FaRPs) are short peptide neurotransmitters that have been found throughout the animal kingdom. FaRPs have been shown to have many general functions, including cardioregulation, muscle control, pain modulation, and learning. Our lab uses *Caenorhabditis elegans* as a model system to study FaRPs. In *C. elegans*, at least 50% of the neurons express FaRPs, which are encoded by at least 23 *flp* (*F*MRFamide-*l*ike *p*eptides) genes. Expression of most *flp* genes has been confirmed by the isolation or identification of peptides, cDNAs, or EST sequences. In addition, genetic analysis of *flp-1* has shown that at least one of *flp* genes has unique functions in *C. elegans*.

To determine the cell-specific expression of the *flp* genes in *C. elegans*, transgenic animals carrying a *gfp* (green fluorescent protein) reporter construct under the control of each *flp* promoter are being generated by germ-line transformation. To date, the cell-specific expression patterns of 18 *flp* genes have been determined. Each *flp* gene is expressed in distinct, but sometimes overlapping, sets of cells, suggesting that *flp* genes function in both unique and overlapping pathways. Furthermore, several genes show stage-specific or sex-specific expression patterns, suggesting that some FLP peptides may function during development and in reproductive behaviors. The cell-specific expression patterns of the remaining *flp* genes are being determined.

To determine the function of the *flp* genes in *C. elegans*, several *flp* deletion mutants were isolated by screening libraries of chemically-mutagenized *C. elegans*. All deletion mutants are still being characterized. We are continuing the screen for new deletion mutants.

To identify genes that may regulate expression of the *flp* genes, we are using transgenic animals expressing a *gfp* reporter construct under the control of a *flp-1* or *flp-12* promoter in genetic screens. These strains are mutagenized, and mutants showing altered expression of these markers are isolated. Several mutants that show no, faint, or strong GFP expression have been isolated. The genes corresponding to these mutations are being mapped, characterized, and cloned.

112. SUE-1: a novel *C. elegans* neuropeptide that regulates muscle contraction

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In a screen for suppressors of the *sma-1* defect in embryonic elongation, we isolated a set of 13 Sue (**su**ppressor of **e**longation defects) mutants that alter regulation of muscle contraction as well as increasing elongation of *sma-1* embryos. Sue mutants display multiple phenotypes associated with misregulated muscle contraction: constitutive egg laying and lengthened defecation cycles as well as hyperactive body movement and pharyngeal pumping. Recessive *sue* mutations that map to different loci fail to complement, suggesting that these genes act in a common pathway. *sue* mutations also fail to complement *goa-1* mutations. GOA-1 is the alpha subunit of Go, a G protein also involved in the regulation of egg laying, defecation, pharyngeal pumping and movement but that does not affect embryonic elongation.

We identified the *sue-1* gene product by rescue of its Egl-c phenotype. Using extrachromosomal arrays generated by microparticle bombardment, we rescued the *sue-1(ru23)* phenotype with a 100bp fragment located within an intron of the predicted gene F07A5.5. This fragment contains a single open reading frame encoding a putative 23 amino acid peptide required for rescue; a truncated peptide generated by a single base pair change does not rescue. The full length peptide contains a short, 9 amino acid signal sequence followed by a di-lysine cleavage site and a potential secreted neuropeptide. Using a *sue-1* promoter GFP construct, we observe expression in the HSN and VC neurons, which regulate vulval muscle contraction. We propose that the *sue-1* Egl-c phenotype is the result of loss of SUE-1 function in these neurons. In addition, we observe expression in head and tail neurons that may identify additional SUE-1-expressing neurons. Based on its sequence, SUE-1 is not a member of the FaRP neuropeptide family. Since the *sue-1* gene encodes only a single copy of the SUE-1 peptide, it would also not have been identified in a genome neuropeptide search that relied on the presence of nearly identical amino

acid repeats (1). These data suggest that SUE-1 is a novel *C. elegans* neuropeptide.

We are cloning two additional *sue* genes that contain the gain-of-function mutations *sue-2(ru27)* and *sue-3(ru36)*. We are able to phenocopy the Egl-c phenotypes of these mutations by overexpression using extrachromosomal arrays. We find that *sue-2(ru27)* can be phenocopied by overexpression of a 2.7 kb fragment of cosmid R05F9; the *sue-3(ru36)* defect in egg laying can be phenocopied by overexpression of a 9 kb fragment of the cosmid H09G03. We are interested in determining whether these genes encode additional neuropeptides or other components of a neuronal signaling pathway or whether they regulate muscle contraction by a different mechanism.

(1)Li, C. *et al* . 1999. Ann. NY Acad. Sci. 897: 239-252.

113. Identification and characterization of *nic-1*, a gene involved in regulation of the nAChR.

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Long-term exposure to nicotine causes long-lasting changes in behavior which reflect in part changes in the abundance and activity of nicotinic acetylcholine receptors. The molecular mechanisms underlying this adaptation to nicotine are not well understood in any organism. To address these questions, we have been investigating nicotine adaptation in *C. elegans*. Acute treatment with nicotinic agonists such as nicotine and levamisole leads to body muscle hypercontraction and paralysis, increased pharyngeal pumping, and stimulation of egg laying under conditions inhibitory for egg laying. Long-term treatment with nicotine confers recovery from these effects, a process we have termed adaptation: worms regain movement, decrease their rate of pharyngeal pumping, and fail to be stimulated to lay eggs under inhibitory conditions. At least some of these behavioral changes (in particular those related to egg-laying) occur at least in part through downregulation of nicotinic receptor abundance.

To identify molecules involved in the regulation of nicotinic receptor abundance or activity, we have conducted genetic screens for mutants that are hypersensitive or fail to adapt to nicotine. Among the genes identified in these screens is a new gene, *nic-1*. *nic-1* mutants are strongly nicotine hypersensitive: they undergo paralysis and are stimulated to lay eggs at doses of nicotine three-fold lower than wild type. In addition, these mutants exhibited phenotypes that support *nic-1*'s possible role in the regulation of cholinergic neurotransmission. For example, *nic-1* animals were hyperactive for egg-laying and showed an egg-laying pattern reminiscent of wild-type animals treated with

cholinergic agonists. Moreover, they displayed abnormalities in locomotion and male mating, two behaviors known to be controlled by cholinergic motor neurons. Experiments with UNC-29::GFP chimeras indicated that *nic-1* mutants actually had reduced levels of the levamisole receptor in vulval muscles and normal levels in body muscles; thus, we believe the hypersensitivity of *nic-1* animals reflects a change in receptor activity rather than abundance. To gain more information about the effect of *nic-1* on nicotinic receptor activity, we are collaborating with Janet Richmond to obtain electrical recordings from the body muscle and egg-laying NMJs of *nic-1* animals. We have also mapped *nic-1* to an approximately a 100kb interval on LGX; thus, we are optimistic about the prospect of understanding the molecular identity of *nic-1* very soon, and hope to report our findings at the meeting.

114. Genes that affect the behavioral response of *C. elegans* to ethanol

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Although multiple targets for ethanol in the mammalian nervous system have been proposed, the effects of ethanol that bring about alterations in behavior are not understood. We have taken a genetic approach to test suspected targets of ethanol and identify new targets and pathways that can be affected by ethanol to produce behavioral changes in *C. elegans* with a focus on the behavior of locomotion.

Examination of existing nervous system mutants suggests that regulators of synaptic transmission play a role in sensitivity to ethanol in *C. elegans*. Our genetic screens for new mutations that alter the sensitivity to ethanol have identified multiple mutations in the calcium-activated potassium channel encoded by the *slo-1* gene. Loss of function mutations in *slo-1* result in ethanol resistance. We can restore wild-type ethanol sensitivity to *slo-1* mutant animals by expressing *slo-1* only in neurons but not by expressing *slo-1* only in muscle (many thanks to Mike Nonet for the *slo-1* constructs). Our data suggests that SLO-1 is likely to represent a target of ethanol in the nervous system of *C. elegans* and may be a conserved target of ethanol in mammals (Dopico *et al*. 1999 Neurochem. Int. 35:103-106).

We have also shown that *C. elegans* displays acute tolerance, the process by which animals adapt from an intoxicated state to a sober state during a single, maintained and prolonged exposure to ethanol. During mapping experiments we noticed that the Hawaiian-isolated wild-type strain CB4856 shows rapid acute tolerance compared with the N2 strain after both strains show a similar level of initial intoxication. de Bono and Bargmann (1998 Cell 94:679-689) identified an allelic variant at the *npr-1* locus in social strains, including CB4856. We found that other loss of function alleles of *npr-1* display rapid acute tolerance and that the rapid tolerance in CB4856 could be mapped to the region of the *npr-1* gene. These data suggest that *npr-1* may

function to limit the rate of acute tolerance. de Bono and Bargmann showed that *npr-1* encodes a neuropeptide Y (NPY) receptor-like molecule so it is of significant interest to us that variation in NPY levels in mammals can alter ethanol sensitivity and tolerance (Thiele *et al*. 1998 Nature 396:366-369).

115. State-Dependency of Olfactory Adaptation

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Many learned responses are dependent on the context in which the learning occurs. In vertebrate learning studies, effective memory retrieval can depend on the similarity of the external context or internal physiological state of the organism present during testing to that present during learning. State-dependent learning is thought to drive aspects of addictive behavior, particularly those associated with alcholism.

Worms become intoxicated by ethanol in a manner similar to that of most other organisms tested (see abstracts by A. Davies and H. Kim, this meeting). We have sought to study the mechanisms of ethanol-induced state-dependent learning in worms.

Olfactory adaptation in *C. elegans* is a decrease in the chemotaxis response to an odorant as a result of prior exposure to the odorant (Colbert and Bargmann (1995) *Neuron* **14** 803). We demonstrated a form of state-dependency in worms by pairing olfactory adaptation and ethanol administration: Worms exposed to an odorant while intoxicated by ethanol will only show subsequent adaptation to the odorant if ethanol is again administered during chemotaxis testing. If the odorant is presented without ethanol during testing, the animals behave as naïve animals and fail to alter their behavior based on their previous experience or prior exposure to the odorant.

Further, we have demonstrated that the state-dependent effects of ethanol require normal dopaminergic function. The dopamine-defective *cat-1* and *cat-2* mutants are able to adapt to volatile odorants, however, they do not show state-dependency when they are adapted to volatile odorants while intoxicated by ethanol. These results suggest that there is a conserved role of dopamine in the modulation of behavioral responses to ethanol. Other mutations tested so far, including *glr-1*, an AMPA-type glutamate receptor, have not

disrupted the state-dependency of olfactory adaptation.

We have begun to screen for animals that are unable to pair olfactory adaptation and ethanol intoxication. From a small pilot screen (<1000 haploid genomes), we have isolated a single mutant, *eg160*, that adapts to benzaldehyde in an ethanol-independent manner. We have begun to characterize this mutation, and have found that *eg160* mutant animals are normal for dopaminergic function, indicating that *eg160* disrupts a novel component of the pathway. The *eg160* mutation is completely recessive, and has no visible phenotype. Using the snipSNP method of mapping (thanks to Wicks and Plasterk), we have tentatively assigned *eg160* to the left arm of Chromosome I, and are continuing to identify recombinants in order to map it more finely. Our success thus far in finding and mapping *eg160* suggests that we will be able to use this assay to identify proteins required for this form of learning in *C. elegans*. Further screens are underway.

116. Activity of nociceptive neurons is required for social behaviour in *C. elegans*

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Wild isolates of *C. elegans* show one of two foraging behaviours. Solitary strains, like N2, feed alone. By contrast social strains aggregate and feed together (R. Cassada, wbg 9(3): 29; Hodgkin and Doniach, 1997; de Bono and Bargmann 1998). This natural variation in behaviour is principally due to differences at the *npr-1* locus. *npr-1* encodes a 7 TM receptor of the neuropeptide Y family, and null mutants at this locus display strong social behavior.

To explore the signals required for social behaviour, we asked whether social strains aggregate in the absence of food. We find that even when dispersal is prevented, natural social isolates and *npr-1(null)* mutants do not aggregate without food. To ask whether all food sources induce social behaviour, we grew *npr-1* mutants on a variety of soil bacteria. When grown on NGM, all bacteria tested induce aggregation. Surprisingly however, if bacteria are grown on simple carbon sources aggregation is strongly suppressed, regardless of the bacterial strain tested.

To gain insights into the neuronal circuitry regulating aggregation we looked for mutations that abolish social behaviour. Mutations in *ocr-2* and *osm-9*, genes that encode subunits of a capsaicin receptor-like channel, completely abolish social behaviour. OCR-2 and OSM-9 are co-expressed in 6 pairs of neurons. We expressed the OCR-2 subunit in each of these neurons in an *ocr-2; npr-1* background. We find that expression of OCR-2 in ASH or ADL, but not other neurons, is sufficient to restore social behaviour.

Two other genes that suppress social behavior are *odr-4* and *odr-8*. *odr-4* encodes a novel transmembrane protein required for localizing odorant receptors to the cilia of sensory neurons. Expression of ODR-4 in ADL, but not in any

other neuron tested, restores social behaviour to *odr-4; npr-1* animals.

The ADL and ASH neurons have been shown to mediate avoidance behaviour. Their involvement suggests that a repulsive cue plays a role in social behaviour, and the requirement for *odr-4* suggests that a G protein-coupled receptor senses this cue. The carbon source experiments suggest that the bacterial food itself is a source of a repulsive cue. A role for repulsive cues in social behaviour was originally suggested by Davis and Avery (WBG 11(5): 69). *E. coli* is also a source of attractive cues, particularly water-soluble cues, which may also impinge on the social response.

Together these results suggest that social behaviour is induced partly in response to aversive stimuli sensed by ADL and ASH.

117. The *C. elegans* G-protein gamma subunit *gpc-1* is involved in adaptation to water-soluble attractants.

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Heterotrimeric G proteins form a first intracellular step in many signal transduction cascades. *C. elegans* has 21 Galpha, 2 Gbeta and 2 Ggamma subunits. Based on functional analyses the Galpha subunits can be divided into 2 groups. The conserved alpha subunits are ubiquitously expressed and regulate muscle and neuron activity. At least 14 other Galpha's play a role in sensory transduction. A similar discrimination can be made between the 2 G-protein gamma subunits, *gpc-1* and *gpc-2*. This latter gamma subunit is quite ubiquitously expressed. By contrast, *gpc-1* is specifically expressed in only 6 pairs of amphid neurons and 1 pair of phasmid neurons, suggesting a function in sensory signaling.

However, *gpc-1* is not essential for the detection of various environmental cues, such as odorants or salts. To test if GPC-1 is involved in other types of sensory plasticity we developed a water-soluble compound adaptation assay. The behavior of wild-type animals in this assay confirms the idea that prolonged exposure to water-soluble compounds can abolish chemo-attraction to these same water-soluble compounds. This decrease in chemotaxis is time and concentration dependent, salt specific and reversible. *gpc-1* mutant animals show clear deficits in their ability to adapt to three water-soluble compounds, NaAc, NaCl and NH_4Cl. Also *adp-1* and *osm-9*, two loci previously implicated in odorant adaptation, are involved in adaptation to these salts. Our findings clearly indicate that G proteins are involved in the perception of salts. It is still unclear where in the signaling cascade these molecules function.

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118. EGL-47 is a putative G protein-coupled receptor that controls egg-laying behavior

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A screen to identify mutations that effect the functioning of the neurons that control egg-laying identified five genes, *egl-10, egl-42, egl-47, egl-49* and *egl-50* (Desai and Horviz, 1989). Mutants in these genes are egg-laying defective and insensitive to the serotonin reuptake inhibitor imiprimine, while having an anatomically normal egg-laying system, and normal sensitivity to serotonin. These characteristics are thought to result from functional defects in the HSN neurons that control egg laying. EGL-10 has been identified as a regulator of G protein signaling (RGS) protein, while the remaining genes have not been cloned. We chose to focus on *egl-47* as it has the strongest egg laying defective phenotype. *egl-47* is defined by two independently isolated dominant alleles *n1081dm* and *n1082dm*. Two intragenic revertents of *n1082dm* have a wild-type phenotype. High resolution mapping using single nucleotide polymorphisms (SNPs) from the strain CB4856 identified a 97 kb interval on chromosome V containing *egl-47*. A PCR product generated from *n1081dm* from within this interval and containing only one predicted ORF, C50H2.2, was able to confer the Egl phenotype when transformed into wild-type worms, suggesting C50H2.2 is *egl-47*. Comparable PCR products generated from wild-type worms and injected at higher concentrations were also able to confer the Egl phenotype to wild-type worms. This demonstrates that the dominant *egl-47* alleles cause a gain of normal EGL-47 function. 5' RACE revealed that *egl-47* uses two promoters and generates transcripts encoding proteins with different N termini and a common C terminus. The common C terminus appears to contain seven transmembrane spanning domains, suggesting *egl-47* encodes two G protein-coupled receptors. Sequencing of the dominant alleles revealed an alanine to valine mutation in a putative transmembrane domain. The same base change is present in both dominant alleles. The two revertant alleles contain additional missense mutations. An Alanine to Valine mutation in a transmembrane domain of a G protein-coupled receptor commonly constitutively activates the receptor. Perhaps constitutively active EGL-47 leads to an increase in signaling through the G alpha protein GOA-1, which inhibits egg laying. We have isolated a null allele of *egl-47* by screening a library of deletion mutants. We are currently characterizing *egl-47*, using both gain and loss of function alleles in epistasis experiments with known G protein signaling mutants. We are analyzing the localization of EGL-47 to determine if this protein is involved in the function of the HSN.

Reference Desai, C. and Horvitz, H.R. (1989). Genetics, 121:703-721.

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119. UNC-58 IS AN UNUSUAL POTASSIUM CHANNEL OF THE TWIK FAMILY

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unc-58 was first identified by dominant mutations that cause hypercontracted body-wall and egg-laying muscle in *C. elegans*. *unc-58(dm)* animals are rigidly paralyzed and egg-laying constitutive. *unc-58(dm)* animals also frequently flip around their longitudinal axis.

Putative loss-of-function *unc-58* alleles have been isolated as revertants of the *unc-58(dm)* phenotype. These alleles have a much milder and distinct phenotype, suggesting that *unc-58(dm)* mutants result in an inappropriately activated gene product.

We have cloned *unc-58* and found that it encodes a member of the TWIK potassium channel family. TWIKs are a distinct family of potassium channels having four transmembrane domains (M1 to M4) and two pore domains. Potassium-selective currents have been recorded from TWIKs from both mammals and worms.

The hypercontracted phenotype of *unc-58(dm)* is in sharp contrast to that of all other activated potassium channel mutants, which lead to muscle relaxation caused by excessive inhibitory currents. Two scenarios can explain this hypercontraction. First, UNC-58(dm) may produce excessive potassium currents in the inhibitory motorneurons. However, this does not account for the hypercontraction of the egg-laying muscle, which is not innervated by inhibitory motorneurons. Second,, UNC-58(dm) may act in excitatory neurons and/or muscle to conduct an excitatory current. A precedent for that is the Ih channel from the human heart, which is related to potassium channels by sequence, but is permeable to both potassium and sodium [Ludwig et al, Nature 393, 587-591]. To distinguish between these two possibilities, we are expressing UNC-58(dm) in *Xenopus laevis* oocytes to determine its ion selectivity and using GFP fusions to determine the UNC-58 site of action.

Both the flipping and the hypercontarction phenotypes of *unc-58(dm)* are partially rescued by the drug endosulfan, best known as an antagonist of GABA-gated chloride channels (B. Wightman and G. Garriga, personal communication; our unpublished data). This rescue is allele specific, and is not affected by mutants in GABA-ergic neurotransmission. We plan to use electrophysiology to determine whether this rescue is by direct channel block.

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120. *adc-1* MUTANTS FORAGE WHILE MOVING BACKWARDS

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We have identified a *C. elegans* gene encoding a putative L-aromatic amino acid decarboxylase (*adc-1*) similar to human and *Drosophila melanogaster* histidine and dopa decarboxylases. Histidine decarboxylase synthesizes histamine. Dopa decarboxylase is required for serotonin and dopamine synthesis. To analyze the function of *adc-1* we obtained three independent deletion mutations in the *C. elegans adc-1* gene (gifts from M. Dong and M. Koelle). These deletions disrupt the conserved catalytic domain and likely represent functional nulls. These *adc-1* mutations perturb coordination between the head and body during backward movement. Specifically, while wild-type worms move backwards in a smooth sinusoidal wave, *adc-1* mutants appear to forage while moving backwards (Fwb).

To identify *adc-1*-expressing cells we generated antibodies against bacterially-expressed ADC-1. These antibodies label several cells in wild-type worms. No staining is observed in *adc-1(n3420)* mutants. This antibody staining colocalizes with P_{adc-1}::GFP. We have identified two stained cells in the lateral ganglion as the RIM neurons. The RIMs receive sensory input from the amphid sensilla and make gap junctions with AVAR/L, the backward command neurons. RIMs synapse onto AVBR/L, the forward command neurons, and the RMDs, motor neurons that synapse onto head muscles and contribute to the control of foraging. RIMs also synapse directly onto head muscles. Given the position of the RIMs within the neural circuitry, we postulate that these neurons act to inhibit foraging during backward locomotion. In addition we see staining in a single neuron within the ventral ganglion, and in four cells near the vulva that may be either the gonadal uv1s or the uv2s. These cells have lateral nuclei that extend thin processes ventrally. Interestingly, the uv1s contain dense core vesicles suggesting they may act as neurosecretory cells (J. White, personal communication).

Although ADC-1 is similar to dopa decarboxylase, both dopamine and serotonin levels appear to be normal in *adc-1* mutants. Furthermore, worms defective in serotonin and dopamine biosynthesis are not Fwb. However, we found that *cat-1(e1111)* and *cat-1(n733)* animals are Fwb. *cat-1* encodes a vesicular monoamine transporter required for the transport of biogenic amines into synaptic vesicles. We suggest that it is the transport of a biogenic amine other than dopamine or serotonin that accounts for the Fwb phenotype of *cat-1* mutants. Interestingly, the biogenic amine histamine has been shown to compete with serotonin for transport by CAT-1 (Duerr et al., J. Neurosci.19: 72-84, 1999). ADC-1 may synthesize histamine, which is then transported by CAT-1.

To identify new genes that may function with *adc-1* to inhibit foraging during backwards locomotion we screened the F2 progeny of EMS-mutagenized worms for the Fwb phenotype. F2 worms were scored by touching each with an eyelash to initiate backing. Our pilot screen of 5,250 genomes yielded 17 mutants, including a new allele of *adc-1*. We are mapping the remaining mutants, dividing them into complementation groups and conducting a larger screen.

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121. Analysis of the Sex-Specific Death of the Male-Specific CEM Neurons

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Programmed cell death (PCD) is an important process during animal development and tissue homeostasis to clear away unnecessary or abnormal cells. One notable example is the sexually dimorphic process in which cells or organs that are needed only in one sex are eliminated in the opposite sex by apoptosis.

In *C. elegans* two sets of sex-specific neurons, four cephalic companion neurons (CEMs) and two hermaphrodite specific neurons (HSNs), undergo sexually dimorphic PCDs. Although all are born embryonically in both males and hermaphrodites, the CEMs survive in males and undergo PCD in hermaphrodites. Conversely, the HSNs survive in hermaphrodites but undergo PCD in males. The sex determination pathway has been studied extensively in *C. elegans* but very little is known about what controls the sex-specific PCDs of these two sets of neurons. We are interested in identifying genes that operate at the intersection of the sex determination and PCD pathways.

In *C. elegans* males the CEMs are located bilaterally between the two bulbs of the pharynx: two on the dorsal side and two on the ventral side. We have used a transgenic strain, *pha-1(e2123ts); him-5(e1490); syEx313* (kindly provided by Paul Sternberg), in which GFP is specifically expressed only in the CEMs and a few tail neurons in males, to screen for mutations that cause ectopic PCD of CEMs in males or survival of CEMs in hermaphrodites. We have screened 8500 haploid genomes and have thus far isolated four mutations that affect CEMs in males and nine mutations that affect CEMs in hermaphrodites. These include (1) three mutations in two genes required for all programmed cell deaths, *ced-3(sm120, sm144)* and *egl-1(sm157)*, (2) mutations (*sm117 and sm150*) in two genes that affect CEM cell fate determination, and (3) mutations (*sm119 , sm130,* and *sm146*) in three genes that specifically affect the life vs. death fate of the CEMs or HSNs.

We have focused on studying the third class of mutants (*sm119 , sm130,* and *sm146*). In *sm130* mutant animals, the dorsal CEMs but not the ventral CEMs undergo ectopic PCDs in males, which can be blocked by loss-of-function mutations in *ced-3*. *sm130* is thus a dorsal CEM-specific cell death mutant. Both *sm119* and *sm146* mutations cause partial reversal of CEM/HSN cell fates: ectopic deaths of HSNs and inappropriate survival of CEMs in hermaphrodites. *sm119* and *sm146* may define genes that control the life vs. death fates of both HSNs and CEMs. We are in the process of mapping and cloning these three genes.

Two mutations (*sm117* and *sm150*) in the second class are also of interest to us. In *sm117* mutants, 100% of males lose all four CEMs. This cannot be blocked by mutations in *ced-3*, indicating that the absence of the CEMs is not due to their ectopic PCD but rather to abnormal cell fate transformation. *sm117* turns out to be an allele of *unc-86*, which has been previously implicated in the cell fate determination of the HSNs but not the CEMs. *sm150* is another interesting mutation that not only causes survival of the CEMs in 58% of the hermaphrodites but also appears to cause sister cell transformations in several other cell lineages. Specifically, in *sm150* mutants several pharyngeal cells (e.g. I2 and MC cells) undergo ectopic PCD taking on cell fates that originally belong to their sister cells. Consistent with this hypothesis, in *sm150; ced-3* double mutants, the deaths of I2 and MC pharyngeal cells are prevented. We are in the process of mapping *sm150*.

In summary, we have identified several genes that specifically affect the life vs. death fate of the CEM neurons. Further characterization of these genes should help reveal how sexually dimorphic apoptosis is regulated and executed in nematodes and may shed light on the regulation of sexual dimorphic cell deaths in general.

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122. The *cfi-1* gene inhibits expression of CEM cell fate in other *C. elegans* neurons.

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We are interested in understanding cues that determine cell fate in *C. elegans*. Specifically, we aim to understand how cell fate determinants activate or inhibit programmed cell death (PCD). To this end we have been studying cues affecting differentiation and sex-specific survival of the male-specific CEM neurons. We showed that CEM death requires the cell death genes *egl-1*, *ced-3* and *ced-4* and is inhibited by the cell death gene *ced-9*. Mutations in *tra-2* and *tra-1*, which promote male identity in XX animals, prevent CEM death. We also isolated mutants in which CEM neurons expressing a *pkd-2*::GFP transgene (from M. Barr and P. Sternberg) survive inappropriately in hermaphrodites. From a screen of 9500 genomes we identified 25 independent mutants. 15 specifically affect survival of the CEMs and no other discernable aspect of development. Five contain mutations in general programmed cell death genes and three affect sex determination. The remaining two mutants fail to complement and result in the transformation of some head neurons (probably the IL2s) into CEM-like neurons. Transformed cells express CEM-specific markers, exhibit a nuclear morphology unique to the CEMs, and their cell bodies are sometimes located where CEMs' normally reside. We have named the gene affected in these mutants *cfi-1* (for CEM fate inhibitor). The CEMs die appropriately in these mutants, but ectopic CEM-like neurons survive in both sexes, suggesting that neuronal identity and survival are separable aspects of CEM fate.

To understand the molecular mechanism underlying *cfi-1* function we are cloning the gene. We have mapped the gene to a 0.5 map unit interval on chromosome I, and have obtained rescue using a single cosmid. Our results will be presented at the meeting.

To further understand determinants that regulate CEM neuronal identity we have been studying regulation of *pkd-2*::GFP expression in these cells and in CEM-like neurons generated in *cfi-1* mutants. CEM-like cells express *pkd-2*::GFP at higher levels in male than in hermaphrodite *cfi-1* mutants. Similarly, *pkd-2*::GFP expression in CEMs is stronger in male vs. hermaphrodite *ced-3* mutants. Thus, the sex determination pathway can impinge on CEM development at several levels; by regulating PCD and by regulating cell-type specific gene expression. Mutations in either *lin-32*, which encodes a bHLH transcription factor, or *unc-86*, a POU-domain transcription factor, strongly inhibit *pkd-2*::GFP expression in CEM-like cells in *cfi-1* hermaphrodites and in normal CEMs in males. These results suggest that *pkd-2*::GFP expression, and perhaps CEM neuronal identity, are promoted by the absence of *cfi-1* activity, presence of *lin-32* and *unc-86* activities, and male sexual identity.

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123. Dissection of DNA damage checkpoint regulation in C. elegans and cloning and initial characterisation of the novel rad-5/ clk-2 checkpoint gene

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We started to use the nematode worm *C. elegans* to study DNA damage-induced checkpoint responses. Our experimental system is based on our previous observation that DNA damage checkpoint responses can be analysed in the *C. elegans* germ line 2).

In contrast to the somatic tissues of the worm, the germ line continues to proliferate throughout life. In addition to giving rise to differentiated gametes a high proportion of germ cells is eliminated by programmed cell death 1). To determine whether germ cell death can be used as a model to study the mechanisms underlying DNA damage-induced apoptosis we determined the effects of genotoxic agents on germ cells. We found that germ cells, in contrast to somatic cells, respond to genotoxic stress by programmed cell death and by inducing a transient cell cycle arrest. These two responses are spatially separated. Mitotic germ cells respond by cell cycle arrest, whereas pachytene-stage meiotic germ cells undergo apoptosis. Interestingly, the integrity of meiotic recombination and chromosome pairing is also monitored by checkpoints that lead to the apoptotic demise of compromised cells. Checkpoint induced apoptosis needs the same, well-characterized apoptotic core pathway as utilized to execute somatic cell death in the worm 2).

To elucidate the pathways leading to radiation induced apoptosis we are following two experimental approaches. In a reverse genetic approach, to generalize findings obtained from yeast and other systems, we inactivated conserved DNA damage genes by RNAi based techniques. We show that *C. elegans* homologs of fission yeast *rad-1, rad-3, rad-9, hus-1, tel-1* are required for worm checkpoint signalling and are therefore likely to be universally used in metazoans. In addition, we also find a distant worm homolog of the mammalian p53 gene that is required for DNA damage induced apoptosis.

To define novel, potentially metazoan-specific checkpoint genes, we initiated a genetic screen for mutants defective in DNA damage-induced apoptosis. One previously uncharacterised checkpoint gene is *C. elegans rad-5*. We show that *rad-5* is evolutionarily conserved and that it is related to budding yeast *tel2*. Interestingly *rad-5 mn159* is allelic to the clock mutation *clk-2 qm37* suggesting multiple functions of *rad-5/clk-2*. Both *rad-5* and *clk-2* alleles are checkpoint defective and ts lethal. In addition, both alleles lead to a slight increase in telomere length.

1) Gumienny et al., 1999. Development 126, 1011-1022

2) Gartner et al., 2000. Mol. Cell 5, 435-443

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124. A Genetic Pathway Involved In Apoptotic DNA Degradation In Nematodes

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Programmed cell death (apoptosis) is a tightly regulated cell disassembly process, which includes shrinkage and fragmentation of both dying cells and their nuclei as well as fragmentation of chromosomal DNA into internucleosomal repeats, a biochemical hallmark of apoptosis. The execution of these systematic and orderly cell disassembly processes is regulated by apoptotic caspases, which trigger these events by cleaving critical protease targets, most of which remain to be identified. To identify genetic components that act downstream of, or in parallel to, the *C. elegans* caspase CED-3, we have designed and carried out a sensitized GFP-based genetic screen to isolate suppressors of a constitutively activated CED-3 mutant (Ledwich et al., 1999). Using this screen we have isolated 64 suppressor mutations which define at least 8 new cell death genes, which we named *cps* genes (CED-3 protease suppressors). Further genetic and phenotypic analyses of *cps* mutants indicate that loss-of-function mutations in *cps* genes result in either a partial suppression of programmed cell death (*cps-1, 2*) or a delay in the timing of programmed cell death (*cps-3* to *cps-8*).

We have focused on studying the possible role of the *cps* genes in the apoptotic DNA degradation pathway in *C. elegans*. Using the TUNEL assay which labels 3-OH DNA breaks generated during apoptosis (Wu et al., 2000), we found that mutations in four *cps*genes result in accumulation of TUNEL-positive staining in mutant embryos, indicating that these mutants are defective in resolving TUNEL-positive DNA ends. Further genetic analysis indicates that these four genes are likely to function in the same apoptotic DNA degradation pathway. We have characterized one of these genes, *cps-6*, in detail and have found that it encodes a specific *C. elegans* mitochondrial endonuclease, whose role in apoptosis appears to be evolutionarily conserved. CPS-6 is thus the first mitochondrial protein identified to be directly involved in programmed cell death in *C. elegans*, underscoring the conserved and important role of mitochondria in the execution of apoptosis. Our studies also provide the first genetic evidence that the apoptotic DNA fragmentation process is important for proper progression of apoptosis. We are now investigating how CPS-6 is released from mitochondria and activated during apoptosis to mediate the nuclear DNA fragmentation process. We are also examining how the activity of *cps-6* is regulated by other *cps* genes or by previously identified cell death genes. Finally, we are in the process of cloning three other *cps* genes in the same pathway to further understand their roles and functions in mediating apoptotic DNA degradation. These studies should reveal the molecular and biochemical mechanisms that regulate the apoptotic DNA fragmentation process in *C. elegans* and in general.

Ledwich, D., et al. (1999). Identification and Characterization of Downstream Targets of the Cell-Death Protease CED-3. 12th International *C. elegans* Meeting, 112.

Wu, Y. C., et al. (2000). NUC-1, a caenorhabditis elegans DNase II homolog, functions in an intermediate step of DNA degradation during apoptosis. Genes Dev *14*, 536-48.

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125. CED-12, A COMPONENT OF A RHO/RAC GTPASE SIGNALING PATHWAY, REGULATES CYTOSKELETAL REORGANIZATION AND CONTROLS CELL-CORPSE ENGULFMENT AND CELL MIGRATION IN *C. elegans*

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During animal development, cells undergoing programmed cell death, or apoptosis, are rapidly engulfed and degraded by neighboring cells. The engulfment of apoptotic cells by their neighbors is an evolutionarily conserved process. In *C. elegans*, mutations in genes that define two partially redundant pathways block the engulfment of cell corpses, causing cell corpses to persist abnormally. In one of these pathways, *ced-1*, *ced-6* and *ced-7* appear to act together to control cell-corpse recognition and to initiate phagocytosis. CED-1 is a transmembrane receptor similar to a mammalian scavenger receptor. CED-1 recognizes and clusters around cell-corpses to mediate their engulfment. *ced-7* encodes an ABC transporter that promotes the recognition of cell corpses by CED-1, possibly by exposing phospholipids on the outer surface of the cell corpses. *ced-6* encodes an adaptor-like protein that acts in engulfing cells. In the other engulfment pathway, *ced-2* CrkII, *ced-5* DOCK180, and *ced-10* Rac are part of a Rac GTPase signaling pathway proposed to mediate cytoskeletal reorganization.

We identified a new engulfment gene, *ced-12*, in a large-scale genetic screen for engulfment-defective mutants. Like *ced-2*, *ced-5*, and *ced-10*, *ced-12* functions in both cell-corpse engulfment and distal tip cell migration. Genetic interaction studies showed that *ced-12* acts in a pathway with *ced-2* CrkII, *ced-5* DOCK180, and *ced-10* Rac GTPase in cell-corpse engulfment. We cloned *ced-12* and found that it encodes a novel protein with a candidate SH3-binding motif. The CED-12 protein has both *Drosophila* and human counterparts. The *Drosophila* homolog *Dced-12* could partially replace the function of *ced-12* in *C. elegans*. CED-12 functions in engulfing cells to mediate cell-corpse engulfment. A CED-12::GFP fusion protein is localized to the cytoplasm in *C. elegans*. CED-12 interacts physically with CED-5, which contains an SH3 domain. When expressed in Swiss 3T3 fibroblast cells, CED-12 induced formation of filamentous actin structures in a manner dependent on the GTPase Rho. We propose that CED-12 is recruited to a CED-2/CED-5 protein complex through its interaction with CED-5 and regulates or effects Rho/Rac GTPase signaling, thereby leading to cytoskeletal reorganization by a mechanism that is evolutionarily conserved. We have performed a yeast two-hybrid screen for *C. elegans* proteins that interact with CED-12 and have identified a number of positive clones. Currently we are studying the functions of these potential CED-12 interacting proteins in the context of programmed cell death, cell-corpse engulfment, and cell migration.

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126. *mec-4(d)*-induced necrotic-like cell death in *C. elegans* requires calreticulin and regulators of ER-mediated Ca^{2+} release

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Identifying molecules critical for necrotic cell death is a major objective for therapeutic intervention with deleterious consequences of stroke, ischemia and degenerative disease. Unlike apoptotic cell death, little is understood of molecular mechanisms of necrosis. Our interest is in elaborating necrotic-like cell death mechanisms in *C. elegans*. A variety of different insults can initiate necrotic-like cell death in this nematode, leading to similar morphological changes in the affected cells. Necrosis-inducing genes include dominant alleles of the *mec-4* and *deg-1* degenerins which encode ion-channel subunits with similarity to mammalian epithelial Na^+-ion channels (ENaCs), a dominant allele of the *deg-3* acetylcholine receptor α-subunit, and a transgenicially expressed constitutively activated mutant of the G-protein subunit $G\alpha_S$. That channel hyperactivating mutations induce necrosis is reminiscent of the initiation of excitotoxic cell death in humans.

We study necrotic neurodegeneration with a focus on channel-hyperactivating mutations in the *mec-4* gene as the death-initiating event. The MEC-4 protein is expressed in the six touch neurons in *C. elegans*, where it normally participates in the formation of mechanically gated ion channels and is involved in the transduction of mechanical stimuli. The dominant *mec-4(d*) allele encodes a hyperactive channel that leads to necrotic demise of the touch receptor neurons. We established a behavioral assay that allowed us to isolate suppressor mutations that block *mec-4(d)*-induced neurodegeneration. Ectopic expression of *mec-4(d)* in the ventral nerve cord leads to the appearance of a number of swelling and degenerating cells visible as vacuoles during the larval stage L1. As a consequence of this cell death, worms are severely paralyzed. We mutagenized 45,000 haploid genomes and isolated 24 suppressors with reconstituted locomotion. Such strains harbor candidate suppressors of *mec-4(d)*-induced neurodegeneration.

One strong suppressor locus maps to chromosome V and is represented by 4 alleles. Mutations affecting this locus can also suppress defects associated with hyperactivated mutants of degenerins *unc-8*, *unc-105* and *deg-1*. Interestingly, strong alleles also strongly suppress paralysis consequent to expression of human $A\beta_{1\text{-}42}$ fragment in *C. elegans* muscle (see Link, 1995, PNAS 92: 9368). We observe modest suppressor effects on $G\alpha_S$-induced degeneration. We cloned this locus and found it to encode calreticulin. The strongest suppressor alleles are nonsense alleles that appear to be null (antibody staining performed by the lab of J. Ahnn, Kwangju Institute of Sci. and Tech., Korea). Calreticulin is a Ca^{2+} binding protein of the ER that plays critical roles in chaperone function and in maintenance of Ca^{2+} homeostasis. Both functions may contribute to suppression of neurodegeneration. Prompted by an interest in the contributions of Ca^{2+} release in neurotoxicity, we found that mutations in ER Ca^{2+} release channels *itr-1* (IP3 receptor) and *unc-68* (ryanodine receptor) also significantly suppress *mec-4(d)*-induced degeneration. Conversely, pharmacologically-induced release of ER Ca^{2+} in the *crt-1; mec-4(d)* mutant can bypass the *crt-1* block of cell death. Our data suggest release of ER Ca^{2+} stores is a critical step in necrotic cell death in *C. elegans*. Since ER Ca^{2+} release has been implicated in excitotoxic cell death, our data provides one of the first molecular indications that necrotic death mechanisms may be conserved from nematodes to humans.

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127. A Novel Pharmacogenetic Model for Parkinson's Disease: Dopamine Neurodegeneration in C. elegans

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Current animal models for idiopathic Parkinson's Disease rely on inducing nigrostriatal damage in mammals with the neurotoxin 6-OHDA or MPTP. The dopamine transporter DAT, which is the target for many psychoactive drugs, provides the cellular gateway for the accumulation of the neurotoxin that evokes neuronal death and Parkinson-like syndrome. We have previously cloned the C. elegans DAT (DAT-1), and have shown that it is functionally similar to mammalian DATs and expressed exclusively in the dopamine (DA) neurons (Jayanthi et al. 1998, Nass et al. 1999, 2000). We have also developed WT and dat-1 knockout (see abstract by Duerr et al.) transgenic lines which target a dat-1 promotor-gfp fusion to all 8 DA neurons in the hermaphrodite. Exposure of the reporter line to 6-OHDA results in DA neuronal blebbing, soma deformation, and loss of GFP expression. DA transport by DAT-1 is found to be blocked by 6-OHDA, antidepressants, and amphetamine in vitro. These latter agents block the effects of 6-OHDA on DA neurons, and a dat-1 deletion line is insensitive to the neurotoxin. Ultrastructural analysis of the worm DA neurons show significant signs of neurodegeneration including small, dark, and rounded cell bodies, as well as vacuolated and loss of neuronal processes following 6-OHDA exposure. Interestingly, although these phenotypes are characteristic of apoptosis both in mammals and worms, the effect appears to be independent of the classic apoptotic pathway, since the caspase-deficient ced-3 and ced-4 backgrounds still display the toxin-induced neuronal blebbing and loss of GFP expression. Finally, studies with DA deficient lines suggest that endogenous DA production plays a role in the neurotoxicity. These studies as well as our early progress on toxin-based genetic screens for regulators of DAT function, localization, and the toxin-mediated cell death will be presented.

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128. MPTP-based test system for Parkinson´s Disease in *C. elegans*

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The neurotoxin MPTP is known to cause Parkinson´s disease (PD)-like symptoms in vertebrates by selectively destroying dopaminergic neurons in the substantia nigra. MPTP-based animal models (mice, primates) are used to test pharmacologically active compounds for their therapeutic potential in the symptomatic and neuroprotective treatment of PD. These assays are usually expensive, time-consuming and not suitable for the screening of large compound libraries. Therefore, we have established a test system based on the rapidly developing and easily screenable nematode *C. elegans*. Incubation of animals with MPTP resulted in developmental as well as behavioural defects like reduced mobility, twitched coiling and retarded development. These phenotypes were strongly reduced by the treatment with L-DOPA, dopamin or other anti-PD drugs. Antioxidants also reduced the MPTP-induced phenotypes, indicating that the neurotoxin caused oxidative stress in *C. elegans* as in vertebrates. MPTP-treatment of various transgenic strains expressing GFP behind different neuronal promoters resulted in specific reduction of fluorescence in dopaminergic neurons, but not in serotonergic, sensoric, inter- or motoneurons, suggesting specific degeneration of dopaminergic neurons in our model. Our experiments have shown, that the effects of MPTP on *C. elegans* are very similar to those on vertebrates: behavioural and mobility phenotypes, specific degeneration of dopaminergic neurons, and rescue of the phenotypes by treatment with L-DOPA and other known anti-PD drugs. However, our *C. elegans*-based tests are much easier, faster and cost-effective to perform. We conclude that *C. elegans* is an excellent animal model for developing new drugs for the treatment of PD.

129. The Gln-Ala repeat transcriptional activator CA150 interacts with huntingtin: neuropathologic and genetic evidence for a role in Huntington's disease pathogenesis

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Huntington's disease (HD) is a neurodegenerative disease caused by polyglutamine (polyQ) expansion in the protein huntingtin (htt). Pathogenesis in HD appears to involve the formation of ubiquitinated neuronal intranuclear inclusions containing N-terminal mutated htt, abnormal protein interactions, and the aggregate sequestration of a variety of proteins (noticeably transcription factors). To identify novel htt-interacting proteins in a simple model system, we used a yeast two-hybrid screen with a Caenorhabditis elegans activation domain library (R. Barstead, ORMF, Oklahoma City, OK). We found a

predicted WW domain protein (ZK1127.9) that interacts with N-terminal fragments of htt in two-hybrid tests. A human homologue of ZK1127.9 is CA150, a transcriptional coactivator with a N-terminal insertion that contains an unperfect (Gln-Ala)38 tract encoded by a polymorphic repeat DNA. CA150 interacted in vitro with full length htt from lymphoblastoid cells. The immunohistochemical expression of CA150 was markedly increased in human HD brain tissue, in comparison to normal age-matched human brain tissue, and showed aggregate formation with colocalization to ubiquitin-positive aggregates. In 432 HD patients, the CA150 repeat length explains a small but statistically significant amount of the variability in HD onset age. Our data suggest that abnormal expression of CA150, as mediated by interaction with polyQ-expanded htt, may alter transcription, and have a role in HD pathogenesis. Our data illustrate the value of using C. elegans for the direct identifcation of novel biochemical and genetic modifiers of human neurodegenerative disease pathogenesis [Holbert et al. (2001) Proc. Natl. Acad. Sci. USA 98 1811-1816].

130. HEAT SHOCK TRANSCRIPTION FACTOR (HSF) ACTIVITY REGULATES THE APPEARANCE OF POLYGLUTAMINE PROTEIN AGGREGATES

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Disruption of protein folding homeostasis resulting in protein misfolding and aggregation is associated with many human diseases including Huntington’s disease where expansion of a polyglutamine (polyQ) tract to greater than 37-40 residues leads to the formation of protein aggregates, neuronal dysfunction and premature cell death. The expression of expanded chimeric polyglutamine (polyQ) -YFP proteins as extrachromosomal arrays in body wall muscle cells results in the formation of visible protein aggregates associated with a10-fold reduction in motility. Immunohistochemical studies of Q82-expressing animals reveal displacement of actin and myosin filaments. In contrast, neither visible aggregates nor altered motility were detected in animals expressing Q19 or Q29-YFP. Animals expressing an intermediate polyQ tract (Q40) exhibit striking polymorphism, as different muscle cells in the same animal contain soluble or aggregated polyQ. The severity of motility defect in Q40 animals corresponds to the proportion of cells with aggregates. Our laboratory and others have previously identified individual molecular chaperones as modulators of the aggregation phenotype. As chaperones are co-regulated during the heat shock response by HSF, we have examined whether altering the levels of HSF by RNAi and tissue-specific overexpression would impact the biochemical events that influence protein aggregation. Expression of a full length *Ce-hsf cDNA* from the *unc-54* promoter results in more rapid activation of an *hsp-16::lacZ* reporter in body wall muscle cells, confirming overexpression of functional HSF. In *unc-54::Q82-yfp; myo-2::Q82-gfp; unc-54::hsf* animals, we observe the appearance of soluble patches of fluorescence in body wall muscle, but not in the pharynx. However, expression of a

C-terminal HSF fragment that lacks DNA binding and trimerization domains has no effect on activation of the heat shock response or distribution of Q82-YFP. Injection of dsRNA transcribed from a full-length *hsf* cDNA inhibits activation of the heat shock response in heat shocked *hsp-16::lacZ* animals, confirming inactivation of HSF by RNAi. Inhibition of HSF in Q19 or Q29 animals leads to sporadic formation of aggregates similar to those observed in Q82 strains. Consistent with the transient nature of RNAi phenotypes, F1 progeny of injected animals were affected, but F2 animals exhibit normal activation of the heat shock response and distribution of Q19. Normal activation of the heat shock response and distribution of Q19 in animals injected with *unc-22* dsRNA demonstrated the specificity of *hsf* RNAi effects. These data suggest that Q19 and Q29 solubility is maintained by an active process that requires HSF activity. The heat shock response is considered an acute response to changes in environmental conditions, and the requirement for HSF activity to maintain Q19 and Q29 solubility in the absence of stress reveals a novel role for HSF. We are currently testing whether manipulations of HSF levels that influence aggregate formation are associated with altered motility.

131. Identification of C. elegans proteins that directly interact with the human beta amyloid peptide.

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Although the beta amyloid peptide (Abeta) has been implicated as causal in the etiology of Alzheimer's disease (AD), the mechanism of its cell toxicity has not been established. Constitutive or inducible expression of Abeta in C. elegans muscle leads to paralysis. To gain insight into the molecular mechanisms underlying this toxicity, we have sought to identify proteins that directly interact with Abeta in vivo, using a novel co-immunoprecipitation protocol. These proteins may be involved in transducing or ameliorating Abeta toxicity. For our initial experiments, we have investigated whether HSP16 directly associates with Abeta. HSP16 is specifically induced in transgenic animals with constitutive or induced Abeta expression, and closely co-localizes with intracellular Abeta in muscle or neurons as assayed by immunohistochemistry. (The human homologs of HSP16, HSP27 and alpha-B-crystallin, have also been reported to be upregulated in AD brains.) Immunoblot analysis of preparations from transgenic animals demonstrates that HSP16-2 co-immunoprecipitates with Abeta, and Abeta co-immunoprecipitates with HSP-16-2. Immunoprecipitation of synthetic Abeta added to heat-shocked wild type animal lysate also co-immunoprecipitates HSP16. Fractionation of immunoprecipitates from transgenic animals on one dimensional polyacrylamide gradient gels reveals >10 protein bands not observed in control immunoprecipitations. Several of these proteins have been identified by MALDI-TOF mass spectrometry combined with database searching. We are currently trying to establish a role for these proteins in Abeta toxicity by RNAi experiments. Co-immunoprecipitation followed by mass spectrometry may be an effective general approach for identifying interacting proteins in C. elegans

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132. *zag-1*, a Zn finger-homeodomain gene needed for axon guidance

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Migrations of cells and growth cones along the dorsoventral and anteroposterior body axes establish the pattern of the *C. elegans* nervous system. We are undertaking several screens for axon guidance mutants to identify genes needed specifically for longitudinal axon growth as well as genes required generally for all axon extension and pathfinding.

PVQ is a pair of bilaterally symmetric neurons located in the tail and each extends a single axon that enters the ventral nerve cord and continues to the nerve ring in the head. PVQ can be visualized in living animals using a *sra-6::gfp* transgenic strain and fluorescence microscopy. To identify mutants defective in the growth of the PVQ axons, we treated *sra-6::gfp* animals with either the mutagen EMS or ENU, isolated behavioral and morphological mutants in the F2 generation, and then examined their progeny for axon growth defects. To date, we have recovered over ninety mutants with axon outgrowth and pathfinding defects. While some mutations cause the PVQ axons to terminate their growth prior to reaching the nerve ring, most cause both axon outgrowth and pathfinding defects, such as the inappropriate extension along the dorsal nerve cord and lateral midline or across the ventral midline. We found over thirty mutants with defects in either cell fate, cell migration, cell position or programmed cell death. We also performed a screen to identify genes needed for the growth of the AVA, AVB and AVD axons. The roughly 150 mutations found in these two screens define over forty genes, including around twenty-five previously known genes. We have begun the molecular analyses of several new genes discovered in our screens (see below and abstract by Yam & Clark).

zag-1 mutations cause axons to defasciculate and branch inappropriately as well as neurons to extend ectopic axons. Both *zag-1* alleles are nonsense mutations, suggesting that they greatly reduce or eliminate *zag-1* function. We cloned *zag-1* and found that it encodes a protein containing multiple zinc finger domains and a homeodomain (F28F9.1). ZAG-1 is similar in sequence and structure to the products of the *Drosophila zfh-1* gene, which is expressed in mesoderm and the CNS and functions in mesoderm differentiation, and the vertebrate ZEB genes. Like these proteins, ZAG-1 likely acts as a transcriptional repressor, as it possesses a conserved sequence motif needed for association with the CtBP corepressor protein. A transcriptional *zag-1::gfp* transgene is expressed in both neuronal and nonneuronal cells. *zag-1::gfp* expression is observed in ventral cord neurons in a *zag-1* mutant yet not in wild type, indicating that *zag-1* regulates, either directly or indirectly, its own expression in some neurons. Our results suggest that *zag-1* regulates the expression of genes involved in axon formation and guidance. We are currently investigating how *zag-1* activity is regulated as well as the genes that act downstream of *zag-1*.

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133. The homeodomain protein CePHOX2/CEH-17 controls antero-posterior axonal outgrowth

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The paired-like homeobox genes *Phox2a* and *Phox2b* are involved in neuronal subtype specification in the mouse (1,2). The *Phox2* orthologue in *C. elegans*, called *ceh-17*, encodes a protein with 88% identity in the homeodomain to murine Phox2a and Phox2b (3). CEH-17 is expressed in five head neurons, ALA and the 4 SIAs, which project axons towards the tail along the lateral and sublateral cords respectively. Expression starts at the late proliferative stage, and is strongest during axonal elongation from the comma to the three-fold stage. The function of these five neurons is still unknown, and their synaptic connectivity incompletely resolved.

Abrogation of *ceh-17* function disrupts posterior axonal elongation of both ALA and SIA axons beyond the mid-body region. A fully penetrant stalling phenotype was observed for the ALA axons with more than 80% of them stopping within one body diameter of the gonad primordium. Conversely, ectopic expression of *ceh-17* in the mechanoreceptors, using the *mec-3* promoter, leads to exaggerated longitudinal axonal outgrowth. The PLM axons, instead of stopping at the level of the ALM cell body, reached a position anterior to the AVM cell body, and were occasionally more than twice their normal length, extending as far as the nerve ring. This ectopic outgrowth did not fasciculate with the sublateral ventral or the lateral cords, but pioneered its own extension. As to the AVM axon, most often its exaggerated anteriorward progress was stopped only when it reached the nose, whereupon it frequently turned back and grew posteriorly. Another phenotype was the growth of a posteriorly directed axon from the ALM cell body. We also ectopically expressed *ceh-17* using the *glr-1* promoter, which directs expression in several head and tail neuronal classes. In *pglr-1::ceh-17* animals, an unidentified *glr-1* + head neuron was seen projecting abnormally beyond the nerve ring towards the head, sometimes as far as the nose. In addition, PVQ acquired a posterior projection never seen in wild type. Altogether these gain-of-function data show that ectopic *ceh-17* is sufficient to induce cell-autonomous excessive longitudinal axonal outgrowth, both anteriorly and posteriorly, in many neurons.

A hypothesis, compatible with both gain and loss-of-function phenotypes is a role of *ceh-17* in blinding the growth cone to a stop signal. Thus, in the absence of *ceh-17*, an otherwise cryptic signal from the mid-body region would induce the ALA and SIA growth cones to stop. Conversely, in the presence of *ceh-17*, the mechanoreceptors would ignore the stop signal(s) which normally locate the tip of their axons at precise positions on the antero-posterior axis. It is conceivable that, whatever the signal and its source, there is a generic stalling process at play inside every neuron -possibly related to growth cone collapse- that *ceh-17* impinges on.

We are currently screening for suppressors of the *ceh-17* gain-of-function in *glr-1* neurons to identify co-factors and targets of CEH-17. The screen is based on the recovery of wild type movement in *pglr-1::ceh-17* worms which paradoxically go backward after a tap on the tail.

(1) Morin et al. 97, Neuron, 18, 411-423 (2) Pattyn et al. 99, Nature, 399, 366-370 (3) Pujol et al. 00, Development, 127, 3361-3371.

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134. A possible repulsive role for FGF signaling in CAN migration

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Fibroblast growth factor (FGF) can act as an important guidance cue in attracting migrating cells to their appropriate destination. In *Drosophila*, FGF signaling directs cell migration during tracheal branching, while in *C. elegans*, FGF guides the migration of the sex myoblasts to the gonad. We have found that mutations in the *C. elegans* FGF homolog *egl-17* and the FGF receptor *egl-15* also influence the migration of the canal-associated neurons (CANs) from the head to the mid-body region, but only in a sensitized background. *egl-17* and *egl-15* mutants enhance the CAN migration defects of a *vab-8* mutant. This enhancement suggests a role for the FGF pathway in CAN migration.

In order to determine if FGF provides instructional or permissive information for CAN migration, we expressed a constitutively active form of *egl-15* in a *soc-2* mutant background and a *soc-2*; *vab-8* mutant background. The *soc-2* mutation does not affect CAN cell migration but rescues the lethality associated with constitutive EGL-15 activity. Expression of a constitutively active *egl-15* construct in a *vab-8* background enhances *vab-8* CAN migration defects to a similar extent as an *egl-15* loss-of-function mutation. This result is consistent with EGL-15 activity providing direction to CAN migration, since constitutively active EGL-15 could mask directional information provided by regulated EGL-15, possibly its asymmetric activation. Thus, the FGF pathway can likely act as a guidance mechanism for migrating CANs.

To test if EGL-17 can act as an attractant or repellent to direct CAN migration, we misexpressed *egl-17* in the head and mid-posterior region of *C. elegans*. Expression of *egl-17* in the head from the *lim-4* promoter partially rescues the defects of *vab-8; egl-17* double mutants. In contrast, expression of *egl-17* in the mid-posterior region from the *mab-5* promoter enhances CAN migration defects of a *vab-8* mutant. These results suggest that *egl-17* can act as a repellent to direct CAN migration. The observation that an *egl-17* promoter fused to GFP expresses in only two head cells during the time of CAN migration is also consistent with the model of *egl-17* repelling the CANs.

Our results implicate FGF in a new role as a repellent for migrating cells. Several molecules have been shown to act as both attractants and repellents, and our results suggest that FGF should be added to this growing list.

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135. Does UNC-119 mediate collagen-based axon guidance signal?

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UNC-119 is crucial for the correct development of the nematode nervous system. Worms mutant for the neuronally-expressed *unc-119* gene exhibit behavioral abnormalities that are explained by structural defects resulting from aberrant nervous system development.

Unc-119 mutants have a variety of neurite outgrowth defects including both ventral and dorsal elongation defects within the nerve ring. Examination of young animals suggests that fully-elongated but misguided axons are initially extended but are then retracted when their normal synaptic partners are not contacted. Retraction is followed by supernumerary branch formation. A comparison of the nerve ring defects in several other mutants places the *unc-119* phenotype in a unique category.

Defects in the anteroposterior position of commissures suggests a polarity defect in sprouting of the initial axon. However, there are no circumferential pathfinding defects in motor neurons, similar to those seen in *unc-6* , *unc-5* or *unc-40* mutants. Indeed, although some cell bodies are misplaced in *unc-119* mutants, their axons are often correctly targeted, implying that turning at choice points is somewhat independent of other pathfinding mechanisms.

Localized expression of an UNC-119::GFP fusion protein in neural subsets rescues the neural defects only in the cells in which it is expressed. Ectopic expression in muscles does not rescue any structural or behavioral defects. Thus UNC-119 acts cell-autonomously. The rat homologue, RRG4, has been shown to be associated with synaptic vesicles of photoreceptor ribbon synapses. We have raised antibodies to amino, middle and carboxyl portions of UNC-119 and will describe localization of the protein in the worm.

Yeast two-hybrid experiments suggest that UNC-119 is involved in a novel neurite development signaling pathway that mediates interaction between a basement membrane collagen (LET-2) and an uncharacterized zinc-finger protein . We suspect this interaction may be mediated by the NC1 (non-collagenous) domain of LET-2. This domain has 50% identity and 75% similarity to the human angiogenic inhibitors/tumor suppressors arresten, canstatin and tumstatin but no receptors are yet known. A domain analysis of the UNC-119/LET-2 interaction will be presented.

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136. Genetic Analysis of the Kallmann Syndrome Protein Ortholog *Cekal-1 in C. elegans*

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Kallmann Syndrome is a hereditary disease that exists in both X-linked and autosomal forms and is characterized by anosmia (inability to smell) and infertility. Additionally, affected patients display kidney malformations or even lack one kidney. Detailed analyses of the anosmic phenotype showed a specific defect in olfactory axons that fail to make connections with their interneuronal targets while their pathfinding seems unaffected. For the X-linked form a gene called *Kal-1* was identified (1,2) that codes for a secreted protein containing FNIII modules and a protease inhibitor domain. However, the genetic lesions responsible for the autosomal forms, that could reside e.g. in a putative receptor gene, remain elusive.

We identified *Cekal-1*, an ortholog of the human Kallmann Syndrome gene *Kal-1*, in *C. elegans*. CeKAL-1 shows a high structural homology to human Kal-1 and is expressed in subsets of neurons in *C. elegans* as well as in non-neuronal tissues e.g. the excretory cell. To identify loci that genetically interact with *Cekal-1* we employed a two step approach. We first generated a gain-of-function (*gf*) phenotype by over/misexpressing the *Cekal-1* cDNA and then searched for mutations that modified this phenotype by both testing candidate mutations and performing a genetic screen.

When we overexpressed *Cekal-1* in AIY, a pair of interneurons, in a strain carrying a GFP reporter that labeled these neurons, 100% of the animals displayed ectopic neurites originating from the cell body and/or the axons of AIY. We tested whether these neurites were specific for *Cekal-1* by expressing a truncated form of CeKAL-1 in AIY. This, as well as expression of related proteins had no effect in AIY interneurons suggesting the phenotype in AIY to be specific for expression of CeKAL-1.Mis/overexpression using a pan-neuronal promoter also leads to visible neuroanatomical defects in many but not all neurons (see poster by Berry *et al.*).

The AIY specific neurite outgrowth defect could not be modified by mutations in genes required for axonal pathfinding including *unc-6, unc-5, unc-40, unc-73, sax-3, vab-1,* and *ina-1* indicating that these genes do not have a role in the generation of the observed neurites. However, some *exc* genes that had previously been identified in a screen for animals with defects in the excretory canal (3) efficiently suppressed our *gf* phenotype. This is insofar of significance as the excretory canal is believed to be the functional equivalent of the mammalian kidney. Thus, the biological function of the Kallmann Syndrome protein in neuronal guidance as well as development of kidney-like structures could be conserved throughout evolution

To identify additional interacting loci we performed a suppressor/modifier screen of the *gf* phenotype. In a small screen we identified at least 6 mutants (*ot16-ot20, ot24*) in 4 complementation groups that efficiently suppressed the sprouting phenotype to various degrees between 25-80%. Interestingly we also found one mutant (*ot21*) that qualitatively enhanced the axonal sprouting phenotype. We assessed the specificity of the modifier mutations by constructing double mutants of these mutations with *ttx-3(ks5*) or *sax-2(ot10),* both mutants that lead to neurite outgrowth phenotypes in AIY or sensory neurons. Our modifier mutations did not modify these phenotypes suggesting specific modification of the *Cekal-1* dependent neurites.

We have started to characterize the isolated modifier mutations molecularly. Using a combination of 3-factor mapping, deficiencies and single nucleotide polymorphisms we were able to map two of the strong suppressor mutations *ot16III* and *ot17X* to a small intervals. *ot16III* maps to a six cosmid interval between *unc-79* and *emb-5* on LGIII and *ot17X* to seven cosmids between *lon-2* and *unc-97* on LGX. We are currently attempting tranformation rescue by injecting cosmids of the respective regions.

1. Franco B, Guiloli S, Pragliola A, *et al.* (1991) *Nature*, 353:529-536
2. Legouis R, Hardelin JP, *et al.* (1991) *Cell*, 67:423-435
3. Buechner *et al.* (1999) Dev. Biol. 214:227-241

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137. A conserved interaction between integrins and a GCK kinase required for axonal navigation in *C elegans*

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Integrins are the main transmembrane receptors for extracellular matrix (ECM) molecules. They have a crucial function by mediating the signalling originating from the ECM as well as by participating to the dynamics of the cytoskeleton during cell adhesion and migration. To gain insight into the mechanisms of integrin signalling *in vivo*, our aim is to identify and characterise cytoplasmic molecules that interact with integrin cytoplasmic domains, and may thus be part of the same pathways. Although our main interest is to analyse integrin function during mouse development, we will illustrate below that *C. elegans* can be a very useful test-tube to understand the function of vertebrate proteins. Using a mouse embryo (E9.5-E12.5) cDNA library in a two-hybrid screen, we have identified a serine-threonine kinase of the GCK family as a putative partner for the cytoplasmic domain of the integrin-beta1 chain. By *in situ* hybridization of E12.5 and E14.5 mouse embryos, we have shown that this kinase is expressed in several tissues, particularly in the nervous system. A myc-tagged fusion of the ORF is found colocalized with actin-containing structures in stable NIH-3T3 transfected cells. To assess the functional significance of the interaction *in vivo*, we turned to *C. elegans*, which possesses a unique beta-integrin chain, PAT-3, and a well-conserved homologue of this GCK kinase, MIG-15. PAT-3 can form heterodimers with two alpha-integrin chains, PAT-2 and INA-1: PAT-2 is involved in muscle anchoring, while INA-1 is required for axon fasciculation and neuronal migration. Although a full description of *mig-15* has not been published yet, previous abstracts from the Hedgecock lab suggested that it is required for hypodermal development, excretory canal outgrowth, Q neuroblast migration and muscle arm targeting. First, using a two-hybrid assay we have shown that an interaction also occurs between PAT-3 and MIG-15. Using RNA interference (RNAi) against *mig-15*, we did not observe a Pat phenotype, such as the one reported for *pat-3* and *pat-2*, nor Q migration or excretory canal defects, such as those reported for *mig-15* alleles. However, we observed a weak kinker Unc phenotype, reminiscent of certain neuronal Uncs. To characterise further this phenotype, we performed RNAi in a strain carrying a GFP expressed in GABAergic neurons and axons (*unc-47::GFP*). We observed axon defasciculation defects (as in *ina-1* mutants) and premature branching of commissures (present at a low penetrance in *ina-1* mutants), often at a sub-dorsal position when commisssures should find their ways between muscles. To determine if *ina-1/pat-3* and *mig-15* act in the same pathway, we performed RNAi into the weak *ina-1(gm119)* background and observed a very significant aggravation of the commissural outgrowth defects. A *mig-15(full-length)::gfp* construct showed that *mig-15* is present in most tissues at different levels, including in the ventral nerve cord, muscles, vulva and the epidermis, raising the possibility that it might not act cell-autonomously. To address this issue, we specifically induced RNAi against *mig-15* under the control of a neuronal (*unc-25*), muscle (*unc-54*) or hypodermal (*lin-26*) promoter and observed commissural outgrowth defects only with the *unc-25* promoter, arguing for cell-autonomy. Using the same strategy, we showed that *unc-25::pat-3(RNAi)* leads to the same axon navigation defects. This provides the first evidence for a conserved interaction between beta-integrins and a GCK kinase likely involved in axonal navigation. We are pursuing this work by the identification of signals that could link the cytoskeleton to the MIG-15 kinase.

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138. Interacting proteins with UNC-51, UNC-14 or UNC-33

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unc-51, *unc-14* and *unc-33* genes are required for axonal elongation, fasciculation and guidance of many neurons. Previously, we reported that *unc-51* encodes a novel serine / threonine kinase and that UNC-51 directly interacts with a novel protein UNC-14. On the other hand, we isolated CRMP-62 (colapsin response mediator protein) and found that the protein shares homology with UNC-33. These genes are expressed in many neurons, suggesting that these molecules regulate axon guidance in neurons. However, their functions are largely unknown.

In order to analyze their functions, we newly identified interacting proteins with UNC-51, UNC-14 or UNC-33 by using a yeast two hybrid system. In this screening, UNC-73 (a Trio homolog) and VAB-8 (a kinesin like protein) were identified as UNC-51 interacting proteins. A *C. elegans* homolog of filamin 1 (FLN1) was identified as a UNC-33 interacting protein. UNC-73 is a GEF (guanine nucleotide exchange factor) and is required for axon guidance and cell migrations. The GEF domain activates Rac GTPase and stimulates actin polymerization. We examined genetic interaction between *unc-51* and *unc-73* on circumferential axonal defects of GABAergic DD/VD neurons. We found that *e936* (a hypomorphic allele of *unc-73*) strongly enhanced the defects of *e369* (a strong allele of *unc-51*), suggesting that these molecules function at the same signaling pathway, probably for regulation of actin polymerization. The defects of *e369; e936* double mutants were severer than *gm40* (a null allele of *unc-73*), suggesting that UNC-51 regulates other pathways as well. In *unc-51* mutants, abnormally large varicosities are observed in presynaptic axons of ASI (Crump et al., Neuron, 29, 115-129, 2001). We examined presynaptic morphology of the ASI in *unc-14(e57)* and *unc-73(e936)* mutants, and found that, at low penetrance, similar large varicosities were also observed in *unc-14(e57)* mutants. We think that UNC-14, in corporation with UNC-51, regulates presynaptic formation as well. We did not detect such large varicosities in *unc-73(e936)* mutants. UNC-73 may not cooperate with UNC-51 on presynaptic formation. Analysises of other interacting proteins are in progress. We will discuss possible mechanisms and functions of these molecules on regulation of axon guidance and presynaptic formation.

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139. VAB-8 AND UNC-51 PROTEIN INTERACTIONS MEDIATE AXON OUTGROWTH

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In *C. elegans* most posteriorly directed cell and growth cone migrations require *vab-8*, a gene that encodes at least two protein products known as VAB-8L and VAB-8S. VAB-8L is a 1066 amino acid protein that contains an N-terminal kinesin-like motor domain and functions in *vab-8*-dependent growth cone migrations. VAB-8S is colinear with the C-terminal half of VAB-8L, and lacks the kinesin-like motor domain. VAB-8S is necessary for certain *vab-8*-dependent cell migrations.

To identify VAB-8-interacting proteins, we conducted a yeast two-hybrid screen using full length VAB-8L as bait. One protein identified was UNC-51, a serine/threonine kinase required for proper axon outgrowth (Ogura et al., 1994). UNC-51 was found to interact with another novel protein, UNC-14 (Ogura et al. 1997). We have found that both VAB-8 and UNC-14 bind the C-terminal 100 amino acids of UNC-51.

Several observations suggest that VAB-8, UNC-14 and UNC-51 also interact in *C. elegans*. First, *vab-8, unc-14* and *unc-51* mutants display axon outgrowth defects. Second, all three genes are expressed in neurons that require *vab-8* function. We have determined that *vab-8* and *unc-51* act cell autonomously for CAN axon migrations. Finally, misexpression of the UNC-51-binding domain of VAB-8L under control of the *ceh-23* promoter results in a highly penetrant CAN posterior growth cone migration defect, a *vab-8* phenotype, presumably by interfering with UNC-51 binding with wildtype VAB-8L. Misexpression of the VAB-8-binding domain of UNC-51 also produced CAN axon outgrowth defects, however, the defects extended to both anterior and posterior migrations. We are in the process of determining whether the misexpression phenotype can be suppressed by simultaneous misexpression of both protein fragments.

The protein interactions and similar mutant phenotypes suggest that VAB-8 and UNC-51 act in the same pathway. Overexpression of *vab-8* suppressed the posterior axon outgrowth defects of an *unc-51* mutant, indicating a positive regulatory relationship between the two proteins. Overexpression of *unc-51* in the CAN caused its axons to terminate prematurely (the loss-of-function phenotype) and to branch (a novel phenotype). We will test whether *vab-8* mutations can suppress this branching phenotype. Suppression would suggest that *vab-8* acts downstream of *unc-51*. We are also taking biochemical approaches to determine the order of VAB-8 and UNC-51 action. We are testing whether UNC-51 can phosphorylate VAB-8 or whether VAB-8 can regulate UNC-51 autophosphorylation.

Ogura et al. (1994) *Genes & Dev.* 8: 2389-2400.

Ogura et al. (1997) *Genes & Dev.* 11: 1801-1811.

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140. A genetic network involving unc-53, unc-5, unc-71, unc-51, unc-14, apr-1 and bg7 is controlling the dorsoventral outgrowth of the excretory canals.

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The excretory canals reflect the migration of their growth cones during development: first the cell sends out two branches dorsally, which, when reaching the lateral midline, split to form an anterior and a posterior branch, which first pass the region of the gonad primordium and grow then further into the tail.

Previous genetic analysis of single and multiple mutants showed that *unc-53*, *unc-5* and *unc-71* represent genes of different pathways that interact in the dorsoventral growth cone steering of the excretory canals. Mutations in the three genes strongly enhance each other: whereas in *unc-53(n152)* less than 5% of the animals show ectopic ventral canals and in *unc-71(e541)* and *unc-5(e53)* there are 22% and 35% of them, respectively, we find up to 94% of ventral canals in the triple mutant strain. Based on this enhancement we designed forward genetic screens in the *unc-53* background to find mutants identifying new genes in the other two pathways.

As an alternative approach to identify components of these genetic pathways we performed a Yeast-Two-Hybrid screen for UNC-5 interactors and functionally analyzed the genetic interactions of the positives with *unc-53* and *unc-71*. We expect an enhancement comparable to that by *unc-5* (see above). We found 10 positives, all of which were unknown to be involved in the pathway previously. Here we focus primarily on *apr-1* and on *unc-14*, together with its direct interactor *unc-51* (Ogura et al., 1997). The phenotypes of the doubles of *unc-14* and *unc-51* mutants with *unc-53* and *unc-71* confirmed the positions of the former in the netrin pathway downstream of *unc-5*.

During the migration of a growth cone, the extracellular environment is explored by filopodia extended in three dimensions. The filopodium encountering the most favorable environment is then stabilized, a process regulated by microtubules (Bentley and O'Connor, 1994). APR-1 and UNC-53 are the only known proteins to be located at the microtubule plus ends, this could be the point where the directional information from different pathways converges to the final decision the cell takes to stabilize a growth cone in a specific direction.

Two of the mutants isolated in the genetic screen are new alleles of *unc-71*, thus validating the screen. One other mutant, *bg7*, strongly enhances the ventral excretory canal phenotype of *unc-53* and moreover on itself it shows not only ectopic ventral canals but also ectopic dorsal canals. This mutant points towards another mechanism controlling dorsalward migration.

Our genetic analysis reveals that the apparently simple dorsalward migration of the excretory canals is in fact a complex decision involving an extended genetic network, composed by partially redundant genes and pathways. The identification of UNC-5 interactors on the other hand gives a first insight in the genes acting in the pathway downstream of this netrin receptor.

Ogura,K., Shirakawa, M., Barnes, T.M. , Hekimi, S., Ohshima, Y., Genes Devel., 11(1997), 1801-1811

Bentley, D. and O'Connor, T.P., Curr. Opin. Neurob. 4(1994), 43-48

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141. MAX-1, a novel conserved protein, may function in UNC-5 mediated axon repulsion

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Axon guidance uses mechanisms that are conserved from worms to mammals. The laminin-related molecule UNC-6/Netrin and its receptor UNC-5 have been shown to mediate axon repulsion. It is currently not known what the signaling events downstream of UNC-5 are. We report here a novel, multi-domain containing, conserved protein, MAX-1 (motor axon guidance) that appears to function to modulate and transduce UNC-5 signal.

In wild type worms, the ventral cord GABAergic DD and VD neurons grow circumferential commissures to connect the dorsal and ventral nerve processes. This dorsal directed growth cone movement require the UNC-5 receptor and the UNC-6/Netrin. Loss of function mutations in *max-1* cause fully penetrant but variable defects in DD and VD commissural guidance. On average, 20% of the DD and VD commissures stop short or wander. Weak alleles of *unc-5* and *unc-6* strongly enhance Max-1 phenotypes, and null alleles of *unc-5* and *unc-6* act as dominant enhancers of *max-1*. In contrast, *unc-40* mutations, which affect another receptor of UNC-6, do not dominantly enhance Max-1 phenotypes. The genetic interactions suggest that MAX-1 functions in UNC-5 mediated axon repulsion.

max-1 encodes a novel 1099 amino acid protein that has homologs in fly and human. MAX-1 has two PH, one Myth4 and one FERM domains. PH domains are known to interact with lipid membrane; FERM domains are also membrane-anchoring domains, originally found in Band4.1 protein and Ezrin/Radixin/Moesin (ERM) proteins and can interact with PDZ domains and the cytoplasmic tails of several transmembrane proteins. The functions of Myth4 domains are largely unknown. MAX-1 functions in motor neurons and is localized to neuronal processes. Based on its mutant phenotypes, genetic interactions and protein domain structure, we propose that MAX-1 may modulate UNC-5 activity by binding to UNC-5 and may also act as a scaffold to transduce UNC-5 signals in axon repulsion.

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142. A Constitutively Active UNC-40/DCC Reveals Downstream Signaling Components

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UNC-6/Netrin attracts and repels circumferentially migrating axons in *C. elegans.* UNC-6/Netrin is expressed ventrally and mediates attraction via the receptor UNC-40/DCC. Ventral migration of numerous axons including the AVM touch cell axon is disrupted in *unc-6* and *unc-40* mutants. In order to identify signaling events subsequent to UNC-40 activation, we have attempted to generate a constitutively active UNC-40 transgene by replacing the extracellular and transmembrane domains with a myristoylation signal. When this transgene, designated MYR::UNC-40, is expressed by the *mec-7* promoter, a variety of phenotypes were induced in the touch cells. These include exuberant axon outgrowth and branching, defective axon guidance, and lamellipodial extensions from cell bodies. These effects are independent of endogenous UNC-6 and UNC-40. Similar phenotypes were also observed when MYR::UNC-40 was expressed in other neurons that utilize UNC-40 in axon guidance. Thus we believe MYR::UNC-40 represents a ligand-independent, gain-of-function receptor.

We have identified several suppressors of MYR::UNC-40 that may represent components of the UNC-40 signaling pathway. The suppressors include loss-of-function alleles of *unc-115* (an actin-binding protein with LIM domains), *unc-34*/Enabled, and *ced-10*/Rac. These three genes have all been implicated in signaling to the actin cytoskeleton. Loss-of-function mutations in all three genes can affect axon guidance, including ventral migration of the AVM touch cell axon. In *unc-40; unc-115* double mutants, the AVM axon guidance defect is not enhanced compared to single mutants, further suggesting that UNC-115 functions in the endogenous UNC-40 pathway. The fact that UNC-34/Enabled may be downstream of UNC-40/DCC is particularly interesting given that Enabled may also be downstream of the SAX-3/Robo receptor, which mediates axon repulsion. This result suggests a possible bifunctional role for UNC-34/Enabled in signaling cascades that both attract and repel axons.

Biochemical and cell biological experiments are currently underway to determine whether UNC-40 physically associates with UNC-115, UNC-34, or CED-10. Structure/function experiments are also being conducted to determine which residues of the UNC-40 cytoplasmic domain are responsible for signaling to its effectors. We are also searching for new components of the UNC-40 signaling pathway by screening for additional suppressors of MYR::UNC-40. One additional mutant of interest is *ky612*, which was identified in a screen for mutants with HSN axon guidance defects. *ky612* is a recessive mutant that phenocopies UNC-40 gain-of-function in HSN. Molecular identification of *ky612* may provide insights into inhibitory components of the UNC-40 signaling pathway.

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143. Roles of *unc-34* and *unc-40* in repellent guidance by *sax-3*

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Attractive and repulsive guidance receptors play key roles in the development of the nervous system by directing growing axons to their proper targets. The signaling mechanisms by which these receptors communicate with the growth cone cytoskeleton are not well understood. We are studying mechanisms of axon repulsion mediated by *sax-3*, a member of the Robo family of guidance receptors. *sax-3* mutants have defects in anterior and posterior cell migrations, in the formation of the nerve ring in the head, and in ventral axon guidance in the body. The *slt-1* gene, a homolog of *Drosophila* and vertebrate Slit, encodes one probable ligand for *sax-3* in several of these guidance decisions.

Members of the Enabled/VASP family of proteins have been shown to be important modulators of actin dynamics and are potential downstream effectors of axon guidance receptors. We find that the *C. elegans* Enabled protein UNC-34 is a mediator of SAX-3 signaling in repulsive guidance from SLT-1. Genetic interactions with gain and loss of function mutations in the *sax-3* pathway demonstrate that *unc-34* works together with *sax-3* in several guidance decisions. UNC-34 associates with the SAX-3 cytoplasmic domain *in vitro*, suggesting that these interactions are direct. These genetic and biochemical interactions are conserved across species, since Robo and Enabled also act together in *Drosophila* midline guidance (Bashaw et al, 2000). These results support a direct role for UNC-34 in repellent guidance.

Unexpectedly, SAX-3 function is also potentiated by an UNC-6/Netrin-independent function of the Netrin receptor UNC-40. Genetic interactions between *unc-40* and *sax-3* are similar to interactions between *unc-34* and *sax-3*, and point to a role for UNC-40 in SAX-3 guidance. Such a role is further supported by the finding that UNC-40 and SAX-3 cytoplasmic domains directly interact. The interaction between UNC-40 and SAX-3 is also observed with their vertebrate counterparts, DCC and Robo, where it is implicated in a different guidance decision (Stein et al, 2001). Our results suggest that SAX-3 and UNC-40 may interact directly in guidance complexes that contribute to repulsive guidance. A combinatorial logic thus dictates alternative functions for UNC-40/DCC, which can act in attraction to UNC-6, repulsion from UNC-6 (with UNC-5), or repulsion from Slit (with SAX-3).

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144. Putative matrix proteins with thrombospondin type I repeats and serine protease inhibitor domains are involved in guidance of the distal tip cell

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In *C. elegans* hermaphrodites, the gonad acquires a U-shape by the directed migration of distal tip cells (DTCs). They start migration at late L2 stage from the ventral mid body of the animal, and by late L4 stage they finish migration. The first phase of DTC migration is longitudinal migration along the ventral muscle bands. The second phase is a circumferential migration, where DTCs migrate from ventral to dorsal along the epidermis, then finally they migrate retrogradely along the dorsal muscle band back to mid-body.

We have characterized two phenotypic classes of *mig-6* mutants. In class 1 *mig-6* mutant, originally described by Hedgecock *et al.* (1), the first longitudinal migration is defective but ventral to dorsal migration is normal, resulting in short and immature gonad, which is sterile. By a large scale F1 screen for DTC migration mutants, we have obtained a new class of *mig-6* mutant (class 2). The heterozygotes of class 2 display ventral reflection of gonad, which indicates that the circumferential migration of DTCs is defective but longitudinal migration is normal, similar to the defects seen in *unc-5, -6, -40,* and *-130* (2, 3). Interestingly, homozygotes of this class 2 show leaky embryonic/early larval lethality, with escapers (average 17) growing to adulthood and their gonads displaying supernumerary turns, indicating disruption of both longitudinal and circumferential guidance of DTC migration. This lethality, but not migration defects, is rescued by maternal effect, since most of homozygote animals from heterozygote parents grow to adults with a complete penetrance of supernumerary turn of DTCs. It seems *mig-6* affects all of the three phases of DTC migration, but separately.

Both classes of *mig-6* are rescued by a genomic fragment containing a single locus. There are two alternative transcripts, designated mig-6a and mig-6b. They have the same exon structure down to exon 10 and have different 3' extensions. The proteins are putative secreted proteins with a signal peptide. The N-terminal half of the proteins has a cluster of thrombospondin type I domains and N-linked carbohydrate attachment sites. The C-terminal half of the proteins has a repeat of Kunitz-type serine protease inhibitor (KU) domains. Both forms of the proteins share the same structural features until the 6th KU domain. The large one has 5 extra KU domains and a immunoglobulin C2 type domain in the end. By designing dsRNA based on transcript specific 3' sequence, each transcript was disrupted independently or together. Quite surprisingly, it was shown that RNAi of mig-6b phenocopied the class 1 mutant, and RNAi of mig-6a alone or together with mig-6b phenocopied class 2 mutant recessive phenotype. All four class 1 alleles are nonsense mutations that affect just the mig-6b transcript. At least one of the class 2 mutations is a missense codon affecting both transcripts.

mig-6 promoter:: gfp reporter showed *mig-6* expressions in various tissues including DTCs, body wall muscles, the head mesodermal cell, coelomocytes, and some neuronal cells. Preliminary results suggest that expression of the two transcripts is under distinct regulation. Hence the expression patterns of the two transcripts, in addition to structural difference may define the class 1 and class 2 phenotypes.

(1) Hedgecock *et al*. (1987) *Development* **100**, 365-382

(2) Hedgecock *et al*. (1990) *Neuron* **2**, 61-85

(3) Nash *et al*. (2000) *Genes & Development* **14**, 2486-2500

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145. Cellular mechanisms governing the specificity and timing of anchor cell invasion into the vulval epithelium

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We are examining the initial steps that regulate the connection of the uterus and vulva during *C. elegans* development. The initial contact between the developing uterus and vulva is established by the anchor cell (AC), which crosses the basement membranes separating both tissues and specifically attaches to the inner descendants of the 1^o fated vulval precursor cell, P6.p, during the mid- to late L3 stage. Following attachment, the AC extends cellular processes from its basolateral surface and invades between the inner descendants of the P6.p. cell, positioning itself at the apex of the developing vulva. Using mutants lacking vulval induction, we show that underlying vulval cells trigger AC attachment. We have further discovered that AC attachment is not stimulated by 2^o fated vulval cells (the vulval cells that flank the centrally located 1^o cells), but rather appears to be specific for the 1^o vulval cells. Using a basement membrane marker, we show that isolated 1^o cells do not break down the basement membrane in the absence of the AC, suggesting that 1^o vulval cells do not stimulate attachment by removing the basement membrane. Instead we provide evidence indicating that the 1^o vulval cells secrete a signal that triggers AC invasive behavior: when the AC is placed at a distance from the 1^o vulval cells, it sends out cellular extensions toward the 1^o vulval cells that are similar to those seen during normal invasion. We further show that the competence of the AC to respond to this signal is regulated: AC attachment is either absent or delayed in the heterochronic mutant *lin-28*, which causes precocious vulval development. To understand the molecular mechanisms that regulate AC attachment and invasion, we are examining many known mutants. Through these studies we have found that the *evl-5* mutant, originally isolated in a screen for sterile mutants with everted vulvae (Seydoux et al., (1993) Dev. Biol. 157(2): 423-36), has a defect in AC attachment to the vulval epithelium. In *evl-5* animals vulval induction and AC positioning over the P6.p cell is normal; however, AC attachment either does not occur or is severely delayed. Visualization of AC behavior in this mutant revealed the extension of cellular processes from the AC toward the vulva, indicating that the AC is still attracted to the developing vulva. However, in many cases the AC processes appeared to flatten or broaden out at the basement membrane of the developing gonad, suggesting that the AC may not be able to cross this basement membrane. We are currently molecularly cloning *evl-5*.

146. Maturation of the C. elegans centromere requires a CENP-C-like protein

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Prior to mitosis, the centromere must by duplicated, sister centromeres resolved from one another and sister kinetochores assembled adjacent to each centromere. This process of maturation results in both sister kinetochores being oriented back-to-back; which may facilitate attachment to both poles. We sought to determine if the holocentric chromosomes of Caenorhabditis elegans had a similar process. Using the centromeric histone H3-varient, HCP-3, as a marker for the centromere, we found that the centromere of C. elegans is composed of many independent elements distributed through out the chromosome. These elements coalesce to form a single aggregate of centromere units that form a line along the entire length of the chromosome. Later in prophase, two centromere lines are observed on opposite faces of the condensed chromosome and sister kinetochores are subsequently assembled adjacent to each. Thus, C. elegans chromosomes undergo both sister centromere resolution and kinetochore assembly similar to the process in mammalian chromosomes. To study this process and to extend our knowledge of holocentric chromosome structure, we identified a CENP-C-like protein, HCP-4. HCP-4 is localized to the centromere early in prophase when only a single centromere line is observed. HCP-4 remained centromere localized until the beginning of decondensation in telophase. The centromere localization of HCP-4 was dependent on HCP-3 and both HCP-3 and HCP-4 are required to localize the kinetochore component, HCP-1. We also found that HCP-4 is required for the process of sister centromere resolution. Thus, HCP-4, and perhaps other CENP-C-like proteins, is essential for the maturation of the centromere.

147. Dissecting the molecular architecture and structure of the kinetochore by genetics and correlated light and electron microscopy

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The kinetochore is a complex and dynamic macromolecular structure that attaches and moves chromosomes within the spindle during mitotic and meiotic chromosome segregation. A partial list of kinetochore proteins now exists and attempts are being made to identify their functions. To define additional kinetochore proteins, we turned to *C. elegans* because the holocentric structure of its mitotic chromosomes is well-suited to identification and localization of these molecules. To characterize kinetochore structure at a macromolecular level of resolution, we are using electron microscopy to investigate chromosomes of wild-type worms and worms depleted of kinetochore proteins. An EM procedure optimized for the preservation of structure revealed that in wild-type spindles, the kinetochore ultrastructure is similar to that seen on mammalian chromosomes, i.e., a plaque represented by a ribosome exclusion zone and an underlying fibrous mat on the poleward face of the chromatin. Microtubules originating from the spindle poles insert orthogonally into this plaque.

We have shown that HIM-10 is an evolutionarily conserved component of the kinetochore required for attachment of *C. elegans* holocentric chromosomes to mitotic spindles. HIM-10 is a coiled-coil protein related to the Nuf2 kinetochore proteins in yeast and humans, and is more poleward facing on mitotic chromosomes than the *C. elegans* centromere protein HCP-3. Depletion of HIM-10 disrupts kinetochore ultrastucture, causes a failure of bipolar spindle attachment, and results in embryonic lethality associated with chromosome nondisjunction or loss in mitosis.

Electron microscopy of HIM-10-depleted spindles reveals that the plaque structure seen in wild-type chromosomes is greatly diminished or absent. This correlates exactly with the loss of HIM-10 immunofluorescence seen in the light microscope, and indicates that HIM-10 is necessary for the proper assembly and function of the kinetochore.

Meiotic chromosomes viewed by EM have a similar kinetochore structure to mitotic chromosomes. This meiotic structure, together with the localization and function of HIM-10 in meiosis, provides the first demonstration that meiotic chromosomes of a holocentric organism possess kinetochores that share molecular, morphological and functional features with those of mitotic chromosomes.

Conservation of kinetochore proteins in *C. elegans* extends to KCM-1, a kinesin-like protein that couples ATP hydrolysis with depolymerization of microtubule plus-ends. KCM-1 localizes to mitotic and meiotic chromosomes in a pattern related to that of HIM-10. Reduction of KCM-1 causes a failure of polar body extrusion, floppy spindles in mitosis and embryonic lethality. The conservation in kinetochore structure and proteins makes the extended kinetochores characteristic of holocentric chromosomes in *C. elegans* a guide to the structure, molecular architecture, and function of conventional kinetochores in organisms such as humans.

148. Analysis of chromosome segregation and kinetochore assembly in the one-cell stage *C. elegans,* embryo

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We have developed live and fixed assays to analyze chromosome movement and kinetochore function in one-cell stage C. elegans embryos. Unlike most eukaryotes, C. elegans has holocentric chromosomes, where the kinetochore is assembled along the length of the chromosome. Despite this dramatic structural difference, the C.elegans genome contains many genes whose homologues are implicated in the formation and function of localized kinetochores of other eukaryotes. Using strains expressing GFP-histone or GFP-tubulin we have examined chromosome segregation and spindle function in worms depleted of proteins involved in chromosome segregation by RNAi. Depletion of the C. elegans homologs of either CENP-A, (HCP-3) or CENP-C (HCP-4) results in an identical "kinetochore-null" phenotype, characterized by complete failure of mitotic chromosome segregation as well as failure to recruit other kinetochore components and to assemble a mechanically stable spindle. Parallel analysis of embryos depleted of the C. elegans homolog of the chromosomal passenger protein INCENP (ICP-1) revealed mitotic chromosome segregation defects different from those observed in the absence of HCP-3 or HCP-4. Defects are observed before and during anaphase but the chromatin separates into two equivalently sized masses. Kinetochore components are recruited normally and mechanically stable spindles assemble that show defects later in anaphase and telophase. These detailed cytological phenotypes are also serving as high resolution "fingerprints" for classifying new genes important for chromosome segregation that are being identified in comprehensive functional genomics screens. So far, we have one novel gene that has a "kinetochore-null" phenotype from a RNAi based screen of the genes on chromosome III.

We have also initiated an analysis of kinetochore assembly in this system. Using antibodies to kinetochore components and RNAi, we have developed a preliminary map of the dependency relationships during kinetochore assembly.

149. Centrosome maturation in *C. elegans*: respective roles for gamma-tubulin and aurora-A kinase

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The centrosome is a dynamic organelle that undergoes a complex series of cell cycle-dependent changes. One of the most striking of these is the maturation of centrosomes that occurs as the cell enters mitosis. During maturation, the accumulation of centrosomal gamma-tubulin and other pericentriolar material (PCM) components results in an increase in centrosome size that correlates with enhanced centrosomal microtubule assembly. We use RNA-mediated interference (RNAi) in *C. elegans* embryos to dissect the pathways that govern maturation and to determine if recruitment of gamma-tubulin is required for increases in the number of centrosomally organized microtubules. Perturbing gamma-tubulin function using either RNAi or a maternal effect mutation results in a cytologically indistinguishable phenotype characterized by a failure to form interphase asters and to assemble a mitotic spindle. Surprisingly, however, as the embryos enter mitosis prominent centrosomal asters assemble. Depletion of the aurora-A kinase, AIR-1, results in a similar mitotic phenotype. We show that AIR-1 is required to recruit additional centrosomal gamma-tubulin as cells enter mitosis. AIR-1 is also required for the accumulation of another centrosomal marker, ZYG-9, suggesting that aurora-A kinases play a central role in the recruitment of PCM during maturation.

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150. SPD-5 is a novel centrosomal protein required for mitotic spindle assembly

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We isolated a mitotic spindle-defective mutant we call *spd-5* in a screen for temperature-sensitive embryonic-lethal cell division mutants. Pronuclear migration fails in *spd-5* mutant embryos, a mitotic spindle does not form, and the first cell division fails. Furthermore, there are defects in anterior-posterior polarity. Indirect immunofluorescence analysis of fixed embryos reveals that astral microtubules associated with the sperm pronucleus are greatly attenuated, although free cytoplasmic microtubules are present in early pronuclear stage embryos. During the first mitosis, microtubules radiate out from the condensed DNA, but no bipolar spindle structure forms. The severe defects in microtubule organization probably result from non-functional centrosomes; γ-tubulin, a critical component of microtubule organizing centers, is not localized to centrosomes in *spd-5* mutant embryos. We positionally cloned *spd-5*, which maps to approximately -.24 map units on LGI, and it corresponds to the Genefinder locus F56A3.4. *spd-5* encodes an approximately 135 kD novel coiled-coil protein. Using antibodies raised against SPD-5, we show SPD-5 normally localizes to centrosomes at all stages of the cell cycle, although the levels are highest during mitosis. We predict SPD-5 may be part of the centrosomal matrix required for both recruitment of other components (e.g. γ-tubulin) to the centrosome and for centrosome activation. Intriguingly, *spd-5* shows allele-specific genetic interactions with another spindle-defective mutant isolated in our screen that probably encodes dynein heavy chain (D. Schmidt, S. Strome, and W. Saxton personal communication). This result suggests that the novel protein SPD-5 may function with the highly conserved microtuble-motor protein dynein to regulate spindle structure and function. Furthermore, consistent with the model that sperm pronucleus-associated asters are important for establishing anterior-posterior polarity in the early embryo, *spd-5* mutants, which lack functional centrosomes and sperm astral microtubules, lack most aspects of anterior-posterior polarity.

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151. *zyg-12* is required for the positioning of *C elegans* centrosomes

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Although the centrosome is associated with the nuclear envelope during interphase in a variety of organisms and cell types, the mechanism of this association is largely unknown. The only protein known to be involved in this process, dynein, has been shown to play only a minor role in the interaction in both *C. elegans* and *Drosophila*. We report that a temperature sensitive (ts) maternal effect mutation, *zyg-12(ct350),* caused completely penetrant dissociation of the centrosome and the nucleus. Embryos at the 1-cell stage from homozygous mutant mothers appeared normal except for the dissociated centrosomes. To address whether *zyg-12(ct350)* is disrupting centrosomal positioning through a disruption of dynein activity, we looked for other dynein dependent activities. Centrosome separation, centrsosome movement away from the cortex and dynein-mediated transport are normal in *zyg-12(ct350)* embryos, suggesting that dynein function is also normal.

Time-lapse optical sectioning of *zyg-12(ct350)* embryos revealed, except for dissociated centrosomes, otherwise normal embryonic development. For example, mitosis and establishment of polarity both appeared normal. This suggests that centrosomes may be able to function normally in the absence of nuclear association. To address whether centrosome function was otherwise normal, I assayed their ability to organize microtubules. I created an integrated ßtubulin::GFP transgene using micro-particle bombardment[1]. Analysis of *zyg-12(ct350)* in the ßtubulin::GFP background indicated that centrosomes appeared completely wild-type in their ability to organize both astral and spindle microtubules. Therefore, *zyg-12* may be specifically required for the association of the centrosome and the nucleus and may help elucidate the molecular mechanism of this association.

[1] Praitis et al. 2001. Genetics. 157: 1217.

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152. *zyg-8*, a gene required for spindle positioning in one cell stage embryos, encodes a Doublecortin-related kinase that promotes microtubule stability

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In wild-type one cell stage embryos, the spindle is displaced slightly towards the posterior during anaphase, leading to an asymmetric cell division. We screened maternal-effect embryonic lethal mutants by time-lapse DIC microscopy and identified a locus called *zyg-8*, which is required for proper anaphase spindle positioning. In most *zyg-8* mutant embryos, the spindle is displaced excessively towards the posterior during anaphase, resulting in aberrant cleavage furrow placement.

Overall antero-posterior polarity is not affected in *zyg-8* mutant embryos, since PAR-3, PAR-2, PAR-1 and P granules are correctly distributed. In contrast, astral microtubules during anaphase are shorter than in wild-type, usually not reaching the cell cortex. This is the likely cause of exaggerated posterior displacement, because a similar phenotype is observed by treating wild-type anaphase embryos with the microtubule destabilizing agent nocodazole. Therefore, *zyg-8* is required for the stability and/or growth of microtubules during anaphase. We identified the molecular nature of *zyg-8* in the course of a large scale RNAi-based functional genomics screen. *zyg-8* encodes a bipartite protein which harbors a kinase domain and a domain related to Doublecortin, a microtubule-associated protein (MAP) affected in patients with neuronal migration disorders. ZYG-8 co-pellets with microtubules *in vitro*, and co-localizes with microtubules *in vivo*. Moreover, HA-tagged ZYG-8 transfected into COS-7 cells colocalizes with microtubules. Importantly, the presence of HA-tagged ZYG-8 protects the microtubule network of COS-7 cells against depolymerization by cold or nocodazole treatment. Taken together, these results demonstrate that ZYG-8 is a MAP that promotes microtubule stabiliy, thus ensuring proper spindle positioning in one cell stage *C. elegans* embryos.

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153. The mitosis-regulating kinase AIR-2 interacts with and phosphorylates the *C. elegans* kinesin-like motor protein BMK-1.

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Eukaryotes have evolved an elaborate, multi-protein cellular machinery, the mitotic spindle, to equally partition the chromosomes into each daughter cell during cell division. Malfunctioning of the mitotic spindle can cause defects in both chromosome segregation and cytokinesis, which can produce cells with abnormal numbers of chromosomes -- a condition often seen in cancerous cells. We are therefore interested in elucidating the regulatory pathways that control mitotic spindle dynamics.

Regulation of mitotic spindle function is not well understood, but the Aurora/Ipl1 kinases are known to be involved. This lab has previously identified two novel *C. elegans* members of the Aurora/Ipl1 kinase family, AIR-1 and AIR-2. By immunofluoresence AIR-1 and AIR-2 were shown to be dynamically associated with mitotic chromosomes and the mitotic spindle. *C. elegans* embryos in which either AIR-1 or AIR-2 expression was abolished by RNA interference (RNAi) displayed defects in mitotic spindle formation and cytokinesis. Exactly how loss of AIR kinase expression disrupts mitosis is not known. Our current goal is to identify the proteins with which the AIR kinases interact to regulate mitotic spindle function.

One candidate AIR-2 substrate is the BimC kinesin-like motor-protein BMK-1. We have shown that *C. elegans* BMK-1 co-localizes with AIR-2 in developing embryos, and that proper localization of BMK-1 is lost in *air-2 (RNAi)* embryos. AIR-2 and BMK-1 interact in a yeast-two hybrid assay, and we show here by GST-pull down and co-immunoprecipitation that recombinant BMK-1 interacts with recombinant AIR-2 *in vitro*. BMK-1 and AIR-2 interact *in vivo* as well, as they co-immunoprecipitate from embryo extracts. In addition, BMK-1 is directly and specifically phosphorylated by recombinant AIR-2 *in vitro*. Thus, our data indicate that the kinesin BMK-1 is a substrate of AIR-2. We are determining the site of phosphorylation within BMK-1 and will explore the requirement for phosphorylation at that site *in vivo.*

We intend to identify novel kinase substrates by using the purified recombinant AIR-2 in an *in situ* phosphorylation screen of a *C. elegans* expression library. We currently are constructing a GST-tagged cDNA expression library for this screen. The function of candidate substrates identified in the library screen will be tested *in vivo* by RNAi. Clones that phenocopy *air-2(RNAi)* will be actively pursued as likely *in vivo* substrates of the AIR-2 kinase.

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154. Biochemical Identification of LIN-5 Associated Proteins with Mitotic Spindle Functions

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We seek to improve our understanding of the events in mitosis by studying the *C. elegans lin-5* gene. Phenotypic characterizations of *lin-5* mutants and *lin-5(RNAi)* embryos have indicated an essential role for *lin-5* in chromosome segregation. Specifically, loss of *lin-5* function causes major defects in chromosome and spindle movements at three levels: incorrect positioning of the meiotic spindle, incorrect positioning and rotation of the mitotic spindle, and defective metaphase alignment and segregation of chromosomes (Lorson *et al*, 2000). The *lin-5* gene encodes a novel 821 amino acid protein marked only by a large central coiled-coil domain and ten candidate phosphorylation sites in the N-terminus and C-terminus. LIN-5 protein localizes to spindle microtubules, centrosomes and the cell cortex. Although these results establish LIN-5 as an essential novel spindle component, the protein structure does not reveal the molecular mechanisms involved in LIN-5 function.

Identification and study of LIN-5-associated proteins may provide insights into the molecular function of *lin-5*. As an alternative to genetic suppressor analyses, we have taken a biochemical approach to identify the components of a LIN-5 protein complex from *C. elegans* embryos. As shown by gel filtration chromatography of N2 embryo lysates, LIN-5 is part of a large protein complex greater than 600 kDa in size. Two-hybrid assays in yeast suggested that LIN-5 may homodimerize, accounting for a small part of this complex. To isolate the other components of this complex for subsequent identification, we used immunoaffinity purification. With LIN-5 specific monoclonal antibodies, we isolated protein complexes from lysates of embryos obtained from large liquid cultures of N2 animals treated with hypochlorite. Immunoprecipitated proteins were analzyed by SDS-PAGE and silver-staining, which revealed six specific protein bands not present in control reactions. These six polypeptides were excised from the gel, digested with trypsin and analyzed by tandem mass spectrometry. A 93 kDa protein was identified conclusively as LIN-5, confirming the specificity of the antibodies. A 60 kDa protein was identified as the product of one of two predicted paralogous genes located on linkage group III. RNA inhibition of either gene has been reported to cause anaphase spindle positioning and rotation defects (Gonczy *et al*, 2000). This phenotype is strikingly similar to that of *lin-5(RNAi)* embryos, indicating these proteins may function in a complex with LIN-5 in mitosis. The other four protein bands are being analyzed by mass spectrometry and their characterizations will be presented. These results support the usefulness of biochemical techniques to complement forward genetics in *C. elegans*.

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155. CDC-42, AGS-3 and heterotrimeric G proteins regulate spindle position and orientation

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Generation of asymmetry in the 1-cell embryo of *C. elegans* establishes the anterior posterior axis and is necessary for the position and orientation of mitotic spindles. Conserved PAR proteins are asymmetrically distributed and are required for these differences. However, how PAR asymmetry is established and how this polarity is translated into spindle position is still an open question. We have identified a series of components involved in these events: the conserved small G protein CDC-42 plays a role in generating PAR asymmetry and heterotrimeric G proteins in conjunction with AGS-3 proteins transduce polarity information to the mitotic spindle.

Cdc42 is a highly conserved small G protein that has been shown to be important for cell polarity during mating and budding in yeast, and for establishing and maintaining epithelial polarity in mammalian cells. We found that *cdc-42(RNAi)* embryos have a phenotype nearly identical to *par-3, par-6, or pkc-3* mutants, and asymmetric localisation of these and other PAR proteins is lost. CDC-42 is required to maintain, but not to establish the asymmetric PAR domains. The anterior actin cap is lost in *cdc-42(RNAi)* embryos as in *par-2* and *par-3* mutants, suggesting that CDC-42 may mediate a transient reorganisation of actin at the anterior which is important to localise factors required for polarity and spindle orientation.
Surprisingly, reduction of *cdc-42* function supresses the *par-2* mutant phenotype indicating that CDC-42 and PAR-2 act antagonistically. We further showed that CDC-42 binds PAR-6 in a two-hybrid assay. One possibility consistent with our data and related mammalian data is that CDC-42 activates the PAR-3/PAR-6/PKC-3 complex, and activation is required for its proper localisation.

How are these early polarity signals translated into correct spindle position and orientation? GPB-1, a Gβ subunit of heterotrimeric G proteins, was previously shown to be required for orientation of early cell divisions in *C. elegans* embryos, but its mechanism of action was unknown. We have shown that two Gα subunits (GOA-1 and GPA-16) function redundantly in the early embryo with GPB-1, but that Gα and Gβ control distinct microtubule dependent processes. Gβ is important in regulating centrosome migration around the nucleus and hence in orienting the mitotic spindle. Gα is required for asymmetric spindle positioning in the 1-cell embryo. Although loss of Gα results in a symmtetric first cleavage, embryonic polarity appears to be normal. The uncoupling of spindle asymmetry from general polarity suggests that Gα might be responsible for translating embryonic polarity from the PAR proteins into mitotic spindle positioning.

The link between PAR polarity and the heterotrimeric G proteins may involve homologues of AGS3, a mammalian activator of G protein signaling. AGS3 contains GoLoco domains implicated in binding Gα. *C .elegans* contains three AGS3 homologues, called *ags-3.1, ags-3.2,* and *ags-3.3.* Embryos simultaneously depleted for *ags-3.2* and *ags-3.3* display a phenotype identical to Gα depleted embryos suggesting that they are regulators of this heterotrimeric G protein. Interestingly, a *Drosophila* homologue of AGS3, PINS (Partner of Inscuteable), is essential for polarity and spindle orientation in the neuroblast and is found in a complex with Inscuteable, Bazooka (the *Drosophila* PAR-3 homologue) and a Gα. We suggest that in *C. elegans* an equivalent complex exists which contains PAR-3, Gα and AGS-3.2 or AGS-3.3 and that this complex translates polarity into spindle position. We are currently investigating this possibility.

We propose a model in which CDC-42 controls the localisation and activity of PAR proteins. PAR proteins are in turn responsible for the activatation of the heterotrimeric G protein via the AGS-3 proteins, and thus for controling spindle orientation and position.

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156. *pom-1* is Required for Anterior/Posterior Polarity and Microtubule Dynamics in 1-Cell Embryos, and is Related to a Regulator of Cell Polarity in *S. pombe*

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Polarization of the anterior/posterior (A/P) axis in the 1-cell embryo depends on a group of cortically-enriched proteins (PARs) that become asymmetrically localized in response to a spatial cue dependent on the sperm asters. The PAR proteins regulate the asymmetric localization of the spindle and several cytoplasmic factors, including PIE-1 and P granules; however, the mechanisms by which the PAR proteins regulate these asymmetries are unknown.

To investigate these mechanisms, we have conducted an RNAi screen for new regulators of A/P polarity. Unlike previous screens, which relied on mutants with symmetric cleavages to identify polarity genes, our screen uses a PIE-1:GFP fusion to monitor A/P polarity. Because PIE-1 asymmetry is visible in the zygote before the first cleavage, this approach avoids biases against genes with cytoskeletal functions, such as spindle formation or cytokinesis. We reasoned that genes involved in A/P polarity are likely to be maternally expressed and therefore began by screening a set of 766 genes shown by microarray analysis to be enriched in the germline (Reinke et al., 2000).

One candidate gene identified in our screen is F49E11.1, which we have named *pom-1* (see below). We find that *pom-1(RNAi)* zygotes exhibit severe defects in the localization of PIE-1 and P granules. In contrast, immunolocalization experiments with PAR-3 and time lapse movies of embryos expressing a PAR-2:GFP fusion protein indicate that, initially, PAR asymmetry is correctly established in *pom-1(RNAi)* zygotes. Our observations suggest that POM-1 functions downstream of PAR-2 and PAR-3 to regulate P granule and PIE-1 asymmetry in early zygotes. During mitosis, *pom-1(RNAi)* embryos exhibit several cytoskeletal defects, including premature mitotic spindle assembly, mispositioning of the mitotic spindle, and abnormal cytokinesis. A POM-1:GFP fusion localizes to numerous small foci on the cortex during interphase, to centrosomes and midzone microtubules during mitosis, and to P granules in later stages. Together, these observations suggest that POM-1 is a good candidate for a molecule that links the PAR polarity machinery to downstream events required for PIE-1/Pgranule asymmetry and spindle positioning.

Consistent with this hypothesis, POM-1 is related to Pom1p, a protein kinase in *S. pombe* that has been proposed to regulate cell polarity by interacting with the actin and microtubule cytoskeletons (Bahler and Pringle, 1997). Our findings reveal a previously unrecognized parallel between A/P polarity in *C. elegans* and cell polarity in fission yeast and suggest that POM-1 may function to transmit positional information from the cell cortex to the cytoplasm and the spindle during polarization of the one-cell embryo.

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157. The NED-8 ubiquitin-like conjugation pathway regulates the microtubule and microfilament cytoskeleton in *C. elegans*.

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Ubiquitin (UBQ) and ubiqutin-like proteins are a family of small conserved polypeptides that become covalently attached to other cellular proteins. Conjugation of ubiquitin usually targets proteins for degredation, while modification by UBLs can instead affect the subcelluar localization of target proteins, or their conformation and association with other proteins. Related pathways mediate the attachement of UBQ and UBLs to target proteins. In reactions that require ATP, UBQ and UBLs are covalently atached to cognate E1 activating enzymes. Following activation, UBQ/UBLs are transferred to E2 conjugation enzyme that, directly or with the help of an E3 ubiquitin-ligase mediate final transfer of UBQ/UBLs to target proteins. The only known targets of the NED-8 subfamily of UBLs are cullin proteins, which are thought to be subunits of SCF or SCF-like ubiquitin-ligase complexes. Conjugation of NED-8 to the cullin subunit seems to be essential for the assembly of those complexes. The E1-like activating enzyme for NED-8 consists of a heterodimer of two proteins, called UBA3 and ULA1 in yeast. UBA3 is highly homolgues to the C-terminal half of the monomeric E1 ubiquitin activating enzyme UBA1, while ULA1 corresponds to the N-terminus. The yeast *ned-8* homologue *rub1p* has been shown to be involved in cell cycle progression.

In our screens we have identified a temperature-sensitve maternal effect lethal mutation in the C. elegans orthologue of the E1-like activating enzyme UBA3. We termed this gene *rfl-1* (*ruffle-1*) based on its phenotype, which includes highly penetrant ectopic membrane contractions during the first embryonic cell divisions. We also detect spindle orientation defects, a partially penetrant cytokinesis defect, mispositioning of interphase nuclei and cell cycle delays in *rfl-1* mutant embryos. Inactivation of the other Ned-8 pathway components *ula-1*, *ubc-12* and *ned-8* by RNAi results in similar defects than the ones observed in *rfl-1* mutant embryos. The possible NED-8 target CUL-3, also shared defects with *rfl-1*, suggesting that the NED-8 conjugation pathway is intact in *C. elegans*.

The observed cytoskeletal defects in *rfl-1* mutant embryos represent a novel role for this post-translational modification pathway. Our analysis of the membrane defects suggest that the ectopic membrane contractions observed in *rfl-1* mutant embryos might result from the initiation of multiple cleavage furrows during cytokinesis. This is supported by the finding that ectopic furrowing is suppressed in *rfl-1* mutant embryos if genes required for the formation of the cytokinetic furrow are inactivated by RNAi. Additionally, the contractile ring component CYK-1, which is only detectable at the tip of ingressing cleavage furrows in wildtype, is ectopically localized to the sites of extra pinching. We also detected severe defects in spindle structure in *rfl-1* mutant embryos. Astral microtubules often terminate prematuraly and appear bent before reaching the cortex. A partially penetrant chromosome segregation failure also suggests defects in spindle function. Consistent with the requirement of Ned-8 conjugation for cell cycle progression in other organisms, we detect interphase delays in *rfl-1* mutant blastomeres of approx. 50% in both AB and P1.

Based on the observed phenotypes, we concluded that Ned-8 conjugation regulates the microfilament as well as the microtubule cytoskeleton in the early embryo, in addition to being required for progression of the cell cycle.

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158. The role of *C. elegans* Rho-binding kinase and myosin phosphatase in cytokinesis

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Rho-binding kinase and myosin phosphatase have been implicated in cytokinesis in higher eukaryotes, however, this was determined with dominant-negative and chemical inhibitor studies. We have addressed the *in vivo* role of Rho-binding kinase and myosin phosphatase in cytokinesis using loss-of-function mutations. Previously, we had determined that *let-502* (Rho-binding kinase) and *mel-11* (myosin phosphatase) control the contraction of epidermal cells driving embryonic elongation, when the embryo is transformed from a ball of cells into a tube-like structure. *let-502* mutants fail to elongate and *mel-11* mutants display hypercontraction of the epidermal cells[1,2]. *let-502* and *mel-11* mutants suppress one another and are redundant with a *fem-2* (PP2c phosphatase)-mediated pathway[2]. Recently, we identified loss-of-function *let-502* alleles that display embryonic lethality due to the failure of another contractile event, cytokinesis[2]. All embryos derived from *let-502* (RNAi) in *let-502* mutant mothers die from failed cytokinesis, however not every cell division fails. In addition, *let-502* mutant embryos fail to undergo pseudocleavage and in cells that successfully divide, cell cleavage is slower than in wild-type embryos. Interestingly, *mel-11* mutants display opposite phenotypes to *let-502*. They have ectopic furrows during pseudocleavage and cell divisions and early cell cleavage is faster than in wild-type embryos, indicating that *mel-11* also may control the rate/force of furrow contraction. As with elongation, *mel-11* suppresses *let-502* cytokinetic defects. Previously, Shelton *et al*. (1999) showed that *mlc-4* (non muscle myosin light chain) was required for cytokinesis consistent with biochemical evidence that MLC is a target of the Rho-binding kinase/myosin phosphatase pathway in smooth muscle contraction[3].

Antibody localization to study the molecular epistasis between components of the cytokinetic pathway demonstrated that actin, NMY-2 (non-muscle myosin heavy chain) and MLC-4 (non-muscle myosin light chain) act upstream of LET-502 and MEL-11 whereas CYK-1 (formin homology protein) and CYK-4 (RhoGAP) act downstream. Although *let-502* mutant embryos fail to undergo some cell divisions, some cell divisions still occur. This implies that low levels of LET-502 are still present or that LET-502 is partially redundant. RNAi was performed to several genes that may be potentially redundant with *let-502* and that may affect cytokinesis, such as the citron-like genes. However, none revealed any cytokinetic defects. We have proposed a pathway in which LET-502 and MEL-11 regulate MLC-4 activity to fine-tune the force/rate of cleavage furrow contraction in combination with other (unknown) factors. 1. Wissmann *et al*., 1997. Genes Dev. **11**: 409-422. 2. Piekny *et al*., 2000. Genetics **156**: 1671-1689. 3. Shelton *et al*., 1999. J. Cell Biol. **146**: 439-451.

159. OOC-5, required for polarity in the 2-cell embryo, encodes a AAA+ ATPase related to the human disease protein Torsin A

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During intrinsically asymmetric divisions, a polarized parent cell divides to produce daughters that are different. We previously showed that *ooc-5* and *ooc-3* mutations result in defects in asymmetric divisions in early *C. elegans* embryos. In particular, mutations in these genes specifically affect the reestablishment of asymmetric PAR protein domains in the P1 cell at the 2-cell stage. The PAR proteins are required for polarity and the asymmetric localization of cell fate determinants. We have cloned the *ooc-5* gene and find that it encodes a predicted ATPase that is most closely related to the Torsin family of proteins. Torsin-related proteins are conserved in *C. elegans*, *Drosophila*, mouse and human, and the gene for human Torsin A is mutated in individuals with early onset dystonia, a neuromuscular disease. OOC-5 and the Torsins are members of the recently expanded AAA+ family of ATPases. Although the cellular roles of AAA+ family members are quite diverse, a large number of these proteins have chaperone activity; that is, they participate in the remodeling of proteins, either to facilitate the folding of an individual protein or assembly of a complex, or to facilitate unfolding or disassembly. Our immunolocalization studies show that OOC-5 colocalizes with a marker of the endoplasmic reticulum in all blastomeres of the early *C. elegans* embryo, in a pattern indistinguishable from that of OOC-3 protein. Furthermore, we have found that OOC-5 colocalization with the ER marker depends on the normal function of the *ooc-3* gene. Based on these results, we propose that OOC-5 functions as an ER chaperone in the secretion of proteins required for the establishment of asymmetric domains in the P1 cell, and that OOC-3 is required for the subcellular distribution of OOC-5.

160. Analysis of gut epithelial polarity in the C.elegans embryo

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At present, several proteins are known to affect epithelial cell polarity during development. However, their sig-nalling pathways and mechanisms are still elusive. In C. elegans the gut forms a simple tube made up of 20 cells arranged in a series of nine rings (1). To perform its function, the cells have to develop an apico-basal axis that is manifested in their internal architecture, surface polarity and the formation of specialised junctions. In C. elegans the zonula adherens (ZA) forms a continuous belt around the apex of epithelial cells, defining the border between apical and baso-lateral domains. The ZA is the only type of junction which has been detected in the em-bryo at the ultrastructural level so far (2,3). Our primary interests are to determine which components of this junction are required for the integrity of the gut epithelium in C. elegans and which adaptor molecules mediate the interaction of polarity cues with the executive cytoskeleton.

dlg-1 (discs large) is restricted to the ZA of all embryonic epithelia (4), which contrasts with the localisation of the Drosophila and vertebrate homologues in septate and tight junctions, respectively. The molecular nature of DLG-1 makes it a likely candidate to participate in the organization of protein scaffolds that control the assembly of junction components into the ZA. We have started to screen for direct binding partners of DLG-1, using two different domains as baits in a yeast two-hybrid screen. Proper localization of DLG-1 requires the baso-lateral LET-413 protein (3), the Drosophila scrib orthologue (5), but is independent of the catenin-cadherin system (6). High quality immunofluorescence analysis of dlg-1 and catenin/cadherin knockout embryos revealed severe defects in epithelial integrity, demonstrating the necessity of both sytems for correct development. Analysis of band-4.1/ERM (ezrin-radixin-moesin) proteins, a family that is defined as membrane-cytoskeleton linkers, has

revealed an essential function of erm-1 for correct localization of the junctional complex in the gut epithelium of C.elegans.

(1) Sulston et al., 1983, Dev Biol 100, p64; (2) Leung et al., 1999, Dev Biol 216, p114; (3) Legouis et al., 2000, Nat Cell Biol 7, p415; (4) Bossinger et al., 2001, Dev Biol 230, p29; (5) Bilder and Perrimon, 2000, Science 289, p113; (6) Costa et al., 1998, JCB 141, p297.

161. Establishment of apical junctions in epidermal cells during *C elegans* embryogenesis: compaction by the LAP protein LET-413 and protein clustering by the MAGUK protein DLG-1.

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Specialised sub-apical junctions play a critical role in maintaining epithelial cell polarity and tissue integrity, and provide a platform for intracellular signalling. We have analysed the roles of *C elegans* genes *let-413* and *dlg-1* in the assembly of the *C elegans* apical junction (CeAJ) and provide a first characterisation of this structure.

The LET-413 protein is a member of the new LAP family of proteins which contain 16 leucine-rich repeats (LRR) and PDZ domain(s). LET-413 localises to the basolateral membrane of epithelial cells

DLG-1 is a MAGUK protein homologous to the *Drosophila* protein *lethal discs large* and localises at CeAJs with the junctional protein JAM-1.

Immunohistochemistry and electron microscopy analysis of epidermal cells in *dlg-1* and *let-413* mutants have revealed that :

1) DLG-1 and JAM-1 are part of a complex in CeAJ which does not colocalise with the HMP-1/HMP-2/HMR-1 complex (alpha-catenin/beta-catenin/cadherin). This indicates that CeAJs are composed of at least 2 distinct units.

2) At the onset of epidermal differentiation the CeAJ markers DLG-1, JAM-1 and HMP-1 are spread along the lateral membranes. Later on they become restricted to their precise and definitive sub-apical localisation.

3) Loss of *dlg-1* activity leads to JAM-1 mislocalisation and the disappearance of the electron dense component of the CeAJs, but only mild adhesion and polarity defects.

4) In contrast, loss of *let-413* activity leads to the formation of basally extended discontinuous CeAJs and strong adhesion and polarity defects. Localisation of CeAJ markers is reminiscent of the situation observed in wild type immature epidermal cells.

We conclude that the primary function of LET-413 is to correctly position CeAJ components at a discrete sub-apical position. Furthermore, we propose that DLG-1 is required to aggregate JAM-1 and other proteins forming the electron dense CeAJ structure. This study is the first characterisation of the dynamic of assembly of apical junctions during *C. elegans* epidermal differentiation.

We are currently investigating which domains of LET-413 are responsible for its basolateral localisation. This is achieved by making specific deletions in a functional *let-413::*GFP construct and testing for GFP localisation. Recent results will be presented.

162. Cooperative regulation of JAM-1 by Discs large and LET-413 controls junctional tightness of *C. elegans* epithelia

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Epithelial morphogenesis depends on specialized cell-cell junctions that maintain an occluding seal between neighboring cells and limit the permeability of the cell sheet. This function has been attributed to septate junctions in arthropods and tight junctions in vertebrates. In *C. elegans* epithelia, neither of these junctional domains has been observed ultrastructurally.

Our TEM analysis of *C. elegans* epithelia identified an apical junctional domain that forms a continuous tight connection between epithelial cells. We present evidence that this domain might serve as an occluding seal in *C. elegans*. We have identified the novel coiled-coil protein JAM-1, which is recognized by the MH27 antibody, and show it localizes to apical junctions by immunogold analysis. Confocal analysis of JAM-1 and HMP-1 localization suggests that JAM-1 occupies a domain immediately basal to the HMR/HMP (cadherin/catenin) complex. In *jam-1* mutant embryos, the apical junctional domain is separated intermittently, compromising the continuity of the apical seal. Embryos arrest at the 2-3-fold stage of elongation; preliminary

results suggest that epithelia in mutants have increased permeability, based on passage of size-specific dyes. We provide evidence that JAM-1 is a physical target of DLG-1, the homologue of *Drosophila* Discs large, which is a critical component of septate junctions. DLG-1 localizes to apical junctions and controls JAM-1 localization but not general cell polarity, while the LAP protein LET-413 regulates the localization of both DLG-1 and JAM-1. Loss of *dlg-1* and *let-413* function results in an almost complete loss of JAM-1 from apical junctions in embryos, while HMP-1 localization is only mildly affected. Dynamic analyses of DLG-1 and JAM-1 localization using multi-photon microscopy suggest that LET-413 is required for rapid accumulation of DLG-1 and JAM-1 at apical junctions. We conclude that in *C. elegans* epithelia junctional permeability is controlled by a distinct apical domain containing JAM-1, which is cooperatively localized to junctions by DLG-1 and LET-413.

163. Analysis of VAB-9, a Putative Integral Membrane, Cell Junction Protein

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vab-9 encodes a putative four-pass transmembrane protein with a predicted topology similar to the claudin family of tight junction proteins. *vab-9* mutants have tail and body shape defects and are variably Dpy and Egl. We previously reported that VAB-9-GFP localizes to the cellular junctions of all epithelia, and to the cell membrane of larval body wall muscles. Using VAB-9 antibodies we have determined that in embryos VAB-9 co-localizes with HMP-1 (α -catenin) at adherens junctions, is apical to JAM-1 (junction-associated molecule 1), and is basal to PAR-3 and PAR-6. These results suggest there are three distinct regions of protein localization at the epithelial cell-cell junction and that VAB-9 is most closely associated with the adherens junction protein HMP-1. One hypothesis is that each protein localization domain represents a functionally distinct region of the epithelial cell junction. JAM-1 and DLG-1 (the homologue of *Drosophila* Discs large) are thought to maintain the tightness of the paracellular space, a function commonly attributed to septate or tight junctions (see abstract by Köppen, *et. al.*). Thus, JAM-1 and DLG-1 may define a septate junction-like region in *C. elegans* epithelia and this region may be distinct from the adherens junction. It was surprising therefore that VAB-9, which is similar to the tight junction claudin proteins, did not co-localize with JAM-1. To determine the functional relationship between these genes, we constructed double mutants. *vab-9* mutations enhance the phenotypes of both *jam-1* and *dlg-1(RNAi)* embryos. Specifically, *jam-1* and *dlg-1(RNAi)* embryos arrest at two-fold and do not rupture, whereas *vab-9; jam-1* and *vab-9; dlg-1(RNAi)* animals rupture prior to this stage. HMP-1 and PAR-3 are properly localized in *vab-9; jam-1* animals, indicating there are no general defects in epithelial polarity in these embryos. One possibility is that VAB-9 and DLG-1/JAM-1 localize independently to distinct regions of the lateral membrane that together regulate

epithelial tightness. Consistent with this hypothesis, we find that *let-413* mutations, which disrupt junctional localization of DLG-1 and JAM-1, do not similarly disrupt the localization of HMP-1 and VAB-9. We previously reported that *vab-9* is required for the organization of the circumferential actin filaments in epithelial cells required for coordinated elongation. Thus, VAB-9 may be a novel junctional protein with multiple roles in regulating cell morphology and adhesion. Surprisingly, we found that VAB-9 localizes along the muscle quadrant tracks in embryos. In contrast, other epithelial cell-junction proteins (*e.g.* HMP-1, JAM-1 and DLG-1) are not expressed along muscle quadrants. Co-staining with muscle markers suggested that this muscle quadrant-associated VAB-9 is expressed by the hypodermis. We tested this possibility (and the possibility that *vab-9* has a role in muscle morphogenesis), by using Andy Fire's tissue-specific RNAi technique. RNAi of *vab-9* in the hypodermis eliminated all VAB-9 expression in the embryo. These embryos arrest during elongation and ultimately are paralyzed. This phenotype is more severe than that of the four existing alleles of *vab-9*, which are viable and fertile. We are currently examining the localization of muscle components in these embryos. VAB-9 localization along muscle quadrants, at muscle cell membranes and at epithelial cell junctions suggests that VAB-9 may have a general role in cell adhesion.

164. The gap junction protein INX-3 is essential for embryonic morphogenesis.

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The invertebrate family of innexin proteins is functionally equivalent to the vertebrate connexin family of gap junction proteins, although the two families have no significant amino acid sequence similarity. Gap junctions mediate the direct intercellular communication of small molecules, such as ions, metabolites and second messengers. Although the properties of gap junction channels have been studied in some detail, their role in development is poorly understood. To analyze the function of gap junctions during embryogenesis, we are investigating the worm innexin gene, *inx-3*. We have examined *inx-3* expression with GFP fusions and anti-INX-3 antibodies. INX-3 can be detected beginning at the 2-cell stage, a time when embryonic cells become dye-coupled (Bossinger and Schierenberg. 1992. *Dev. Biol.* 151:401-409). Expression of INX-3 is ubiquitous throughout early embryogenesis but becomes more restricted at the onset of morphogenesis. Double-labelling with MH27 and anti-INX-3 indicates that during morphogenesis INX-3 is expressed strongly in the developing intestine, hypodermis, and pharynx, although other tissues may express *inx-3* as well. At hatching INX-3 is present principally in the posterior pharynx (isthmus and terminal bulb), at the anteriormost tip of the pharynx, near the posterior intestine, and briefly in the hypodermis. Several transient patterns of INX-3 accumulation are striking, although we do not yet know their developmental significance. As mentioned, during embryogenesis INX-3 is present strongly but briefly in the intestine, and hypodermal expression is limited to embryonic and early L1 stages. During the late L1 stage INX-3 is detectable briefly in the post-embryonic motor neurons descended from the Pn.a cells and in the immediate descendants of the M blast cell (first two cell divisions). At the L3/L4 stages the developing sex muscles express INX-3 strongly, then expression is reduced in adults such that only a few INX-3 plaques are detectable.

To determine what role gap junctions may play during embryogenesis we have begun to analyze *inx-3* mutants. RNAi analysis indicated that loss of *inx-3* is lethal. To isolate *inx-3* mutants, lethals linked to the *inx-3* region were identified, and embryos were stained with anti-INX-3 antibodies to identify candidates. Two deficiencies and one point mutant with a premature stop, which we believe represents a null, were identified. Mutant embryos appear to progress normally through embryogenesis until morphogenetic stages. The earliest defect detected in *inx-3(lw68)* embryos, at nearly 100% penetrance, is the loss of several cells from the anterior of the embryo just prior to anterior hypodermal enclosure. Other less penetrant mutant phenes that occur during embryonic morphogenesis appear to involve the hypodermis. These defects include mispositioning of lateral seam cells, altered ventral P cell contacts, problems with body elongation, as well as ventral or anterior rupture of the hypodermis. Most embryos that complete embryogenesis display highly penetrant pharyngeal defects that include a foreshortened pharynx and failure of the pharynx to attach to the nose. When embryos are isolated that lack both maternal and zygotic INX-3, the severity and penetrance of mutant phenes are the same as for embryos lacking only zygotic INX-3. We hypothesize that INX-3 is required to form gap junction channels that coordinate activities between coupled cells necessary for proper morphogenesis.

165. A Role for Inositol 1, 4, 5-Triphosphate Receptor During the Process of Ventral Enclosure in *Caenorhabditis Elegans*

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During embryogenesis, epithelial cells migrate and change shape in order to generate three dimensional structure. During the process known as ventral enclosure in *C. elegans*, epithelial cells that originate on the dorsal surface of the embryo migrate to the ventral midline, forming adhesive junctions. One mutant that is defective in ventral enclosure is *jc5*.

jc5 is a cold sensitive maternal effect mutant that displays defects during varying stages of embryogenesis. At least 20% of embryos arrest because migration of epithelial cells from the dorsal side never occurs. Approximately 20% die during enclosure. Often in these embryos, the epithelial sheet initiates migration towards the ventral midline, fails to seal, and eventually retracts back to the dorsal surface. Others display partial enclosure phenotypes. Approximately 40% of dead embryos enclose properly, but develop severe body shape defects during elongation.

Using RNA interference, we have been able to phenocopy the *jc5* phenotypes with RNA corresponding to the inositol 1, 4, 5-triphosphate receptor-1 gene, *itr-1*. In addition , we have obtained rescue of the *jc5* phenotype by injection of genomic DNA corresponding to the *itr-1* gene.

It is known that ITRs control intracellular calcium levels, and a recent paper has shown a role for calcium in the regulation of actin in growth cone turning in another system(Gallo et al, Current Biology 9, 490). From work done in our laboratory, we know that the ventral hypodermal cells extend actin-rich filopodia before they migrate (Williams-Masson et al, Development 124, 2889). We hypothesize that

ITR-1 regulates cytoskeletal rearrangement during ventral enclosure through localized calcium release. To test this, we are planning to study calcium dynamics in *itr-1(jc5)* mutants using the calcium indicator fluo-4.

Additional experiments must be done to explore how ITR-1 is functioning to regulate the cytoskeleton of hypodermal cells. To this end, we are analyzing alpha-catenin-GFP dynamics during migration in epithelial cells using multiphoton microscopy. Preliminary data suggest that in some cases filopodia are misdirected, failing to migrate to the proper contralateral partner cell. In addition, preliminary phalloidin staining suggests that actin is disorganized in epithelial cells that have halted migration in mutants. Currently, we are sequencing the lesion in the *itr-1*, gene as well as eliminating *itr-1* activity in epithelial cells using antisense expression to test for epithelial-specific defects. In order to find other proteins that may affect ITR-1 function either directly or indirectly, we have initiated a suppressor screen for rescue of the *itr-1(jc5)* phenotype. Thus far, we have screened 1100 genomes and have found 5 candidate suppressors.

166. The LAR-like Receptor Tyrosine Phosphatase PTP-3 and the VAB-1 Eph receptor tyrosine kinase have partly redundant functions in morphogenesis

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Mutations in the *C. elegans* Eph receptor tyrosine kinase, VAB-1, and the ephrins, EFN-1/VAB-2 and EFN-4/MAB-26, cause defects in neural and epidermal morphogenesis. These defects are incompletely penetrant, suggesting that other pathways may function in parallel. To identify components of such parallel pathways we investigated whether *vab-1* mutations displayed synthetic lethality with other morphogenetic mutants. A mutation in a *C. elegans* receptor tyrosine phosphatase, *ptp-3(op147)* , previously known as *ptp-1*, causes mild morphogenetic defects similar to those of weak *vab-1* alleles. We have found that *ptp-3(op147)* synergizes with *vab-1* mutations. *ptp-3(op147)* also synergizes with mutations in ephrin genes *efn-1/vab-2* and *efn-4/mab-26* suggesting that PTP-3 may have redundant functions with Eph signaling to regulate morphogenesis. *ptp-3* does not display genetic interaction with other RTK mutants or morphogenetic mutants tested.

PTP-3 is most similar to LAR-like receptor protein tyrosine phosphatases. We have found that the *ptp-3* locus encodes two isoforms, PTP-3A and PTP-3B. PTP-3A contains 3 Ig-like domains, 8 fibronectin type III repeats, and two tandem cytoplasmic phosphatase domains. PTP-3B contains only 4 FNIII repeats and the two phosphatase domains. PTP-3 isoforms are expressed in many tissues in early embryogenesis, and later become localized to neuronal processes and to epithelial adherens junctions. Interestingly, mutations that specifically delete the PTP-3A isoform (isolated by the *C. elegans* Gene Knockout Consortium)

display wild type morphogenesis and do not synergize with Eph signaling mutants. PTP-3A and PTP-3B may have overlapping functions in morphogenesis and loss of one or the other may not cause significant defects (the *op147* mutation affects both isoforms).

We have analyzed the phenotypes of *ptp-3* single mutants and *vab-1 ptp-3* double mutants using 4D microscopy. *ptp-3* mutants have gastrulation and ventral closure defects similar to those of Eph signaling mutants. *vab-1 ptp-3* double mutants show the same defects, only at a higher frequency. Our data suggest that PTP-3 and Eph signaling function redundantly. We are currently using tissue specific promoters to ask whether PTP-3 and VAB-1 function redundantly in the same tissues.

167. *kal-1*, the *C. elegans* homolog of the X-linked Kallmann syndrome gene, is involved in epidermal morphogenesis and neuronal growth.

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The molecular mechanisms through which cells and neuronal growth cones migrate, adhere to the substrate and find their targets are varied, appear to often work in redundant pathways, and appear to be conserved in evolution. Although many molecules have already been identified and studied, especially using the invertebrate model systems, it is clear that many other molecules involved in these processes still need to be discovered, especially the components of the extra cellular matrix (ECM) that play a role the final steps of morphogenesis. We have focused our study on *kal-1*, the *C. elegans* homolog of the X-linked Kallmann syndrome gene. The syndrome is characterized by anosmia and hypogonadism and is apparently due to a failure in the migration or proper targeting of olfactory axons in the olfactory bulbs. The human gene responsible for the X-linked form of the disease has been identified. It codes for a secreted protein of still elusive function that contains a cysteine rich N-terminal domain, a four disulfide core domain, WAP, and three fibronectin type III domains. The *C. elegans* gene is composed of 6 exons and codes for a 700 aa protein with a domain topology similar to that of vertebrates. Using reporters we have determined that *kal-1* is expressed by a subset of neuroblasts/neurones beginning early in embryogenesis. To understand the function of *kal-1* in *C. elegans*, we generated and studied a null deletion mutant, *kal-1(gb503)*, as well as worms overexpressing KAL-1 from transgenic extrachromosomal arrays. The phenotypes shown by *kal-1* mutants include:

i) male tail defects; in general the whole structure appears scrawny or distorted and the sensory rays are variously altered with reductions and losses of rays, presence of extra rays, fusions and inversions of the position of rays. The male tail defects of the loss of function mutant *gb503* are recessive and represent the most penetrant phenotype observed. The defects are rescued in *gb503* worms carrying a wild type copy of the gene as a transgene on an extra-chromosomal array. **ii**) embryonic lethality and morphological abnormalities of newly hatched larvae; affected *kal-1* mutant embryos are defective in ventral enclosure and rupture ventrally with cells protruding out of the embryonic mass. Some embryos appear to present later enclosure defects resulting in head and tail abnormalities of the hatched larvae. **iii**) incompletely penetrant neuronal growth defects with extra-branching of processes. Experiments using specific reagents to visualize epithelial cell boundaries in mutant worms indicate that the defects, caused by a reduction or an increase of *kal-1* function, are mediated by dramatic effects on shape, position, adhesion and migration of epidermal cells undergoing active morphogenesis in the proximity of *kal-1* expressing neurons. Since *kal-1* reporters are expressed in neuronal cells while the cells most affected by mutants are epithelial cells, we propose that CeKAL-1 acts non-cell-autonomously to modulate, in concert with other molecules, the adhesion of cells and growth cones to the matrix and to other cells. The combination of phenotypes we have observed in *kal-1* mutants is strongly reminiscent of that presented by mutants in other genes which affect epithelial morphogenesis and axonal growth, such as *mab-20* (Ce-Sema-2a), *vab-2* and *vab-1* (ephrin and ephrin receptor) and others. It is possible that *kal-1* acts in the same pathways in which these genes function.

168. LAD-1, the *C. elegans* L1CAM homolog, participates in cell migration, and is a substrate for FGFR pathway-dependent phosphotyrosine-based signaling

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LAD-1, the sole C. elegans homologue of the L1CAM family of neuronal adhesion molecules, is required for proper development of the germline and the early embryo, as well as morphogenesis and migration of the distal tip cells of the gonadal arms. In addition, the ubiquitously-expressed LAD-1, which binds ankyrin, may function as a general receptor for the UNC-44 ankyrin in C. elegans. Finally, we present evidence that LAD-1 is phosphorylated, in an *egl-15* FGFR pathway-dependent manner, on a tyrosine residue in the highly conserved ankyrin-binding motif FIGQY, which was previously shown to abolish L1CAM binding to ankyrin in cultured cells.

Immunofluorescence studies revealed that FIGQY-tyrosine phosphorylated LAD-1 does not co-localize with non-phosphorylated LAD-1 or UNC-44 ankyrins, but instead is localized at sites that experience mechanical stress including the apical surface of the pharynx, the adherens junctions in the hypodermis, intestine, vulva, and rectum, and axon-muscle junctions. These findings suggest a novel ankyrin-independent role of LAD-1 in cell domains subjected to mechanical stress.

Taken together, these results indicate L1CAMs constitute a family of ubiquitous molecules, which participate in fundamental processes to promote cell migration and tissue integrity in metazoans.

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169. A tale of one gene encoding two proteins, one required to anchor muscles the other to maintain epidermal integrity

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We are interested in analysing the process of embryonic morphogenesis, and in particular genes required to maintain the integrity of epidermal cells. We have previously performed a chromosomal deficiency screen to identify loci required for morphogenesis. Following the approach used to identify *let-413* (see abstract by Legouis), w*e* have decided to investigate the region covered by *hDf17* (LGI), which completely prevents morphogenesis. By analysing the phenotype of lethal mutations mapping under *hDf17*, we identified *h1483*, which is a new *hmr-1* allele leading to ventral enclosure defects, and *h1356*, which proved to be a *vab-10* null allele preventing elongation. Using a candidate gene approach and RNAi, we cloned *vab-10* and showed that it encodes different members of the plakin protein family via a complex pattern of alternative splicing. Specifically, we potentially identified 10 isoforms that share a common N-terminus (circa 1000 aa) with a predicted actin-binding domain and different C-termini. Isoforms can be grouped into two major classes: those (circa 3500 aa) related to human plectin, and those (circa 5000 aa) related to fly kakapo/ human macrophin. We found that a vab-10::gfp fusion common to most isoforms is expressed in the hypodermis and localised in circumferentially-oriented stripes that correspond to areas of contact between the hypodermis and body-wall muscle quadrants. Furthermore, we demonstrated that MH5, a mAb known to mark muscle attachment structures within the hypodermis (fibrous organelles), recognises a *vab-10*-encoded epitope. Fibrous organelles are anchored to the cuticle and coupled via myotactin to ECM, and subsequently to the dense bodies in mucles.

RNAi experiments against specific isoforms coupled with eletron microscopy analysis and immuno-staining with muscle-specific (MH3, MH24, MH25, NE8-4C6, 5.6.1 mAbs) or hypodermal-specific (MH4, MH46, MH27, anti-LIN-26 Abs) markers, suggest that the plectin-specific and kakapo-specific isoforms have different functions. Removal of plectin-like isoforms result in embryonic lethality, producing 1.5-fold embryos in which only the tips of the head and tail have elongated. Muscle cells are initially positioned correctly but fail to attach, sinking into the embryo, while hypodermal cells seem structurally normal. EM studies revealed the accumulation of ECM material basal to the hypodermis, a severe disorganisation of muscle fibres and cuticle defects. In contrast, removal of kakapo-like isoforms produces embryos that can elongate slightly beyond the two-fold stage and hatch; the resulting larvae are lumpy and misshapen and remain very small even if they survive to the L2/L3 stage with additional moulting problems. In embryos lacking kakapo-like isoforms, muscle fibers are locally disorganised in areas where the structure of hypodermal cells seems affected (as inidcated by the localised accumulation of hypodermal nuclei and lumpiness). The phenotype of *h1356* is very similar to that of embryos lacking plectin-like isoforms.

We propose that plectin-like isoforms are components of the *C. elegans* hypodermal fibrous organelle, and that their loss results in a breakdown of muscle-hypodermis-cuticle attachments. We suggest that the function of kakapo-like isoforms is more directly related to hypodermal cytoskeletal integrity with some indirect affects involving muscles. Plectin in vertebrates and kakapo in flies are also part of anchoring structures in the epidermis (hemi-desmosomes) or tendon cells (muscle attachments), respectively. The *vab-10* locus could correspond to an ancestral form of the main anchoring structural gene caught in the act of divergence.

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170. *mua* genes function within the hypodermis, and may be regulated by the EKLF homolog MUA-1

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Mutations in the *mua* (for *mu*scle *a*ttachment) *genes* result in a use dependant failure of the linkages that transmit force between skeletal muscle and cuticle[1]. Force generated by the body wall muscles is transmitted through an intervening basal lamina and the hypodermis to the cuticle driving locomotion. Complexes of matrix receptors and associated cytoskeletal proteins are assembled by both muscle and hypodermal cells forming mechanical link between the tissues at the sites of force transmission In *mua* animals initial assembly of these structures during embryogenesis is apparently normal, however postembryonic failure of these linkages results in detachment of muscle from the cuticle.

At least five of the *mua* genes appear to be involved in maintaining integrity of the hypodermal portion of this force transmission pathway. It has been previously reported that *mua-3* encodes a novel matrix receptor protein, and *mua-6* encodes IFA-2, both proteins are expressed in hypodermis where they associate with the fibrous organelles (hemidesmosomes) involved in muscle force transmission[2]. TEM examination of *mua-3* mutants show attachment failure between the apical hypodermis and the basal cuticle. Antibodies against hypodermal proteins and/or GFP tagged MUA-6 were used to show that mutants in *mua-2* and *mua-10* result in loss of cuticle adhesion and fragile IFs respectively, suggesting that they also are expressed in the hypodermis.

mua-1 encodes a C2H2 zinc finger protein of the EKLF class. These proteins are known to act at transcriptional modulators of tissue specific expression, and play a variety of important roles in vertebrate tissue specific differentiation. The phenotype of *mua-1* suggested that it may play a similar role in the terminal differentiation of either muscle or hypodermis, with the Mua phenotype resulting from failure to produce adequate levels of differentiated gene products. Given the similarity of the *mua-1* phenotype to the phenotypes of the other *mua* genes it was predicted that *mua-1* would be hypodermally expressed.

In order to determine the cells in which *mua-1* is expressed, the promoter and first intron of *mua-1* were fused in frame with GFP. When re-introduced into wild-type animals the fusion protein was expressed almost exclusively in hypodermal nuclei bordering the seam. Expression was occasionally observed in hypodermal nuclei of the ventral cord. Significantly, no expression was observed in body-wall muscles. These observations are consistent with a model in which *mua-1* expression is activated in nuclei that join the hypodermis postembryonically to drive expression of tissue specific gene products. We are currently in the process of testing this hypothesis.

[1]Plenefisch et al., (2000) Development 127: 1197

2 Bercher et al., submitted; Hresko, et al ., submitted.

171. From a tetraspanin mutation, hopeful monsters

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Mutations have been isolated that disrupt the completion of molting. Among these are three that confer unusual phenotypes. *sv3* homozygotes arrest growth in the L3 stage and are constricted by unshed cuticle for most of the middle part of the body. Cuticle is shed from the extremities, producing worms that resemble dumbbells. Genetic mapping has placed *sv3* within a few cosmids of *lin-2* on the X chromosome. The *sv3* phenotype is mimicked by an unlinked mutation, *sv9*, suggesting that at least two genes function in a biochemical or signal-transduction pathway that is required for proper synthesis or shedding of part of the cuticle. Located near *unc-29* on LGI, *sv15* confers dumpiness, molting defects, blistering of the cuticle, and the growth of small protuberances from the cuticle. Present at low penetrance are worms disfigured by large protuberances. The *sv15* mutation has been rescued. Subcloning has indicated that *sv15* is a mutation in a tetraspanin gene, a supposition proved by DNA sequence analysis. Mosaic analysis indicates that hyp7 is the focus of gene activity. More detailed analysis of the *sv15* mutant phenotype and the isolation of interacting mutations have the potential to expand our knowledge of these enigmatic integral membrane proteins.

172. *tlp-1* is involved in the control of cell polarity and male tail tip morphogenesis during *C. elegans* development

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We are interested in how cell polarity and morphogenesis are controlled during metazoan development. Mutations in *lin-44/Wnt* and *lin-17/frizzled* affect the polarity of certain cells in the tail called TL and TR. We have isolated mutations defining a new gene, *tlp-1*(T cell lineage defective and leptoderan tail). Cell lineage analysis has revealed that *tlp-1* mutations cause defects in T cell division patterns consistent with defects with cell polarity. Specifically, we observed defects in the divisions of the T.p and T.ap cells. In addition, *tlp-1* males have abnormal tails in that they have a posterior protrusion reminiscent of leptoderan nematode species. In *wild-type* males, retraction of the tail tip hypeodermis involves a temporally ordered set of cell fusions and changes in cell shape. *jam-1::gfp* expression in *tlp-1* males showed that, as compared to *wild-type* males, fusions of the tail tip hypodermal cells were heterochronically delayed. We cloned *tlp-1* by transformation rescue and found sequence changes in all three *tlp-1* mutant alleles. DNA sequence analysis revealed that *tlp-1* encodes a zinc-finger protein with weak similarity to *NocA* which is required for the embryonic brain and the adult ocellar development in *Drosophila*. Phylogenetic analysis suggests that *tlp-1* may be distantly related to the Sp family of transcrioptional factors. A *tlp-1::gfp*transgene, in which GFP was fused in frame with TLP-1, rescued the T cell polarity defect and was expressed in the nuclei of the posterior intestine cells, the tail hypodermal cells, the T cells and certain T cell descendents. Specifically, we observed *tlp-1::gfp* expression in T, T.p and T.ap, suggesting that TLP-1 may be asymmetrically expressed or segregated during the divisions of the T cell lineage. Expression of *tlp-1::gfp* in *lin-44* and *lin-17*, as well as T cell lineage analysis of *lin-17(n671); tlp-1(mh17)* and

lin-44(n1792); tlp-1(mh17) double mutants suggest that *tlp-1* may respond to *lin-44* and *lin-17* gene in the control of T cell polarity. These results suggest that *tlp-1* encodes a transcription factor required for cell polarity and patterning in the *C. elegans* tail.

173. An innexin, *lep-1*, is required for male tail tip morphogenesis

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We are interested in the genetic and molecular mechanisms that control the assembly and function of male tail tip morphogenesis. The male tail tip of *C. elegans* undergoes morphogenesis during the fourth larval stage (L4 stage) of post-embryonic development. The four hypodermal cells that form the tail tip coordinately fuse and retract in the males, generating a blunt-ended (peloderan) tail tip in the adult. In some species related to *C.elegans*, the tail tip cells fail to retract, producing a pointy (leptoderan) tail tip. This suggests that tail tip morphogenesis can be regulated independently from general male tail morphogenesis.

To further understand the genetic mechanisms that underlie male tail tip morphogenesis in *C.elegans*, we undertook a genetic approach. We are characterizing fourteen new mutants in which the tail tip cells fail to retract. The tail tip of these mutants resembles that of leptoderan nematodes (Lep phenotype) in both morphology and development. The first gene cloned from this screen is *lep-1*. The *lep-1* gene, encoding a potential innexin, controls male tail tip morphogenesis in *C.elegans* (Yang, 2000). LEP-1 might be part of a signaling pathway involved in tail tip morphogenesis since it is a predicted gap junction component. Members of the innexin protein family are structural components of invertebrate gap junctions and are analogous or possibly homologous to vertebrate connexins.

To estimate the loss of function phenotype of the *lep-1* gene by RNA interference, *in vitro* transcribed *lep-1* double stranded RNA was injected into wild type worms (*him-5* background). Our results indicate that RNAi is ineffective to generate a phenotype for the *lep-1* gene. To further investigate the function of *lep-1* during post-embryonic development we are in

the process of examining the expression patterns of GFP translational and transcriptional fusions. For the translational fusion, a 7.3 kb genomic region of *lep-1* (rescuing fragment) is being fused to a *gfp* promotorless vector. For the transcriptional fusion, 3.6kb of the upstream region of *lep-1* will be fused to *gfp*. Current functional studies include *Xenopus* oocyte coupling experiments to determine the ability of LEP-1 to influence cell-cell communication. Overall, these studies will help us to understand the role of this innexin in tail tip morphogenesis.

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174. Regulation of Pn.p Cell Fusion in *C. elegans*

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Cell fusion is a common process in *C. elegans*. The *C. elegans* epidermis consists of several multinucleate syncytia that are generated by the fusion of cells throughout development. The largest such syncytium is hyp7, which spans most of the length of the worm and which contains more than 130 nuclei. To study the formation of this syncytium, we isolated mutations that prevent fusion of one set of cells that fuse with hyp7, the 12 Pn.p cells that line the ventral surface of the worm late in L1. We identified several mutations that affect the pattern of Pn.p cell fusion and have been characterizing two mutations that affect Pn.p cell fusion by altering Hox protein activity.

The fusion decision of the 12 Pn.p cells is controlled by two Hox genes, *lin-39* and *mab-5*. *lin-39* is expressed in the mid-body [P(3-8).p] and in hermaphrodites prevents fusion of these cells. *mab-5* is expressed more posteriorly [in P(7-11).p] in both sexes, but is not active in hermaphrodite Pn.p cells. In *ref-1(mu220)* (REgulator of Fusion) hermaphrodites, P9.p and P10.p fail to fuse with hyp7. This is due, at least in part, to inappropriate activation of MAB-5 in *ref-1* mutant hermaphrodites. *ref-1* encodes a gene with two basic helix-loop-helix DNA binding domains of the *hairy/E(spl)* subfamily.

In males, *lin-39* and *mab-5* each individually prevent Pn.p cell fusion in P(3-6).p and P(9-11).p, respectively. However, in P7.p and P8.p, where both Hox genes are expressed in the same cell, the two Hox proteins neutralize one another's activities, so that P7.p and P8.p fuse with hyp7. In *ref-2(mu218)* males, P7.p and P8.p fail to fuse with hyp7, perhaps because LIN-39 and MAB-5 fail to cancel each other's activities. *ref-2(mu218)* is a dominant mutation in a non-coding region near a zinc finger transcription factor. This mutation affects the pattern of Pn.p cell fusion by affecting this transcription factor, perhaps by altering its expression. To determine the role of *ref-2* during development, we carried out RNAi of *ref-2* and also ectopically expressed *ref-2* using a heat shock promoter. These experiments demonstrate that the *ref-2* gene product is

required for the generation of the Pn.p cells and also to keep the Pn.p cells unfused. We are currently trying to determine the effect of the *mu218* mutation on *ref-2* expression and are also trying to define the relationship between *ref-2* and the Hox genes.

175. Functions of *lin-40* MTA, synMuv, and Ras in regulating the competence of the vulval precursor cells during vulval fate specification

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The vulval fate in the Pn.p cells is specified in two sequential steps. During L1 and L2, a *lin-39* Hox-dependent mechanism blocks the fusion between six Pn.p cells (P3.p through P8.p, collectively referred to as the vulval precursor cells) and the hypodermal syncytium, hyp7, so that these Pn.ps are competent to adopt the vulval fate during the second step. Later in L3, the vulval fate is induced in three (P5.p through P7.p) of the six Pn.ps by the cooperation of the RTK/Ras/MAPK pathway, the synMuv genes, and *lin-39*. We will report our studies of another regulator of vulval fate specification, *lin-40* MTA, and discuss previously uncharacterized roles of the synMuv genes and the Ras/MAPK pathway in the early fusion event.

Several *C. elegans* homologues of the nucleosome remodeling and histone deacetylation (NuRD) complex components have been shown to function in the class B synMuv pathway, together with *lin-35* Rb, to repress vulval induction [1-3]. *lin-40* (also known as *egr-1*) encodes a homologue of the metastasis-associated factor-1 (MTA-1) in mammals, which was identified as another component of the NuRD complex. We found that severe loss-of-function mutations in *lin-40* led to a Muv phenotype, indicating that *lin-40* negatively regulates vulval induction. However, using a weak loss-of-function mutation and RNAi of *lin-40*, we did not identify the typical synergistic interaction between *lin-40* and either class of the synMuv genes. These results suggest that *lin-40* represses vulval induction independently of the class B synMuv pathway. Our studies reveal that *lin-40* affects the fusion between Pn.ps and hyp7, and therefore regulates the competence of the vulval precursor cells (VPCs) for vulval induction. Using a JAM-1::GFP reporter to examine the fusion frequency, we found that in a *lin-40* mutant, P3.p remained unfused at a higher frequency than in WT. Similar effect was also observed in the posterior P9.p through P11.p cells in an

egl-27 background. In addition, a *lin-40* mutation caused P3.p, as well as P4.p and P8.p, to adopt the vulval fate at an elevated frequency in a *let-60* Ras*(n1046 gf)* background, indicating that the competence of these Pn.ps is enhanced by the *lin-40* mutation. This elevated competence of Pn.ps in *lin-40* mutants was suppressed by a *lin-39(n709ts)* mutation. Furthermore, we show that *lin-39* expression was upregulated in *lin-40* mutants. Taken together, these results suggest that *lin-40* represses the competence of Pn.ps for vulval induction by promoting cell fusion between these cells and hyp7 during L1/L2, and this inhibitory function may be carried out by down-regulating *lin-39* Hox expression.

We further show that the Ras/MAPK pathway and the synMuv genes also regulate the fusion of Pn.ps during L1/L2. The Ras/MAPK pathway positively regulates the competence of these cells for vulval induction by preventing the fusion, whereas the class B synMuv genes have the opposite effect. The class A synMuv mutations did not appear to affect the fusion of Pn.ps on their own, but were able to enhance the effect of the class B mutations. The *lin-39(n709ts)* mutant phenotype in the Pn.p cell fusion was epistatic to the ones caused by mutations in the synMuv genes and the Ras/MAPK pathway, suggesting that *lin-39* is a pivotal regulator downstream of multiple pathways in controlling the competence of the VPCs.

1. Lu, X. and H.R. Horvitz, Cell, 1998. 95(7): p. 981-91.

2. Solari, F. and J. Ahringer, Curr Biol, 2000. 10(4): p. 223-6.

3. von Zelewsky, T., et al., Development, 2000. 127(24): p. 5277-84.

176. HOW HOX WORKS? *eff-1* is the name, effector of cell fusion is the game

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Throughout evolution the Hox genes are responsible for establishing regional identity in the antero-posterior axis. In the ventral midbody of *C. elegans* the Hox gene *lin-39* specifies which of the Pn.p cells will become vulval cells, and which of them will fuse to the hypodermis. *lin-39* activity is necessary both at the L1 stage [for P(3-8).p] and later, at the L3 stage [for P(5-7).p] in order for the cells to remain unfused and to be further induced to form the vulva [1]. In *lin-39*(-) mutants all the Pn.p cells undergo fusion leading to a vulvaless phenotype (Vul). In *P. pacificus* the activity of *lin-39* is also needed for vulval fates but lack of this activity results not in cell fusion but rather in programmed cell death. This regulation is achieved by the repressive activity of *lin-39* on the apoptotic effector *ced-3* [2]. We have asked whether cell fusion of Pn.p cells in *C.elegans* is regulated in the same manner through repression of a "fusion effector". Albeit the importance of the Hox gene regulation, there are almost no identified Hox target genes that participate in actual cellular events including cell fusion.

We isolated *eff-1(hy21)* (**e**pithelial **f**usion **f**ailed*)*, a mutant in which all epithelial cells fail to fuse. Our results suggest that *eff-1* may be the first isolated gene in *C.elegans* encoding a fusogen- a protein that mediates cell-cell fusion [3]. The vulva in *eff-1(hy21)* single mutants is functional even though cell fusion during vulva formation is defective. In order to test whether *eff-1* could serve as the "fusion effector" regulated by *lin-39*, we investigated worms that are marked at their adherens junctions by the JAM-1:GFP construct. We found out that in *lin-39(n1760); eff-1(hy21)* double mutants lack of *eff-1* activity completely suppressed the Vul phenotype of *lin-39(-)*. This was evident at the L1 stage as all the Pn.p cells in the double mutant escaped fusion to the surrounding hypodermis and also at the L3 stage as the Pn.p cells remained unfused and continued proliferating to form the vulva primordium. Thus, *lin-39* inhibits cell fusion in *C. elegans* by

repressing the fusogenic activity of *eff-1* in the vulva equivalence group.

To test whether the role of *lin-39* in vulva formation is restricted to fusion repression, we analyzed the vulva structure formed in the *lin-39(n1760); eff-1(hy21)* double mutants. Although the vulval cells were able to migrate and form a stack of rings in the double mutant, the final structure of these rings was completely abnormal leading to a non-functional vulva in contrast to the vulva of *eff-1(hy21)* single mutants. Thus, *lin*-39 activity is required not only for inhibiting the fusion of vulva precursor cells, but also for induction of proper vulva organogenesis. We will discuss our studies on the relationship between *eff-1* and *lin-39* as well as other Hox genes in *C. elegans* and our experiments with worms carrying *hs-lin-39* constructs in order to test directly LIN-39 inhibition of *eff-1(hy21)* activity.

[1] Maloof and Kenyon (1998) Development 125 : 181

[2] Sommer et al. (1998) Development 125: 3861

[3] Shemer et al. (2001) 13th International *C. elegans* meeting

[4] Mohler et al. (2001) 13th International *C. elegans* meeting

177. Microevolution of vulval cell lineages within two nematode genera : *Caenorhabditis* and *Oscheius*.

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The cell lineage of *Caenorhabditis elegans* and some other nematodes is mostly invariant. Given this invariance, one can wonder how a cell lineage can evolve. We have started a microevolutionary approach by observing lineage variations of vulva precursor cells (VPCs) in different natural nematode populations : 22 strains of the *C. elegans* species and 5 different species in the *Caenorhabditis* genus, as well as 38 strains belonging to 9 different species of the *Oscheius* genus.

Our results show that, within both genera, lineage variations between species and even between strains of the same species occur mostly for VPCs that do not participate to the vulval invagination (VPCs with a 3° non-vulval fate). These cells are probably not under strong selection pressure and this could explain the large variations observed at a small evolutionary timescale.

In the reference strain *C. elegans* N2, the lineage of the P3.p cell is not invariant (Sulston and Horvitz, 1977). In 50% of N2 animals, P3.p is a true VPC : it is competent to form the vulva and divides once before fusing with the surrounding hypodermis. In the other 50%, P3.p is not competent, does not divide and fuses with the hypodermis. The percentage of occurrence of P3.p division varies between strains of the *C. elegans* species (from 10% to 60%) and between species of the *Caenorhabditis* genus (from 0% to 100%). In the *C. elegans* N2 strain, P3.p division is correlated with the competence of the cell. However, the correlation does not hold in the *C. elegans* strain CB4857, where P3.p divides in only 15% of the animals whereas it is competent in about 60% (as determined by ablation of P(4-8).p in the L1 stage). P3.p competence varies between *Caenorhabditis* species, suggesting that the size of the competence group has evolved between closely related species, but that the program of cell division can vary even within a species.

Species of the *Oscheius* genus can be easily found in soil samples from around the world (much more easily than *Caenorhabditis* species). Variations mostly concern P4.p and P8.p lineages (which adopt a 3° non-vulval fate in these species) : these cells divide twice, once, or not at all, but are always competent to replace the vulval cells P(5-7).p. In this genus too, intra-species polyporphism in cell lineages is amplified between closely related species. Thus, cell lineage variations observed between species could be due to fixation of variants that segregate within species.

Variations observed between strains are larger within the species *Oscheius* sp.1 than in *C. elegans*. Therefore, we have undertaken a genetic analysis between two pairs of strains of *Oscheius* sp. 1 that show distinct P4.p and P8.p lineages. For each pair, at least three loci are involved in the phenotypic differences. We also find that variation at one locus has a relatively strong effect on the phenotype (instead of small additive effects of many genes).

We have also detected "errors" at a very low frequency (0.1-1%) in the fate and lineage of cells which do form the vulva. These frequencies give the level of precision of vulval patterning mechanisms and point to a selection pressure for maintenance of a large vulval equivalence group. Such "errors" are more frequent in the *Oscheius* group than in *Caenorhabditis* species ; thus the robustness in vulval patterning may be stronger in the latter genus.

178. ORDERING THE synMUV CLASS A PROTEINS: LIN-15A MAY BE IMPORTANT FOR THE NUCLEAR EXPRESSION OF LIN-56

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The receptor tyrosine kinase/Ras pathway essential for vulval induction in *C. elegans* is negatively regulated by two redundant pathways, A and B. Hermaphrodites mutant in only one of these two pathways appear wild-type for vulval induction. Hermaphrodites mutant in both pathways exhibit the synthetic Multivulva (synMuv) phenotype: cells that normally adopt a hypodermal fate instead adopt a vulval fate and generate ectopic protrusions of vulval tissue along the ventral side of the animal. Various screens for multivulva animals have defined four genes in the synMuv class A pathway: *lin-8, lin-15A, lin-38*, and *lin-56*. Of these genes, only the *lin-15A* locus was cloned previously. *lin-15A* encodes a novel protein with no recognizable functional or structural motifs. The class B synMuv genes antagonize Ras-mediated vulval development via an RB/E2F/DP-mediated pathway. This inhibition is thus likely effected by transcriptional repression of genes required for vulval development. The class A synMuv genes function in parallel to this Rb pathway, but the molecular mechanism by which they inhibit vulval development is not known.

To further our understanding of the process by which the class A synMuv genes antagonize the Ras pathway, we have cloned *lin-56* and *lin-8*. Both are predicted to encode novel highly-charged proteins. LIN-8 belongs to a family of highly similar but apparently uncharacterized proteins in *C. elegans*. We have identified molecular lesions associated with both *lin-56* alleles and with 18 *lin-8* alleles. Antibodies directed against LIN-56 indicate the protein is localized to the nuclei of many, if not all, cells throughout development. This wild-type pattern is maintained in *lin-8* and *lin-38* mutant animals. By contrast, preliminary observations suggest that LIN-56 nuclear staining is reduced in *lin-15A* mutants, indicating a potential role for LIN-15A in the expression or nuclear localization of LIN-56.

lin-15A may therefore function upstream of *lin-56* in the synMuv class A pathway. Antibodies have also been generated against LIN-8, and the expression pattern and subcellular localization of this protein are currently being analyzed.

179. Activation of Wnt signaling bypasses the requirement for Ras signaling during vulval induction

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During vulval induction, activation of the Ras and Notch pathways causes the vulval precursor cells (VPCs) to adopt induced cell fates and divide to form the vulva. The *bar-1* gene encodes a beta-catenin/armadillo-related protein and is required for expression of the Hox gene *lin-39* in the VPCs. Loss of LIN-39 causes VPCs to sometimes adopt the F fate, which is to fuse with the hypodermis without dividing. Loss of *bar-1*activity and the APC homologue *apr-1* can also cause the VPCs to adopt F fates, suggesting that a Wnt pathway regulates fate specification in the VPCs via LIN-39. To determine whether the Wnt pathway plays an instructive or a permissive role in VPC fate specification, we determined the effect of overactivating the Wnt pathway. First, we used a *pry-1* mutant which was identified in the Kenyon lab as a negative regulator of Wnt signaling during Q_L neuroblast migration. We found that for 54% of *pry-1* mutant animals too many VPCs adopt vulval fates. We also created a version of BAR-1 in which the amino terminal region was deleted, since such deletions have been shown to stabilize beta-catenin and activate Wnt target genes in other systems. Following heat shock, in 70% of worms carrying the *HS::delNTbar-1* construct too many VPCs adopt vulval fates. These results suggest that activation of the Wnt pathway causes VPCs to adopt induced cell fates. Previous work has demonstrated that activation of the Ras pathway is necessary for VPCs to adopt induced cell fates and that the Ras pathway can also regulate LIN-39 levels. To determine if activation of the Wnt pathway could bypass the requirement for Ras signaling, we compromised the activity of either *let-23 (RTK)* or the *let-60 (Ras)* genes in animals carrying either the *pry-1* mutation or the *HS::delNTbar-1* construct. In each case, in the resulting animals too many VPCs adopt vulval cell fates, indicating that activation of the Ras

pathway is not essential for VPCs to adopt induced cell fates when the Wnt pathway is activated. This suggests that Wnt signaling may play an instructive role in vulval induction and that vulval development may involve more extracellular signals than previously believed.

180. Docking sites on substrate proteins direct extracellular signal-regulated kinase (ERK) to phosphorylate specific residues.

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During vulval development the primary cell fate is induced by a signaling pathway that includes *mpk-1*, the *C. elegans* homolog of extracellular signal-regulated kinase (ERK), a member of the mitogen activated protein (MAP) kinase family. ERK phosphorylates S/TP sites in many different protein substrates including *C. elegans* LIN-1. We identified gain-of-function mutations in *lin-1* that affect an FQFP motif that functions as a docking site for ERK. We characterized the sequence of amino acids that can constitute the FXFP motif using peptide and protein substrates. Substitutions of the phenylalanines at positions 1 and 3 had significant effects, indicating that these phenylalanines provide substantial binding affinity, whereas substitutions of the residues at positions 2 and 4 had less effect. LIN-1 also contains a different ERK docking site called the D-domain. The FXFP and D-domain docking sites were analyzed in a variety of positions and arrangements in the proteins Elk-1 (a vertebrate homolog of *C. elegans* LIN-1) and *C. elegans* KSR-1. Our results indicate that the FXFP and D-domain docking sites form a flexible, modular system that has two functions. First, the affinity of a substrate for ERK can be regulated by the number, type, position, and arrangement of docking sites. Second, in substrates with multiple potential phosphorylation sites, docking sites can direct phosphorylation of specific S/TP residues. In particular, the FQFP motif of Elk-1 is necessary and sufficient to direct phosphorylation of serine 383, a residue that is important for Elk-1 transcriptional activation, whereas the D-domain directs phosphorylation of other S/TP sites in Elk-1.

181. Two *enhancers of raf, eor-1* and *eor-2*, function in parallel to *sur-2, lin-25*, and *lin-1* to positively regulate Ras signaling

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In *C. elegans*, the Ras/Raf/MEK/ERK signaling pathway is required for many processes. To identify Ras pathway modifiers, our lab conducted a screen for mutations that alone cause Ras-like defects at low penetrance but that in combination with a weak *lin-45raf* allele, *ku112*, cause Ras-like defects at high penetrance (see abstract by Rocheleau et. al.). This screen has isolated loss-of-function mutations in two previously uncharacterized *enhancers of raf, eor-1* and *eor-2*, each defined by several alleles.

eor-1 and *eor-2* do not show genetic interactions with each other but show strong genetic interactions with *sur-2, lin-25*, and *lin-1*, genes encoding transcriptional regulators that genetically act downstream of *mpk-1*. Our data suggest that *eor-1* and *eor-2* function together to positively regulate Ras signaling at a similar step but in parallel to *sur-2, lin-25*, and *lin-1*. *eor-1/eor-2* and *sur-2/lin-25* are redundantly required for multiple outputs of Ras signaling. We propose that these two sets of genes may be the key positively acting downstream transcriptional regulators that are controlled by Ras signaling in several tissues. Consistent with *eor-1* and *eor-2* acting as transcriptional regulators, *eor-1* encodes a BTB/Zinc finger protein, similar to human promyelocytic leukemia zinc finger protein (hPLZF). PLZF can bind corepressor complex components and mouse knockouts of PLZF show decreased Hox gene expression. We are testing whether EOR-1 binds corepressor complex components and whether *eor-1* and *eor-2* regulate Hox gene expression.

182. Protein Phosphatase 2A is a positive regulator of the Ras pathway.

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sur-6 is a positive regulator of the ras pathway and acts between *ras* and *raf*. It encodes the PR55 regulatory subunit of the heterotrimeric protein phosphatase 2A (PP2A). By several genetic interaction criteria, *sur-6* mutants are very similar in their behavior to those of another positive regulator *ksr-1* that encodes a raf-like kinase. We present data to show that the protein phosphatase 2A (PP2A) holoenzyme is a positive modulator of the Ras pathway.

Depending on the cellular context, the PR55 regulatory subunit can potentiate or inhibit the activity of the PP2A holoenzyme. We wished to understand whether SUR-6 activated or inhibited the catalytic activity of PP2A in the context of vulval fate specification. Work by the Kohara and Baillie labs showed that *let-92* encodes the catalytic subunit of PP2A and that the strong *let-92(s504)* mutation causes worms to die as young larvae before vulval development commences. We have shown that *let-92(s504)* is a strong dominant (haplo-insufficient) enhancer of two *let-60(dn)* alleles, *n2031* and *sy100*, for their vulval induction defects. The strong *lin-45 Raf(oz166 lf)* mutation has a similar dominant genetic interaction with *let-60(n2031)*. Additionally, *let-92* soaking RNAi causes a weak vulvaless phenotype in a sensitized genetic background. Therefore reducing *let-92* function impairs the ability of VPCs to adopt vulval fates. Finally *let-92(s504)* is also a dominant lethal in a *sur-6* background, indicating that *sur-6* mutants have reduced PP2A activity. Taken together these data imply that the catalytic activity of PP2A is positive regulator of the Ras-MAPK phosphorylation cascade and that its action is potentiated by SUR-6. Its likely role would be to remove inhibitory phosphates that repress the activity of other positive regulators of the pathway.

Two candidate substrates of PP2A are KSR-1 and LIN-45 Raf. We predicted that ablation of sites for inhibitory phosphorylation in presumptive targets would bypass the need for PP2A in the vulva and transgenes bearing the relevant mutations could rescue *sur-6* mutants. We are testing variants of KSR-1 lacking various phosphorylation sites in this manner. We have shown that ser312 in the first 14-3-3 site of LIN-45 is a likely site of inhibitory phosphorylation since WT animals bearing a *lin-45(S312A)* transgene are multivulva.

183. Mechanism and role of LIN-12 downregulation in response to Ras activation during VPC fate specification.

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P5.p, P6.p and P7.p are the Vulval Precursor Cells (VPCs) that give rise to the vulva. As a consequence of the inductive and lateral signalling events, P5.p, P6.p and P7.p adopt a 2°-1°-2° pattern of fates. The inductive signal, LIN-3, produced by the anchor cell, activates a receptor tyrosine kinase/Ras/MAP kinase cascade that causes P6.p to adopt the 1° fate. One consequence of the inductive signal is to activate the expression or function of the lateral signal in P6.p, so that LIN-12 is activated in P5.p and P7.p, leading to the 2° fate.

Another consequence of the inductive signal is to influence LIN-12 protein accumulation. In wild-type hermaphrodites LIN-12::GFP is reduced specifically in P6.p and its daughters in response to vulval induction. Activation of Ras appears to be both necessary and sufficient to cause LIN-12::GFP downregulation.
Furthermore, mutations in *sur-2* and *lin-25*, two genes that appear to be targets of the Ras pathway and are required for lateral signalling, abolish downregulation of LIN-12::GFP in P6.p. These observations suggest that LIN-12 downregulation in the presumptive 1° cell may play a role in lateral signalling and/or proper execution of the 1° fate.

We would like to understand both the mechanism of LIN-12 downregulation in response to inductive signalling and also the role of LIN-12 downregulation in VPC patterning. The approach we have taken has been first to identify the mechanism of LIN-12 downregulation and then to use this information to engineer a form of LIN-12 that cannot be downregulated, but can still signal in response to ligand.

In order to elucidate the mechanism, we first showed that downregulation occurs posttranslationally, and that the target sequence is within the intracellular domain of LIN-12. Interestingly, downregulation of LIN-12 only occurs when the intracellular domain is associated with the plasma membrane via a transmembrane domain (TM). We have identified a region of the intracellular domain of LIN-12 that is both necessary and sufficient to target TM-GFP for downregulation in P6.p. Further dissection of this region identified a 15 amino acid segment that when deleted does not affect LIN-12 signal transduction but does abolish downregulation. We are currently conducting an alanine scan of the 15 amino acid segment to find point mutations with the same properties, and using the somewhat larger region as bait in a yeast two-hybrid screen.

Deletions and point mutations that abolish LIN-12 downregulation without affecting signal transduction activity should allow us to construct a full-length LIN-12 protein that would not be downregulated in P6.p during vulval induction. Such a construct would help elucidate the role that LIN-12 downregulation in response to Ras activation may play during VPC specification. We hope to have completed the analysis of such a construct by the meeting.

184. Regulation and function of *lin-11* in vulval development

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C. elegans vulva is an excellent model system to study the mechanisms of cell fate specification during animal development. Of the six vulval precursor cells (VPCs) - Pn.p (n=3-8), three - P5.p, P6.p and P7.p - are induced by *lin-3/let-23* mediated *ras*-singaling pathway to adopt the 2^o-1^o-2^o fates, respectively. These VPCs then undergo three rounds of cell division to produce 22 progeny that differentiate to form the 7 specific cell types in adult vulva. We are interested in identifying and studying the function of genes that regulate the terminal differentiation process of vulval cells. In the past several years many such genes have been identified including *lin-11, lin-17* and *lin-18*. While the molecular nature of these genes is known, their biology is poorly understood. We are currently working on the *lin-11* gene and its regulation in vulval development.

LIN-11 is a founding member of the LIM Homeodomain family of proteins. The loss of *lin-11* gene function causes a fully penetrant egg-laying defect (Egl). Pertaining to this, two defects at cellular level have been reported - an abnormal 2^o vulval lineage and a failure of uterine development, namely utse formation. Our work on the *lin-11* gene shows that it is required at multiple times during vulval development and functions in both 1^o as well as 2^o cell lineages. We have molecularly characterized its regulatory region and find distinct *cis*-elements for controlling the gene expression in the vulval cells and uterine pi cells. We further show that both elements are necessary to rescue the *lin-11* Egl defect; thus *lin-11* is required in both vulva and uterus for the development of a functional egg-laying system. In an attempt to identify the genes that regulate *lin-11*, we have done epistasis experiments with a set of known vulval genes and find that *lin-17* functions upstream of *lin-11*. In addition, we have also carried out a genetic screen using *lin-11::gfp* transgenic strain and have identified three class of mutants that enhance the vulval *gfp* expression. We are

currently working on these loci to get a better understanding of their role in *lin-11* regulation.

185. Rac and candidate Rac-pathway genes control patterning and morphogenesis of secondary vulval cells

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Rac is a Rho family GTPase that controls actin cytoskeletal dynamics and cell migration and is a major target of Ras during oncogenic transformation. We are using vulval development as a model system to study Rac function as it involves several different processes potentially influenced by Rac, including Ras-MAP kinase signaling, cell proliferation and cell migration.

We had previously reported that two *rac* genes, *mig-2* and *ced-10* , function redundantly during vulval morphogenesis, that *mig-2(gf)* alleles have dominant negative characteristics and that UNC-73 seems to be the major GEF for MIG-2 and CED-10 in this process . In either *mig-2(gf*), *mig-2(lf);ced-10(RNAi)* or *unc-73(lf)* mutants, P5.p and/or P7.p descendants failed to join the central invagination (migration defect) and in a low percentage of the animals , failed to adopt vulval fates. Further, Nomarski analysis of the final vulval-specific division in *mig-2(gf)* animals showed a change of the T polarity of the secondary vulval cell descendants to L or O so that these cells show NLLL or NOLL polarity instead of the wild-type NTLL. Cells exhibiting this changed polarity also showed a migration defect indicating that the two phenotypes may be related. Vulval development thus offers a good system to identify and distinguish between the different rac-associated phenotypes.

Alleles of *mig-15*, a worm ortholog of Drosophila *misshapen* and a *ste-20* family member (1) showed similar phenotypes. Based on the similarity of phenotypes and the molecular nature of these genes, *mig-2* and *ced-10* may act in the same or in a parallel pathway with *mig-15* to control migration of P5.p and P7.p descendants and additionally may interact with genes that specify the secondary fate.

We have generated transgenic animals expressing the activating (*mig-2* G16R), dominant negative (*mig-2* T21N) and WT *mig-2* constructs under the *lin-31* promoter to circumvent the pleiotropies associated with the *mig-2* alleles. This experiment also showed that expression of these constructs in the vulva mimicked the vulval phenotypes of *mig-2(gf)* and *mig-2(lf);ced-10(*RNAi) mutants. We are currently using these transgenics to test the interaction of *mig-2* with *unc-73* and *mig-15* as well as with genes known to be involved in patterning of secondary vulval cells.

(1) Zhu, X., and Hedgecock, E., WBG 14(5): 76, 1997.

186. Analysis of genes involved in late patterning of the vulva

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Based on EM reconstructions, two and five terminally differentiated cell types are generated by the primary and the secondary vulval lineages, respectively (Sharma-Kishore et al. 1999). We have assembled a panel of *gfp*, *cfp*, and *yfp* markers that label subsets of cell types, and together enable us to distinguish between six of these cell fates. We are using these marker lines to screen for new mutations that affect the patterning of these cell fates, and to analyze previously isolated mutations that affect the secondary VPC lineage execution. The process is complex and likely involve multiple cell signaling events and transcription factors.

Previous analysis based on lineage have suggested a role of a Wnt signaling pathway in the P7.p secondary lineage patterning. Mutations in *lin-17* (Frizzled homolog) and *lin-18* (homolog of Ryk = related to receptor-tyrosine kinases; W. Katz and P. W. S.) cause a phenotype interpreted to be the anterior-posterior reversal of the P7.p (secondary) lineage. Preliminary examinations of marker expression patterns in these mutants suggest a more complex defect. We are analyzing the defects in these mutants using additional *gfp* markers. We are also investigating how *lin-18* Ryk is involved in the Wnt signaling pathway.

A number of transcription factors are also implicated in late patterning. *egl-29*, which affects expression of *egl-17::gfp* (M. Wang and P.W.S.) was found to be allelic with the heterochronic gene *lin-29*, encoding a zinc-finger transcription factor. Re-interpretation of previous results and examination of additional *gfp* markers suggest that *lin-29* mutations affect the timing of marker expression. Another gene under investigation is *cog-1*, encoding a GTX homeodomain protein (R. Palmer and P.W.S.). A missense allele, *cog-1(sy275)* causes ectopic expression of *egl-17::gfp* (M. Wang and P.W.S.) and *ceh-2::gfp* in the outer cells (vulE) of the primary lineage. A partial deletion allele *cog-1(sy607)* (generated by D. Sherwood)

causes loss of *ceh-2::gfp* and *cdh-3::gfp* expression from cells of the secondary lineage. By lineage analysis, *sy275* delays or eliminates the terminal division of the inner primary cells (vulF), whereas *sy607* eliminates the terminal division of secondary cells P5.ppa and P7.pap (vulC). *cog-1* encodes two alternative transcripts, and the differences in the phenotypes of two alleles possibly reflect different functions of the two transcripts.

187. The *Caenorhabditis elegans evl-20* Gene Encodes a Developmentally Conserved ARF-like Protein Involved in Cytokinesis and Morphogenesis

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We describe the phenotypic analysis and molecular characterization of a *C. elegans* gene *evl-20*. *evl-20* was identified in a screen for mutants that phenocopy *lin-12* loss-of-function phenotype and produce sterile animals with everted vulva (Seydoux et al., 1993). We have cloned *evl-20* and shown that it encodes a developmentally conserved ARF-like (ARL) protein most closely homologous to human ARL2. ARFs are small guanidine-nucleotide binding proteins that belong to the Ras GTPase superfamily, and our analysis represents the first mutational study of a metazoan member of the ARF gene family. We show that a mutation in *evl-20* results in a highly abnormal vulval lineage. The lineage defects are not caused by misspecification of vulval cell fates since *evl-20* fails to show genetic interactions with the Ras signaling pathway responsible for vulval induction and expression of a vulval differentiation marker, *egl-17::gfp*, appears normal in *evl-20* mutant animals. *evl-20* mutants also display underproliferation of somatic gonad and germ line tissues, as well as male tail morphogenesis defects. Results of phenotypic and germ line mosaic analyses combined with data obtained in intestinal cell DNA quantification experiments demonstrate that the underproliferation phenotype of *evl-20* mutants is not caused by DNA replication or karyokinesis defects, but is produced by defective cytokinesis. Germ line mosaic analysis also shows that *evl-20* is not required for germ line proliferation, progression through meiosis, or sperm and oocyte development, and that the germ line defects result from aberrant somatic gonad development. Maternal contribution is necessary for embryogenesis, and elimination of both maternal and zygotic functions of *evl-20* by RNAi disrupts several aspects of embryonic development including proliferation, hypodermal enclosure and elongation. A GFP

expression reporter construct driven by *evl-20* promoter is expressed in the affected tissues and in most if not all neurons, which show no detectable phenotype in *evl-20* animals. Using yeast two hybrid system, we also show that EVL-20 ARL interacts with several components of focal adhesions. Based on our data, we propose that *evl-20arl* plays a conserved role in cytokinesis and morphogenesis and may function in regulating actin- or microtubule-based cytoskeleton dynamics.

Seydoux, G., Savage, C., and Greenwald, I. (1993). Isolation and characterization of mutations causing abnormal eversion of the vulva in Caenorhabditis elegans, Dev Biol *157*, 423-36.

188. A PROTEOGLYCAN BIOSYNTHETIC PATHWAY INVOLVED IN *C. elegans* EMBRYOGENESIS AND VULVAL MORPHOGENESIS AND IN HUMAN AGING DISORDERS

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Mutations in eight *sqv* (squashed vulva) genes can result in several developmental abnormalities, including defective vulval invagination and maternal-effect lethality. We report the cloning of two *sqv* genes, *sqv-2* and *sqv-6*, and discuss their roles in a molecular genetic pathway defined by the six previously cloned *sqv* genes. We also report the characterization of *sqv-5* and *sqv-7* expression. The molecular identities of the eight *sqv* genes suggest that the molecular basis for the *sqv* defects lies in the disruption of the biosynthesis of glycosaminoglycans (GAGs) of the structure (Serine residue in the protein core)-Xylose-Galactose-Galactose-Glucuronic acid-(X-Glucuronic acid)$_n$, where X is either N-acetylgalactosamine or N-acetylglucosamine.

The biosynthesis of GAGs requires the synthesis of nucleotide sugars in the cytoplasm and the translocation of nucleotide sugars into the endoplasmic reticulum (ER) and/or Golgi, where polymerization of sugars is catalyzed by glycosyltransferases. SQV-4 is a UDP-glucose dehydrogenase, a key enzyme in UDP-Glucuronic acid synthesis. SQV-7 is an atypical multi-pass transmembrane protein that transports UDP-Glucuronic acid, UDP-N-acetylgalactosamine and UDP-Galactose from the cytoplasm into the ER and/or Golgi. Mammalian homologs of *sqv-3*, *sqv-6* and *sqv-8* encode glycosyltransferases necessary for the biosynthesis of the GAG-protein linkage region of proteoglycans: SQV-6, SQV-3 and SQV-8 are similar to Xylosyltransferase, Galactosyltransferase I, and Glucuronyltransferase I, respectively. SQV-1 is a cytoplasmic protein with weak similarities to nucleotide-sugar modifying enzymes, and SQV-2 and SQV-5 are both type II transmembrane proteins and candidate glycosyltransferases. We postulate that *sqv-1*, *sqv-2* and *sqv-5* are components of the same

GAG biosynthesis pathway and that the GAGs are important for cell-cell or cell-matrix interactions in embryonic and vulval development.

Preliminary antibody staining using rabbit polyclonal antibodies against SQV-4, SQV-5 and SQV-7 shows localization of these proteins consistent with their deduced functions. SQV-4 appears to localize broadly to the cytoplasm of many tissues, including the vulva, consistent with its function in the biosynthesis of UDP-glucuronic acid in the cytoplasm. SQV-5 shows punctate cytoplasmic localization in the vulva and germline, consistent with our model that SQV-5 functions as a glycosyltransferase in the Golgi. SQV-7 also shows punctate cytoplasmic localization in the hypodermal seam and gonadal distal tip cells, consistent with its function in transporting nucleotide sugars into the Golgi. We plan to characterize the subcellular localization of SQV-5 and SQV-7 further using immuno-electron microscopy.

Mutations in the human homolog of *sqv-3* are implicated as the cause of a progeroid variant of the connective-tissue disorder Ehlers-Danlos syndrome. All eight *sqv* genes have close human counterparts, suggesting that a common pathway for modifying important cell surface and/or extracellular GAGs is present in humans and in *C. elegans*. Defects in the human counterparts of other *sqv* genes therefore may be responsible for aging disorders and connective tissue diseases.

189. A broad transcriptional requirement for CDK9/CyclinT *in vivo* involves overcoming elongation inhibition by SPT4/SPT5.

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Biochemical and drug sensitivity analyses indicate that in metazoans the kinase CDK9 can promote the elongation phase of mRNA transcription, and can phosphorylate RNA Polymerase II (Pol II) on its C-terminal domain (CTD). During the transcription process, the CTD repeat YSPTSPS is first phosphorylated on Ser 5 by the CDK7 kinase, then Ser 2 phosphorylation is observed on elongating polymerases. At most genes, CTD phosphorylation is essential for transcription elongation, and for coupling transcription to mRNA processing steps. CDK9 can phosphorylate the CTD on Ser 2 *in vitro*, but whether CDK9 is important for Ser 2 phosphorylation in vivo is unknown. The complex of CDK9 with its partner CyclinT is also recruited directly by the human immunodeficiency virus (HIV) protein Tat, which binds to the nascent HIV RNA and is required for production of full-length viral transcripts. It is an important question whether CDK9 is broadly essential for transcription *in vivo* or has a specialized role.

We have addressed this question by studying CDK9/Cyclin T functions in the *C. elegans* embryo. *C. elegans* encodes a single *cdk-9* gene, and two cyclin T *(cyt)* orthologs. Similarly to *ama-1(RNAi)* (Pol II) embryos, *cdk-9(RNAi)* embryos arrest development at around 100 cells without any evidence of differentiation. Staining with antibodies against Pol II CTD phosphoepitopes indicates that in *cdk-9(RNAi)* embryos, CTD Ser 5 phosphorylation is essentially normal, but Ser 2 phosphorylation is undetectable. In *cdk-9(RNAi)* embryos we have also not detected expression of any GFP reporters that correspond to early embryonic genes, and have obtained essentially identical results when both cyclin T genes are inhibited. We conclude that CDK9/Cyclin T is essential

for mRNA transcription, and may phosphorylate CTD Ser 2 *in vivo*.

Biochemical and some yeast genetic evidence indicates that the protein complex SPT4/SPT5 can inhibit transcription elongation, and that this effect may be counteracted by CDK9, which can phosphorylate SPT5 *in vitro*. To test this model, in *C. elegans* we have inhibited *spt-4* and *spt-5* by RNAi simultaneously with *cdk-9*. We find that for the most part, lack of *spt-4* and *spt-5* does not overcome the requirement for CDK9/Cyclin T for transcription. Strikingly, however, inhibition of *spt-4* and *spt-5* can relieve this requirement at heat shock genes, which in yeast are regulated distinctly in that they can be transcribed in the absence of the CTD. We conclude that CDK9/CyclinT can overcome the inhibitory effects of SPT4/SPT5 *in vivo*, but that this aspect of its function is insufficient at most genes, which depend also upon its phosphorylation of the CTD. Efforts are now underway to determine whether CDK9/Cyclin T is required *in vivo* not only for transcription elongation, but also for efficient mRNA splicing.

190. Nuclear hormone receptor *nhr-23*(CHR3) is a critical regulator of all four larval molts of *Caenorhabditis elegans*

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nhr-23 (CHR3, NF1F4), the homologue of *Drosophila* DHR3 and mammalian ROR/RZR/RevErbA nuclear hormone receptors, is important for proper epidermal development and molting in *C. elegans*. Disruption of *nhr-23* function leads to developmental changes including incomplete molting and a short, fat (dumpy) phenotype.

Here, we studied the role of *nhr-23* during larval development using expression assays and RNA-mediated interference (RNAi). We show that the levels of expression of *nhr-23* cycle during larval development and that reduction of *nhr-23* function during each intermolt period results in defects at all subsequent molts. Assaying candidate gene expression in populations of animals treated with *nhr-23* RNAi has identified *dpy-7* as a potential gene acting downstream of *nhr-23* . Contrary to that, other collagen genes, *col-12* and *dpy-13* were not affected by *nhr-23* RNAi. We also show that *nhr-25*, which inhibition leads also to a molting defect, is not downstream in *nhr-23* regulatory pathway as is expected from similarity to the *Drosophila* homologue FTZ-F1. These results define *nhr-23* as a critical regulator of all *C. elegans* molts and begin to define the molecular pathway for its function.

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191. Functional Analysis of C. elegans Nuclear Hormone Receptors

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Nuclear hormone receptors (NHRs) comprise a large family of metazoan transcription factors that participate in numerous developmental and metabolic processes. NHRs target their transcriptional regulation to specific genes through interaction with DNA sequences called hormone response elements (HREs) and the type of transcriptional regulation carried out by an NHR at these genes is often determined by interaction with a small hydrophobic ligand and/or various protein cofactors. Sensitivity to regulation by these modulators allows NHRs to be quite useful regulators of development, as their activity can be specifically modified by environmental signals to orchestrate distinct transcriptional effects at different genes and in various cell types. Additionally, these responses can be modified by regulatory factors in a target-gene-specific fashion over the course of development.

In order to understand the utility of NHRs in metazoan development, we are investigating NHRs in C. elegans. Our goals are to understand how NHRs are required for worm development and to identify the C. elegans factors that serve as determinants of NHR activity. Initially, we plan: (i) to isolate C. elegans NHRs and characterize their physiological roles, (ii) to identify DNA binding sites and target genes for individual NHRs, (iii) to characterize C. elegans proteins that function as NHR cofactors and (iv) to purify small molecules that function as NHR ligands. Thus far we have cloned over 20 NHRs from C. elegans and have characterized their RNA interference phenotypes by bacterial feeding and/or by injection. Using yeast transcriptional reporter assays we have identified the DNA binding sites of many of these NHRs and have quantified their ligand independent transcriptional activities. Our results suggest that C. elegans NHRs function similarly to mammalian NHRs in regards to DNA binding site recognition and transcriptional activation. We have found that, like mammalian NHRs, C. elegans receptors recognize their DNA binding site using a conserved amino acid stretch in the zinc-finger DNA binding domain called a P-box. Additionally, some C. elegans NHRs also utilize an additional sequence located at the C-terminal end of the DNA binding domain called the CTE to recognize their DNA binding site. Our results show that C. elegans NHRs with conserved P-boxes recognize DNA binding sites that are identical to their mammalian homologs. Many C. elegans NHRs, however, possess novel P-box elements that mediate the recognition of novel DNA binding sites. We are currently using this DNA binding site information to carry out computational searches for regulatory elements that support regulation of target genes by NHRs in vivo. While some C. elegans NHRs have strong activity in yeast in the absence of ligand, we have found that others have little activity, suggesting the these receptors likely require interaction with a ligand and/or cofactors in order to affect transcription. Thus we have extended our yeast system to identify ligands and proteins that serve as regulators of C. elegans NHR activity. Indeed, we have found that the mammalian NHR coactivator GRIP can also serve as a functional coactivator of the C. elegans receptor CHR3 (nhr-23). Mutations that interrupt the predicted coactivator interface in CHR3 block the ability of GRIP to coactivate, arguing that the basic NHR coactivator binding interface is conserved in C. elegans. We are currently conducting yeast screens using a C. elegans protein expression library to identify such C. elegans coactivators.

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192. From Binding Sites to Target Genes: Molecular Characterization of the Intracellular Receptor Daf-12 Function

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The intracellular receptor *daf-12* regulates dauer larva formation, developmental age, and affects *C. elegans* lifespan. *Daf-12* alleles with distinct protein sequence alterations partially uncouple its phenotypic effects. We would like to understand the molecular mechanisms of Daf-12 action, including the basis for differential phenotypic outcomes of specific *daf-12* mutants.

We developed an *In Vitro* Genomic Selection method that yielded a series of specific *C. elegans* genomic DNA fragments containing Daf-12 binding sites. We identified DNA sequences within these fragments that were required for specific Daf-12 binding *in vitro*. Daf-12 selectively activated transcription of the reporter gene in yeast from these *C. elegans* genomic fragments. The specific Daf-12 binding sites within these fragments were necessary and sufficient for Daf-12-dependent transcriptional activation in *Saccharomyces cerevisiae*.

We inserted some of these fragments into *C. elegans* reporter plasmids and showed tissue-specific activity of the reporter gene that depended on Daf-12 binding site sequences within the fragments. Hence, *C. elegans* genomic DNA fragments bound specifically by Daf-12 *in vitro* display Daf-12 response element activity *in vivo* in both yeast and *C. elegans*.

In principle, *In Vitro* Genomic Selection should also enable the identification of potential target genes. We are currently investigating whether Daf-12 binding site-containing *C. elegans* genomic fragments are linked to *daf-12*-regulated target genes. We are furthermore investigating tissue specificity and activities from the *C. elegans* reporter gene with Daf-12 binding sites in various distinct *daf-12* mutant strains.

During the course of our studies, we found that a C-terminal truncated Daf-12 derivative was a much stronger transcriptional activator than full length Daf-12 in the yeast reporter system. The transcriptional activity of this truncated Daf-12, which lacks its putative ligand-binding domain, implies that a small molecule ligand may control Daf-12 activity in *C. elegans*.

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193. A pharyngeal muscle specific enhancer from *ceh-22* is targeted by PHA-4 and other factors

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C. elegans pharyngeal muscle development involves *ceh-22*, an NK-2 family homeobox gene structurally and functionally related to genes controlling heart development in other species. *ceh-22* is the first gene known to be expressed as cells commit to a pharyngeal muscle fate. We are studying how *ceh-22* expression is regulated to understand the early steps in pharyngeal muscle differentiation.

ceh-22 is expressed in a subset of pharyngeal muscle cells, beginning at the bean stage of embryogenesis. Its expression is regulated at the level of transcription, and within the *ceh-22* promoter we have identified two transcriptional enhancer regions, which we call the distal and proximal enhancers. The proximal enhancer is a pharyngeal muscle specific autoregulatory element that becomes active during embryogenesis shortly after the endogenous *ceh-22* gene, and we suggest it maintains *ceh-22* expression. The distal enhancer has a broader cell type specificity, but it becomes active in the pharynx at the bean stage of embryogenesis, coincident with expression of *ceh-22*. We believe that the distal enhancer is involved in initiating *ceh-22* expression. At least three separable subelements we call *DE1*, *DE2*, and *DE3*, contribute to distal enhancer activity. *DE3* appears to be most active and is sufficient to enhance abundant gene expression specifically in the *ceh-22* expressing muscles.

We have identified two short segments of *DE3*, which we call *de199* and *de209*, that together are sufficient to reproduce the activity of *DE3*. *de199* appears to be a more general regulatory site that alone activates transcription in pharyngeal muscles, epithelial cells, marginal cells, as well as body wall muscle. In contrast, *de209* is a more specific regulatory site that activates transcription predominantly in the pharyngeal muscles. *de209* contains a consensus GATA factor binding site and a candidate binding site for PHA-4, a winged helix transcription factor that is expressed in all pharyngeal cell types and is required for *ceh-22* expression. Disruption of the PHA-4 site reduces *de209* activity in the pharynx, while ectopic expression of PHA-4 results in expression of a *de209* regulated reporter outside the pharynx. These results suggest that PHA-4 directly regulates *ceh-22* through *de209*.

A mutation outside the PHA-4 and GATA sites also disrupts the activity of *de209*. We hypothesize this mutation affects a site for a factor that works with PHA-4 to activate *ceh-22* expression specifically in the pharyngeal muscles. We are currently performing a one-hybrid screen in yeast to identify this factor.

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194. The novel factor PEB-1 functions during C. elegans pharyngeal organogenesis

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The *C. elegans* pharynx is a complex organ consisting of five distinct cell types: muscles, glands, epithelial cells, neurons, and marginal cells. Development of each of these cell types requires PHA-4, a winged-helix transcription factor expressed in all pharyngeal cells. However, it is unknown how organ-wide expression of PHA-4 contributes to cell type-specific differentiation. In the pharyngeal muscle-specific *myo-2* gene, PHA-4 targets the *C*-subelement, a site of organ-specific regulation. The *C*-subelement also binds the novel factor PEB-1 at a site overlapping the PHA-4 binding site. We are examining PEB-1s role during pharyngeal development and characterizing how PEB-1 and PHA-4 contribute to *C*-subelelment activity.

In embryos, PEB-1 protein accumulates most abundantly in pharyngeal nuclei beginning at the comma stage of embryogenesis, although it is also detectable in hypodermal cells and near the rectum. After hatching PEB-1 remains detectable in the pharynx until mid larval development. PEB-1 protein is also expressed post-embryonically in some tissues outside the pharynx, including the pharyngeal-intestinal valve cells, cells near the rectum and vulva, hypodermal cells in the head and tail, and in the germline.

The PEB-1 expression pattern in the pharynx is distinct from other known pharyngeal regulatory factors. PEB-1 is expressed in many pharyngeal cell types, including the muscles, marginal cells, epithelial and gland cells, but, in contrast to PHA-4, it is undetectable in the pharyngeal neurons. PEB-1 expression requires PHA-4 but does not depend on either PHA-1 or CEH-22. This expression pattern suggests PEB-1 functions during pharyngeal development at an intermediate step between organ specification and terminal differentiation of specific cell types.

Reduction of PEB-1 activity by RNAi does not cause embryonic lethality but does result in slow growth and a severely constipated phenotype. *peb-1(RNAi)* animals display defects in the somatic tissues where PEB-1 is expressed, including the pharynx, vulva, and tail. Defects in the pharynx are most prominent in the g1 gland cells, which have enlarged processes that often exhibit abnormal projections. We are currently generating a *peb-1* null mutant and characterizing the effect of expressing dominant-negative forms of PEB-1 to further examine its role in pharyngeal development.

In addition, we are examining the effect of ectopic PEB-1 and PHA-4 expression. PEB-1 does not strongly activate a *C*-subelement-regulated reporter gene, whereas PHA-4 can activate this reporter. Co-expression of PEB-1 with PHA-4 blocks this activation, suggesting that PEB-1 and PHA-4 do not function synergistically and that PEB-1 may interfere with activation by PHA-4.

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195. The transcriptional Mediator complex functions as a switch of cell fates during vulval development

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Transcriptional regulation of gene expression is the final step in various signaling pathways. Transcription must be properly repressed or activated depending on status of signaling. The precise mechanism for transcriptional regulation by various transcription factors remains to be elucidated, especially *in vivo*. During vulval development, it has been shown that the LIN-31 protein activates transcription in P5.p, P6.p and P7.p cells (Tan et al. Cell 1998). In contrast, in the other vulval precursor cells, the LIN-1-LIN-31 complex functions as a repressor of transcription.

psa-7 mutants were identified in a screen for mutants defective in asymmetric division of the T cell (Sawa et al. 2000 Japanese Meeting abstract). In addition to the T cell phenotype, the mutants often have ectopic vulva (Muv). Genetic analyses indicate that *psa-7* acts downstream of *lin-1* in repression of transcription. We have cloned *psa-7* to find that it encodes a homolog of human TRAP240, a component of the transcriptional Mediator complex. The Mediator was identified in mammals as a large complex that can interacts with both various transcription factors and RNA polymerase. One of the components of Mediator is a homolog of SUR-2 in *C. elegans* that is also involved in vulval development (Singh & Han G&D 1995). In contrast to *psa-7*/TRAP240 that is likely to be involved in transcriptional repression, *sur-2* is required for activation of transcription mediated by Ras signaling. Double mutants between *psa-7* and *sur-2* show strong Muv phenotype, indicating that *psa-7* acts downstream or in parallel to *sur-2* in the Mediator complex. These results suggest that the Mediator complex functions as a switch of transcription while integrating information from transcription factors. Roles of other components of the Mediator complex in vulval development will also be reported.

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196. Functional analysis of splicing factors by RNAi using a nuclear retention assay

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A novel use of RNAi has allowed us to explore interacting factors that recognize the 3 splice site in *C. elegans* and to uncover aspects of the relationship between splicing and nuclear retention of mRNA. An alternatively spliced exon from the *uaf-1* gene (encoding U2AF65, an essential splicing factor that binds to the 3 splice site in an early intron recognition step) was inserted into the 3 UTR of a *gfp* reporter gene. This 216 nt exon contains 10 repeats of a heptameric sequence that matches the consensus for a 3 splice site, UUUUCAG/R. Inclusion of this exon sequence results in nuclear retention of the mature *gfp* reporter mRNA. We hypothesized that splicing factors remaining bound to the splice site-like sequences (but without splicing) keep the mRNA from leaving the nucleus. We tested this idea by using RNAi to lower the levels of individual splicing factors and then looked for release of the retained mRNA to the cytoplasm with a resulting increase in GFP expression in the injected animals. We have performed RNAi against a number of splicing factors and have found that both U2AF subunits are indeed required for nuclear retention, as expected based on their demonstrated UUUUCAG/R binding specificity. However, they are not sufficient: PUF60 and p54, two RNA-binding proteins (previously identified in mammals and flies) that form a complex with each other and associate with U2AF to promote splicing, are also required for nuclear retention. RNAi targeting any of the four polypeptides resulted in increased GFP levels. Furthermore this does not result from a reduction in splicing, since RNAi against six other splicing factors failed to relieve nuclear retention. We showed that the *C. elegans* homolog of TAP, *nxf-1*, an essential gene required for mRNA export, is required for the *gfp* mRNA transport stimulated by *uaf-1* RNAi. The most interesting results were observed when we performed RNAi against the splicing factor UAP56, a member of the DexH/D box family of helicases previously shown to interact directly with U2AF65. Our results support a model in which UAP56 acts to remove bound U2AF, resulting in TAP-mediated RNA transport, and that the PUF60/p54 complex may prevent UAP56 action. This model can be formally described by the epistasis relationships depicted in the figure. RNAi against UAP56 reduced basal levels of GFP expression, suggesting it is required for transport. When U2AF levels were lowered by RNAi, GFP was expressed, even when UAP56 levels were lowered simultaneously, suggesting the role of UAP56 is superfluous in the absence of U2AF. However, when the levels of PUF60 and p54 were lowered by RNAi, simultaneous lowering of UAP56 levels resulted in a lack of GFP expression, indicating that the PUF60/p54 complex is not required for nuclear retention when UAP56 levels are lowered. Thus, these factors may act by countering UAP56. The model suggests the possibility that a presumed RNA helicase, UAP56, may act to dissociate a protein/RNA complex in a step required for spliced RNAs to leave the nucleus.

PUF60/p54 -----| UAP56 ------| U2AF ------| TAP ---- mRNA nuclear export

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197. A complex containing CstF and the SL2 snRNP connects 3’ end formation and trans-splicing in *C. elegans* operons

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Polycistronic pre-mRNAs from *C. elegans* are processed by 3’ end formation of the upstream mRNA and SL2-specific trans-splicing of the downstream mRNA. These processes occur very close together, usually within an ~100 nt region. Our previous work demonstrated that the processes are coupled; but until now the mechanism by which SL2 is specifically chosen has been unknown. In other animals, the site of 3’ end formation is determined by the binding of cleavage and polyadenylation specificity factor (CPSF) to the AAUAAA just upstream of the site of cleavage and of cleavage stimulation factor (CstF) to a U-rich sequence just downstream of the site of cleavage. We have identified a U-rich sequence in the intercistronic region that is likely to represent a CstF binding site, based on its location and sequence, and have shown that this sequence is required for SL2-specific trans-splicing. We now report the existence of a complex in *C. elegans* extracts containing CstF and the SL2 snRNP, which suggests a mechanism for coupling 3’ end formation and SL2-trans-splicing. The complex is immunoprecipitated with anti-CstF-64 antibody, and contains SL2 snRNP, but not SL1 snRNP or other U snRNPs. Previous in vivo mutational studies have shown that some sequences of SL2 RNA, including the second stem and third stem/loop, are required for SL2 trans-splicing. A mutation in the second stem of the SL2 RNA prevents all trans-splicing, based on its inability to trans-splice to the SL2-accepting *gpd-3*, mRNA or to rescue the SL1 RNA deletion mutant, *rrs-1*. Nevertheless, this mutant SL2 RNA can complex with CstF. In contrast, a mutation in the third stem/loop still allows the mutant SL2 RNA to function in trans-splicing based on *rrs-1* partial rescue, but it fails to splice to SL2-accepting trans-splice sites, suggesting it has lost its identity as an SL2 snRNP. Concomitantly it has lost its interaction with CstF. Thus, third stem/loop sequences in SL2 RNA are not required for trans-splicing, but are required for both SL2 identity and formation of a complex containing CstF. This finding suggests that the machinery for SL2-trans-splicing is associated with the complex that promotes 3’ end formation, thus providing the coupling that ensures SL2 trans-splicing specificity for downstream genes in operons.

198. Operon Resolution, Usage and Diversity of SL2-like spliced leaders in the phylum Nematoda

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Nematodes represent an extremely diverse phylum with five major clades containing both free living and parasitic representatives (Blaxter et. al. 1998). The genome of the clade V nematode *Caenorhabditis elegans* contains many gene clusters which are transcribed as polycistronic pre-messenger RNAs. Unfortunately there is currently no information on the genomic organization of any nematodes other than the free living rhabditids *Oscheius brevesophaga, C. elegans* and *C.briggsae* (Evans et. al. 1997). One particularly interesting question is whether these operon-like structures are conserved in other nematode species and if they are do they utilize SL2-like spliced leaders to resolve the polycistronic transcripts. We have employed a directed approach using ribosomal protein operons found in the *C. elegans* genome and the Nematode EST datasets (Parkinson et. al. 2001) to identify a potential operon in a selection of clade V, IV, and III nematodes. In the clade III filarial nematode *Brugia malayi* we have determined that the rpL27a and rpP1 are transcribed as a polycistronic unit. However, the second transcript in the unit (rpP1) exclusively receives SL1 when it is transpliced. Conversely in the clade V diplogasterid *Pristionchus pacificus* we have found that a diverse set of SL2-like sequences transpliced to rpP1. In a survey of the genome sequence of *C. elegans* we identified several additional SL2-like sequences that had not been previously described. This data indicates that while SL2 usage may have evolved in a clade V ancestor, there have been independent expansions of SL2-like sequences in the rhabditids and diplogasterid. We are currently examining the rpP1 of the clade IV nematode *Strongyloides ratti* to determine if it utilizes SL2-like spliced leaders.

199. RNA editing is important for normal development and behavior in C. elegans

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Adenosine deaminases that act on RNA (ADARs) convert adenosines to inosines in highly base-paired RNA. When these RNA editing enzymes target codons, the amino acid specified by the codon is often changed. Thus, ADARs create diversity by allowing more than one protein isoform to be synthesized from a single mRNA. Many of the mRNAs targeted by ADARs code for proteins involved in neurotransmission, and our goal is to correlate specific RNA editing events with specific behaviors. Given its well-characterized and relatively simple nervous system, *C. elegans* seems ideal for such studies.

We determined that there are two ADARs in *C. elegans*, *adr-1* and *adr-2*. Expression of GFP by the *adr-1* promoter indicates that *adr-1* is expressed in most cells of the nervous system as well as in the vulva, during morphogenesis. We obtained deletions in the two ADAR genes, designated *adr-1(gv6)* at the H15N14.1a/b locus and *adr-2(gv42)* at the T20H4.4 locus. Strains homozygous for the single or double deletions are viable, exhibiting subtle but interesting phenotypes that correlate with the observed expression patterns. For example, a small percentage (7%) of double mutants have a protruding-vulva phenotype (Pvl), usually associated with an improperly formed gonad. We also analyzed the function of two chemosensory neurons, the AWA and AWC neurons, which are involved in the detection of volatile chemicals. When compared to wild-type animals, *adr-1;adr-2* double mutants tracked poorly to chemicals detected by the AWA and AWC neurons, showing consistently lower chemotaxis indices.

Incubation of synthetic dsRNA with extracts from the two knockout strains reveals diminished deaminase activity in the *adr-1(gv6)* strain and a complete lack of activity in the *adr-2(gv42)* strain. Examination of known editing sites within endogenous ADAR substrates confirms these results. RNA isolated from *adr-2(gv42)* worms is completely unedited at every site examined. Interestingly, the *adr-1(gv6)* deletion affects editing sites in three ways: some sites are edited normally, some sites show decreased editing, and some sites show increased editing.

Our results illustrate the importance of RNA editing for normal development and behavior in *C. elegans*, and set the stage for correlating specific editing events with specific behaviors.

200. GLD-2, a Putative Cytoplasmic Poly(A) Polymerase, is Required for Both Germline Development and Embryogenesis

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Numerous germline and early embryonic fates are controlled at the post-transcriptional level, including proliferation, sex determination, progression through meiosis and embryonic polarity. Although several RNA binding proteins essential to these processes have been identified (e.g. FBF), the mechanism by which they control fates is not known. Here we report the molecular characterization of *gld-2*, which controls commitment to meiosis with *gld-1* as well as progression through meiosis on its own (1). By RNAi, we find that *gld-2* is also essential for early embryogenesis: *gld-2(RNAi)* embryos appear multinucleate with no obvious membrane boundaries. GLD-2 is expressed in the germ line and early embryo; it is predominantly cytoplasmic, being abundant in the pachytene region of the germ line and co-localizing with P granules in the early embryo.

GLD-2 contains a predicted nucleotidyltransferase domain found in several nucleic acid polymerases, including poly(A) polymerase (PAP), the enzyme that adds poly(A) to mRNAs in the nucleus. GLD-2 binds specifically to GLS-1, a KH protein that also regulates germline fates and early embryogenesis (also see C. Eckmann abstract). Several lines of evidence suggest that GLD-2 is a GLS-1-dependent poly(A) polymerase *in vitro*. First, when co-expressed with GLS-1 by *in vitro* translation, GLD-2 stimulates *in vitro* incorporation of radiolabeled ATP into an RNA substrate for polyadenylation. Second, this incorporation requires both GLD-2 and GLS-1 and is observed only with ATP (not GTP, UTP

or CTP). Third, incorporation requires a key asparate in the predicted GLD-2 active site. Fourth, the *gld-2(h292)* missense mutation, which abrogates *gld-2* function *in vivo* and prevents the GLD-2/GLS-1 physical interaction, eliminates the ability of GLS-1 to stimulate GLD-2 enzyme activity. Although GLD-2 thus appears to have poly(A) polymerase activity, its level of activity is less than that observed with a conventional PAP. Experiments are in progress to characterize the products of the GLD-2-catalyzed reaction definitively.

We suggest that GLD-2 is a cytoplasmic poly(A) polymerase and that GLD-2 controls the activity of specific mRNAs by polyadenylation. We also suggest that GLD-2 gains specificity for particular mRNAs by interacting with RNA-binding proteins, such as GLS-1. The GLD-2 in P granules may provide an enzymatic activity by which P granules and their integral RNA-binding proteins regulate maternal RNAs and cell fates.

1 Kadyk and Kimble (1998) *Development 125*, 1803-1813

201. *cdl-1*, whose mutation cause defects in programmed cell death and morphogenesis, encodes a homologue of stem-loop binding protein, and may contribute to proper core histone expression.

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In *cdl-1* (cell death lethal) mutants, several embryonic defects are observed: 1) delay in appearance and elimination of cell corpses, 2) variable defects in body elongation, 3) failure in elongation of the pharynx toward the buccal cavity. To understand the function of *cdl-1*, we cloned the gene. *cdl-1* turned out to encode a homologue of vertebrate stem-loop binding proteins(SLBP)/hairpin binding proteins(HBP), which bind to the 3'-stem-loop of histone mRNAs. Mature replication-dependent histone mRNAs in metazoa have a stem-loop structure instead of a poly-A tail. SLBPs have been implicated in post-transcriptional regulation of histone mRNAs via the binding to the stem-loop structure. In *C. elegans*, 57 core histone genes contain a conserved stem-loop sequence in their 3'-UTR. We confirmed interaction of the full-length CDL-1 protein and the conserved stem-loop structure in histone mRNAs by yeast three-hybrid system. This suggests that CDL-1 may function in the post-transcriptional regulation of core histone expression.

cdl-1(RNAi) embryos showed defects in karyokinesis in addition to the phenotypes of the original *cdl-1* mutants . The embryos defective in karyokinesis showed less-condensed chromosome during metaphase to telophase, and had chromosome bridges between daughter nuclei. To examine whether the *cdl-1* phenotypes are the results of shortage of core histone proteins, we performed RNA interference for each of the core histone genes. RNAi for histone H2B, H3 and H4 respectively reproduced phenotypes similar to *cdl-1(RNAi)* embryos. Thus, we presume that histone protein

production is decreased in *cdl-1* embryos. It is likely that *cdl-1* regulates replication-dependent core histone expression at the post-transcriptional level as suggested for other SLBPs.

We observed subcellular localization of CDL-1 protein in embryos. CDL-1 appeared to localize in all nuclei throughout embryogenesis. In several other organisms, SLBPs have been demonstrated to localize mainly in Cajal (coiled) bodies. Cajal bodies are nuclear organelles, whose morphology and composition appear to be highly conserved throughout evolution. Cajal bodies associate with specific gene loci including histone gene clusters, and they also contain several factors implicated in histone mRNA transcription and processing other than SLBPs, suggesting that Cajal bodies are involved in assembly of histone mRNA transcription and processing machineries. Thus, it is plausible that CDL-1 also localizes in Cajal body-like structures and on histone gene loci.

202. The *lin-4* RNA is neither necessary nor sufficient for a post-initiation translational regulatory mechanism in the heterochronic pathway

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The heterochronic gene *lin-4* encodes a 22-nt RNA that represses the expression of *lin-14* and *lin-28* via their 3' untranslated regions after the L1. This activity of *lin-4* is necessary for the succession of diverse somatic developmental events through the first three larval stages. However, *lin-14* and *lin-28* can be repressed at the normal time even in the absence of *lin-4* activity if either one of the two genes is inactivated by mutation. Thus there is a *lin-4*-independent repression of *lin-14* and *lin-28* that mediates a positive regulatory relationship between them. Interestingly, this 'feedback loop' is also stage-specific. Like the *lin-4* repression, it is not observed in the early L1 but is evident by the L2.

We have found that the *lin-4*-independent repression of *lin-28* shows the same unusual phenomenon seen for wildtype regulation of *lin-14* and *lin-28*. We have observed that despite a decrease in LIN-28 protein level of about 10-20 fold, no change in the mRNA level nor its association with polyribosomes occurs from early to late stages. This is true both in wildtype and in *lin-4 (0); lin-14(ts)*. We and others have interpreted the unusual nature of this regulatory phenomenon in the heterochronic pathway to mean that the repressive event occurs after translation initiation, during peptide synthesis or shortly after (1). The fact that this phenomenon occurs in the absence of *lin-4* indicates that the 22-nt *lin-4* RNA is not a core component of the mechanism. We believe that *lin-4* rather initiates or potentiates the post-initiation repression. The *lin-4*-independent repression of *lin-28* apparently involves sequences in the *lin-28* 3' untranslated region that are distinct from the *lin-4* site.

We also sought to determine whether *lin-4* activity is sufficient to repress *lin-28* when the *lin-4*-independent mechanism is not active. We have found by immnuoblot of synchronized

late-stage populations that LIN-28 protein is as high in *lin-14(n355sd)* and *daf-12(rh61)* as it is in *lin-4(e912)*. We have determined for the *lin-14(n355sd)* strain that the *lin-4* RNA is indeed expressed. Thus, in strains in which *lin-4* is present, *lin-28* is not repressed if *lin-14* levels are high.

We have attempted to establish an *in vitro* translation system for *C. elegans* in which to investigate the mechanism of this regulation. We have been successful in creating a mixed system in which *C. elegans* polyribosomes translate in a reticulocyte extract. With this system we have obtained evidence that the heterochronic gene mRNAs are actively translated at late stages.

1 Olsen and Ambros, 1999, and K. Seggerson, L. Tang, and E.G. Moss, submitted.

203. Touch Cell Proteins Needed for the Formation of Amiloride-sensitive Ion Channels

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Touch sensation along the body of *C. elegans* depends on six specialized sensory neurons called touch cells (ALM, AVM, PVM, PLM). Genetic screens for touch insensitive mutants identified twelve *mec* (*mec*hanosensory abnormal) genes needed for the activity, but not the development, of the touch cells. Most of these genes have been cloned. Except for *mec*-5, all of the cloned genes are expressed in the touch cells. The protein encoded by *mec*-4 is touch-cell specific and belongs to the DEG/ENaC superfamily of proteins that form amiloride-sensitive ion channels. The *mec*-10 gene encodes another DEG/ENaC channel subunit expressed in touch cells and four other mechanosensory neurons. These channel subunits are hypothesized to be the core of a mechanotransduction complex.

A long-standing question is whether the putative ion channel subunits, MEC-4 and MEC-10, form channels similar to other DEG/ENaC channels. To address this question we measured whole cell currents in Xenopus oocytes injected with cRNAs encoding the channel subunits. We found that heterologous expression of MEC-4 and MEC-10 bearing activating mutations that cause degeneration *in vivo* fails to produce any current. However, robust amiloride-sensitive currents are observed when either MEC-2 (a protein related to human stomatin) or MEC-6 (a protein related to human paraoxonase) is added. MEC-4, but not MEC-10, is required to produce amiloride-sensitive currents. These currents were Na^+-selective, inwardly-rectified, effectively time-independent, and exquisitely sensitive to amiloride. The largest currents were observed when both MEC-2 and MEC-6 were present together with the channel subunits; MEC-2 and MEC-10 altered affinity for amiloride. The expression pattern data, genetic interaction data, and now these oocyte expression data suggest that all four of these

proteins (and possibly others) are needed for the formation of a mechanotransduction complex *in vivo*.

204. The complete *C. elegans* family of *osm-9*/capsaicin receptor related genes: a conserved family of putative ion channel subunits with roles in nociception and chemosensation.

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C. elegans has a family of four putative ion channel subunits that are closely related to the *C. elegans osm-9* gene. *osm-9* encodes a putative ion channel with homology to the vertebrate capsaicin receptor, a cation channel expressed in pain-sensing neurons and gated by the active component of chili peppers. In the amphid neuron AWA, OSM-9 localizes to sensory cilia, suggesting a direct role in sensory transduction. *osm-9* mutants are defective in a wide range of sensory functions in *C. elegans* including chemosensation, mechanosensation, osmosensation, and certain forms of olfactory adaptation.

Four relatives of *osm-9* are each expressed in subsets of *osm-9*-expressing cells. We call these genes *ocr* (*osm-9*/capsaicin receptor-related) genes and have generated mutations in two of them. Based on GFP fusions, *ocr-1* is expressed primarily in AWA, *ocr-2* is expressed in AWA, ASH, ADL, ADF, PHA, and PHB, *ocr-3* is expressed in rectal gland cells, and *ocr-4* is expressed in OLQ mechanosensory neurons. All *ocr* genes are expressed in subsets of *osm-9*-expressing cells. We hypothesized that the OCR channels might coassemble with OSM-9 to form heteromultimeric complexes. Like OSM-9, OCR-2 localizes to sensory cilia. Proper localization of OCR-2 requires *osm-9*, suggesting that these proteins may indeed associate. We propose that different combinations of subunits may account for the distinct functions of *osm-9* in different sensory neurons.

Mutations in *ocr-2* recapitulate a subset of *osm-9*''s defects: there are dramatic defects in AWA-mediated chemotaxis and ASH-mediated avoidance behaviors. By contrast, *ocr-1* mutants do not have obvious sensory defects. However, both *ocr-1* and *ocr-2* appear to contribute to activity-dependent gene regulation in AWA (A. Kahn and C.I.B.), so both proteins are probably functional.

Although heterologous expression of VR1 does not rescue *ocr* behavioral phenotypes in *C.elegans*, mammalian VR1 is functional and responsive to capsaicin in the *C. elegans* nociceptive neuron ASH. We have used this observation as the basis for a method of reversible, drug-inducible activation of sensory neurons in *C. elegans*. Animals expressing VR1 in ASH reverse in response to capsaicin. Capsaicin bypassed several ASH molecules normally required for signaling, suggesting that the channel depolarizes the neurons directly. The effect of VR1 in ASH required the endogenous vesicular glutamate transporter EAT-4. The introduction of a channel with a novel pharmacology into sensory neurons may have general utility for answering temporal questions about neuronal activity.

205. Neuropeptide modulation of touch sensitivity and locomotion

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Secretion of neuromodulatory peptides has often been proposed as a mechanism for regulating synaptic efficacy and for producing adaptive changes in behavior. At synapses, neuropeptides are released with other classical neurotransmitters and are thought to modulate the impact of the co-released neurotransmitters. Neuropeptides are packaged as proproteins into dense core vesicles or secretory granules, where they are enzymatically processed into biologically active peptides. To study the role of neuropeptides in modulating behavior, we have analyzed mutations in two neuropeptide processing enzymes. Proprotein convertases (PCs) cleave proneuropeptides at dibasic residues and carboxypeptidase E (CPE) removes the dibasic residues from the carboxy termini of the cleaved peptides. We previously reported that the *egl-3* gene encodes the worm homolog of PC2 (Kass and Kaplan IWM99) and that *egl-21* encodes the worm CPE (Carey and Kaplan WCWM00). The genome sequence predicts three other PC genes and two additional carboxypeptidase genes; therefore, these mutants do not represent null mutations for neuropeptide biosynthesis.

We have shown that the *egl-3* PC2 regulates the efficacy of sensory-interneuron synapses. Nose touch sensitivity (mediated by ASH sensory neurons) is defective in mutants lacking GLR-1 glutamate receptors (GluRs); however, mutations eliminating the *egl-3* PC2 restored nose touch sensitivity to *glr-1* GluR mutants. Mutants lacking the *egl-3* PC2 had increased sensitivity to nose touch whereas transgenic animals over-expressing the *egl-3* PC2 had decreased touch sensitivity. These results suggest that *egl-3* PC2 processed peptides regulate the gain of mechanosensory responses, perhaps by regulating the efficacy of ASH-interneuron synapses.

We also show that *egl-3* PC2 and *egl-21* CPE both regulate synaptic efficacy at body wall neuromuscular junctions (NMJs). Both mutants are resistant to the paralytic effects of the acetylcholinesterase inhibitor aldicarb but are fully sensitive to the acetylcholine (ACh) agonist levamisole. These results suggest that *egl-3* PC2 and *egl-21* CPE mutants have decreased ACh release at NMJs. We will discuss candidate neuropeptides that may account for the effects of these mutations on touch sensitivity and locomotion.

206. Avoidance and bitter taste in *C. elegans* : evidences for a head-tail chemotopic sensory map

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The ability to recognize, avoid and reject toxic compounds in the environment is crucial for survival. In humans, oral exposure to poisonous compounds often results in the association of sets of aversive responses with the perception of bitter taste. Using the drop test as an assay (12[th] International *C. elegans* Meeting 1999, abstract 392), we have observed that *C. elegans* senses as repellent many water soluble substances that are toxic or related to toxic compounds and that also taste very bitter to humans, suggesting a conserved response to toxicity and an evolutionary relationship between avoidance and bitter taste. Through laser ablation of specific neurons we have identified sensory neurons responsible for detection, in the drop test, of quinine, SDS, Cu^{++}, high osmotic strength and low pH. Our results suggest that the polymodal sensory neuron ASH, which has already been shown to mediate avoidance responses to high osmotic strength using different avoidance assays, plays also a central role in the avoidance of all water soluble repellents tested. We have found that ASK, described as responsible for chemotaxis toward the attractant lysine, is also involved in water soluble avoidance, suggesting a new double-opposite role for this cell. We will also present evidences that PHA and PHB, the two sensory neurons of the phasmid, the tail sensillum of Secernentea nematodes, function in *C. elegans* as modulators of the avoidance response to SDS. To complement the laser ablation data, we developed a genetic cell specific rescue using the mutants strain *osm-6*. These mutants fail to respond to many repellent tested because of defects in sensory cilia formation. *osm-6* has been shown to act cell autonomously (1). We used different neuronal

specific promoters to drive the expression of the wild type *osm-6* gene in a subset of neuronal cells, in order to see if it was possible to recover their cilia defect and their function. We observed anatomical rescue of the cilia structure in those cells where the w.t. *osm-6* gene expression was directed. Behavioral assay, using the drop test, have shown that worms in which ASH, ADL and ASK where structurally recovered, rescued also their ability to respond to high osmotic strength, quinine, Cu^{++} and SDS. Thus the anatomical rescue correlates with the functional rescue and the results are consistent with those obtained by laser ablation. An EMS mutagenesis screening has been performed to isolated worms unable to avoid quinine hydrochloride. We found 5 strains who showed the quinine non avoiders phenotype and were non-Dyf (abnormal dye filling) indicating that their sensory cilia were intact. One of them, *gb 404*, had the most severe phenotype in relation to quinine response although still able to respond to mechanical stimuli and to a subset of other repellents. Complementation analysis revealed that *gb 404* and *gb 408* were alleles of the same gene we called *qui-1* (QUInine non-avoiders). Mapping data using SNPs placed the mutation on the IV chromosome. Recently, we have discovered the molecular nature of *qui-1*.

1. Collet, J., Spike, C.A., Lundquist, E.A., Shaw, J.E. and Robert K. Herman. Analysis of *osm-6*, a gene that affects sensory neuron function in *Caenorhabditis elegans* . 1998. Genetics 148:187-200.

207. The novel conserved protein OCA-1 is required for chemosensory and osmosensory response by the ASH circuit.

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It is unclear how a single sensory neuron can distinguish between a variety

of external stimuli and how the sensory circuit can generate distinct behavioral responses. The *C. elegans* ASH sensory neurons are required to detect volatile repellent chemicals (1-octanol and benzaldehyde), high osmolarity, and touch to the nose. We hope to reveal the sensory and synaptic mechanisms involved by studying the ASH sensory circuit. To address these questions, we focus our attention on modality specific genes which are required for response to one or two stimuli but not required for response to other stimuli detected by the ASH neurons.

We conducted a behavioral screen to identify genes required for octanol avoidance. *octanol avoidance defective-1(rt68)* (*oca-1*) animals are severely defective in their response to hyperosmolarity, octanol or benzaldehyde, but their response to nose touch is normal. *oca-1(rt68)* animals habituate to nose touch and they can respond to odorants and soluble compounds detected by AWA, AWC, and ASE sensory neurons. *oca-1* encodes a small, previously uncharacterized protein with putative homologs in invertebrates and vertebrates. We are in the process of elucidating the function of OCA-1 using cellular, biochemical and genetic approaches.

Interestingly, response to octanol is modulated by serotonin. Another modality specific mutation, *rt6*, shows transient defects in octanol avoidance. *rt6* mutant animals respond as well as normal animals to octanol on the bacterial lawn. After 10 minutes off the bacterial lawn, *rt6* mutant animals are severely defective in their response to octanol, but they can respond

to

octanol after 60 minutes off food. The *rt6* mutant phenotype can be rescued by the presence of food and 5mM serotonin. In *C. elegans* and other organisms, serotonin acts as neurotransmitter or neurohormone to regulate behavior. The *rt6* transient defect is surprising as the feeding status of *C. elegans* normally does not affect response to octanol. We have found that *C. elegans* sensitivity to octanol is increased in the presence of food. Consistent with our results, *tph-1(m280)* and *dgk-1(nu62)* mutant animals, which are defective in serotonin mediated signal pathway(Sze JY et al., Nature. 403:560-4, 2000. Nurrish S et al., Neuron 24:231-42, 1999), show decreased octanol sensitivity even in the presence of food. These mutant animals mimic the behavior of normal animals off food. The application of exogenous serotonin fails to rescue the defective octanol response of *eat- 4* , *osm-9* or *odr-3* mutant strains. As the corresponding genes encode a vesicular glutamate transporter, a TRP ion channel, and a G-alpha protein which are all necessary for ASH-meditated sensory signal transduction, we conclude that serotonin modulates synaptic signaling in the ASH circuit.

208. TAX-6 calcineurin acts as a negative regulator of sensory signal pathways to modulate excitability of sensory neurons

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Calcineurin, calcium/calmodulin-dependent phosphatase, is involved in a variety of calcium signaling cascades. In the absence of calcium, the phosphatase activity of calcineurin A subunit is inhibited by its own autoinhibitory domain. In the presence of calcium, binding of calcium/calmodulion and calcineurin B subunit to the A subunit releases the autoinihibition and triggers the drastic increase of the catalytic activity. Despite the abundant expression of calcineurin in mammalian brain, its neuronal function is poorly understood. We found that *tax-6* gene encodes sole calcineurin A subunit in *C. elegans* and that its function is essential for the sensory systems. A reduction-of-function mutation, *tax-6(p675)*, was originally isolated as chemotaxis-defective mutant for NaCl (1) and was also found to show thermophilic (heat-seeking) phenotype in thermotaxis (2).

TAX-6 calcineurin was mainly expressed in sensory neurons, such as thermosensory neuron AFD, chemosensory neuron ASE, AWA, AWC, and osmosensory neuron ASH. The specific expression of *tax-6* cDNA in AFD thermosensory neurons of *tax-6* mutants rescued AFD-mediated defective thermotaxis, but did not rescue ASE-mediated defective chemotaxis, indicating that *tax-6* functions cell-autonomously in AFD neurons. Interestingly, either killing AFD or expressing constitutively activated TAX-6 in AFD results in cryophilic and/or athermotactic phenotype. These results suggest that thermophilic phenotype exhibited by *tax-6* mutation is caused by hyper-activation of AFD neurons. Consistent with the hyper-activation of sensory neurons by the loss of TAX-6 function, *tax-6* mutants also showed hypersensitive osmotic avoidance mediated by the ASH neurons. To address how and where TAX-6 calcineurin functions in

sensory signaling, we behaviorally analyzed animals doubly mutant for *tax-6* and a mutation that is proposed to be required for sensory signaling in AFD and ASH neurons. Our results are consistent with the model, in which TAX-6 calcineurin is activated by calcium influx through the sensory cation channel, TAX-4/TAX-2 cyclic nucleotide-gated channel or capsaicin-receptor-like OSM-9 channel, and negatively regulate the sensory signal transduction pathway. In contrast to our intuitive prediction, *tax-6* mutants however showed partially defective olfactory responses to AWA- and AWC-sensed odorants. We then explored olfactory responses further and found that partially defective olfactory responses of *tax-6* mutants are caused by hyper-activated olfactory adaptation (3). First, *tax-6* mutants were hyper-adaptable to isoamyl alcohol (iaa) sensed by AWC neurons. Second, *tax-6; osm-9* double mutants, where *osm-9* is known to be completely defective in adaptation to iaa, showed normal responses to iaa as in wild type. Thus, it is likely that TAX-6 normally inhibits adaptation machinery in AWC neurons. Altogether, we suggest that TAX-6 calcineurin controls the efficiency of sensory neuronal activity and excitability, by negatively modulating signal transduction pathways in sensory neurons.

(1) Dusenbery et al (1975), Genetics 80; 297-309.

(2) Hedgecock and Russell (1975), PNAS 72; 4061-4065.

(3) Colbert and Bargmann (1995), Neuron 14; 803-812.

209. Analysis of TTX-4 nPKC-epsilon required for sensory signaling

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C. elegans is capable of sensing various environmental stimuli and modifying its behavioral responses. We are particularly interested in molecularly dissecting temperature-evoked behavior (thermotaxis), because of its experience-dependent plasticity. In order to identify molecular components that regulate thermotaxis, we have isolated many thermotaxis-defective mutants (see abstract by Okumura *et al.*). Of these, *ttx-4(nj1, nj3, nj4)* mutants showed thermophilic phenotype. In addition, *ttx-4* mutants were found to be defective in chemotaxis to ASE-sensed NaCl, AWA- and AWC-sensed odorants, and in ASH-mediated osmotic avoidance. We found that *ttx-4* gene encodes a novel Protein Kinase C (nPKC)-epsilon homologue. nPKCs consist of a regulatory domain in the amino terminus and a catalytic domain in the carboxyl terminus. In the regulatory domain, there are two conserved regions, C2-like region and C1 region. Unlike conventional PKCs (cPKCs), nPKC activation is Ca^{2+}-independent, but is known to be DAG-dependent due to the presence of C1 region. However, *in vivo* substrates phosphorylated by nPKC are not well understood (see abstract by Kimura *et al.*). *ttx-4(nj1)* and *ttx-4(nj4)* mutants have missense mutations in kinase domain, and the *ttx-4(nj3)* mutant has nonsense mutation in C1 domain. *ttx-4* mutants are all genetically recessive and *ttx-4(nj1)* mutant was rescued by a low concentration of wild type *ttx-4* transgene, suggesting that *ttx-4* mutations are loss of function mutations. The functional GFP-tagged TTX-4 protein was expressed in many sensory and interneurons, and some motorneurons. These neurons include AFD thermosensory neuron, AIY and AIZ interneurons, all of which are essential for thermotaxis. TTX-4 protein was also expressed in AWA, AWC and ASH neurons. Cell-specific expression of *ttx-4* cDNA in AFD or AWC of *ttx-4* mutants rescued abnormal thermotactic or chemotactic behavior,

respectively, implicating that the function of TTX-4 nPKC in sensory neurons is sufficient for normal behavioral responses to sensory stimuli.

Previous laser-killing experiments and genetic studies demonstrated that animals whose AFD neurons are inactive or barely active show cryophilic phenotype. As TTX-4 functions in AFD neurons and loss of function mutations of *ttx-4* cause thermophilic phenotype, it is likely that TTX-4 functions as negative regulator of temperature signaling in AFD neurons. This negative regulation resembles the proposed role of TAX-6 calcineurin in AFD (see abstract by Kuhara *et al.*). To explore the functional relationship in temperature signal transduction between TTX-4 and TAX-6, if any, we are in the process of analyzing thermotactic phenotypes of *ttx-4(lf)* ; *Ex[TAX-6(gf)]* and *tax-6(lf)*; *Ex[TTX-4(gf)]* animals. *tax-6* mutants show partial olfactory responses, which turned out to be caused by hyper-sensitivity to olfactory adaptation. By contrast, *ttx-4* mutants are completely defective in chemotaxis to odorants. To investigate whether these severe olfactory defects in *ttx-4* mutants are caused by hyper-sensitivity in olfactory adaptation as in *tax-6* mutants, we are analyzing primary olfactory responses to AWC-sensed odorants in *osm-9(ky10);ttx-4(nj1)* mutants, where *osm-9* mutation is expected to suppress olfactory adaptation to isoamyl alcohol and butanone.

210. Behavioral and genetic analysis of starvation-induced neural plasticity in thermotaxis

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C. elegans can associate cultivation temperature with feeding state and modify behavioral outputs in response to environmental changes. After the animals were grown under well-fed condition, they migrate to cultivation temperature on a temperature gradient. We found that animals that were conditioned to thermotax to cultivation temperature become to avoid that temperature after starved under uncrowded condition for only 20 minutes (for 25C-grown animals) or for 2.5 hours (for 17C-grown animals). We also found that recovery from the starved state occurs very quickly: after animals that had been conditioned to avoid cultivation temperature were re-cultivated with food only for 10 minutes, they are fully capable of migrating to cultivation temperature (for both 25C- and 17C-grown animals).

Like in the case for egg-laying, pharyngeal pumping and locomotion activity, exogenous serotonin and octopamine were found to mimic well-fed and starved state, respectively, in thermotaxis. To further address the importance of serotonin signaling in thermotaxis, we assayed thermotaxis of serotonin-defective mutants (*cat-1*, *cat-4*, *bas-1* and *tph-1*). Surprisingly, these mutants showed almost normal thermotactic responses in any aspects. We noticed however that *cat-1* mutants recover from the starved state 10 minutes more slowly than the other mutants and wild type animals. This delay in *cat-1* mutants implicates the involvement of synaptic transmission in thermotaxis, since CAT-1, vesicular monoamine transporter, is thought to be required for neurotransmitter release at synaptic terminal. We also showed that killing NSM serotonergic neurons in the pharynx did not affect thermotaxis. Altogether, these results led us to propose two models. Although exogenous serotonin can substitute food signal in thermotaxis, other *in vivo* component might be

important for food-temperature association. Alternatively, endogenous serotonin that could be still synthesized even at low level in serotonin-defective mutants sufficiently works as food signal in thermotaxis.

To identify genes and neurons required for associating temperature memory with feeding state, mutants were sought that can respond to feeding states normally, but are defective in starvation-induced temperature avoidance. By screening about 5,300 genomes for animals that constitutively migrated to cultivation temperatures despite starvation experience, we obtained several mutants designated *aho* (abnormal hunger orientation). These *aho* mutants responded normally to food in locomotion assay, indicating that they can feel feeding states properly. The dominant *aho-1(nj5)* mutation maps to chromosome I and leads to abnormal defecation as well as pharyngeal pumping that is reflected in EPG (T. Niacaris and L. Avery, pers. comm.), although *aho-1(nj5)* mutants exhibited normal chemotaxis to Na^+ ion and odorants, and normal olfactory adaptation. The recessive *aho(nj15)* mutation maps to the center of chromosome I, and *aho(nj15)* mutants showed normal chemotaxis to Na^+ ion and odorants. We suggest that *aho* mutants are the first example of novel thermotaxis-defective mutants, in which modulation of thermotaxis by the feeding state is specifically impaired. This also implies that the process for modulatory aspect of thermotaxis is separable from the process for primary thermosensation and temperature memory formation. The *hen-1* mutant defective in integration of aversive and attractive stimuli, and chemotaxis-based learning paradigm (see abstract by Ishihara et al.) also showed Aho phenotype. It is thus likely that mutants generally defective in learning are included among our *aho* mutants. We are currently testing this possibility.

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211. The Role of *srf-6* in Chemosensory Control of Surface Antigen Switching

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Nematodes restrict the expresssion of specific surface molecules to a particular time or developmental stage and can switch surface molecules in response to environmental changes. Our study of surface antigen switching in *C. elegans* has led to the discovery that it is modulated in response to environmental signals, and this response requires functional chemosensory neurons.

Previously, we identified a surface antigen switch in which wild type *C. elegans* is induced to display an L1 surface epitope at a later larval stage (inducible larval display or ILD) when grown under special conditions. We also identified mutations that result in nonconditional display of this epitope on all four larval stages (constitutive larval display or CLD). These include mutations in a new gene, *srf-6*, and in previously identified dauer-constitutive (*daf-c*) genes involved in signal transduction during dauer larva formation.

Surface antigen switching and dauer formation are controlled differently. For example, *srf-6* mutations apparently do not affect dauer formation. Examination of double mutants combining *srf-6(yj13)* with ts mutations in *daf-c* genes suggested that *srf-6* acts in parallel with the TGF-β signaling pathway defined by some *daf-c* genes, but might act in the same pathway with *daf-11*.

ILD requires intact sensory cilia. Cilium structure mutations such as *che-3*and *osm-3* resulted in greatly reduced ILD. All sensory cilia are abnormal in *che-3* mutants, while *osm-3* mutations affect only the chemosensory neurons that detect water-soluble substances (taste). A *che-3; srf-6* double mutant showed no

CLD, indicating that intact sensory cilia are required for *srf-6* to affect phenotype. By contrast, mutations that affect olfaction, but not taste, had no effect on ILD. Taken together, these results suggest that ILD requires some ciliated sensory nerve endings, but not the olfactory neurons.

In chemotaxis assays, *srf-6(yj13)* showed greatly reduced attraction to both volatile and nonvolatile substances that attract wild type. Taken together, our results are consistent with a model in which *srf-6* activity is required in a chemosensory neuron to inhibit a downstream component that activates expression of the L1 surface epitope at later larval stages.

212. Lamin-dependent localization of UNC-84, a protein required for nuclear migration in *C. elegans*

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Mutations in the *C. elegans unc-84* gene cause defects in nuclear migration and anchoring. We show that endogenous UNC-84 protein co-localizes with Ce-lamin at the nuclear envelope, and that the envelope localization of UNC-84 requires Ce-lamin. We also show that during mitosis, UNC-84 remains at the nuclear periphery until late anaphase, similar to known inner nuclear membrane proteins. These findings suggest that UNC-84 is a nuclear lamina-associated protein. UNC-84 protein is first detected at the 26-cell stage and thereafter is present in most cells during development and in adults. UNC-84 is properly expressed in *unc-83* and *anc-1* lines, which have phenotypes similar to *unc-84*, suggesting that neither the expression nor nuclear envelope localization of UNC-84 depends on UNC-83 or ANC-1 proteins. The envelope localization of Ce-lamin, Ce-emerin, Ce-MAN1 and nucleoporins are unaffected by the loss of UNC-84. UNC-84 is not required for centrosome attachment to the nucleus because centrosomes are localized normally in *unc-84* hyp7 cells despite a nuclear migration defect. Models explaining the involvement of UNC-84 in nuclear migration and anchoring are discussed.

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213. Translational control of maternal *glp-1* mRNA by POS-1 and its interacting protein PIP-1

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A maternal factor POS-1 regulates cell fate determination of early embryos (Tabara et al., Development, 126, 1-11, 1999). POS-1 has two TIS11 type (CCCH) zinc finger motifs, and is present in cytoplasm of posterior blastomeres, being a temporary component of P granules. At the previous meeting (12th IWM, abstract 83, 1999), we reported that POS-1 binds to PIP-1 that has an RNP type RNA binding motif. PIP-1 is present in cytoplasm of oocytes and early embryos, also being a temporary component of P granules. RNAi analysis showed that PIP-1 is also required for cell fate determination of early embryos.

This time, we have isolated a deletion null mutant *pip-1(tm291)* and found that the phenotypes are identical to those of RNAi, and that POS-1 and PIP-1 regulate maternal *glp-1* mRNA translation. Translation of the maternal *glp-1* mRNA is temporally and spatially regulated by the 3' UTR (Evans et al., Cell, 77, 183-194, 1994). In wild type embryos, GLP-1 begins to be detected in anterior AB blastomeres at two-cell stage. At four-cell stage, GLP-1 is only detected in anterior blastomeres ABa and ABp, but not in posterior EMS and P2. We found that GLP-1 was not detected in *pip-1* embryos, and that GLP-1 was ectopically detected in the posterior blastomeres in *pos-1* embryos. At four-cell stage embryos of *pos-1*, GLP-1 was detected in posterior EMS and P2 in addition to anterior ABa and ABp. GLP-1 was not detected in *pos-1* oocytes and one-cell stage embryos, suggesting that the temporal regulation is normal. We examined POS-1 localization in *pip-1*, and PIP-1 localization in *pos-1*, and found that their localizations were unchanged. These results suggest that PIP-1 is a positive regulator and POS-1 is a negative regulator of the translation of maternal *glp-1* mRNA. In *pos-1; pip-1* double RNAi embryos, the GLP-1 localization was identical to that of *pos-1* embryos, suggesting that *pos-1* is genetically epistatic. Finally, by using the three hybrid system, we found that POS-1 specifically interacted with spatial control region (SCR) identified in the 3' UTR of *glp-1* mRNA (Rudel and Kimble, Genetics, 157, 639-654, 2001), but PIP-1 did not. We will discuss possible mechanisms of the translational regulation of maternal *glp-1* mRNA.

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214. Mutations in *pos-1* dominantly suppress the Mex phenotype of *efl-1*, *dpl-1* and *mex-5* mutant embryos

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EFL-1 and DPL-1 are C. elegans proteins related to vertebrate E2F and DP proteins that form a heterodimeric transcription factor. Progeny from *efl-1* and *dpl-1* mutant mothers have anterior misexpression of the transcription factor SKN-1 at the 2-cell stage of embryogenesis, resulting in a "Mex" (muscle excess) terminal phenotype. The Mex phenotype of *efl-1* and *dpl-1* embryos can be suppressed by reducing the level of Ras/Map kinase signaling in the mother, and oocytes of *efl-1* and *dpl-1* mutant mothers show higher than normal levels of activated Map kinase. Thus, normal embryonic polarity is dependent on wild-type levels of Ras/Mapk.

Reducing Ras/Map kinase activity also supresses the Mex phenotype of the previously described *mex-5* mutant. The *mex-5* gene encodes a novel protein with zinc-finger motifs and is asymmetrically distributed to the anterior of newly fertilized eggs. The MEX-5 expression pattern is complimentary to that of germline-localized proteins such as POS-1, a protein that also contains zinc-finger motifs similar to those found in MEX-5.

We isolated a collection of dominant suppressors of the temperature sensitive Mex phenotype of *efl-1*, one of which has a mutation in *pos-1*. We subsequently determined that loss-of-function mutations in *pos-1* dominantly suppress not only *efl-1*, but also *dpl-1* and *mex-5* mutant embryos. Because MEX-5 activity prevents the anterior expression of at least one posterior-localized protein, we examined whether POS-1 plays a complimentary role in preventing the posterior expression of any anterior-localized protein. We found that GLP-1, which is normally expressed in anterior blastomeres, is present in both anterior and posterior blastomeres in *pos-1* mutant embryos. Our results suggest that MEX-5 and POS-1 have mutually antagonistic functions and that the asymmetric distribution of proteins in the 2-cell embryo relies on a balance between these two proteins.

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215. MEX-3 Interacting Proteins Link Cell Polarity to Asymmetric Gene Expression

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The KH domain protein MEX-3 is key to spatial and temporal control of PAL-1 expression in the *C. elegans* early embryo. *pal-1* encodes a Caudal-like homeodomain protein that specifies the fate of posterior blastomeres (3).While *pal-1* mRNA is present throughout the oocyte and early embryo, PAL-1 protein is only detected in posterior blastomeres starting at the 4-cell stage. This spatial and temporal patterning of PAL-1 is dependant on the *pal-1* 3'UTR and the putative RNA binding protein MEX-3, whose localization correlates with PAL-1 repression (2). MEX-3 localization and activity is dependent on the early-acting *par* genes. In *par-1* and *par-4* mutants, MEX-3 in all cells is coincident with failure to express PAL-1 in any cell (1). In contrast, 4-cell *par-3* embryos express PAL-1 despite high levels of MEX-3 in all cells, indicating that MEX-3 is not sufficient to repress PAL-1 at the 4-cell stage.

To identify co-factors that work with MEX-3 or regulatory factors that modulate MEX-3 activity, we conducted a two-hybrid screen using MEX-3 as bait. We identified two MEX-3 Interacting Proteins (MIPs), MEX-6 and PIP-1, that regulate MEX-3 expression levels and control spatial but not temporal patterning of PAL-1. *mex-6* encodes a CCCH type zinc finger protein that shares high sequence identity and partial functional redundancy with *mex-5* (6). In fact, *mex-6*(RNAi) reduces the function of both *mex-5* and *mex-6*. Anteriorly localized MEX-5,6 prevent premature degradation of MEX-3 and repress anterior PAL-1 expression. ZC404.8 encodes an RNA recognition motif protein also identified as a POS-1 interacting protein, *pip-1* (5), and as a mutant, *spn-4* (4), that affects spindle rotation. *pip-1* is required to repress weak anterior PAL-1 expression starting at the 4-cell stage and for the timely degradation of MEX-3.

Genetic analysis suggests that MEX-6 and PIP-1 define parallel pathways that control MEX-3 stability and activity. First, *mip; par* double mutants with *par-3* or *par-1* are similar to the *mip(RNAi)* single mutants, indicating that *par-3* and *par-1* function upstream of *mex-5,6* and *pip-1*. In contrast, *par-4* mutations affect the RNAi phenotype of both *mex-6* and *pip-1*, suggesting that *par-4*, *mex-5,6*, and *pip-1* act in parallel pathways. In *pip-1(RNAi) par-4* mutants, MEX-3 accumulates abnormally as in *pip-1*(RNAi) embryos, but like *par-4* mutants, PAL-1 is not expressed. *mex-6(RNAi); par-4* embryos express PAL-1 like *mex-6*(RNAi) embryos, but contain stable MEX-3 like *par-4* embryos. In all cases in which *mex-5,6* are absent, PAL-1 is ectopically expressed, suggesting that *mex-5,6* are absolutely required for PAL-1 spatial repression. In all cases in which *pip-1* is absent, MEX-3 persists through the 16-cell stage, suggesting that *pip-1* is absolutely required for the timely degradation of MEX-3. In all cases in which *par-4* is absent, MEX-3 persists at least through the 8 cell stage, suggesting that *par-4* plays a role in MEX-3 inactivation and degradation.

We suggest that *par-4* normally acts to both inactivate MEX-3 and mark it for *pip-1*-mediated degradation. In the anterior, *mex-5,6* activate MEX-3 and protect it from inactivation and degradation. Furthermore, we suggest that a small amount of inactive MEX-3 in the anterior of *pip-1*(RNAi) embryos, which is normally degraded, interferes with active MEX-3, causing weak PAL-1 expression. From these results we propose that MEX-3 and the MIPs act through multiple independent pathways to translate the polarity information provided by the early acting *par* genes into the asymmetric distribution of PAL-1.

1. Bowerman, Ingram and Hunter (1997) Development 124, 3815-3826.
2. Draper, Mello, Bowerman, Hardin and Priess (1997) Cell 87, 205-216.
3. Hunter and Kenyon (1996) Cell 87, 217-226.
4. Gomes, Swan, Shelton and Bowerman (2000) West Coast Worm Meeting
5. Ogura and Kohara (1999) International Worm Meeting
6. Schubert, Lin, Plasterk and Priess (2000) Mol. Cell 5, 671-682.

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216. Early zygotic *pal-1* expression in the AB lineage depends on the LAG pathway

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Embryonic *pal-1* expression in the AB lineage appears initially in two cells, ABplpappp and ABplppppp, at about the 250-cell stage. These cells move from lateral positions to meet their contralateral lineal homologs at the ventral midline, divide, and close the posterior end of the ventral cleft during late gastrulation. After closure, the daughters of these cells as well as those of the right homolog ABprpppppp express *pal-1*; these six cells and their descendants continue to express through morphogenesis and early L1, forming the rectal-associated muscles and some tail hypodermis (Edgar et al., Dev. Biol. 229:71-88, 2001). Two findings indicate a critical role for these cells, and their expression of *pal-1*, in rectal induction and morphogenesis. First, laser ablation of one or two (of the initial six) cells just after ventral cleft closure results in L1 animals with no rectum. Second, partial rescue of the Nob phenotype of *pal-1(ct224)* embryos with a short-promoter genomic construct gives the same rectal-less L1 phenotype, and a GFP reporter construct with the same promoter is not detectably expressed in these AB cells.

We are currently investigating what initiates such a specific expression of *pal-1* in two non-sister cells on only one side of the embryo. Because the rectal-less phenotype produced by ablation or partial rescue resembles the Lag phenotype of mutants in the *lin-12-glp-1* (Notch-like) signaling pathway (Lambie and Kimble, Development 112:231-240, 1991), we followed expression of a *pal-1:GFP* reporter in both *lag-1(q385)* and *lag-2 (q411)* embryos. *pal-1:GFP* was expressed normally in subsets of the C, D, MS, and E lineages, but not in the AB cells contributing to rectal structures. Thus it appears that the Lag pathway initiates *pal-1* expression in these AB cells.

The construct giving "rectal-less rescue" of *ct224* embryos contains 1.1 kb of 5’ sequence, while a longer genomic construct with 5 kb of 5’ sequence rescues the rectal defect as well as the Nob phenotype. The *pal-1* gene includes several predicted LAG-1 binding sites (Christensen *et al.*, Development 122:1373-1383, 1996), (two in the 5’ region, one in exon 3, one in exon 4, and two in intron 4), but only one lies within the 4 kb of 5’ sequence that is present in the longer but not the shorter rescuing construct. We are mutagenizing this site and making both GFP and genomic constructs to test whether it is critical for expression of *pal-1* in the AB mesectodermal lineage and for *pal-1* function in rectal formation.

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217. The role of PLP-1 in mesendoderm specification and in other developmentally asymmetric cell divisions

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The EMS blastomere divides to produces MS, a progenitor of mesoderm, and E, the endoderm precursor. The generation of unequal daughters by this asymmetric division requires both the Wnt and MAP kinase pathways, which are activated in the endoderm lineage by inductive signals from the neighboring P_2 blastomere. As a result of these signals, the levels of the POP-1 transcription factor are reduced in the nucleus of E compared to MS, and POP-1 is converted from a repressor to an activator of the endoderm-determining *end-1* and *-3* genes (Kasmir and Rothman, IWM,1999). We are investigating the regulation of *end-1* in an effort to learn more about the mechanisms that regulate developmentally asymmetric cell divisions.

Using affinity purification methods and mass spectrometry we identified several factors that bind to key regulatory sites in the *end-1* promoter (Witze and Rothman, WCWM, 2000). One such factor, PLP-1 (**p**ur alpha **l**ike **p**rotein), is similar to the mammalian transcription factor pur alpha. RNAi of the *plp-1* gene results in impenetrant phenotypes suggesting a role in the asymmetric division of EMS: in mutant embryos we periodically observe derepression of endoderm in the MS lineage, reminiscent of the *pop-1(-)* phenotype. However, unlike *pop-1* mutants, we also observe a gutless phenotype. While the latter phenotype is quite impenetrant, we find that it is greatly enhanced by mutations in the Wnt pathway: for example, *mom-2*; *plp-1(RNAi)* doubles are nearly fully penetrant for the absence of gut. Interestingly, this synergy is not seen with mutations in the MAP kinase pathway, suggesting that PLP-1 may act in the MAPK pathway. Such a possibility is supported by our finding that early embryonic extracts can phosphorylate recombinant PLP-1 protein and that phosphatase treatment of extracts containing PLP-1 eliminates its *in vitro* binding to the *end-1* promoter.

We found that PLP-1 immunoreactive protein is present throughout all nuclei of early embryos up to the 100 cell stage and also associates with P granules. However, it distributes asymmetrically between nuclei of dividing cells: staining is more intense in the future posterior nucleus during telophase in EMS and in many other dividing cells, including the AB granddaughters (which undergo a POP-1-dependent developmentally asymmetric cell division) and even in the zygote, P0. Early in the subsequent cell cycles, staining once again equalizes in the two sister cells. This transient asymmetry in PLP-1 levels may reflect a role in specifying differences in sister cells: for example, *plp-1* mutant embryos do not express the AB-specific component of *lag-2* expression, consistent with a possible conversion in fate of the anterior AB great-granddaughters to that of their posterior sisters.

These results lead us to propose that PLP-1 may collaborate with POP-1 to establish differences between daughter cells recursively throughout *C. elegans* development.

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218. Control of hox gene expression might be more conserved between *Caenorhabditis elegans* and *Drosophila melanogaster* than previously thought.

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Null mutants of the *C. elegans labial/Hox1* type gene *ceh-13* display severe morphological defects, predominantly in the anterior, and cell adhesion defects[1]. Since spaciotemporal *ceh-13* expression appears to be controlled at the transcriptional level we have undertaken a promoter analysis. Interestingly, the *ceh-13* promoter region is capable of driving reporter gene expression in *Drosophila melanogaster* in a pattern highly reminiscent of *labial (lab)* expression. As for endogenous *lab,* this expression depends on the presence of *lab* function and on *wingless (wg)* signaling. In the worm, important features of comma stage expression also depend on a highly conserved auto-regulatory input that requires the activities of *ceh-13* and *ceh-20,* the worm *extradenticle* homolog. Consistent with that, a 450bp long enhancer fragment (enh450) that drives GFP expression in most cells that express *ceh-13* at this stage contains a 10 bp element identical or very similar to the so called auto-regulatory elements of mouse *hoxb1* and *Drosophila lab* respectively. Point mutations in this element abolish the activity of enh450 (see also abstract by Takacs-Vellai et al.).

A different regulatory element of 740bp (enh740) is sufficient to drive correct early embryonic *ceh-13* expression. Interestingly, this fragment acts as a strong *wg* responsive element in fly embryos. *In vitro* this element binds POP-1, the downstream effector of the Wnt/WG signaling pathway in *C. elegans,* indicating that it might also act as a target for this type of signaling in the worm. This is supported by our finding that in *pop-1* (RNAi) and in *lit-1* mutant early embryos CEH-13::GFP is absent from most of the cells that normally express it. However, POP-1 may not act simply as an activator of *ceh-13*. In the AB lineage e.g. onset of *ceh-13* expression occurs in the posterior daughters of a/p dividing cells that have lower POP-1 levels than their anterior sisters that dont express *ceh-13*. This indicates that POP-1 might act to activate or repress *ceh-13* depending on its concentration or phosphorylation status. Furthermore, sub-fragments of enh740 that interact efficiently with POP-1 *in vitro* but lack the most upstream portion are not capable of activating reporter gene expression suggesting that additional spaciotemporal cues are required for correct activation of *ceh-13*.

We are currently further investigating the regulatory elements within the *ceh-13* promoter using a combination of deletional analysis, *in vitro* binding assays and comparative sequence analysis of *ceh-13* from *C. elegans, C. briggsae* and *C. remanei*.

1) Brunschwig et al. (1999) Development 126:1537-1546

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219. 4D-analysis of a collection of maternal effect mutants of *C. elegans*.

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We have prevously identified 389 maternal-effect embryonic lethal mutations on LG. IV and V. They define 156 genes. 48 of those genes are represented with more than one allele. Instead of picking single genes and studing them in multiple mutants and embryos, we now try to explore the phenotypic spectrum by analysing as many genes as possible. After classifying the mutants as "interesting" according to the terminal phenotypes we immediately undertake a complete lineage analyis using a new digital 4D-microscope. We believe that this method is more efficient than any other approach. Until now 11 genes have been analysed and grouped in the following categories.

Metabolism: *t2129* embryos accumulate yolk bubbles during development without showing any other significant defects. Embryos appear starved. **Cell migration:** *t1903* embryos are unable to define the normal regions in the embryo. In a *t1855* embryo two descendants of the ABpla lineage migrate in a few minutes over the dorsal side of the embryo to join the ABpra descendants. **Cell death:** *t1875* is a new maternal effect lethal allele of *ced-10*. **Morphogenesis:** Embryos mutant for *t1777* or *t1831* both show a normal lineage, and regions are correctly formed, but fail to undergo normal morphogenesis. Embryos from mothers homozygous for the mutation *t2160* have a "small" pharynx cluster which looks like those in weak alleles of *glp-1*. However we did not find any lineage defect so far. The gene may thus have a function in the morphogenesis of the pharynx. **Cell fate:** Three *t1724* emrbryos showed aberrant C and D lineages. Two *t1930* embryos showed a left-right transformation of at least the ABara lineage. Two *t2121* embryos displayed a E to MS transformation as observed in mutants of the wnt pathway. In a *t1881* embryo the size of the pharyns cluster is reduced since the descendants of ABalp do not contribute as in normal embryogenesis to the pharynx. Because of their genetic position many of these genes may define new players in interesting pathways. We initiated the cloning of the genes corresponding to *t1903* (cell migration), *t1724* (C and D lineage defect) and *t2121* (E transformed into MS).

220. Variations in pattern formation and cell specification during nematode embryogenesis

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We have found that the cephalobid nematode A. nanus expresses considerable differences in the pattern of cell specification and spatial arrangement of cells during early embryogenesis compared to C. elegans (Wiegner and Schierenberg, Dev. Biol.,1998, 1999). Based on these results we have begun to analyze other free-living and parasitic nematodes in this respect.

Here we report our findings on representatives belonging to the same family as C. elegans (Rhabditidae). We find major differences in the duration of cell cycles, early zygotic transcription, cellular pattern formation and cell-cell interactions required for proper fate assignment. In contrast, species with only distant relationship to C. elegans may nevertheless show more similarities in this respect. This indicates that variations cannot be explained by phylogenetic position alone. We suggest that the observed differences reflect alternative developmental strategies going along with the occupation of different ecological niches.

221. Metaphase-to-anaphase transition-defective mutants occur in Anaphase Promoting Complex genes and exhibit phenotypes in both oocyte and spermatocyte meiosis in *C. elegans*.

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The Anaphase Promoting Complex or Cyclosome (APC/C) is a multisubunit ubiquitin ligase, which causes the destruction of cell-cycle regulators leading to the segregation of chromosomes at anaphase and exit from mitosis and meiosis. Previously, we described 32 temperature-sensitive *C. elegans* mutants that defined five genes, three of which were novel. At the non-permissive temperature of 25 degrees, all 32 mutant adults produce embryos arrested at the 1-cell embryonic stage, with their sperm and oocyte chromatin highly condensed, while polar bodies are absent. The phenotypes of these mutants indicates that they are arrested at the metaphase-to-anaphase transition of meiosis I in oocytes. We termed the three novel genes "mat" (metaphase-to-anaphase transition-defective). In addition, each *mat* gene is represented by some alleles that exhibit germline mitotic and somatic defects.

Genetic mapping demonstrated that all three *mat* genes mapped near *C. elegans* orthologs of APC subunits. To reveal the molecular identity of these genes, we determined the DNA sequences of the appropriate APC subunit genes in several alleles of each complementation group. The results indicate that in all cases, a given *mat* mutation occurred in the predicted APC subunit gene. Further, RNA interference using the predicted APC subunit genes gave the same 1-cell meiotic arrest phenotype as the mutants at the non-permissive temperature. We conclude that *mat-1*, *mat-2*, and *mat-3* are all alleles of *C. elegans* APC subunit genes.

We also analyzed sperm development and function in Mat mutant males. For these experiments, we focused on *mat-3*, because this complementation group represents the largest allelic series of the three. Although all thirteen *mat-3* alleles exhibited similar oocyte meiosis defects, they differed in their sperm meiosis and mitotic germline defects. In L3 upshift experiments, four categories of sperm meiosis defects were observed: fully fertile males (3 alleles); weakly fertile males (1 allele); infertile males with aneuploid sperm (3 alleles); infertile males with largely anucleate sperm (6 alleles). The five alleles that exhibited germline mitosis defects in L1 upshift experiments produced infertile males in L3 upshift experiments, but the alleles did not split evenly between the aneuploid and anucleate categories.

Finally, we identified *som-1*, an extragenic suppressor of the *mat-3* oocyte meiosis phenotype. Suppression in *mat-3*; *som-1* animals was only partially penetrant within a given brood, giving rise to only 3%-15% viable embryos at the non-permissive temperature. This suggests that, along with other phenotypes, aberrant chromosome segregation is occurring in order to allow *mat-3* animals to reproduce. Characterization of the *som-1* gene will be discussed. We postulate that *som-1* encodes a protein that physically interacts with the protein encoded by *mat-3*.

222. Making sperm without APC/C

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Temperature-sensitive mutations in subunits of the anaphase promoting complex (APC/C) cause metaphase to anaphase transition (mat) defects in C. elegans meiosis. In adult upshifted mat hermaphrodites, the fertilized primary oocytes accumulate at metaphase I and consequently arrest development at the meiotic one cell stage. Likewise, adult upshifted mat males produce primary spermatocytes that accumulate in metaphase of meiosis I. However, despite a strong metaphase I meiotic cell cycle block, the mutant mat primary spermatocytes continue to develop and produce spermatozoa (Golden & Sadler et al., 2000). Like wild type secondary spermatocytes, metaphase I arrested mat primary spermatocytes progress normally through cellular morphogenesis and individualization. All of the events of the budding process occur in the presence of a stable meiosis I metaphase plate and spindle. This analysis of mat spermatogenesis suggests that late APC/C function, specifically the stimulus of M-phase exit, might be dispensable for the budding division in wild type spermatogenesis. To test this hypothesis, we analyzed wild type secondary spermatocytes for indicators of cell cycle exit and found that, like the mat primary spermatocytes, they not only exhibit persistent meiotic spindles but they also lack several key indicators of M-phase exit. Does this new understanding of C. elegans spermatogenesis suggest that it is advantageous for spermatocytes to remain in a M-phase-like state during spermiogenesis? Perhaps C. elegans spermatocytes have evolved a unique way to circumvent the requirement for late APC/C function and thus discovered a faster way to make a sperm cell.

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223. Null mutations in wee1.3 are recessive lethal but rare dominant mutations specifically arrest the sperm cell cycle

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We have undertaken a genetic analysis of three gain of function *spe* mutants. All of these mutants map close to one another and show a striking and unique phenotype. EM analysis shows that these gain of function mutants arrest the sperm cell cycle but allow many aspects of sperm differentiation to continue. Oocytes and the soma are unaffected by these mutants. A suppressor screen was designed to identify intragenic suppressors of these dominant mutations and showed that the null phenotype of this gene is embryonic lethal. Physical mapping allowed the use of a candidate gene approach, and sequencing of 10 intragenic suppressor alleles revealed that our dominant mutations (and all suppressor mutations) were located in the *wee-1.3* gene. All three dominant mutations were G to R missense mutations clustered in a region of unknown function. *Wee-1.3* is an ortholog of the metazoan specific *Myt1*, an intracellular transmembrane member of the wee1 family of kinases. *Myt1* regulates entry into mitosis by altering the phosphorylation state of *cdc2*. Most suppressors alter conserved residues in this kinase domain, suggesting the original dominant mutation functions by altering this kinase activity. Hypomorphic suppressor alleles make functional sperm, suggesting that a presumed down regulation of *wee-1.3* is permissive for sperm function. Together, these data suggest that the dominance is neomorphic in nature, allowing the kinase to be active at an inappropriate time or place. Northern analysis and GFP promoter fusion experiments both show that *wee-1.3/ Myt1* is not expressed in a sperm-specific manner. Therefore, some aspect of the regulation of *wee-1.3* must be sperm-specific. All dominant missense mutants surround a serine residue in the C-terminal tail of this protein, so perhaps regulatory phosphorylation of this residue might explain the dominance of these mutants. We are currently determining if phosphorylation of this domain occurs, as well as identifying which genes are responsible for regulating this kinase in a sperm-specific fashion.

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224. *lin-9, lin-35* and *lin-36* negatively regulate G1 progression in *C. elegans*

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Coordination of cell division with growth and differentiation is critical to the development of multicellular organisms. The decision to enter or withdraw from the cell-cycle is made during G1 phase. The importance of this regulation is underscored by the finding that most tumor cells have defects in G1 control. In a screen for positive regulators of G1 progression, we identified mutations in *cyd-1*, the only *C. elegans* D-type cyclin, and *cdk-4*, the single kinase related to mammalian Cdk4 and Cdk6. Analysis of the mutant phenotype confirmed the previously published conclusion that *cyd-1* and *cdk-4* are required for G1 progression in postembryonic cell divisions (Park & Krause, Development 1999).

Members of the Rb tumor suppressor family are thought to be important targets of Cyclin D-dependent kinases. The synMuv class B gene *lin-35* is the single Rb-related gene in *C. elegans* (Lu & Horvitz, Cell 1998). However, a role in cell-cycle regulation has not been described for *lin-35*. Using double mutant combinations, we observed that *lin-35* inactivation overcomes the G1 arrest normally observed in *cyd-1* and *cdk-4* mutants. Thus, *lin-35* Rb is an important inhibitor of the G1/S transition and, by analogy with other systems, likely a major target of *cdk-4* and *cyd-1*. Interestingly, cell divisions are not restored to wild-type levels in *lin35;cyd-1* or *lin-35;cdk-4* double mutants, indicating that *cyd-1* and *cdk-4* have additional targets. We have identified a candidate additional target in a screen for suppressors of the *cyd-1;lin-35* cell cycle defects.

As several synMuv genes encode homologs of known cell-cycle regulators, we tested synMuv class A and B genes for a role in regulating G1 progression. Loss of function of two class B genes, *lin-9* and *lin-36*, partially rescued the defects of *cyd-1* and *cdk-4* mutants. These results demonstrate a role for *lin-9* and *lin-36* as novel negative regulators of G1 progression.

As *lin-35* does not appear to provide the only level of control over G1 progression, we examined whether the Cdk inhibitors of Cip/Kip family cooperate with *lin-35* in controlling cell-cycle entry. The C. elegans genome encodes two Cip/Kip family members, CKI-1 and CKI-2. Combined inactivation of *lin-35* with *cki-1,2* dramatically increased the number of divisions of the intestinal nuclei compared to *lin-35* or *cki-1,2* mutants alone. Interestingly, a similar synergistic effect was observed between *lin-36* and *cki-1,2*. Our data shows that *cki-1,2* Cip/Kip cooperates with *lin-35* Rb and *lin-36* in controlling G1/S progression. Based on the overlap in phenotype of *lin-35* and *lin-36* mutants, it is likely that these two genes act in a linear pathway for cell-cycle control, perhaps even in a complex.

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225. A novel synthetic approach to identify additional roles for lin-35/Rb in cell growth and proliferation.

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The Retinoblatoma gene product (Rb) is commonly regarded as the prototype for tumor suppressor genes. Numerous studies have indicated roles for Rb in cell-cycle regulation, transcriptional control, differentiation, and apoptosis. The *C. elegans* genome encodes for a single Rb-like protein, LIN-35 (Lu and Horvitz, 1998). *lin-35*/Rb was identified as a class B synthetic multivulval (SynMuv) gene . Loss-of-function mutations in SynMuv genes lead to a "multivulval" phenotype whereby affected animals contain multiple ectopic egg-laying structures. However, this phenotype is only obtained with specific combinations of SynMuv mutations, and the majority of SynMuv mutants, including *lin-35*/Rb, show no obvious defects on their own.

This finding is surprising as it would seem to indicate that *lin-35*/Rb carries out no essential function in worms, at least on its own. Such results contrast greatly with the known functions of Rb in other systems including Rb knockout studies in flies and mice where loss of Rb is lethal. In addition, because *lin-35*/Rb was found to be expressed in essentially all cell-types during *C. elegans* development (Lu and Horvitz, 1998), the question becomes, what is the function of *lin-35*/Rb in all these cells and how can such functions be identified? This question can be broadened to ask: How can functions be ascribed to genes where mutations show no obvious phenotype? This issue will likely arise with increased frequency as the genome becomes saturated for mutations with clear plate phenotypes.

We have designed and initiated a novel genetic screen with two basic goals: 1) to determine what additional biological functions *lin-35*/Rb may carry out during nematode development; and 2) to identify genes that may cooperate with Rb in carrying out these functions. Such genes may have conserved functions in higher eukaryotes and could conceivably play a role in Rb-mediated carcinogenesis. The screen is based on the premise that any additional functions of *lin-35*/Rb will require cooperation with a second site mutation (a synthetic interaction), similar to its known role in the SynMuv pathway. The screen utilizes a strain that is homozygous for a strong LOF mutation in *lin-35* (*n745*) but carries an extrachomosomal array containing both *lin-35* rescuing sequences and *sur-5::*GFP. In this way, green *lin-35+* animals can be readily distinguished from non-green *lin-35* animals. The screen seeks to identify phenotypes that are distinct to the non-green (*lin-35*) population of animals, thereby implying a phenotype that is specifically synthetic with *lin-35*/Rb.

Using the above approach we have identified at least seven *slr* mutations (for Synthetic with *lin-35*/Rb) defining at least six different genes. These mutations in conjunction with *lin-35*/Rb lead to pleiotrpic defects including lethality, slowed growth rates, decreased size, and infertility. One allele, *slr-1(ku298)*, shows a striking synthetic lineage defect with *lin-35*/Rb, resulting in generalized excess cell divisions. Importantly, this result indicates that hyperproliferation in *C. elegans* can follow the same genetic pattern as multi-step carcinogenesis in humans. Interestingly, a hyperproliferation phenotype is not observed when *slr-1* is combined with a number of other SynMuv genes tested, indicating a specific role for *lin-35*/Rb in this process.

We will describe our substantial progress towards mapping and identifying the affected gene products; six mutations have been mapped to chromosomal sub-regions and cosmid rescue experiments are underway for several of these. In addition, details of our screen will also be presented. We are encouraged that our technical approach will prove to be of general use for those wishing to identify mutations that may interact synthetically with their gene of interest.

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226. CUL-4 functions to prevent DNA re-replication

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DNA replication is normally strictly controlled to occur only once per cell cycle. Our studies show that CUL-4 is required for the regulation of this process. CUL-4 is a member of the cullin protein family. Other cullins have been found to function as ubiquitin ligases.

We inactivated *cul-4* by RNAi in order to study its cellular function. The majority of *cul-4* RNAi animals arrest as L2-stage larvae with huge seam nuclei. DNA quantitation revealed that these cells can have more than 100c DNA content. A similar phenotype of increased DNA content was also observed in P cells and somatic gonadal cells.

There are three possible mechanisms that can give rise to polyploidy. First, failed mitoses, in which cells enter mitosis normally but anaphase fails to occur, and cells subsequently enter interphase with doubled DNA content. Second, endoreplication, in which cells replicate their genomes in S phase, bypass mitosis, and double their DNA content again in the next S phase. And finally, DNA re-replication, in which cells arrest in S phase and reinitiate DNA replication continuously.

We employed three criteria to test which mechanism gave rise to the polyploidy in cul-4 cells. First, we tested whether cells entered mitosis. Using phospho-histone H3 antibody, an early mitosis marker, we found that *cul-4* animals have significantly less mitotic seam cells (0.09% in *cul-4* vs. 3.8% in control). This result was confirmed by a lineage study that indicated no mitotic entry in *cul-4* seam cells. These data suggest that the failed mitosis model is unlikely. Our second criteria was to measure the length of S phase to determine if cells were entering and leaving S phase normally. Using the S phase marker rnr:GFP (a kind gift of R. Roy and V. Ambros), we found that *cul-4* seam cells entered their first S phase at the normal time but then remained in S phase through the entire subsequent larval stage. This S phase arrest phenotype favors the DNA re-replication model. Finally, our third criteria was to measure the DNA content of the *cul-4* cells to determine if the increase in DNA content was quantized. We observed that the DNA content of *cul-4* cells increases continuously rather than increasing in multiples of 2N as is found for endoreplication. Taken together, these data indicate that *cul-4* cells undergo an S phase arrest with continuous DNA re-replication.

In summary, CUL-4 is required to limit DNA replication to once per cell cycle, which implicates CUL-4 in regulating the licensing of DNA replication. To our knowledge, *cul-4* is the first known metazoan gene whose inactivation causes a DNA re-replication phenotype.

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227. C. elegans DNA damage checkpoint mutants mutate

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The DNA damage checkpoint is composed of a group of proteins that sense DNA damage and induce either cell cycle arrest and DNA repair (which mends the damage) or programmed cell death (which removes the damaged cell). Although many cancer cells are checkpoint-defective, efforts to show that mammalian cells with defects in the DNA damage checkpoint are mutating spontaneously have produced controversial results. In addition, it is unclear which DNA damage checkpoint protein physically binds to damaged DNA. We have been addressing both of these issues using the nematode *C. elegans*.

Three *C. elegans* DNA damage checkpoint mutants have been identified to date: *mrt-2 (e2663)*, *op241*, and *rad-5 (mn159)* (1,2). The rates and nature of spontaneous mutation in these mutants have been examined using a variety of tests. We find that all of these DNA damage checkpoint mutants display a spontanaeous mutator phenotype, and that most of these spontaneous mutations are deletions. Although *mrt-2 (e2663)* and *op241* mutate at all loci tested, *rad-5 (mn159)* is mutating primarily at a single locus. This surprising result agrees with the observation that *rad-5 (mn159)* is Temperature-sensitive and is therefore unlikely to be a null allele (3). Since *rad-5 (mn159)* is mutating mostly at a single locus, *rad-5 (mn159)* may be defective in sensing a particular kind of spontaneous DNA damage that occurs at this locus. Thus, the RAD-5 checkpoint protein may be a primary 'sensor' of DNA damage, a protein whose identity has eluded the DNA damage checkpoint community for many years.

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228. Genes that ensure genome stability in *C. elegans*.

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Organisms have evolved a number of mechanisms to maintain genome stability during their lifetime; loss of these systems (e.g. witnessed by somatic repeat instability) is a frequent cause of human cancers. One mechanism is mismatch repair (MMR), which corrects mismatches and frameshifts that arise during DNA replication. Sporadic somatic MMR mutations are indeed responsible for many human cancers. In the *C. elegans* genome four MMR genes are present: *msh-6, msh-2, mlh-1* and *pms-1*. We used a reverse genetics approach to knock out *msh-6*.

We found that *msh-6* mutants have a 25 times increased germline mutation frequency and that both the male and the female gametes are equally protected by *msh-6*. We sequenced mutants that occurred in an *msh-6* strain and found primarily single basepair substitutions and frameshifts in short repeats. In addition, we observed a germline DNA repeat instability phenotype; after 10 generations germline DNA repeats are growing or shrinking in *msh-6* mutants (but not in the wild type strain).

To monitor somatic DNA repeat instability, we constructed transgenic *C. elegans* containing a high copy array of a LacZ reporter gene fusion downstream of a DNA repeat that puts this monitor gene out of frame. Constructs that acquire a frameshift will express the reporter gene. Using these transgenic worms, we can now easily see frameshift mutations and other deletions or insertions. In an *msh-6* mutator strain we see blue patches. Also RNAi with *msh-6* and *msh-2* results in somatic repeat instability. Using these strains for genome wide RNAi screens, we identified additional genes involved in genome stability. We characterized several new mutator genes on chromosome 1, such as *cdc-5* and *rpa-2*. We expect that some of the genes thus identified may turn out to be orthologs of thus far unknown mutator genes responsible for human cancers.

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229. Heterochromatin binding protein 1 (HP1.1) in *Caenorhabditis elegans*: cell cycle analysis of chromatin structures and protein interactions

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HP1 is a heterochromatin-associated protein, which was first described in Drosophila. It plays an important role in the silencing of genes located next to heterochromatin. HP1 interacts with important architectural proteins of the nucleus like the lamin B receptor (LBR), the origin recognition complex protein (orc2), the inner centromere protein (INCENP) and the SET domain protein Su(var)3-9.

We are interested in analysing the cytological function of HP1 in *C. elegans*, an organism that has either no or only small amounts of heterochromatin. Two HP1 homologues can be identified in *C. elegans*: HP1.1 (K08H2.6) and HP1.2 (K01G5.2a). We created integrated transgenic lines which express either HP1.1::GFP or a mixture of HP1.1::YFP and histone H1.1::CFP. The latter serves as a living color stain of chromosomes and chromatin. Cell cycles of embryonic cells were recorded as time series of confocal laser scanning images. During interphase, HP1.1 is prominently concentrated in a number of distinct spot-like structures (mostly 6) in the nuclear periphery. During mitosis this static localization of HP1 disappears and HP1.1 relocates to chromosomal sub-structures in a very dynamic way. In metaphase a fraction of HP1.1 is concentrated at the telomeres of the chromosomes, and another fraction is co-localized with H1.1::CFP. During anaphase HP1.1 separates from the chromatin and forms a thin layer between the chromosomes and the mitotic spindle. This corresponds to the outer part of the holocentric centromeres. Beginning with the 60-cell stage of embryogenesis, HP1.1::GFP is expressed in most tissues. dsRNA interference with HP1.1 expression showed multiple and variable defects including embryonic death, slow growth, dumpy-like animals, larval arrest, and sterility. Interestingly, HP1.1 is not involved in the prominent chromatin silencing in the germ line of *C. elegans* as shown by HP1.1 RNAi in LET-858::GFP reporter animals. The molecular mechanisms of HP1.1 localization to the spot-like structures in interphase were analyzed by RNAi with established HP1 interacting proteins in the HP1.1::GFP reporter strain. RNAi with the set domain proteins C41G7.4 and C15H11.5 relocated HP1.1::GFP to the cytoplasm. RNAi with the lamin B receptor (B0250.7) led to a diffuse intranuclear distribution of HP1.1::GFP in the nuclei. RNAi with orc2 (F59E10.1) expression resulted in misregulation of the quantity of HP1.1 expression and a modified intranuclear distribution. We conclude that the distinct spot-like localization of HP1.1 in the interphase nucleus is dependent on all four potential HP1 interacting proteins.

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230. Open-reading-frame sequence tags (OSTs) support the existence of at least 17,300 genes in *C. elegans*

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The genome sequences of *Caenorhabditis elegans*, *Drosophila melanogaster* and *Arabidopsis thaliana* have been predicted to contain 19,000, 13,600 and 25,500 genes, respectively. Before this information can be fully used for evolutionary and functional studies, several issues need to be addressed. First, the gene number estimates obtained *in silico* and not yet supported by any experimental data need to be verified. For example, it seems biologically paradoxical that *C. elegans* would have 50% more genes than *D. melanogaster*. Second, intron/exon predictions need to be experimentally tested. Third, complete sets of open reading frames (ORFs), or ORFeomes, need to be cloned into various expression vectors. To simultaneously address these issues, we have designed and applied to *C. elegans* the following strategy. Predicted ORFs are amplified by PCR from a highly representative cDNA library using ORF-specific primers, cloned by Gateway recombination cloning, and then sequenced to generate ORF sequence tags (OSTs), as a way to verify identity and splicing. In a sample (n=1,222) of the nearly 10,000 genes predicted *ab initio* (that is, for which no expressed sequence tag (EST) is available so far), at least 70% were verified by OSTs. We also observed that 27% of these experimentally confirmed genes have a different structure from that predicted by GeneFinder. We now have experimental evidence that supports the existence of at least 17,300 genes in *C. elegans*. Hence we suggest that gene counts based primarily upon ESTs may underestimate the number of genes, in human and in other organisms.

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231. The worm transcriptome project

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As of March 2001, 16175 yk clones have been sent to researchers around the world, in answer to 3463 requests. The main progress since the last worm meeting has been the isolation of full length cDNA libraries, selected by the cap and stage specific. All 83,000 cDNA clones and 9000 sequences collected from other sources including public databases were coaligned using the Acembly program on the 99.4 Mb of genome sequence (many thanks to the Consortium). We confirm the very high quality of the genome and estimate, from the unaligned clone proportion, that we are now missing only 0.7 to 1.2 Mb of genome. The basecall of the cDNAs was edited, and cloning artifacts, such as mosaic clones, internal deletions or inversions, internal oligo dT priming or unspliced RNA / DNA, were flagged and treated with appropriate care: these amounted to 1% of the clones from the first yk libraries, 5% of the capped clones and 2% of the clones in Genbank.

As of today, we have cDNAs in Acembly/AceView for just over half of the C.elegans genes: 10126 genes produce 14154 transcripts through alternative splicing or alternative polyadenylation. We have started to annotate the proteins and to submit the results to the public databases. We will submit in priority the genes you ask for. We use names, such as 1K18 (mec-8), that contain chromosome, megabase (letter), kilobase number and strandedness (even/odd) and allow easy distinction from the predictions.

Indeed, contrary to the outstanding quality of the genome sequence, the Wormpep de novo predictions are usually incorrect: in a sample of 111 newly hit genes, only 33% were exactly correctly predicted: 7% were not predicted, the first and last exons were incorrect in 35% and 28% cases respectively (in particular 9% of genes touch two predictions), and finally, in the common region, 33% of the genes had at least one internal exon incorrectly predicted. Gene prediction remains a difficult problem.

The new capped libraries are a huge step forward for the transcriptome project. More than 80% of the 12000 capped clones actually contain the entire mRNA, from 5’ cap to polyA. Transpliced leaders are present in up to 65% of the genes, the remaining 35% genes are not transpliced (for example, collagens or surface proteins). We identify 12 types of transpliced leaders, possibly encoded by 30 SL genes clustered in 15 loci: each SL gene is followed in the genome by a strong donor site. Among the genes transpliced, 76% are transpliced to SL1 exclusively, the remaining 24% are transpliced to SL2/SL12. We confirm Tom Blumenthal et al ’s hypothesis relating minor SLs to operons: the 700 genes with SL2/12 are most often located less than 300 bp downstream of an expressed gene in cis. The proportion of pure SL1 genes with such a close neighbor is 20 times lower, and, for genes not transpliced, 10 times lower. The specific transpliced leader used appears to be stage dependent: in particular, usage of the minor leaders increases from 5% in embryos to more than 25% in adults. In a compact genome such as C.elegans’, close genes tend to be cotranscribed. Such physical constraints for coexpression of close genes during development could be tuned by controlled availability of minor leaders.

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232. A snip-SNP map of the *C. elegans* genome: Applications to positional cloning.

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Single nucleotide polymorphisms (SNPs) are valuable genetic markers of human disease. They also comprise the highest potential density marker set available for mapping experimentally derived mutations in model organisms such as *C. elegans*. To facilitate the positional cloning of mutations we have identified polymorphisms in CB4856, an isolate from a Hawaiian isle that shows a uniformly high density of polymorphisms compared to the reference Bristol N2 strain. Based on 5.4 Mbp of aligned sequences, we predict 6222 polymorphisms. Furthermore, 3457 of these markers modify restriction enzyme recognition sites (snip-SNPs) and are therefore easily detected as RFLPs. Of these, we have experimentally confirmed 493 by restriction digest to produce a snip-SNP map of the worm genome (ref 1).

A mapping strategy using snip-SNPs and bulked segregant analysis (BSA, ref 2) is outlined. CB4856 is crossed into a mutant strain, and exclusion of CB4856 alleles of a subset of snip-SNPs in mutant progeny is assessed with BSA. The proximity of a linked marker to the mutation is estimated by the relative proportion of each form of the biallelic marker in populations of wildtype and mutant genomes. This step bounds the mutation between flanking snip-SNPs. These flanking markers can be used to detect recombination in individual animals, and only recombinant strains need be phenotyped. By mapping the recombination points in individual animals, it is possible to rapidly zoom in on the site of a mutation. The advantages and limitations of this approach will be discussed.

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233. WorfDB: The *C. elegans* ORFeome database

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WorfDB, (http://worfdb.dfci.harvard.edu**) the database of the *C. elegans* ORFeome cloning project, was created to integrate and disseminate the data from our attempt to clone the 19,000 predicted open reading frames (ORFs).**

WorfDB serves as a central data repository enabling the scientific community to search for available clones and find information about their quality. The database contains all available ORF sequence with the data pertinent to the cloning process. This information includes: the names and sequences of the primers used to amplify the ORFs, the success of the PCR amplification, photographs of the resulting PCR products separated on agarose gels and ORF sequence tags (OSTs) when available. The database is searchable by gene name, prediction name or blast searches. A list of ORFs can also be searched for availability and amplification. The database currently contains PCR amplification data for about 10,000 ORFs.

234. The BioKnowledge Library™: an Integrated Collection of Databases for Model Organism and Human Proteomes

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The BioKnowledge Library™ is a database of protein information designed to integrate biological knowledge from the scientific literature with genomic information. The BioKnowledge Library contains several interconnected species-specific volumes maintained in a relational database format and presented through a Web-based platform, and is updated weekly with new information. Versions of the BioKnowledge Library are available to industry researchers through subscription, and a Web-based version is available free to academic scientists at http://www.proteome.com, including: PombePD™ (*S. pombe*), WormPD™ (*C. elegans*), YPD™ (*S. cerevisiae*), and soon to be added, MycoPathPD™ - a volume of information regarding Candida albicans and 16 other fungal pathogens of humans. In addition, the BioKnowledge Library contains Public HumanPSD™, the Human Proteome Survey Database of over 10,000 human proteins that encompasses Title Lines, biochemical functions, cellular and organismal roles and subcellular localization, as well as Gene Ontology classification terms. New features are being introduced into WormPD. First, the coverage of functional genomic literature has been incorporated, including major enhancements in the presentation of transcription profiles, allowing users to browse all published microarray results in one Web platform. Conclusions from transcription profiling and other high-throughput, functional genomic experiments are included in individual protein reports, making them easily accessible and fully integrated with other curated literature information. Second, information collected for uncloned genetic loci,never before available in WormPD, will be displayed. Third, Gene Ontology terms will be applied to the proteins in

WormPD, as well as the other volumes of the BioKnowledge Library, to provide a complete, consistent and searchable classification for all proteins in the BioKnowledge Library.

235. Recently duplicated genes are less than half as likely to drive reporter gene expression: implications for genome annotation.

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The ultimate aim of *C. elegans* research is to understand how the genome, via the developmental programme, generates the adult worm, but as yet there is little functional knowledge for the vast majority of genes predicted in this genome. A detailed understanding of spatial and temporal gene expression is essential for a full understanding of genome function.

We have been generating reporter-gene expression pattern data, and so far, have analysed expression for 600 genes, of which 290 drive reporter gene expression to an observable level. The genes assayed were selected at random, without consideration of homology, or other genetic or molecular information. Our expression pattern data are made available via our web pages (http://bgypc086.leeds.ac.uk) and through ACeDB and Wormbase.

An analysis of our data indicates that *C. elegans* genes that have been recently duplicated (40% of those assayed), are less than half as likely to drive reporter gene expression to an observable level, than genes that are either unique to *C. elegans* (but with no recent duplicate), or have closer homologues in other organisms (i.e. *Drosophila*, yeast or human). One explanation of this observation is that many recently duplicated genes, which are currently annotated as real genes, are in fact pseudogenes, and extrapolation from our data suggests that up to 4000 predicted genes may fall into this category. Such a large number of apparently non-functional genes is not inconsistent with data from other sources, such as microarrays, reverse genetics, EST data, or RT-PCR. An alternative explanation, that expression of recently duplicated genes is more likely to be dependent upon specific environmental situations, and therefore, is not observed under standard conditions, could account for, at most, half of the effect. This would still leave, at least,

10% of predicted genes as apparently non-functional.

236. Towards a physical and genetic map of *Pristionchus pacificus*

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We are studying the evolution of cell fate specification, using vulva development as a model system and compare *C. elegans* with *Pristionchus pacificus*. We performed various genetic screens to isolate a number of mutants defective in vulva formation, many of which result in phenotypes unknown in *C.elegans*. To facilitate the molecular characterization of these mutants, we have initiated a physical and genetic map project of *Pristionchus pacificus*. The *Pristionchus* genome is approximately 100 MB in size. We have constructed a BAC library (with the help of Keygene N.V., Netherlands) with approximately 14 fold coverage of the *Pristionchus* genome and have performed BAC end sequencing (in collaboration with the Genome Sequencing Center, MPI Tuebingen) for half of the clones. We are using the sequence information from the BAC ends to create a polymorphism map using the strain *Pristionchus pacificus* var. Washington. It has been observed that the Washington strain shows a high degree of polymorphism with respect to *Pristionchus pacificus* var. California. The AFLP analysis indicated 55% of the bands (368 of the 630 bands) to be polymorphic between the two populations (Srinivasan *et al*, 2001). More than 1,000 BAC ends were tested for polymorphisms using the Single Stranded Conformation Polymorphism (SSCP) technique. 200 of the polymorphic markers were used to construct a genetic linkage map. The two parental strains from California and Washington were crossed and genetic marker segregation was followed in 48 random F2 offspring for each of the 200 polymorphic markers thereby generating a genetic linkage map. We also have initiated to construct a restriction fingerprint map of *Pristionchus*. Combining the polymorphism data, the genetic linkage map and the restriction fingerprint map will facilitate the cloning of mutants in *Pristionchus pacificus*.

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237. Phylogenomics: Analysis of EST datasets from species across the phylum Nematoda

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Phylogenomics: Analysis of EST datasets from species across the phylum Nematoda

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The complete genome sequence of *Caenorhabditis elegans* is the blueprint for a single organism. How did the genome get to be the way it is, and how does the gene content of *C. elegans* reflect both gain and loss of particular genes? We have embarked on a programme to generate significant EST datasets for a number of nematode species that represent the full diversity of the phylum Nematoda. These ESTs can be used to define the genes expressed in each species, and cross comparisons can be organised using a molecular phylogeny of the Nematoda. The species we are examining are all parasites of humans and domesticated animals. A sister-project at Washington University Genome Sequencing Center is examining additional parasitic and free-living species. We have organised the data using a custom clustering algorithm, and present it to the world wide web using a new SQL database NEMBASE (see http://www.nematodes.org). We find both conserved, nematode specific gene families that have unknown function, and genes that are present in some but not all species. Significantly, some genes are found in the parasites, and in other animals, but not in *C. elegans* . The "other nematode" ESTs are a resource for further annotating the *C. elegans* genome (confirming "predicted" genes, correcting splice predictions, etc), and for

exploiting *C. elegans* for developing novel parasitic nematode control strategies.

238. Genes from Other Nematodes: A Progress Report on the GSC Parasitic Nematode EST Project

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To build upon knowledge gained from the genome of *C. elegans,* we have begun generating Expressed Sequence Tags (ESTs) from parasitic (and free-living) nematodes. This project will generate >225,000 5' ESTs from 14 species by 2003. Additionally, the Sanger Centre and Edinburgh Univ. will complete 80,000 ESTs from 7 species. Through these combined efforts, we anticipate the identification of >80,000 new nematode genes. At the GSC, approximately 35,000 ESTs have been generated to date including sequences from *Ancylostoma caninum, Heterodera glycines, Meloidogyne incognita* and *javanica, Parastrongyloides trichosuri, Pristionchus pacificus, Strongyloides stercoralis* and *ratti, Trichinella spiralis,* and *Zeldia punctata*. We will report on our progress in sequence analysis, including the creation of the NemaGene gene index for each species by EST clustering and consensus sequence generation, identification of common and rare transcripts, and identification of genes with orthologues in *C. elegans* and other nematodes. All sequences are publicly available at www.ncbi.nlm.nih.gov/dbEST. NemaGene sequences and project details are available at WWW.NEMATODE.NET.

We would like to thank collaborators who have provided materials and ideas for this project including Prema Arasu, David Bird, Rick Davis, Warwick Grant, John Hawdon, Doug Jasmer, Andrew Kloek, Thomas Nutman, Charlie Opperman, Alan Scott, Ralf Sommer, and Mark Viney. This work is funded by NIH-AI-46593, NSF-0077503, and a Merck / Helen Hay Whitney Foundation fellowship.

239. Rates and Patterns of Mutation in the Nuclear and Mitochondrial Genomes of *Caenorhabditis elegans*

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Mutations are the ultimate source of genetic disorders and offer important data for phylogenetic, forensic and population genetic studies. We directly assayed the rates and patterns of mutation in a set of 74 *Caenorhabditis elegans* mutation accumulation (MA) lines, where mutations accumulate over time in an effectively neutral manner as each MA line is propagated across generations as a single, random hermaphrodite (Vassilieva and Lynch, 1999). We performed a uniquely direct assay for the underlying rates and patterns of mutation in the MA lines with large-scale DNA sequence collection and analysis from both the nuclear and mitochondrial genomes. The mitochondrial DNA assays revealed a mutation rate that is two orders of magnitude higher than previous indirect estimates, a highly biased mutational spectrum, multiple mutations affecting coding function and mutational hotspots at homopolymeric nucleotide runs (Denver *et.al*., 2000). Mutational hotspots have also been identified in the nuclear DNA sequence assays. Furthermore, we have analyzed the same mitochondrial and nuclear loci in *C. elegans* natural isolates to evaluate the relative roles of mutation and natural selection in shaping molecular variation observed in natural populations.

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240. Changes in Gene Expression Associated with Developmental arrest and Longevity

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Gene expression in the developmentally arrested, long-lived dauer stage was compared with non-dauer (mixed-stage) populations using Serial Analysis of Gene Expression (SAGE). 152,314 dauer and 148,324 non-dauer SAGE tags identified 11,130 of the predicted 19,100 C. elegans genes. Genes implicated previously in longevity were expressed abundantly in the dauer library and new genes potentially important in dauer biology were discovered. 2,618 genes were detected only in the non-dauer population, whereas 2,016 genes were detected only in the dauer, showing that dauer larvae exhibit a surprisingly complex gene expression profile. Evidence for differentially expressed gene transcript isoforms was obtained for 162 genes. H1 histones were differentially expressed, raising the possibility of alternative chromatin packaging. The most abundant tag from dauer larvae (20-fold more abundant than in the non-dauer profile) corresponds to a new, unpredicted gene we have named tts-1 (transcribed telomere-like sequence), the RNA of which may interact with telomeres or telomere-associated proteins. In addition to

providing a robust tool for gene expression studies, the SAGE approach has already provided the advantage of new gene/transcript discovery in this metazoan model.

241. Global profile of gene expression during aging

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Aging is a physiological phenomenon characteristic of metazoan life. As animals age, they suffer a broad functional decline and an exponentially increasing probability of death. Numerous gerontogene mutants have been identified in the nematode Caenorhabditis elegans. These mutants suggest several models by which aging may be slowed and life span prolonged. This study of global transcription during the chronological aging of the worm is a first step to testing several of these models including the stress response, metabolic rate and programmed aging models.

DNA microarrays can be used to profile the expression levels of a large number of genes in parallel. Using our array, which contains a PCR product representing every C. elegans gene, we are profiling expression patterns during the normal aging process. We isolated RNA from synchronized cultures of sterile animals at various ages from young adult to old age. We hybridized labeled cDNA made from this RNA to DNA microarrays, and find that 212 ($p<.001$) genes show statistically significant changes with chronological age. This is a relatively small number of genes relative the number with significant changes during development, those specific for male and hermaphrodite developmental programs, and those involved in reproduction. We find that transposons show progressively increasing transcript levels as the worms age, and several gene classes including histone and mitochondrial genes exhibit progressive down-regulation as the worms age. Many oocyte genes maintain transcript levels past the reproductive period, declining only in old worms. This global transcript profile is the first detailed and complete molecular profile of aging in the worm.

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242. Global Analysis of Gene Expression Patterns in the Dauer Larvae of *Caenorhabditis elegans*

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We are using the full-genome microarrays to profile gene expression differences associated with exit from dauer larvae. In principle, by examining gene expression differences on the whole-genome level, we will be able to illuminate the complete set of genes that are implicated for dauer-specific attributes. Identification of dauer-enriched genes would provide a framework for understanding dauer-specific characteristics, such as altered energy metabolism, certain aspects of aging, stress resistance, and the coordinated execution of a complex morphological change.

To identify genes involved with dauer exit, we performed DNA microarray experiments with RNA isolated at different time points after addition of food to a dauer population. Each RNA sample was compared to a common reference RNA, and multiple RNA samples were prepared for each time point. Since some of the genes regulated upon dauer exit might be in response to the introduction of food rather than a dauer-specific developmental response, we also examined the feeding of starved L1's over a similar timecourse.

We have identified and begun to analyze about 2700 and 1900 genes that are reproducibly altered during dauer exit and L1 feeding, respectively. Examination of the kinetic profiles of these genes reveals several temporally distinct expression groups, defining a developmental cascade of events that occur during dauer exit or L1 feeding. We find about 400 genes that have similar expression patterns in both timecourses and that constitute a common feeding response. Approximately 900 genes have different expression profiles in the two timecourse. Most are regulated only in the dauer timecourse. These likely represent the dauer-specific developmental response.

We have separated the genes, identified above, into different groups based on their expression patterns by hierarchical clustering. Since genes that function together in a common process tend to be placed in the same cluster, we are currently pursuing in-depth analysis of the different clusters to ascertain what types of processes occur during dauer exit and L1 feeding.

We found that stress genes (such as heat shock proteins and superoxide dismutases) are highly expressed in the dauer larvae. We also find that detoxification genes are enriched in the dauer larvae.

Another interesting and unexpected discovery is that some Major Sperm Proteins (MSPs) are expressed in dauers. MSPs were previously reported to be only expressed in males or L4 and young adult hermaphrodites and to function only as components of sperm. Expression of MSPs in the dauer larvae may reveal an alternative function for a subset of the MSPs.

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243. Genome-wide analysis of transforming growth factor-ß-regulated genes in *Caenorhabditis elegans* by using microarrays

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Dauer formation in *Caenorhabditis elegans* is regulated by several environmental stimuli, including a pheromone and temperature. Dauer induces various biochemical and physiological responses in the worm. Three parallel pathways, including a transforming- growth-factor-β-like pathway, regulate dauer formation. Members of the TGF-ß superfamily play critical roles in cell growth, differentiation, and embryogenesis. We used a cDNA microarray containing genomic PCR products corresponding to 19,099 full-length *C. elegans* genes to identify dauer inducible genes. Global transcription analysis of wild-type N2 (L3 stage) and *daf-7* (dauer stage) mutants was used to identify genes whose messenger RNA levels varied as a function of the *daf-7* mutant. In total, 1769 (9% of the genome) genes were regulated (either up or down) more than two-fold, 354 genes were regulated more than 4-fold and 81 genes were regulated more than ten-fold. More than 90% of the regulated genes have not been reported previously as dauer regulated. The regulated genes encode a variety of protein types. We see upregulation of genes with protective functions, such as superoxide dismutase. We also see upregulation and downregulation of a variety of interesting signaling molecules, including several types of transcription factors, cell surface and cytoplasmic signal transducers. We also see many up-regulated cytochrome P450s, which may serve to protect the worms from toxins, or to control signaling by modifying steroid hormones. Interestingly, *daf-9* encodes a cytochrome p450 that is required for normal dauer formation (Jia, Albert and Riddle; Midwest Worm Meeting, 2000). We will discuss our analysis of gene regulation patterns, and our use of bio-informatic analysis of promoters to identify regulatory elements. Finally we will discuss our plans to sort out genes that are directly regulated by the TGF-β pathway and genes that are indirectly regulated. We hope to use this data as a starting point to connecting daf-7 signalling to final morphological and physiological changes in target tissues.

244. Signals from the reproductive system that regulate lifespan and resistance to stress

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Signals from the reproductive system regulate aging in *C. elegans*. The germline exerts a life-shortening effect and the somatic tissues of the gonad appear to send a counteracting, life-prolonging signal. Through the use of laser microsurgery, genetic analysis and RNAi we have found that mitotic proliferation of germ cells in the adult animal produces a negative effect on lifespan, possibly by interacting with specific metabolic pathways. This effect is independent of fertility or reproduction. In addition, we found that the reproductive system has a direct effect on stress-resistance in the worms. We have also investigated how specific genes are required for the action of both the germline and gonad signals and we are presenting a new model that integrates those signals and the *daf-2/daf-16* insulin-like lifespan regulatory pathway.

245. Effects of the gene disruption of two Mn-SOD isoforms on oxidative stress sensitivity and life span in *Caenorhabditis elegans*

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Reactive oxygen species (ROS) are implicated to be involved in the deteriolating processes in aging. Superoxide dismutases (SOD) catalyze the removal of a potent ROS, superoxide radical. Eukaryotes have various types of SODs: cytoplasmic CuZn-SOD, mitochondrial Mn-SOD and extracellular CuZn-SOD. Differently from other species, *C. elegans* has two isoforms of Mn-SOD (*sod-2* and *sod-3*) whose functions are not well understood. To elucidate the biological roles of two Mn-SOD isoforms, we isolated deletion mutants of *sod-2* and *sod-3* by using TMP/UV mutagenesis (Yandell et al 1994, modified by Moulder et al). A 0.45kb deletion of *sod-2* covers the first exon and the first intron. A 0.76kb deletion of sod-3 covers from exon 3 through 3' UTR. The growth and fecundity of the *sod-2* mutant were strongly suppressed by hyperoxia compared with the *sod-3* mutant and the wild type. The double mutant of *sod-2* and *sod-3* was lethal under hyperoxia. Severity of paraquat toxicity was as follows: *sod-2; sod-3* > *sod-2* > *sod-3* > wild. The life spans of *sod-2*, *sod-3* and double mutants were slightly but significantly shorter than that of the wild type. Microinjection of *sod-2* or *sod-3* genes rescued these traits in oxidative stress. The gene expresion of *sod-3* was remarkably induced in the *sod-2* deletion mutant. These results suggest that two isoforms of Mn-SOD mutually function as antioxidant defenses.

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246. *daf-2* Kinase Domain Alleles Prevent Hypoxic Death

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C. elegans has been critical in identifying mediators of programmed cell death. However, to our knowledge, hypoxic death has not been studied in *C. elegans* despite the fact that heart attacks and strokes, the results of cellular hypoxia, cause the majority of human deaths. Towards the goal of developing a forward genetic model of hypoxic death, we have screened through 71,438 mutagenized genomes and 74 existing mutant strains and found several mutants resistant to hypoxic death (Hyp - hypoxic death abnormal). The most Hyp strains were found among previously isolated mutants of the *daf-2* gene, which codes for an insulin/insulin growth factor receptor homolog. The hypoxia resistance was profound; for example, 5.9 +/- 2 % of *daf-2(e1370)* were dead versus 92.8 +/- 2% of N2 after a 24 hr recovery from hypoxic incubation. With shorter hypoxic times, all of the N2 live but have permanent behavioral defects, suggestive of neuronal death. *e1370* behaves normally even after incubation times that kill all N2. The *e1370* animals are paralyzed while in the hypoxia chamber demonstrating that they were indeed hypoxic, and gaseous anesthetics affect *e1370* with the same time course as N2 indicating the cuticle does not exclude gas exchange. *daf-2(rf)*'s Hyp phenotype was recessive, markedly allele specific, and did not correlate with *daf-2*'s other phenotypes including Age, Daf-c, and thermal tolerance. Sequencing of all of the strong Hyp alleles showed that like *e1370* the 3 other strong Hyp alleles carried missense mutations in the tyrosine kinase domain of *daf-2* whereas mutations in the ligand-binding domain produced little or no Hyp phenotype. Utilizing *daf-2(e1370)* strains transformed with *daf-2(+)* driven by tissue specific promoters (thanks to Catherine Wolkow - Ruvkun lab), we found partial rescue of hypoxia resistance by *daf-2*(+) expression in neurons or muscle but not in intestinal cells. The Hyp phene of *e1370* is completely suppressed by *daf-16(null)* and *daf-18(null)*, but not *daf-12(null)*. *age-1(null)* and *pdk-1(null)* are only weakly Hyp, suggesting different pathways mediate the Hyp and Age phenes of *daf-2(rf)*. Ced mutants are not Hyp.

247. *hif-1*, a homolog of mammalian hypoxia-inducible factor-1 alpha, is required for adaptation to low oxygen in *C elegans*

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Hypoxia- inducible factor, a heterodimeric transcription complex, regulates cellular and systemic responses to low oxygen levels (hypoxia) during normal mammalian development and plays a critical role in tumor progression. We have determined that a similar complex mediates the response to hypoxia in *C. elegans*. This complex consists of HIF-1 and AHA-1, two proteins that contain basic-helix-loop-helix and PAS domain motifs. *hif-1* is the *C. elegans* homolog of mammalian HIF-1 α. We screened for deletions in *hif-1* and isolated *hif-1 (ia04)*, a 1,231bp deletion of the second, third, and forth exons that results in a shift of frame. *hif-1 (ia04)* worms exhibit no visible defects under standard laboratory conditions, but they are unable to adapt to low levels of oxygen. While wild type animals survive and reproduce in 1% oxygen, 73% of *hif-1*-defective animals die in these conditions. The *hif-1* promoter directs expression of GFP in all somatic cells. However, the expression of HIF-1 is post-transcriptionally regulated. The level of HIF-1:GFP increases in hypoxic conditions, and the fusion protein is rapidly degraded upon re-oxygenation. HIF-1 can be co-immunoprecipitated with AHA-1. *aha-1* is the *C. elegans* ortholog of mammalian ARNT. In addition to its role in hypoxia response, *aha-1* has essential functions during embryogenesis and larval development. We propose that the mechanisms of hypoxia signaling are conserved among metazoans and that *C. elegans* is an excellent model system for studying hypoxia signaling and response. Additionally, we find that nuclear localization of AHA-1 is disrupted in a *hif-1* mutant. This suggests that heterodimerization may be a prerequisite for efficient nuclear translocation of AHA-1.

248. Signaling pathways in heat acclimation: A lesson from C. elegans mutants

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Persistent exposure to environmental heat stress upregulates protective mechanisms, leading to enhanced endurance during exposure to severe heat stress. This process is defined as 'heat acclimation'. It also leads to the generation of non-thermoregulatory beneficial effects displayed by increased tolerance to a multitude of environmental stressors, such as heavy metals, ischemia, hypoxia, ionized irradiation, etc. The latter protective feature is defined as 'cross-tolerance' (against these stressors). Our data on mammalian species indicate that reprogrammed gene expression, among which are genes coding for heat shock proteins (HSP 70 family) and energy metabolism enzymes leading to decreased heat production, plays a major role. Unfortunately, our knowledge of the "architecture" of acclimatory signaling in mammals is limited. Several previous studies reported on the ability of C. elegans to undergo heat acclimation. The relative ease of genetic and molecular manipulations in C. elegans makes this phylogenetically distant (from mammals) species worthwhile for addressing questions pertaining to mammalian biology. In the present study we aimed to characterize a C. elegans heat-acclimation model, to substantiate the generation of 'cross tolerance' in this model, and to identify selective pathways involved in the evolving adaptive responses.
Heat-acclimated wild-type C. elegans (grown at 25°C for 24 or 96 hrs) had significantly increased heat endurance during exposure to heat stress at 35°C, and upon subjection to progressively increasing cadmium concentrations compared to that of normothermic worms (grown at 20°C). Heat acclimation in the wild type (N2) worms also produced 'cross tolerance' against chemical hypoxia (azide toxication). To identify the mediating signaling pathways, we examined two candidate pathways: the *daf-16* (insulin receptor

pathway, known to affect stress and thermotolerance response), and the *hif-1* (hypoxia inducible factor, affecting metabolic pathways). LD50 of *daf-16* and *hif-1* knockout worms during exposure to 35°C, before and after heat acclimation, was measured. Our data showed that while the wild-type N2 and *daf-16* mutants showed enhanced heat endurance following the acclimation procedure, the *hif-1* knockout worm, although it performed better under normothermic conditions, could not acclimate, and long-term exposure at 25°C interfered with thermotolerance during the heat stress. These findings pinpoint unequivocally the importance of HIF-1 signaling in the acclimation process, and are in conformation with our data from rats that HIF-1 alpha is elevated following heat acclimation (Maloyan, Semenza, Gerstenblith, Stern Horowitz 20011). This may suggest that HIF-1's role in heat acclimation is conserved through evolution.

1 Maloyan,A., Semenza,G., Gerstenblith,G., Stern, M., Horowitz, M. Heat acclimation-ischemia cross-tolerance: Does HIF 1alpha play a role. ISHR 2001

249. P granules, nuclear pores, and RNA

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P granules are cytoplasmic organelles of unknown function that are found in the germ cells of *C. elegans*. Although P granules are present in the cytoplasm of mature oocytes and early embryonic blastomeres, they associate with germ nuclei at all other stages of the life cycle. P granules in the adult gonad are juxtaposed to the surface of germ nuclei, are associated with clusters of nuclear pores, and often contain electron dense material directly opposite the positions of individual pores. In oocytes, where P granules disassociate from nuclei and become cytoplasmic, P granules remain associated with nuclear pore material. This pore material includes a *C. elegans* protein related to RanBP2, a component of the cytoplasmic fibrils of vertebrate nuclear pores. Inhibition of the function of this gene, but not of other nuclear pore genes, by dsRNA prevents P granule association with nuclear pore material in the oocyte cytoplasm. These results suggest there is a functional relationship between P granules and nuclear pores. One possibility that we are examining is that P granules are either directly involved in mRNA export or participate in the exchange of nuclear export factors for cytoplasmic proteins on newly synthesized mRNAs. We have used in situ hybridization to determine that several diverse mRNAs are present in nuclear associated P granules. Thus far, all of the mRNAs that appear enriched in P granules belong to a class of mRNAs described by Seydoux and Fire as class II maternal mRNAs because of their perdurance in the germ cell precursors of embryos.

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250. The splicesomal Sm proteins play a role in P granule localization during early embryogenesis.

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In early C. elegans embryos, development is initiated by asymmetric cell division, which leads to the precise localization cell fate regulators to specific cells. One feature of early asymmetry is the localization of cytoplasmic RNP particles, the P granules, to the germ cell precursors at each of several polarized cell divisions. The composition and function of P-granules is not well understood. Further, the mechanisms that control the localization of P-granules and other factors in the embryo are poorly understood. We used RNA interference (RNAi) to screen a cDNA library for new genes involved in early polarity. Surprisingly, one of the genes identified is the ortholog of human SmE. SmE is one of several Sm proteins that form a multisubunit complex required for snRNP assembly, nuclear import, and mRNA splicing. RNAi of Sm subunits in *C. elegans* caused mislocalization of P granules to somatic sisters of germ cells after the 4-8 cell stages in the early embryo. In addition, Sm RNAi disrupted the subcellular distribution of PGL-1 (a P-granule component) in both mature germ cells and in germ cell precursors after the 8-cell stage. P-granules in wild type germ cells are primarily attached to the nucleus, but in Sm (RNAi) germ cells and embryos, the nuclear attachment of many P-granules is lost. At lower penetrance, GLP-1 protein was inappropriately expressed in posterior cells following Sm RNAi. By contrast, RNAi of the core splicing factors U1 70K and U2AF65, or of RNA polymerase II, had no effect on P granule segregation, subcellular distribution, or GLP-1 asymmetry, although all caused severe embryonic defects. These data suggest that the P-granule and GLP-1 asymmetry phenotypes from Sm RNAi are not likely to result from a general defect in splicing. Therefore, Sm proteins may have a role in P-granule localization that is independent of splicing. Interestingly, antibodies against the Sm complex stain P granules at all stages of development. Sm RNAi attenuates this staining. Therefore, Sm proteins may be P granule components that affect the localization of P granules by controlling their integrity or nuclear attachment in germ cells and their precursors. The Sm proteins are also found in the nucleus of most cells. However, nuclear localization of the Sms is dynamically regulated during oogenesis and germ cell precursor formation, suggesting that regulation of the Sm complex or snRNPs may be important for germ cell development.

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251. IFE-1, an isoform of eukaryotic initiation factor 4E, interacts with PGL-1 and is required for spermatogenesis in *C. elegans*.

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P granules are distinctive ribonucleoprotein organelles that are observed in germ cells at all stages of development. PGL-1, a putative RNA-binding protein, is a constitutive component of P granules. *pgl-1* mutants contain defective P granules and are sterile. Sterility is the result of defects in proliferation and gametogenesis and is highly sensitive to temperature.

Using a yeast two-hybrid screen for proteins that interact with PGL-1, we identified IFE-1, one of the five isoforms of eukaryotic initiation factor 4E (eIF4E) present in *C. elegans*. eIF4E is the cap-binding component of the translation initiation complex. We have confirmed the interaction between IFE-1 and PGL-1 by *in vitro* analysis. Furthermore, native PGL-1 is retained on mRNA cap affinity columns, suggesting interaction with IFE-1 *in vivo* . *ife-1* mRNA and protein are highly enriched in the *C. elegans* germline, and IFE-1::GFP (driven using the *pie-1*-based germline-expression vector) is localized in P granules. This localization is dependent on PGL-1. By RNAi analysis, we found that IFE-1 is required for spermatogenesis in both hermaphrodites and males, and that the requirement is highly sensitive to temperature. At elevated temperature, animals depleted of IFE-1 are delayed in production of sperm, and the sperm formed are defective, resulting in sterility. Consistent with a primary role in spermatogenesis, *ife-1* mRNA levels are highest is regions of the gonad undergoing spermatogenesis. Interestingly, the level of PGL-1 drops to below detectable at the stage of spermatogenesis when IFE-1 function appears to be required, suggesting that the release of IFE-1 from P granules is important for IFE-1 function. Our results suggest that P granules participate in control of translation during germline development.

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252. GLHs associate with a LIM domain-containing protein and two other proteins necessary for fertility

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Four germline-specific RNA helicases, GLHs, have been identified in *Caenorhabditis elegans*. This family of putative ATP-dependent enzymes is localized to the P granules, which are non-membranous, cytoplasmic complexes of protein and RNA. P granules are potential determinants of germline development, segregating with the germline precursor lineage from the first embryonic cell division. Essentially complete sterility results when GLH-1 and GLH-4 are eliminated by RNA interference (RNAi) (Kuznicki et al Development 2000.127:2907). The GLH proteins are similar to the *Drosophila melanogaster* germline RNA helicase VASA; however, the GLHs differ in having multiple CCHC zinc fingers as well as multiple N-terminal, non-charged phenylalanine/glycine-rich repeats. GLH-3 is unique among the GLHs because it does not contain these FGG repeats.

To study the relationships of the GLHs with other proteins, two libraries were screened by the yeast two-hybrid method, using GLHs as bait. Eight *C. elegans* proteins have been identified that interact with GLHs; we have chosen to further study three of these. We have shown by GST pulldown assays that these three proteins bind to GLHs by co-infection of recombinant baculoviruses in insect cells. Ongoing tests consider their function in P granules and in germline development. The GLH binding partners include:

KGB-1: a novel putative kinase that GLHs bind; most closely related to the serine / threonine JNK (Jun N-terminal kinase) MAP kinase family. We generated a KGB-1 deletion strain; it has a temperature-sensitive sterile phenotype. We currently are generating antibodies to KGB-1 and determining if GLHs are substrates for KGB-1s putative kinase activity.

ZYX-1: a LIM domain-containing protein most related to vertebrate Zyxin, a cytoskeleton-associated protein concentrated at focal adhesions. Polyclonal antibodies suggest ZYX-1 is a component of P granules in wildtype worms.

COP-9: closely related to sub-unit 5 of signalosomes, conserved in plants, invertebrates, and vertebrates. RNAi with COP-9 results in worms with small gonads and no oocytes, similar to those produced with *glh-1/4* RNAi.

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253. *cgh-1* , a conserved germline predicted RNA helicase required for gametogenesis and oocyte survival in *C. elegans*

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In *C. elegans* approximately half of all developing oocytes die by apoptosis, primarily during the late pachytene stage of meiosis. This physiological germline cell death is triggered by an unknown regulatory pathway that does not require *egl-1* , and is distinct from mechanisms that induce cell death in somatic cells or in response to genotoxic stress. Evidence suggests that these apoptotic oocytes normally donate their cytoplasm to the survivors, and thereby may function as nurse cells. In mammals, a high proportion of oocytes also die late during the pachytene stage, but it is unknown whether this occurs through an analogous germline-specific mechanism. It is an important goal to identify both normal and abnormal aspects of oocyte function or development that can cause physiological germline apoptosis.

We have determined that in *C. elegans* , the *cgh-1* (conserved germline RNA helicase) gene is required to prevent essentially all developing oocytes from undergoing apoptosis through the physiological mechanism. A single *cgh-1* ortholog appears to be conserved in all eukaryotes, and is expressed in the germline in many metazoans. Remarkably, in yeasts the *cgh-1* ortholog is required for sporulation, suggesting a possibly conserved function. The CGH-1 protein is a predicted DEAD box RNA helicase that is distinct from but related to the translation initiation factor eIF4-A, and is also distinct from the *C. elegans* GLH helicases. We find that in *C. elegans cgh-1* is expressed specifically in the germline. The CGH-1 protein is associated with germline and early embryonic P granules, and is also present in additional granules in the gonad core that persist in the early embryo in a pattern that parallels maternal mRNA. *cgh-1(RNAi)* hermaphrodites and males are sterile. While essentially all *cgh-1(RNAi)* oocytes undergo physiological apoptosis, *cgh-1(RNAi)* sperm appear to develop normally but cannot extend their pseudopods appropriately. When germline apoptosis is blocked by the *ced-3* or *ced-4* mutation, *cgh-1(RNAi)* oocytes similarly are present in appropriate numbers and develop normally in many respects, retaining P granules and reaching the diakinesis stage of meiosis. *cgh-1(RNAi)* ; *ced-3* oocytes cannot be fertilized, however, and do not place the yolk receptor RME-2 on their surface normally. We conclude that *cgh-1* is required for an aspect of germline or maternal gene expression that is essential for gamete function, and that its orthologs may have a conserved function during gametogenesis. We also hypothesize that *cgh-1(RNAi)* oocytes respond to this abnormality by triggering the physiological germline apoptosis mechanism. This response is presumably related to the process by which a significant fraction of normal *C. elegans* oocytes are sacrificed as nurse cells, and may represent a surveillance mechanism that directs developing oocytes to commit suicide in response to certain abnormalities. It is of signficant interest to elucidate the mechanism of this response, and to determine whether it is a conserved aspect of oogenesis.

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254. Control of germline cell fates by FBF, GLS-1, and GLD-2

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In the germ line, cell fate decisions are regulated, at least in part, at the translational level. A clear example is the sperm/oocyte switch in *C. elegans* hermaphrodites, where the sex determination gene *fem-3* is repressed by FBF, a Pumilio-related RNA-binding protein (Puf) (1). More recently, FBF has also been found to regulate the mitosis/meiosis decision (2). To explore the mechanism by which FBF acts in both of these key cell fate decisions, we have been analyzing GLS-1, a protein identified in a two-hybrid screen for FBF-1 interactors. In its N-terminal half, GLS-1 harbors five predicted KH-type RNA-binding motifs that are most similar to *Drosophila* Bicaudal C. The novel C-terminal half of GLS-1 binds specifically to FBF. In addition, the extreme N-terminal 80 amino acids of GLS-1 bind specifically to GLD-2, a regulator involved in both mitosis/meiosis and sperm/oocyte decisions (3, C. Eckmann, unpublished). The *in vivo* importance of the GLD-2/GLS-1 interaction is underscored by *gld-2(h292),* a missense mutant with a defect in germline overproliferation; the binding of GLD-2(h292) to GLS-1 is much reduced *in vitro*. GLS-1, FBF and GLD-2 are all cytoplasmic proteins; GLS-1 is found throughout the germ line and in early embryos, where it segregates with P granules. GLD-2 is abundant in pachytene, maturing oocytes and early embryos, where it too segregates with P granules.

To explore the biological functions of GLS-1, we generated a deletion mutant and also used RNAi both in wild-type and various mutants. The *gls-1* deletion homozygote has no detectable GLS-1 protein, is zygotically fertile, and lays mostly dead embryos, which die in a multinucleate state similar to *gld-2(RNAi)* embryos (4). *gls-1(RNAi)* reveals dramatic cell fate transformations in the post-embryonic germ line, including defects in the mitosis/meiosis and sperm/oocyte decisions as well as defects in progression through meiosis and germ cell survival. Therefore, GLS-1 functions in both the germ line and early embryo.

The physical and phenotypic links between FBF, GLS-1, and GLD-2 suggest that these regulators may function together. GLD-2 and GLS-1 both promote meiosis, whereas FBF promotes mitosis. We suggest that GLS-1 antagonizes FBF and promotes GLD-2 to achieve the transition between mitosis and meiosis. GLS-1 may act by a similar dual mechanism to ensure commitment to other fates as well.

1. Zhang *et al.* (1997) *Nature* 390, 477-484; **2.** J. Bachorik *et al*., abstract; **3.** Kadyk and Kimble (1998) *Development 125*, 1803-1813; **4.** L.Wang et al, abstract.

255. *puf-8*, a member of the puf family of RNA-binding proteins, promotes meiotic divisions and inhibits mitosis in primary spermatocytes.

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Puf proteins are a family of putative RNA-binding proteins, characterized by 8 repeats of a conserved decapeptide, found in a variety of organisms including yeast, *C. elegans*, *Drosophila* and humans. Previously we have shown that five *C. elegans* PUF proteins (*fbf-1, fbf-2, puf-6, puf-7* and *puf-8*) function redundantly during primordial germ cell development. *fbf-1* and *fbf-2* also have an additional function later in development during the switch from spermatogenesis to oogenesis in the hermaphrodite germline (Zhang et al., 1997). Here we report that *puf-8* also has an additional later function: it is required in primary spermatocytes for execution of the meiotic divisions.

Depletion of *puf-8* by RNAi leads to the formation of germline tumors in both hermaphrodites and males undergoing spermatogenesis. These tumors appear in the proximal end of the germline where mature sperm would normally form. Immunostaining experiments using meiotic (HIM-3) and spermatogenesis (MSP, MO) markers reveal that *puf-8(RNAi)* germ cells enter into meiosis and spermatogenesis at the proper stage and proceed normally through the first meiotic prophase. However, instead of undergoing a reductional 1st meiotic division, *puf-8(RNAi)* primary spermatocytes undergo a non-reductional division and their progeny continue to divide by what appear to be standard mitotic divisions, leading to the formation of a tumor in place of sperm.

These observations reveal an unexpected requirement for a PUF protein during the meiotic divisions, and supports the view that PUF proteins control many essential processes during germ cell development.

256. Identification and characterization of multiple mRNA targets of GLD-1, an RNA binding protein required for germ cell development

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GLD-1, a KH motif containing RNA binding protein in *C.elegans*, is a germline specific tumor suppressor that is essential for oocyte development. GLD-1 also functions to promote male sex determination in the hermaphrodite germline and has a redundant function to initiate meiotic development. GLD-1 is abundant in the cytoplasm of early meiotic prophase germ cells in the distal region but absent in developing oocytes in the proximal region. Therefore GLD-1 is thought to regulate the translation and/or the stability of a subset of maternal mRNAs that may have functions in oocyte differentiation, maturation/ovulation and early embryogenesis.

We have identified multiple *in vivo* mRNA targets of GLD-1 by their ability to interact with GLD-1 in cytosol extract. These target mRNAs are preferentially expressed in the germline and as expected, several of them have essential functions in oocyte differentiation, maturation/ovulation and early embryogenesis. Interestingly, subsets of 3 gene families are identified as mRNA targets of GLD-1; chitin binding domain containing, *puf*-5/-6/-7/-10 and *oma-1/-2* TIS11 zinc finger containing. In each subset, single RNAi has no detectable phenotype while double RNAi results in a germline or early embryonic phenotype, suggesting that GLD-1 binds and co-regulates functionally redundant homologs.

Analysis of three mRNA targets (*rme-2, oma-1 and oma-2*), reveals that GLD-1 acts as a translational repressor. Antibody staining of wild-type hermaphrodite germline shows that the corresponding proteins for three mRNA targets are absent from the distal region where GLD-1 is abundant, while they increase in abundance in growing oocytes in the proximal

region where GLD-1 levels fall precipitously. Consistent with GLD-1 functioning as a translational repressor, they prematurely accumulate in the distal region of *gld-1* null hermaphrodites. These data imply that GLD-1 is likely acting as a translational repressor for most mRNA targets. However, two mRNA targets, including T23G11.2, are unstable in *gld-1* null animals, suggesting that GLD-1 binds and stabilizes them. Upon careful inspection, T23G11.2 mRNA has two small upstream open reading frames in 5-UTR and therefore is likely a target of nonsense mediated mRNA decay (NMD). In situ staining shows that the level of T23G11.2 mRNA is high in the distal region of wild-type but is very low in developing oocytes in the proximal region. In contrast, T23G11.2 mRNA accumulates to high levels in developing oocytes of *smg-2* mutants, which lack NMD. In addition, T23G11.2 mRNA is undetectable in *gld-1* null germlines but is present in *gld-1* null *smg-2* double mutant germlines. GLD-1 thus functions to bind and protect T23G11.2 mRNA from NMD, presumably by repressing translation.

At least four GLD-1 binding sites have been identified so far in three mRNA targets. They are located in 5-UTR, 5-end of ORF, and 3-UTR, suggesting that GLD-1 can bind to 5-end, 3-end, or both ends depending on the mRNA. For *rme-2* mRNA, GLD-1 binds specifically to both the 5- and the 3-ends. While a missense mutation (*q361*) in the invariant GXXG residues inside the KH motif completely abolishes the RNA binding activity of GLD-1, another missense mutation (*q126*) in the conserved C-terminal region flanking the KH motif only affects binding to the previously identified target mRNA *tra-2* (Jan *et al*., 1999). This suggests that GLD-1 could have more than one binding specificity or binding could be modified by the formation of complexes with other RNA binding proteins, which could allow GLD-1 to control diverse mRNAs.

257. Two CCCH, zinc finger-containing proteins, OMA-1 and OMA-2, are redundantly required for oocyte maturation in *C. elegans*

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Oocyte maturation is a series of cytoplasmic and nuclear events that release oocytes from prophase I arrest and allow fertilization. The stimulus that initiates maturation is species-specific and has recently been shown in *C. elegans* to be the major sperm protein (MSP). Oocytes in *C. elegans* under optimal conditions do not normally undergo an obvious arrest. However, normal oocyte maturation does require an inductive signal from sperm. We report here the first characterization of a genetic mutant that is defective specifically in the process of oocyte maturation. The phenotype is termed Oma for oocyte maturation defective.

The Oma phenotype results from mutations in two functionally redundant genes, *oma-1* and *oma-2*, required for oocyte maturation. While mutations in either gene alone reveal no obvious phenotype, *oma-1;oma-2* double mutant worms are sterile. Oocytes in *oma-1;oma-2* mutants are fully-grown but remain arrested in diakinesis I. No sperm defect was detected in *oma-1;oma-2* mutants. Oocytes in double mutant animals show initial signs of oocyte maturation; partial nuclear envelope breakdown and cortical rearrangement occur, but the maturation process is not completed. We show that two sperm-dependent molecular events, maintenance of MPK-1 activation and AIR-2 chromosomal association, do not occur in Oma oocytes. These results suggest that OMA-1 and OMA-2 are required for the reception and/or execution of the sperm signal for oocyte maturation.

The prophase arrest of Oma oocytes can be suppressed by RNAi of the *C. elegans* MYT-1 homologue, *wee-1.3*. MYT-1 has been shown in other systems to be a negative regulator of MPF and therefore a negative regulator of meiotic progression. This result suggests that OMA-1 and OMA-2 function

upstream of MPF for prophase progression. Consistent with this notion, we have shown that OMA-1 and OMA-2 are cytoplasmic proteins that are expressed only in the female, but not male, germ line. Using antibodies specific for OMA-1 or OMA-2, expression of both proteins is detected in proximal oocytes and peaks in the maturing oocyte.

oma-1 and *oma-2* each encodes a protein containing two CCCH, TIS11-like zinc fingers, with conserved spacing within zinc finger regions (C-X_8-C-X_5-C-X_3-H, where X is any amino acid). Genome sequencing predicts over 20 TIS11-finger proteins in *C. elegans*. Five of these TIS11-finger-containing proteins, MEX-1, PIE-1, POS-1, MEX-5, and MEX-6, have been previously characterized genetically for their roles in early blastomere fate specification. It has been suggested that proteins containing TIS11-like zinc fingers modulate cell fate via negative regulation of RNA targets. We propose, therefore, that OMA-1 and OMA-2 might negatively regulate an RNA target(s) that represses oocyte maturation in *C. elegans*. We are presently attempting to identify potential OMA-1 and OMA-2 targets.

258. Functional and molecular identification and physiological role of a ClC-2 Cl^- channel ortholog expressed in *C. elegans* oocytes

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Members of the ClC superfamily of voltage-gated Cl^- channels have been found in organisms as diverse as bacteria and humans. Despite their widespread expression and likely physiological importance, the function and regulation of most ClC channels are incompletely understood. The nematode *C. elegans* offers significant experimental advantages for characterizing ClC biology.

Whole-cell patch clamp recordings demonstrated the presence of a small inwardly rectifying Cl^- current activated by strong hyperpolarization in *C. elegans* oocytes. Oocyte swelling dramatically activated this current. The biophysical characteristics of the current including anion selectivity, rectification, pharmacology, and voltage-, volume-, and pH-sensitivity are virtually indistinguishable from mammalian ClC-2.

Analysis of the *C. elegans* genome revealed the presence of six ClC genes termed *clh* (Cl^- channel homologue)-*1-6*. To test the hypothesis that one or more of these genes encoded the oocyte channel, we carried out double strand RNA (dsRNA)-mediated gene interference studies. dsRNAs complementary to the six *clh* genes were microinjected into the distal gonad of adult nematodes. Oocytes were harvested and patch clamped 20-24 h later. *clh-1, 2, 4, 5,* and *6* dsRNA had no effect on whole-cell current. *clh-3* dsRNA inhibited current activity >95%. CLH-3 transcripts were readily detected in single oocytes by nested RT-PCR. Based on these results, we conclude that *clh-3* encodes the oocyte ClC-2-like channel.

ClC-2 and CLH-3 share 40% amino acid identity. The strong conservation of biophysical and structural properties suggests that CLH-3 and ClC-2 may have similar physiological roles and regulatory mechanisms. Because ClC-2 is activated by cell swelling, it has been proposed to function in cell volume homeostasis, a housekeeping process conserved in widely divergent species. However, knockdown of CLH-3 activity by dsRNA has no effect on the rate or extent of oocyte volume regulation.

Basic mechanisms of cell cycle control are conserved in all eukaryotic organisms. We observed that CLH-3 is constitutively active in oocytes undergoing meiotic maturation. These findings suggest that the channel may regulate cell cycle-associated physiological processes important for reproduction. To test this hypothesis, we quantified several reproductive events. Brood size and the timing of meiotic maturation, ovulation and fertilization, and embryonic development were unaffected by dsRNA-induced knockdown of CLH-3.

Meiotic maturation induces ovulatory contractions of gap junction-coupled gonadal sheath cells. Sheath contractions were initiated ~2 min earlier in dsRNA-injected versus buffer-injected control animals. These results indicate that CLH-3 functions in part to modulate ovulatory contractions of gap junction-coupled gonadal sheath cells. We suggest that this modulation occurs by CLH-3-induced membrane depolarization, which in turn may modulate sheath cell Ca^{2+} signaling pathways.

259. DYSTROPHIN AND ASSOCIATED PROTEINS IN C. elegans.

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Dystrophin is the product of the gene mutated in Duchenne muscular dystrophy, a neuromuscular disease leading to muscle necrosis. The function of the dystrophin protein is not known. In mammals, dystrophin is located under the muscle plasma membrane, and is associated with a protein complex (DGC) spanning the membrane. We reported earlier that the C. elegans genome contains a dystrophin homolog named dys-1. Our goal is to understand dystrophin function in C. elegans.

Loss-of-function mutations in the dys-1 gene do not alter the muscle structure, but make animals hyperactive and slightly hypercontracted. In a forward genetics approach, we isolated additional mutations with dys-1-like phenotypes. Mapping and complementation analysis revealed that these mutations account for 4 additional genes. We cloned two of them : dyb-1 and dyc-1. dyb-1 is the C. elegans homologue of dystrobrevins, a family of proteins belonging to the dystrophin complex and known to bind dystrophin directly through coiled-coil domains. dyc-1 has no known homologue. Like DYS-1 and DYB-1, it contains a putative coiled-coil domain. Preliminary data indicate that DYC-1 may bind to DYB-1 in vitro, possibly through the coiled-coil domain. Interestingly dyc-1 has muscular and neuronal isoforms. A gfp protein fusion with the neuronal isoform stains approximately 20 neurons in the animal. Staining is these neurons appears as dots along the neuronal processes. We are currently investigating the nature of these dots. We also created a dystrophin-dependent myopathy in C. elegans by associtaing dys-1 with a mild MyoD mutation that potentializes dys-1. This provides us with a tool to undertake suppresor screens which are under way.

260. Functional analysis of ERM-1, a ryanodine receptor-interacting protein of *C. elegans*

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Ryanodine receptor (RyR) is a calcium release channel that is regulated by many molecules such as calcium, calmodulin, ATP and others. In *C. elegans*, only a single gene, *unc-68*, encodes the CeRyR. Isolated *unc-68* mutant animals are weak Unc and have a thin head even if these are null mutations; *e540* (splice-acceptor mutation), *x14* (Trp153 to stop). This is contrast to that there are three isoforms in mammals and RyR knockout mouse is embryonic lethal. The *kh30* mutant animal, isolated as a ketamine-response abnormal phenotype, causes an amino acid substitution at Ser1,444 to Asn of CeRyR. The Ser1,444-surrounding region having possible PKC phosphorylation sites and high hydrophilicity was useful to raise a specific antibody which stained CeRyR *in situ* (see Hamada et al., this meeting poster). This region could interact with other molecules and thus named as "KH30 domain". We aimed to isolate novel regulatory proteins of CeRyR by using the yeast two-hybrid screening. Using the KH30 domain of CeRyR as a bait, an ERM (ezrin/radixin/moesin) family gene, *erm-1*, was isolated. ERM-1 was most similar to the vertebrate moesin that crosslinks membrane and cytoskeleton. *erm-1* was mapped on chromosome I, and encoded several isoforms under the control of two promoters and an alternative splicing. By using two-hybrid analysis, we found that only the truncated form of ERM-1 could interact with the KH30 domain of CeRyR, and that the alternative form having three amino acid deletion weakened the interaction. *erm-1::gfp* fusion genes were expressed in excretory canals, intestine, pharynx and possibly in body wall muscles. These results suggest that ERM-1 may be a novel regulator of CeRyR and ERM-1/CeRyR interaction could be regulated by cytoskeletal re-organization during development. We are also analyzing *in vivo* function of ERM-1 proteins by RNAi experiment.

261. Functional analysis of the *C. elegans* Sarcoplasmic/Endoplasmic Reticulum Calcium transport ATPase

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A wide range of cellular processes such as muscle contraction, neurotransmitter release, secretion of hormones and cell cycling are regulated by the second messenger calcium. Intracellular calcium stores hold a key position in calcium homeostasis. These stores accumulate and release calcium in order to control cytosolic calcium levels and thus allow rapid establishment of local gradients of calcium. In addition, calcium in the lumen of the stores affects synthesis, folding, proteolytic cleavage and sorting of proteins in the endoplasmic reticulum. Removal of cytosolic calcium and filling of the stores is regulated by the activity of the Sarcoplasmic/Endoplasmic Reticulum Calcium transport ATPase (SERCA). SERCA genes are implied in a number of diseases such as Darier-White disease, Brody myopathy, cardiac hypertrophy, heart failure and type II diabetes. In higher vertebrates, three different SERCA genes exist. Protein diversity is increased by alternative splicing. The *C. elegans* genome contains a single SERCA gene whose transcript undergoes alternative splicing in a manner remeniscent of vertebrate SERCA2. The *C. elegans* and mammalian proteins show 70% identity and 80% similarity. To characterize the *C. elegans* SERCA gene we combined approaches in the fields of molecular biology, genetics, pharmacology and biochemistry.

C. elegans SERCA cDNAs were expressed in COS cells and microsomes representing fragmented endoplasmic reticulum membranes were prepared. Phosphorylation studied showed a calcium-dependent phosphorylated intermediate, an important step in the catalytic cycle of the enzyme. Moreover, Ca^{2+}-uptake

experiments showed that the two isoforms have a 2-fold different affinity for Ca^{2+}.

Inactivation of SERCA by RNAi or gene ablation leads to embryonic or early larval lethality respectively, indicating that embryogenesis requires maternally contributed SERCA. Arrested young larvae lacking SERCA show defects in movement, pharyngeal pumping and defecation. Rescue experiments indicate an additional role for SERCA in gonadal sheath contractility. Similar defects as observed in animals lacking SERCA could be induced pharmacologically using the SERCA-specific inhibitor thapsigargin, indicating conservation of the thapsigargin-binding site.

Together, these results show that SERCA is required for the function of various contractile tissues in *C. elegans*. This notion is supported by strong SERCA expression in all muscle types, the intestine and the gonadal myoepithelium as shown by GFP fusion constructs of both isoforms. Thus, the data presented here open a path to study SERCA function and regulation in *C. elegans*.

262. Third and fourth tropomyosin isoforms of *Caenorhabditis elegans* are expressed in pharynx and intestines and are essential for development

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The tropomyosin gene *tmy-1/lev-11* of *Caenorhabditis elegans* spans 14.5 kilobases and encodes three isoforms by alternative splicing. CeTMI and CeTMII isoforms expresses in body wall from common 5' upstream promoter and CeTMIII isoform expresses in pharynx from an internal promoter. To identify, characterize and compare the genome and tissue expression of a fourth isoform, Rapid Amplification of cDNA Ends and microinjection with *lacZ* and *gfp* fusion plasmid techniques were employed. We elucidated CeTMIV, a fourth isoform of *tmy-1*, which encoded a 256 amino acid polypeptide. CeTMIV isoform had a similar promoter region to CeTMIII isoform, but was alternatively spliced to generate a cDNA that differed in two exons. The *tmy-1::lacZ* and *tmy-1::gfp* fusion genes with 3.2kb upstream promoter region and 1.1kb CeTMIV specific exons were expressed in the pharynx and intestinal cells. Further unidirectional deletion of upstream sequence located the primary promoter region to 853bp upstream from the initial codon. We also show within the fusion gene B and C subelement-like sequences of *myo-2*, which may be utilized to stimulate pharyngeal expression. However despite the presence of a *ges-1* like sequence, we were unable to locate the two GATA factors necessary for the gut expression. Reassessing tissue expression for CeTMIII isoform with newly constructed fusion plasmids, we showed further expressions in the intestinal cells and vulva muscles in addition to pharyngeal expression. Finally to demonstrate that tropomyosin is essential for development, we inactivated isoforms III and IV by RNA-mediated interference and the worms arrested between the 300-cell and comma bean

stage during embryogenesis. These results which confirm the essentiality of tropomyosin in muscle filament assembly and embryonic development in *C. elegans* also illustrates the variant expression patterns associated with tropomyosin isoforms. We are currently trying to elucidate the mechanism underlining the expressions.

1. Kagawa *et. al*., (1995). *J. Mol. Biol*. 251, 603-613.

263. UNC-23 is a member of the BAG family of chaperone regulators and directly interacts with the non-inducible heat shock protein, HSP-1.

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Mutations in the *unc-23* gene cause a bent head phenotype, which is the result of dystrophy and detachment of the anterior body wall musculature (Waterston et al., Dev. Biol. 77:271 1980). Suppression of this phenotype is observed when *unc-23* animals are grown in liquid culture (A. Bullerjahn and D. Riddle, pers. comm.). Attachment of muscle to the underlying hypodermis is still fragile however, as a small amount of pressure applied to the animal results in detachment of the muscle cells from the underlying hypodermis. We have recently cloned *unc-23* and found that it encodes a domain similar to the molecular chaperone regulator BAG-2 (BCL2-associated athanogene 2). In mammals, the BAG family of molecular chaperone regulators, contain a conserved 45 amino acid region near their C termini (the BAG domain). Human BAG-2 and *C. elegans* UNC-23 share 40% amino acid identity and 62% similarity over the BAG domain and its upstream region.

We have characterized the temporal and spatial expression pattern of UNC-23, using a full-length unc-23::GFP construct that is capable of rescuing the *unc-23* phenotype. UNC-23 is expressed in a wide variety of tissues such as the body wall muscle cells, hypodermis, and pharynx.

In mammals, BAG-2 binds the ATPase domain of Hsp70/Hsc70 and regulates the Hsp70 chaperone activity (Takayama et al., The Journal of Biological chemistry 274: 781,1999). Using a yeast two hybrid screen, we have identified the ATPase domain of HSP-1 as an interacting partner with the COOH terminus of UNC-23. We previously had identified two dominant suppressors of *unc-23(e25)* and here demonstrate that they are alleles of *hsp-1*. The molecular lesions that result in HSP-1 suppressor activity are missense mutations

located within the ATPase domain of the molecule. These studies demonstrate an interaction between UNC-23 and HSP-1in *C. elegans*.

264. PAT-4 binds UNC-112 and Functions as an Adapter During Muscle Assembly

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In body-wall muscle cells, attachment structures known as dense bodies and M-lines anchor the contractile apparatus to the basal membrane. In a manner resembling focal adhesion assembly *in vitro*, nascent dense body and M-line assembly initiates following integrin activation and proceeds with the subsequent recruitment of integrin-associated molecules to the growing adhesion sites. To better understand how integrin functions during assembly, we isolated additional genes required for this process, and our work focuses on three of these genes: *pat-4*, *pat-6* (see abstract by X. Lin), and *unc-112* (Rogalski, et al., JCB 150:253). We determined that *pat-4* is the homologue of the vertebrate molecule integrin-linked kinase (ILK). ILK is a focal adhesion component that binds β-integrin *in vitro*. In addition, ILK phosphorylates both GSK-3β and PKB/AKT suggesting that PAT-4 may function during WNT signaling or regulate dauer formation *in vivo*. We determined that PAT-4 co-localizes with PAT-3 (β-integrin) and UNC-112 in dense body and M-line attachment structures *in vivo*. This led us to ask whether these molecules directly interact, and our results from yeast-two hybrid assays indicate that PAT-4 binds UNC-112. The proper localization of PAT-4 requires *pat-2* (α-integrin) and *unc-112*, but not *deb-1* (vinculin). We also observe that *pat-4* is required for integrin to fully assemble into nascent attachments, but *pat-4* does not appear to be required for the assembly of the extracellular matrix component UNC-52/Perlecan. Our genetic analysis of *pat-4* fails to demonstrate an essential role for PAT-4 in either WNT signaling or dauer formation, suggesting that PAT-4 functions as an adapter mediating interactions between integrin and the cytoplasmic components of dense bodies and M-lines. Consistent with this model, we observe that "kinase-dead" PAT-4::GFP can properly localize and fully rescue mutant *pat-4* animals.

However, we do have evidence that *pat-4* is required in tissues other than muscle- when expression of wild-type *pat-4* is restricted to body-wall muscle cells, rescued *pat-4* animals are dumpy, Egl, and have misshapen posterior ends. Current work is attempting to find specific defects in these mosaic animals, as well as, measure protein-protein interactions *in vivo* using fluorescence resonance energy transfer (FRET).

265. PAT-6, a Homlog of Actopaxin, Binds to PAT-4/Integrin-Linked Kinase

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The cytoplasmic domain of the cellular adhesion molecule integrin assembles a plaque of adaptor and signaling proteins that regulates many fundamental cellular functions including adhesion, growth, differentiation, and apoptosis. This protein complex has been most extensively analyzed in the focal adhesions, attachments that are formed by vertebrate cells in culture. *C. elegans* body wall muscle cell dense bodies and M-lines are analogs of focal adhesions that are amenable to genetic analysis. We are contributing to a comprehensive genetic dissection of these structures by studying a subset of the *pat* genes (**P**aralyzed, **A**rrested elongation at **T**wo-fold) that are required for their normal assembly. A partial list of these genes includes *pat-2* and *pat-3*, which are integrin subunit genes (Williams and Waterston, unpubl.; Gettner *et al.*, 1995, JCB 129:1127), *unc-112*, which encodes a novel dense body/M-line component (Rogalski *et al*., 2000, JCB 150:235), *pat-4*, which encodes integrin-linked kinase (see abstract by Mackinnon *et al.*, this meeting), and *pat-6*, which we focus on here.

We molecularly isolated the *pat-6*gene and have found that it encodes the worm homologue of actopaxin, a human actin binding protein discovered very recently in a yeast two-hybrid screen for proteins that bind paxillin, a focal adhesion component (Nikolopoulos and Turner, 2000, JCB 151:1435). Although a recent database search failed to identify any other proteins homologous to the N-terminal half of PAT-6, the C-terminal half includes two calponin homology (CH) domains, a feature of many actin-binding proteins. PAT-6 is located in body wall muscle dense bodies and M-lines. It also colocalizes with integrin at several other sites including the attachments of single sarcomere muscles, within the spermatheca, and within touch neurons.

We are using the collection of *pat* mutants in combination with antisera and/or functional GFP-tagged fusion proteins to investigate the role of *pat-6* in dense body and M-line assembly. To date, we have found that *pat-6* is not required for the normal assembly of UNC-52/Perlecan into the adjacent basal lamina, for the initial polarization of integrin to the basal membrane of muscle cells, nor for the entry of integrin into nascent dense bodies and M-lines. In contrast, *pat-6* is required for maturation of dense bodies and M-lines, including the proper assembly of at least one membrane-distal protein, UNC-89, and for the arrangement of these nascent attachments into a well-ordered striated array. In reciprocal experiments we have addressed the requirements for proper PAT-6 assembly. As predicted, PAT-6 requires UNC-52/perlecan and integrin to assemble normally at the sarcolemma. We were surprised to find that PAT-6 is not detectable in body wall muscle cells when PAT-4 has been removed by mutation. Although this result is consistent a number of interesting possibilities, we currently favor the hypothesis that PAT-4 and PAT-6 interact, perhaps directly, and that this interaction stabilizes the PAT-6 protein. Consistent with this idea, we have found through yeast two-hybrid experiments that PAT-4 binds directly to PAT-6. The kinase domain of PAT-4 and C-terminal CH domain of PAT-6 are both necessary and sufficient for binding. We failed to detect interactions between PAT-6 and any of several other dense body proteins in similar two-hybrid experiments.

In our initial structure/function analysis of PAT-6, we have assayed ability mutant PAT-6 lacking either the N-terminal or the C-terminal CH domain to 1) localize properly in animals that are also expressing wild-type PAT-6, and 2) to rescue *pat-6* null animals. Our results to date show that N-terminal CH domain is not required for localization, but is required for rescue. In contrast, the C-terminal CH domain is required both functions. Current experiments are addressing the function of the N-terminal half of PAT-6.

266. Functional analysis of the 5 LIM domain containing adaptor protein, UNC-97

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unc-97 encodes a phylogenetically conserved adaptor protein consisting entirely of 5 LIM domains. The mammalian ortholog, PINCH, localizes to focal adhesion (FA) sites and has been found to interact with integrin and integrin linked kinase (ILK). UNC-97 has been found to localize to the dense bodies of the bodywall muscle cells, which are analogous to focal adhesions, and to the nucleus (JCB 144:45). Studies of a partial loss of function mutation in *unc-97* demonstrates that the protein is necessary for the integrity of FA-like attachment structures in growing animals. We now have a null allele for *unc-97*. Animals homozygous for the null mutation fail to complete myofilament assembly during embryogenesis and arrest development as Pat embryos. Analysis of integrin and vinculin in *unc-97* null mutants indicates that these molecules localize to dense bodies normally. Myosin and actin however, are not properly assembled, suggesting that UNC-97 functions in attaching the myofilaments to the dense body but not in assembly of the dense body per se.

To further dissect the function of *unc-97*, we have conducted a yeast two-hybrid analysis of full length *unc-97* and 10 different deletion constructs. The aim of this analysis was to identify proteins that interact with *unc-97* and participate in myofilament assembly. Similar to mammalian PINCH, UNC-97 interacts with ILK. Other positives from the 2-hybrid screen are being retested. To identify the regions of PINCH responsible for its localization, we have designed altered UNC-97::GFP reporter constructs which delete one or multiple UNC-97 LIM domains. We have generated six different deletion constructs and are in the process of generating transgenic animals to analyze the subcellular localization of these proteins and their ability to rescue *unc-97* mutants. Thus far, we have two transgenic lines that provide evidence that LIM domain 3 is required for the

nuclear localization of UNC-97. In addition, this construct is capable of partially rescuing the *unc-97* Pat phenotype. When the *unc-97*ΔLIM3 construct is introduced into *unc-97* null mutants, the animals arrest as four fold embryos/L1 larvae and have defects in myofilament attachment. This data and further progress will be presented.

267. *eat-1* Encodes Two Members of the ALP-Enigma Family of Proteins and is Required for Normal Muscle Function in *C. elegans*

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Muscle is required for many vital processes including locomotion, circulation and respiration. Recently, members of the alpha-actinin associated LIM protein (ALP)-Enigma family have been identified as prominent constituents of actin filament anchorage sites in vertebrate muscle. ALP-Enigma family members are comprised of N-terminal PDZ domains linked to one (ALP) or three (Enigma) C-terminal LIM domains. Vertebrates express at least nine different members of this protein family, making it difficult to analyze the roles of the individual proteins *in vivo*. We have identified a single gene in *C. elegans* that, through alternative splicing, encodes both ALP and Enigma isoforms. Consistent with the idea that *C. elegans* ALP-Enigma plays a role in muscle, we determined that GFP-Enigma is expressed broadly in muscle derivatives where it is concentrated at actin anchorage sites such as the dense bodies of body wall muscle. To explore the phenotypic consequences of loss of ALP-Enigma function *in vivo*, we used RNA-interference (RNAi) to perturb gene expression. RNAi directed against ALP-Enigma transcripts results in muscle defects including arrhythmic pharyngeal pumping, sluggish movement, and thin body morphology. Interestingly, mutations at the *eat-1* locus display similar phenotypes to what we observe with ALP-Enigma RNAi and map to the same region as the ALP-Enigma gene. To evaluate whether the *eat-1* alleles result from lesions in the ALP-Enigma gene, we tested the ability of wild-type ALP-Enigma DNA to rescue the *eat-1* mutants. We determined that introduction of exogenous ALP-Enigma genomic DNA fully rescues the *eat-1* mutant phenotype, demonstrating that the *eat-1* gene encodes the *C. elegans* ALP-Enigma family. Our findings illustrate that ALP-Enigma expression is absolutely required for normal muscle function

in *C. elegans*.

268. Mutations in type XVIII collagen perturb fine patterns of matrix protein localization and elements of nervous system and muscle morphology

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cle-1(collagen with endostatin domain) is the *C. elegans* homologue of vertebrate type XV and XVIII collagens, which contain endostatin as their C-terminal domain. At least three mRNAs differing in their 5' ends are produced from *cle-1* (*cle-1A-C*). All three isoforms share the collagenous Gly-X-Y and endostatin domains. CLE-1A and B include thrombospondin-related repeats, and CLE-1A contains UNC-40-related fibronectin type III repeats.

We have examined the tissue localization of CLE-1 using antisera. One of these, CeCol18, shows that CLE-1 localizes evenly within the basement membranes of the pharynx, intestine, and gonad. In addition, it is found in punctate aggregates along the dorsal, ventral and sublateral nerve cords and the nerve ring, as well as in patterned lines under body wall muscle. These fine localization patterns require the C-terminal endostatin domain since specifically deleting it, as in the *cle-1(cg120)* allele, allows accumulation of truncated CLE-1 but disrupts these patterns.

The punctate pattern of CLE-1 along the nerve cords appears to represent concentration at synapses. Double staining for CLE-1 and synaptic markers (UNC-17, SNT-1, or SNB-1::GFP) shows colocalization along nerve cords. Synaptic organization is defective in *cg120* mutants, with fewer, larger, more widely spaced puncta seen using the SNB-1::GFP marker expressed in GABAergic neurons. CLE-1 is thus required for normal synaptic organization. The basement membrane protein NID-1 is also concentrated at synapses and required for normal synaptic organization. These results demonstrate a role for matrix components in organization of *C. elegans*

synapses, as has been shown for the vertebrate neuromuscular junction.

Although *cle-1* and *nid-1* mutants have multiple defects in nervous system organization, they display little or no apparent uncoordination when moving on plates. When placed in liquid, however, their thrashing behavior is substantially defective. Both mutants show a reduced frequency of thrashing and display defects in the normal anterior/posterior coordination of body bends, indicating that their abnormal neuronal organization does result in behavioral defects.

CLE-1 is concentrated along body wall muscle edges and dense body lines, in a pattern similar to the basement membrane molecules NID-1 and perlecan (UNC-52). In *cle-1(cg120)* mutants, NID-1 is absent from dense body lines and its accumulation is reduced along muscle edges. In *cg120* mutants, the normally tight localization of UNC-52 under dense bodies becomes diffuse and its accumulation at muscle edges is weak and patchy. Thus, loss of the CLE-1 C-terminal domain results in subtle disruptions of the localization of other basement membrane molecules.

The *cle-1(cg120*) mutation is synthetic lethal in combination with *nid-1(*null) or *unc-52(gk3)*, an allele that deletes the perlecan domain V. Individually, these mutants are viable and fertile, and display no outward phenotype. These interactions show allele specificity. The *nid-1(cg118)* allele, which deletes the nidogen G2 domain, shows no apparent interaction with *cg120*. Double mutants for the late acting dystrophic *unc-52(e444)* allele and *cg120* are viable, but small and sick, have reduced brood sizes, and abnormal gonad and intestinal morphologies. These data indicate that proper interactions amongst these basement membrane molecules are critical for formation of the nervous system as well as other tissues in *C. elegans*.

We would like to extend thanks to those who shared antisera, GFP markers and worms with us.

269. The *gnd* genes and *tra-1* control assembly and patterning of the gonad primordium

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In *C. elegans*, the gonad primordium forms during embryogenesis when two somatic precursors, Z1 and Z4, migrate posteriorly to flank two central germline precursors. Despite the different polarity of the adult male and hermaphrodite gonads, the four-celled gonadal primordium is morphologically indistinguishable in the two sexes. We have identified four genes that regulate assembly of the gonadal primordium: *gnd-1, gnd-2, gnd-3* and *tra-1*. The *gnd* genes are critical for generation of the primordium and also for patterning within the primordium. Thus, newly hatched animals lacking *gnd* function can be missing Z1 or Z4 entirely, or Z1 and Z4 can be misplaced within the primordium. Therefore, the *gnd* genes either control generation of Z1/Z4 or their migration to the primordium in addition to establishing the symmetry of the primordium. The *gnd-1* gene encodes a bHLH putative transcription factor that is expressed in the MS lineage and becomes restricted to Z1 and Z4 after the primordium forms. Cloning of *gnd-2* and *gnd-3* is in progress.

The role of *tra-1* in gonadogenesis overlaps that of the *gnd* genes: *tra-1* is essential for patterning of Z1 and Z4 within the gonadal primordium of both XX and XO animals. Previous studies showed that *tra-1* promotes female development in somatic tissues and is required for gonadogenesis in both hermaphrodites and males (Hodgkin, 1987). We find that *tra-1* is essential for gonadal primordium formation: Z1 and Z4 are always present in *tra-1* null mutants,

but they are often misplaced. Furthermore, in *tra-1* null mutants, the polarities of Z1 and Z4 divisions are the same with respect to the A/P axis, whereas in wild-type animals, they have opposite polarities. Thus, the *tra-1* gonadal primordium appears to have lost the two-fold rotational symmetry typical of the wild-type primordium in both hermaphrodites and males. Instead, the *tra-1* primordium has an asymmetry that is typical of developing male gonads at a later stage. We conclude that *tra-1* is essential for primordium symmetry in both sexes. Genetic interactions between *gnd* mutants and *tra-1* suggest that they might be part of a common pathway. We are exploring the possibility that the *gnd* genes control patterning of the primordium by regulating *tra-1*. We are also currently examining other sex determination pathway members for a role in this process.

Hodgkin, J. (1987) *Genes & Development* **1**, 731-745.

270. POP-1, *sys* genes and sexual dimorphism during gonadogenesis

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Hermaphrodite and male adult gonads of *C. elegans* have distinct organ polarities: hermaphrodite gonads are symmetrical with two U-shaped gonadal arms, whereas male gonads are asymmetrical with only a single gonadal arm. These sexually dimorphic structures arise from a four-celled gonadal primordium that is morphologically identical in the two sexes. In a screen for mutants defective in early steps in gonadogenesis, we identified mutations in two genes, *sys-1* and *sys-2*, that lead to more severe defects in hermaphrodites than males. We found that the *sys-2* mutations are missense mutations in *pop-1*. The *pop-1(Sys)* mutants are defective in polarity of the first division of Z1 and Z4, resulting in loss of DTCs and production of extra AC/VUs. This phenotype has been previously reported for *lin-17* (1) and *sys-1 (*2). Both *pop-1* alleles affect hermaphrodites more severely than males. Thus, for the strongest *pop-1* allele, 100% of hermaphrodites are missing both DTCs while only 5% of males are missing both DTCs. However, zygotic *pop-1(RNAi)* affects both sexes with equal severity. The *pop-1* and *lin-17* genes are both key components in the Wnt signaling pathway (3,4). Using RNAi, we have found that other components of Wnt signaling also affect this early step in gonadogenesis, including *wrm-1* and *lit-1*, but none of the five Wnt ligands has a detectable effect in mutants or by RNAi. We are continuing to piece together which Wnt pathway members function in early gonad development and evaluate their role in hermaphrodite vs. male gonad development. In addition to *sys-1* and *pop-1*, we have identified mutations in four other genes with a similar gonadogenesis defect. These *sys* genes, including *pop-1*, all genetically interact with *sys-1*, suggesting that they function

with *sys-1* to control cell polarity in the hermaphrodite gonad. All six *sys* genes have been carefully mapped, and to date, only *sys-2/pop-1* maps to a gene encoding a known Wnt pathway component. The *pop-1(RNAi)* phenotype suggests that *pop-1* is part of a common program that controls the asymmetric first division of Z1 and Z4. We suggest that this common program may have been modified during evolution to generate critical regulatory cells in distinct parts of the hermaphrodite and male lineage. The mechanism by which POP-1 is differentially controlled in the two sexes is not known, but may involve the two regions altered by the *pop-1/sys-2* missense mutations, the β-catenin binding site and the HMG box.

1. Sternberg and Horvitz (1988) Developmental Biology 130: 67-73.

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271. The presenilin SEL-12 is required during vulva muscle development for a normal egg laying behaviour in C. elegans.

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The Caenorhabditis elegans SEL-12 protein was shown to be not only structurally but also functionally similar to the human presenilins involved in Familial Alzheimer's disease. sel-12 was initially identified as a suppressor of gain-of-function alleles of lin-12/Notch in C. elegans [1]. Animals mutant for sel-12 exhibit a highly penetrant egg laying defect (egl) and morphological abnormalities that lead to a grossly abnormal protruding vulva (p-vul). Anna Newman [2] has shown that some sel-12 animals fail to specify the p cell fate correctly leading to a defective vulva uterine connection. However strains containing weaker sel-12 alleles only show a weakly penetrant p-vul defect and are able to lay eggs, but become Egl and die of bagging later.This suggests that there might be additional defects responsible for the Egl defect.

We have previously identified a functional defect of sel-12 loss-of-function mutants in selected cholinergic neurons [3]. However, sel-12 is broadly expressed both in neurons and in a variety of muscles at all developmental stages. Therefore we investigated whether the inability of sel-12 animals to lay eggs is also caused by a neuronal defect. Pharmacological and epistasis tests revealed that sel-12 animals behave like a muscle structure mutant. Using different GFP expression constructs that stain the vulva muscles, we are able to show that sel-12 mutants indeed have several defects in their vulva muscles. sel-12 mutant animals display a range of defects. Most animals exhibit misaligned, distorted vulva muscles but we also see muscles which have mismigrated or degenerated. Expression of the sel-12 cDNA under the control of the myo-3 and unc-54 promoters does not rescue the Egl defect of sel-12 which implies that sel-12 activity is not required in mature vulva muscles but rather during their development. In hermaphrodites, the vulva muscles are derived from the

M-lineage. By ablation it was shown that the loss of the vulva muscles is sufficient to cause an Egl defect. Expression of the sel-12 cDNA only in the M-lineage fully rescues the Egl defect of sel-12 mutants in all sel-12 alleles tested so far. In contrast, the vulva structural defect that leads to a p-vul formation was not rescued by this construct. This clearly shows that these defects are independent from each other and can be separated. These data suggest that the different roles of sel-12 during C .elegans development are complex and that sel-12 affects various aspects of development in the nervous system, in muscles, and in epidermal structures. We are particularly interested in understanding the cross talk between the different structures involved in egg laying during their development. As sel-12 was shown to facilitate lin-12/Notch signaling we have further analyzed whether lin-12 function is required for proper vulva muscle development. It is known that in a lin-12 null allele the sex myoblasts (SM) that give rise to the sex muscles are converted into coelomocytes [4]. However in lin-12(n676930ts), a reduction of function mutant that is egl only at 25°C, the vulva muscles are present but display the same variety of defects found in a sel-12 mutant. Therfore we conclude that lin-12/Notch activety is required at various steps during sex myoblast development. This also suggests that sel-12 is acting through lin-12 to influence smooth muscle development in C. elegans.

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[2] 1999 International Worm Meeting

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272. The role of the SEL-12 presenilin in uterine pi cell induction and egg laying

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During larval development of the *C. elegans* hermaphrodite, the uterine and vulval epithelia undergo coordinated morphogenesis to produce a functional connection. The end result is that the uterine and vulval lumens are separated only by the cytoplasmic extension of the utse (uterine seam cell), which is thin enough to be broken when the first egg is laid. The utse is generated by the uterine π cells, which in turn are induced in response to *lin-12*-mediated signaling by the AC.

Presenilins have been implicated in Alzheimers disease pathogenesis and LIN-12/Notch signaling during development. *C. elegans* has the *sel-12* and *hop-1* genes, with approximately 50% and 33% identity to mammalian presenilins, respectively, plus the divergent family member *spe-4*. During a screen for Egl mutants, we isolated a mutant called *ty11* in which the uterus and vulva were separated by thick tissue rather than the thin laminar process of the utse. We found that *ty11* was a missense mutation in *sel-12* resulting in a premature stop codon. While *hop-1* mutants had no apparent defect in uterine development, the uterine morphogenesis defect was more severe in *sel-12; hop-1* than in *sel-12* mutants, implying biological redundancy between the two genes.

Using *lin-11::lacZ* expression studies and cell lineage analysis, we found that *sel-12* mutants had defects in uterine π cell fate induction. This could account for the uterine-vulval connection defects observed. But *sel-12* mutants also have abnormal vulval morphogenesis. So which tissue is the culprit in making the animals unable to lay eggs? We found that expression of wild-type *sel-12* in the presumptive π cells of *sel-12* mutant animals conferred significant rescue of the egg-laying defect, suggesting that *sel-12* function in the π cells is critical. In these experiments, *sel-12* was expressed under the

control of the *egl-13/cog-2* promoter, which drives expression in the uterus but not in the vulva. The *egl-13/cog-2* gene (which encodes a SOX domain transcription factor) appears to function downstream of *sel-12* in the uterus and its expression is dramatically reduced in *sel-12* mutants. Given this, the rescue conferred by the *egl-13/cog-2::sel-12* transcriptional fusion seems quite remarkable. We hypothesize that a positive feedback loop is initiated in which the small amounts of wild-type SEL-12 initially produced increase throughput through the *egl-13/cog-2* promoter, generating more SEL-12 protein, etc. Surprisingly, the presence of an integrated array containing multiple copies of the *egl-13/cog-2::GFP* transcriptional fusion blocked the rescue of the *sel-12* egg-laying defect by *egl-13/cog-2::sel-12* in animals rescued for the marker phenotype. We are currently performing experiments to test the hypothesis that this is due to competition for regulatory proteins that bind to the *egl-13/cog-2* promoter.

273. Analysis of the presenilin complex

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Presenilins are polytopic membrane proteins that play a critical role in facilitating both APP processing and Notch processing. APP is proteolytically cleaved to generate the A-beta peptide, a primary component of plaques found in Alzheimer's disease brains. Upon ligand activation, Notch is proteolytically cleaved in or just adjacent to its transmembrane domain. The Notch intracellular domain (NICD) then translocates to the nucleus where it affects downstream gene expression. There is now mounting evidence that presenilin may be the elusive gamma-secretase, the enzyme responsible for A-beta generation, and the protease that releases NICD. Whether presenilin itself has catalytic activity or not, it is clearly important for enzymatic activity.

Presenilins associate with other proteins in a high-molecular weight complex. The identification of the other components of the complex as well as reconstitution of the complex are important goals in establishing the identity of gamma-secretase. We have used a *sel-12* presenilin rescue assay in combination with an A -beta generation assay in human cells to probe the structure/function relationship of presenilin.

One of the components of the presenilin high-molecular weight complex has been shown to be Nicastrin, the human homolog of *C. elegans aph-2.* The *aph-2* gene is known to encode a cell-surface protein that is required for early embryonic signaling events that are mediated by the Notch homolog *glp-1.* We have extended this analysis to show that *aph-2* is involved in a post-embryonic signaling event that is mediated by *lin-12,* a second *C. elegans* Notch homolog. We have also validated the biochemical association of Nicastrin and presenilin by demonstrating a genetic interaction between *aph-2* and the presenilin gene *sel-12*. Furthermore, we have shown that

aph-2 and Nicastrin are partially functionally conserved and we will present data on our structure/function analysis of this family of proteins.

274. Genes that suppress the Egl defect of *sel-12*, a *C. elegans* presenilin gene

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Presenilins are a family of multiple trans-membrane proteins, specific to plants and animals, that are predominantly localized to the ER and Golgi. Mutations in the two human presenilins dominantly lead to early onset Alzheimer's disease by increasing the production of the 42 amino acid variant of the beta-amyloid peptide. Presenilins also play an essential role in Notch-type signaling throughout the animal kingdom. Studies in a number of systems implicate presenilins in the cleavage of Notch type receptors after ligand binding, but how directly these genes affect this cleavage is unclear. *C. elegans* has two somatically expressed presenilin genes, *sel-12* and *hop-1*, which appear to have partially redundant functions. *hop-1* mutants have no obvious defects however, mutations *sel-12* result in an egg-laying defect. At least two developmental defects underlie the *sel-12* Egl defect, the most penetrant of which appears to be a mispatterning of the sex muscles that control egg-laying (see abstract by Eimer *et al.*). All defects seen in *sel-12* mutants or *hop-1; sel-12* double mutants resemble those seen in mutants with reduced *lin-12* or *glp-1* signaling. This, along with the fact that mutations in *sel-12* suppress *lin-12(gf)* mutations, suggest that the most important, and perhaps only, role of presenilins in *C. elegans* is to regulate Notch-type signaling.

In order to better understand the biological and biochemical role of presenilins in animals, we have screened over 75, 000 mutagenized haploid genomes for suppressors of the Egl defect of *sel-12(ar171)* mutants using either EMS, UV/TMP or a mutator strain. We have identified 26 recessive strong suppressor mutations that fall into at least 6 complementation groups. However most mutations lie in only three genes which we call *ses-1, ses-2* and *ses-3* (for suppressor of the

egg-laying defect of *sel-12*). These strong suppressor mutations completely suppress the egg-laying defect and restore largely wild-type brood sizes to both *sel-12(ar131)* and *sel-12(ar171)* mutants. This indicates that the suppressors are not allele specific and that they must correct both *sel-12* developmental defects. On their own, suppressor mutations display no obvious developmental or behavioral abnormalities, indicating that these mutations are specific suppressors of the *sel-12* defects. We have cloned *ses-1* by a positional cloning strategy and found that *ses-1* encodes for a novel protein with no close similarity to any sequence in the publicly available databases. It contains several C2H2 zinc finger domains and two predicted nuclear localization signals that suggest that the protein may function as a transcription factor. Consistent with this, a Myc-tagged fusion protein is localized to the nucleus in SF9 insect cells. Only one of the seven *ses-1* alleles is clearly non null, suggesting that the function of *ses-1* must be severely reduced or even eliminated to see suppression. All of this evidence suggests that *ses-1* may function as a transcriptional repressor, that acts genetically downstream of *sel-12*. Since another *sel-12* suppressor gene identified by the Greenwald lab, *spr-2*, is also a nuclear gene that may affect transcription (1), we have been looking for possible targets of *ses* gene regulation by Northern analysis. We have examined the expression of many genes involved in *lin-12* and *glp-1* signaling in various *ses* gene mutant strains. Although we have found no clear differences in transcript levels of any gene tested using mixed stage RNA, we have preliminary evidence that *ses-2* and *ses-3* may suppress *sel-12* defects by up-regulating the expression of *hop-1* specifically in the L1 and L2 larval stages. This suggests that we may not be able to bypass completely the need for presenilins in *C. elegans*. At the meeting, the further characterization of *ses-1* and the other *ses* genes will be presented.

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275. Expression and function of the E/Daughterless protein HLH-2 during the AC/VU decision

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The AC/VU decision is a simple example of lateral specification that occurs during the development of the somatic gonad. Two initially equivalent cells, Z1.ppp and Z4.aaa communicate with each other via LIN-12, a receptor of the LIN-12/Notch family, and LAG-2, a ligand of the DSL family. As a result, one of the two cells becomes the anchor cell (AC) while the other becomes a ventral uterine precursor cell (VU). Although one of these cells always becomes the AC, which cell does is random.

Genetic mosaic analysis and *lin-12* and *lag-2* expression data suggested that transcriptional feedback loops reinforce small initial differences in *lin-12* activity between Z1.ppp and Z4.aaa. One feedback loop involves repression of *lag-2* expression in response to LIN-12 activation. As a genetic circuit accounting for repression of *Delta* expression in response to Notch activation has been described in *Drosophila*, we asked whether the same circuitry has been conserved in *C. elegans*.

We have found some similarities and some differences between the two systems. Our most provocative findings concern *hlh-2*, which is homologous to the *Drosophila daughterless* gene and the genes encoding the mammalian E proteins (Park et al., 1998). Several observations suggest that *hlh-2* may be involved in the feedback circuitry or in the execution of the AC/VU decision. (1) HLH-2 is expressed only in the presumptive AC and not in the presumptive VU. (2) Expression of HLH-2 in the presumptive AC occurs prior to the restriction of *lag-2* expression to the presumptive AC; the expression of HLH-2 is thus far the earliest difference detectable between Z1.ppp and Z4.aaa. (3) Preliminary

hlh-2(RNAi) experiments suggest that *lag-2* expression requires *hlh-2* and that *hlh-2* is required for the AC fate. These observations are consistent with a role for *hlh-2* in the circuitry underlying the feedback mechanism. However, RNAi and transgene experiments suggest that *hlh-2* may also play other roles in the AC/VU decision as well; we hope to have more to say about this issue at the meeting.

We want to thank Mike Krause for his generosity with reagents and ideas.

276. Mechanisms that establish anchor cell-specific expression of *lin-3, C. elegans* epidermal growth factor (EGF) homolog

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The C. elegans lin-3 gene is required for multiple aspects of C. elegans development. It is required for vulva development, ovulation, and cell fate specification of the P12 neuroblast of hermaphrodites. Lin-3 is also required for cell fate specification of the B cell lineage in males, and for viability of males and hermaphrodites. LIN-3 has an extracellular domain with one EGF motif, a transmembrane domain, and a cytoplasmic domain that is longer than that of most other growth factors. It expressed in anchor cell in the gonad during vulva induction. The induced LIN-3 binds to LET-23, its receptor in vulval precursor cells, and the binding activates the regulated proliferation of vulval precursor cells and pattern of the developing vulva. We took two different approaches to study mechanisms that establish its AC specific expression. First, we identified the molecular nature of the vulva-specific e1417 mutation of lin-3. Second, the regulatory region of lin-3 for the anchor cell-specific expression was dissected by deletion and site-directed mutagenesis analysis. These approaches identified a 65 bp long cis-element before the 2nd lin-3 promoter that makes lin-3::gfp expressed only in AC in the gonad during vulva induction. We named this element ACEL (anchor cell element of lin-3). The ACEL contains three predicted binding sites for trans-acting factors. All of the sites are essential for expressing lin-3::gfp in anchor cell since mutation of any of the three sites abolished expression. In the L2 stage before AC becomes specified, lin-3::gfp expressed in the four pre-AC and pre-VU cells. By the L2 molt, when the AC/VU cell fates become specified, lin-3::gfp expression is repressed in the three VU cells but remains in the AC. Currently we are looking for proteins that bind to the ACEL and studying mechanisms that regulate lin-3::gfp expression during AC/VU cell fate specification.

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277. Worms, Twist, and human Saethre-Chotzen syndrome

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We are interested in understanding transcriptional regulation during mesoderm development. The *C. elegans* transcription factor CeTwist belongs to the family of basic helix-loop-helix (bHLH) proteins and plays an important role in the development of a subset of mesodermal tissues. We have been studying two alleles in the CeTwist gene, *hlh-8*; a putative null deletion allele and a semidominant allele. Characterization of the defects in these mutant animals reveals that CeTwist is important for embryonic development of the enteric muscles and for postembryonic patterning and differentiation in the mesodermal lineage, M. Among the descendants of M in wild type animals are two sex myoblasts (SMs) that migrate to the developing vulva region and divide to become the sex muscles that are important for egg laying. The SMs behave differently in animals harboring one or the other *hlh-8* allele. In the *hlh-8* deletion allele, the SMs migrate to the vulva region and divide but do not differentiate properly (Corsi et al., 2000). In the *hlh-8* semidominant allele, the SMs migrate but do not divide. In both strains, the sex muscles are not formed properly, and the hermaphrodites cannot lay their eggs.

The phenotype of the semidominant *hlh-8* animals is due to a point mutation in the basic DNA binding domain of CeTwist. The mutation does not abolish DNA binding by CeTwist so we propose that the protein is causing cellular defects by acting in a dominant negative fashion on CeTwist promoter targets. The dominant negative nature of the semidominant allele can be mimicked by expressing *hlh-8* with the point mutation in otherwise wild type animals. The resulting transgenic animals frequently have SMs that do not divide reminiscent of the original semidominant mutation. We observe this same phenotype in animals that express CeTwist with basic DNA binding domain mutations that are analogous to mutations found in the human Twist gene. In humans, these mutations result in Saethre-Chotzen syndrome. This syndrome is characterized by cranium defects (craniosynostosis) and digit anomalies in affected individuals. Similar defects are found in other craniosynostosis syndromes that are associated with downstream Twist target genes that have homologs in *C. elegans*. The study of the molecular mechanism of CeTwist function in target gene regulation is likely to be a good model for human craniosynostosis disorders.

Corsi, A.K., Kostas, S.A., Fire, A., Krause, M. 2000. *Caenorhabditis elegans* Twist plays an essential role in non-striated muscle development. *Development* 127:2041-2051.

278. Mesodermal cell fate specification in the postembryonic M lineage

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We are interested in understanding how mesodermal cell fates are specified. The postembryonic mesodermal lineage, the M lineage, provides a valuable system for these studies. During postembryonic development, the single blast cell M divides characteristically and reproducibly to generate 14 striated bodywall muscles, 16 non-striated sex muscles (vulval and uterine muscles), and two non-muscle cells (coelomocytes). Previous work from our lab and others has shown that correct patterning of the M lineage involves both lineage specific intrinsic factors and cell-cell signaling. We are interested in identifying and characterizing the functions of these factors in M lineage fate specification.

At the start of the M lineage, two Hox cluster genes *lin-39* and *mab-5*, as well as their cofactor, a homeodomain protein CEH-20 specify cellular identity and myogenic potential of M. We have found an overlapping function for *lin-39* and *mab-5* in diversification of different cell fates within the lineage, and have shown that the C. elegans twist ortholog *hlh-8* is a direct target of these factors in the M lineage.

Using gfp-based mutagenesis screens, we have isolated 26 additional mutants that have defects in M lineage patterning (12 of them came from a screen carried out by Judy Yanowitz in the lab). Three of these mutants turned out to be allelic to *sma-9*, and showed intriguing genetic interactions with *lin-12(0)*. We are in the process of characterizing these and other mutants isolated from the screens.

279. Bt toxicity through a genetic lens: *bre-5* and carbohydrate modification

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The naturally occurring protein toxins of *Bacillus thuringiensis* (Bt toxins) are effective insecticides which act through the binding of host gut epithelial cells. Transgenic corn and cotton plants that express these toxins provide continual, environmentally safe protection against insect pests and last year about 20 million acres of Bt transgenic crops were planted. In spite of such extensive use, very little is known about how these toxins actually function, and their long-term effectiveness is threatened by the development of resistant insects. We have established *C. elegans* as the only organism which is both susceptible to Bt toxins and amenable to rapid molecular genetic analysis of toxin resistance. Our reference toxin is Cry5B, and we have identified five *C. elegans* loci which are required for Cry5B toxicity. One of these genes, *bre-5*, has been cloned and characterized. *bre-5* encodes a glycosyltransferase with strong similarity to mammalian beta1,3galactosyltransferases. This result suggests that Cry5B (like the toxins of cholera and other pathogens) requires the presence of specific carbohydrate moieties on host cells, and that the *bre-5* gene product is responsible for such a structure. The presence of lectin-like domains in the three-dimensional structures of other Bt toxins further supports this concept. In testing our hypothesis, we have found that BRE-5 is expressed in the gut (the same cells on which the Cry5B toxin appears to act). Mosaic analysis has shown that wild type *bre-5* is required only in gut cells for animals to be sensitive to the toxin. We have also investigated how toxin interacts with the *C. elegans*gut. Fluorescently labeled Cry5B is rapidly taken up into gut granules, presumably because it binds to receptors and becomes internalized. *bre-5* animals do not show such uptake of fluorescent toxin into their gut. These and other observations strongly suggest that *bre-5* animals block the association and integration of toxin into apical membranes of gut cells. *bre-5*constitutes the first line of

genetic evidence that Bt toxins require host carbohydrate modifying enzymes for their action, and provides the first genetically-backed model for Bt toxin resistance in any organism.

280. A putative GDP-GTP exchange factor is required for development of the excretory cell

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The molecular controls governing the formation of organ structure during development are not well understood. The *Caenorhabditis elegans* excretory cell extends tubular processes, called canals, along the basolateral surface of the hypodermis and mutations in the *exc-5* gene cause tubulocystic defects at the distal tips of the canals.

Here we report that *exc-5* encodes a protein homologous to guanine nucleotide exchange factors (GEFs) and contains, in order, a Dbl/Pleckstrin homology (DH/PH) domain, a cysteine-rich FYVE domain and a second PH element. This motif architecture is similar to that of FGD1, which is responsible for faciogenital dysplasia or Aarskog-Scott syndrome[1]. Ultrastructural analysis suggests that EXC-5 is required for the proper placement of cytoskeletal elements at the apical epithelial surface. *exc-5* interacts genetically with *mig-2*

encoding a Rho GTPase. Overexpression of *exc-5* rescues the apical defect but causes defects at the basolateral surface of the excretory cell. These results suggest that EXC-5 controls the structural organization of epithelia by regulating Rho family GTPase activity.

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281. The ELAV Orthologue EXC-7 Binds *sma-1* mRNA to Regulate Excretory Canal Diameter

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The excretory cell forms the major component of the osmoregulatory epithelium of *C. elegans*, the narrow tubular excretory canals that stretch the length of the organism. The Exc phenotype is characterized by failure of the apical cytoskeleton of this polarized epithelium during development; the lumen of the tubules subsequently swells into a series of fluid-filled cysts. The *exc-7* allele *rh252* is characterized by formation of small cysts throughout the canals, premature cessation of canal outgrowth, and termination of the tubules in a bolus of cysts usually positioned about midway in the body, the position of the posterior termini of the canals at hatch. A synergistic effect on canal structure, resulting in very large cysts at the cell body, is seen in animals doubly mutant for *exc-7* and *sma-1*. *sma-1* has been shown to encode the apically-located cytoskeletal protein β_H-spectrin (McKeown *et al.*, '98).

We previously determined that *exc-7* encodes the nematode homologue of the mRNA-processing protein ELAV. Here we show that the *rh252* mutation eliminates a critical RNA-recognition motif of ELAV, and that EXC-7 protein binds to the 3'UTR of *sma-1* mRNA. In addition, a rescuing GFP construct of *exc-7* is expressed in the excretory cell soon after the cell is born during development, but surprisingly is not expressed during late embryogenesis or early larval stages when the Exc phenotype appears. We believe that EXC-7

is used to build a reserve of *sma-1* spectrin mRNA within the excretory cell for later use in larval development when *sma-1* transcription is insufficient to match the synthetic needs of the rapidly extending canals. Synergistic effects of *exc-7*(*rh252*) on excretory canal diameter are seen with other genes as well, which provides a genetic method for finding other mRNAs regulated by ELAV.

282. Beta-spectrin and synaptic vesicle release

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Spectrin is thought to mediate interactions between proteins and cellular membranes. Specifically, spectrin interacts directly with membranes via its phospholipid-binding PH domain. Spectrin is localized to plasma and other membranes at distinct sites in most cells, and may mediate specific protein-lipid complexes at each of these sites. In *C. elegans*, beta-spectrin is encoded by the *unc-70* gene.

To determine whether beta-spectrin is an essential protein-lipid mediator, we have generated animals that only express mutant forms of beta-spectrin in which the phospholipid-binding PH domain has been deleted (UNC-70ΔPH). *unc-70* null mutants are dumpy, paralyzed and have a severely disorganized nervous system. Surprisingly, UNC-70ΔPH animals are not dumpy and do not have neuronal outgrowth defects. Further, antibody staining shows that UNC-70ΔPH is normally localized. In contrast to several published models, these data indicate that the beta-spectrin PH domain is not required to localize UNC-70 and that this domain is not essential for many beta-spectrin functions.

However, UNC-70ΔPH animals are uncoordinated and hypersensitive to aldicarb, suggesting that the beta-spectrin PH domain is essential for limiting the release of synaptic vesicles from nerve terminals. Interestingly, these phenotypes are identical to the phenotypes of the dominant alleles of *unc-70*. These mutations cause non-conservative substitutions in the 17th spectrin repeat: near, but not in, the PH domain. Thus, the 17th spectrin repeat and the PH domain seem to share a function. How are these mutations causing excess synaptic vesicle exocytosis? Both regions may be required for lipid binding, or both may be required for binding of a specific protein.

To test the lipid-binding model, we are investigating retrograde transport in UNC-70ΔPH animals. Beta-spectrin is thought to function in retrograde transport by linking the dynein/dynactin complex to cargo vesicles via its PH domain. Defects in retrograde transport could lead to the accumulation of synaptic vesicles or other components of release at the nerve terminal. We are using *in vivo* retrograde transport assays and genetic interactions to test for retrograde transport defects in UNC-70ΔPH animals. As expected, these animals are not Dyf, demonstrating that retrograde transport of protein cargos does not require the beta-spectrin PH domain. Finally, we are generating point mutations in the PH domain that specifically abolish lipid binding.

To test the protein-binding model, we are assessing how these mutations affect binding of the several proteins that are known to bind spectrin in this region. We are also testing whether these mutations cause changes in the distribution of synaptic vesicles by quantifying synaptic vesicle distribution in the dominant alleles by serial section electron microscopy. Together, these results should provide insight into how the beta-spectrin PH domain functions in synaptic vesicle release.

283. UNC-16, A JNK signaling scaffold, regulates vesicle transport in *C. elegans*

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Localization of synaptic components must be both precise and dynamic to allow proper information flow between neurons and their targets. In first larval stage (L1) animals, the GABAergic DD motor neurons form synapses with ventral body wall muscle, and the localization of SNB-1::GFP, a synaptic vesicle marker, is restricted to the ventral processes. Loss-of-function mutations in the *unc-16* gene result in the mislocalization of SNB-1::GFP to dorsal dendritic processes of L1 stage DD neurons without a detectable change in a postsynaptic GABA receptor marker in the muscle. The localization of presynaptic markers in many neurons is similarly altered in *unc-16* mutants. SNB-1::GFP is mislocalized to the dorsal dendritic processes of the VD motor neurons and out along the dendritic endings of several sensory neurons in the head. Intriguingly, GLR-1::GFP, a postsynaptic marker, is also mislocalized along the ventral cord and in the anterior endings of some interneurons.

unc-16 encodes a protein homologous to mouse JSAP1/JIP3 and Drosophila Sunday Driver (dSYD). Like its mammalian homologs, UNC-16 physically interacts with JNK signaling components. Deletion mutants of KNK signaling genes result in slight mislocalization of synaptic vesicle markers in L1 DD motor neurons and exacerbate the phenotype of weak *unc-16* alleles. UNC-16 and JNK signaling molecules are expressed in neurons and function cell autonomously in DD neurons. Our results suggest that UNC-16 may regulate synaptic vesicle transport through its combined

interactions with both JNK signaling molecules and motor proteins.

In support of a role for UNC-16 in regulating vesicle transport, *unc-16* mutations partially suppress mutations in *unc-104*, a synaptic vesicle specific kinesin. The suppression is not allele specific, suggesting that the interaction of *unc-16* and *unc-104* may not be direct, but that both UNC-104 and UNC-16 may contribute to a similar process. Mutations in *unc-16* do not suppress *unc-116* kinesin heavy chain mutations. Instead, a weak *unc-116* mutation results in SNB-1::GFP mislocalization in L1 DD motor neurons similar to that seen in *unc-16* mutants. We propose that UNC-16 functions by both forming a linkage between vesicular cargo and motor proteins and serving as a scaffold for signaling molecules that direct cargo selection, motor activity, or motor direction.

284. RME-1, a novel EH domain protein required for endocytic recycling in *C. elegans* and mammalian cells

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The endocytic pathway is essential for the internalization and trafficking of macromolecules, fluid, membranes, and membrane proteins. The steps involved in uptake and endocytic trafficking within the endosomal system have been described, but many of the components mediating these steps at the molecular level remain to be identified. In particular, little is known about the proteins required for endocytic recycling, a critical membrane trafficking event in all cells.

Functional characterization of a new endocytosis gene, *rme-1*, by *C. elegans* genetics, suggests that it functions in endocytic recycling. *rme-1* mutants display endocytosis defects in several cell types. These defects include strongly reduced uptake of yolk proteins by oocytes, a receptor-mediated process. In *rme-1* mutants, yolk receptors accumulate in an abnormally expanded cortical endosomal compartment, implicated in recycling. *rme-1* mutant intestinal cells display an abnormal accumulation of basolateral, but not apical, fluid-phase endocytosis markers in large vacuolated structures. Uptake and trafficking of the membrane dye FM 4-64 from the basolateral membranes of the intestine is unaffected by *rme-1* mutations, suggesting that membrane internalization and late endosomal trafficking are independent of *rme-1*.

RME-1 is a member of a new class of proteins, evolutionarily conserved among multicellular organisms, bearing an amino-terminal P-loop domain, a central coiled-coil domain, and a

carboxy-terminal EH domain. Yeast two-hybrid analysis indicates that the P-loop and coiled-coil regions are required for multimerization. RME-1 is a cytoplasmic protein associated with the periphery of endocytic organelles, consistent with a direct role for RME-1 in endocytic trafficking.

We find that Ce-RME-1 and mammalian Rme-1 (mRme-1) expressed in CHO cells localizes primarily to the endocytic-recycling compartment (ERC). In cells expressing dominant negative mRme-1 G429R, a mutation near the EH domain equivalent to a worm dominant negative *rme-1* mutation, we identified a kinetic slowing of transferrin (Tf) recycling with no apparent alteration of the rate constant for Tf internalization. In addition, TGN38 delivery to the TGN from the ERC was slowed in mRme-1 G429R expressing cells. Together, these data suggest that RME-1 functions specifically in the export of molecules from the ERC.

285. Parallels between Mitochondrial Division and Endocytosis

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Recently it has become clear that mitochondria are filamentous structures that continuously divide and fuse with one another throughout the life of a cell. We have been studying mitochondrial division in the *C. elegans* body wall muscle cells using organelle specific GFP markers. This cell type is ideally suited to look at subtle changes in mitochondrial morphology as the mitochondria are planar and thus easy to focus on using fluorescence microscopy, are sufficiently large in size, and are distributed evenly throughout the muscle cell. We first studied DRP-1, a homologue to the endocytosis protein, dynamin (DYN-1). By expressing both *drp-1*::GFP along with each of several organelle markers under the body wall muscle specific promoter, *myo-3*, we found that DRP-1 was localized to the mitochondria. We then found that expression of a dominant negative form of DRP-1 blocks division of the mitochondrial outer membrane but division of the inner membrane still occurs. Furthermore, overexpressing *drp-1* brings about more mitochondrial division. We concluded that DRP-1 acts to bring about division of the mitochondrial outer membrane. Additional confirmation was given by time-lapse observations of DRP-1 at sites where mitochondrial fission.

Dynamin proteins act in both mitochondrial division and endocytosis. The prominent role of the same type of protein in these processes suggests that they may share a common evolutionary origin. It is not difficult to imagine that the first endosymbiotic bacteria used dynamin of the host cell during entry at the plasma membrane and used it again during the division of its outer membrane. Dynamin may not have only been the only protein used in this process as several additional proteins act along with dynamin in the endocytic process. To investigate whether these or similar proteins also play a role in mitochondrial division, we

have been expressing antisense and snapback constructs of them to see if we can replicate the *drp-1* loss-of-function phenotype observed. In preliminary results we have seen aberrant mitochondria in synaptojanin antisense but not endophilin antisense. However, we have seen abnormal mitochondria with antisense of an endophilin-related protein. The phenotypes seen suggest a mild division defect but we are seeking a stronger division defect through the use of double mutants and snapback constructs as an alternative to antisense. We are then planning to determine at what stage these proteins act in relation to DRP-1.

286. Targeting of rough endoplasmic reticulum membrane proteins and ribosomes to the cell body of *C. elegans* neurons

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The endoplasmic reticulum (ER) is a continuous membrane system that contains specialized domains. The rough endoplasmic reticulum (RER) shares most proteins with the smooth endoplasmic reticulum (SER), but is enriched in a set of proteins that translocate secretory and membrane proteins across the lipid bilayer. We studied targeting of RER membrane proteins within the ER in *C. elegans*. In several cell types fluorescent protein fusions to RER and general ER proteins colocalized, but in neurons RER markers were concentrated in the cell body while general ER markers were found in the neurites as well as the cell body. To determine whether RER membrane proteins were localized to the cell body by immobilization we compared the diffusion of RER and general ER membrane proteins. Surprisingly both types of proteins were equally mobile. We also examined whether the distribution of ribosomes was as restricted as RER membrane proteins. By light and electron microscopy, ribosomes were extremely concentrated in the cell body and rare in neurites. This indicates that, like mammalian axons, *C. elegans* neurites largely exclude protein synthetic machinery.

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287. FUSOMORPHOGENESIS: A TALE OF MONSTERS, ELF TAILS AND COLD FUSION

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In every *C. elegans* hermaphrodite 300 sperm cells fuse with oocytes in the first step of development and 300 somatic cells fuse to invariably form 44 syncytia during organogenesis [1, 2]. To study the mechanisms of cell fusion in *C. elegans* and to use this knowledge to understand the function of cell fusion during animal development we propose to test the fusomorphogenetic hypothesis: membrane fusion facilitates changes in cell shapes that drive pattern formation and generate diversity of development [2].

To identify and characterize intermediates in the complex process of cell fusion we used permeabilized embryos [3,4] that develop to MONSTERS where hypodermal cell fusion was followed using fluorescent probes. We imaged morphogenesis and compared cell fusion in MONSTERS to syncytia formation in intact embryos. In these systems we can block cell fusion using low temperatures, by mutations and by changing the lipids composition of the plasma membranes. These approaches have been successfully used before to arrest viral membrane fusion and we are applying them to study cell fusion in elongating embryos.

Since low temperature is known to affect the fluidity of the membranes and is also an inhibitor of membrane fusion events, we looked for a temperature that blocks cell fusion and uncouple it from embryonic elongation. We found that embryos that were grown at 9° C, develop and elongate normally until they arrest at two-fold with lack of epithelial fusions. We also found that the rate of cell fusion in the dorsal hypodermis is strongly temperature-dependent between 10-25° C. Thus, at temperatures <8° C there is no elongation and no cell fusion in intact wild-type embryos. We will show the characterization of the COLD FUSION intermediate using analysis similar to the characterization of the frozen intermediate in Influenza HA-mediated fusion.

To test whether the rate of embryonic elongation (morphogenesis) is affected in conditional mutants defective in epithelial cell fusion, we measured the rate of embryonic elongation in *eff-1(hy21ts)* and *duf-1(zu316cs)* and compared them to wild-type embryos at the respective restrictive temperatures. We found that the elongation rate of *eff-1* is 2.3-fold slower than in wild-type and that the elongation rate of *duf-1* is 15-fold slower than in wild-type. These and other kinetic and genetic results suggest that cell fusion is required for rapid and efficient embryonic elongation. In addition, newly hatched larvae in *eff-1* backgrounds are lumpy-dumpy and have protuberances in the tail that makes them look like an ELF [5]. In these *eff-1* larvae grown at the permissive temperature there is a dramatic correlation between the presence of lumps and the localization of unfused cells. As predicted by the fusomorphogenetic hypothesis, failure of epithelial fusions results in abnormal morphogenesis and deformities from larvae to adults. Thus, cell fusion is necessary but not sufficient to allow normal morphogenesis. We show that kinetic analysis of cell fusion in intact embryos and permeabilized monsters can be used to characterize morphogenesis in combination with pharmacological and genetic approaches. The intermediates in the process of cell fusion isolated using these methods will be analyzed using electron microscopy and fluorescent techniques [6] to determine what are the rate limiting steps in the process of cell fusion.

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288. THE GENE *eff-1* IS REQUIRED FOR CELL FUSION IN EPITHELIAL SYNCYTIA

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Cell fusion is a major factor in the development of the *C. elegans* body plan. As the molecules and physical mechanisms responsible for cell membrane fusion are unknown in any developmental system, we have focused on *C. elegans* as a uniquely suited model for genetic and structural studies of the process of cell fusion. Some of the advantages of studying cell fusion in the worm are: A) the predictable postion and timing of cell fusion events based on the lineage of precursor cells [1]; B) the amenability of embryos to live-imaging and EM studies of the intermediate structural stages in a cell fusion event [2]; C) the surprising viability of worms with genetic defects in cell fusion [3,4].

In two distinct screens - one for embryonic hypodermal fusion defects and another for vulval morphogenesis/fusion defects - we have isolated recessive mutant alleles of a gene that is required for cell fusion among the precursors of epithelial syncytia [3,4]. Live-imaging experiments confirmed that correctly patterned syncytial precursors are blocked at the earliest stages of cell fusion in these mutants, before formation of an initial cytoplasmic bridge between partner cells. Mutations *oj55* and *hy21* both lie on Chromosome II and fail to complement in the trans-heterozygote. Allele *oj55* was further mapped to postion +0.79, and was rescued by injection of cosmid C26D10. This cosmid also rescues *hy21*, suggesting that both mutations lie within the same gene, which we have named *eff-1* (Epithelial Fusion Failure). Preliminary results from dsRNAi and a single rescue injection experiment appear to confine the functional *eff-1* gene to a 12.5 kb region of C26D10. Confirmation of the identity of the gene and characterization of the molecular lesions underlying *eff-1(oj55)* and *eff-1(hy21)* will be presented at the 13th International *C. elegans* meeting.

The two *eff-1* alleles vary in the numbers of unfused cells per animal and in the overall effects on body morphology: *hy21* has a stronger loss of function than *oj55*. Yet it appears that all morphogenetic defects in both mutants can be attributed to failed cell fusions [3,4]. Other aspects of hypodermal and vulval patterning and differentiation appear normal. We therefore believe that the *Eff-1* gene product functions specifically within the mechanism controlling the fusion of plasma membranes on neighboring cells. To test this hypothesis we will compare the developmental expression pattern of *eff-1* with the known temporospatial pattern of embryonic and larval cell fusions [1]. Furthermore, we will examine the dynamic sub-cellular localization of *Eff-1* protein to assess whether it appears at the known sites for initiation and widening of the opening between actively fusing cells [2].

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289. Chromosome-Wide Regulation of Meiotic Crossing Over

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Reciprocal recombination events between homologous chromosomes, and the resulting chiasmata, are necessary for proper chromosome segregation at the first meiotic division in almost all eukaryotes. Interestingly, however, most organisms allow only a limited number of exchanges per homolog pair per meiosis. This indicates that the process of crossing over must be tightly regulated to ensure that each homolog pair enjoys at least one and only a few crossovers per meiosis. For example, despite a physical size range that varies almost 2-fold, each of the six holocentric *C. elegans* chromosomes has a genetic length of rougly 50 cM, indicating that each chromosome pair normally undergoes only one crossover event per meiosis. The distribution of exchanges along eukaryotic chromosomes is also non-random. On *C. elegans* autosomes, the majority of exchanges occur in the terminal 50% of the chromosome (the "arms") and there is a low frequency of exchanges in the central 50% (the "central gene cluster"). The reduced rate of exchange in the central cluster may be intrinsic - due to the presence or absence of specific sequence elements in the region - or may result from chromosome-wide mechanisms that favor "off-center" exchanges - *e.g.* recombination may be more frequent near chromosomal ends.

We have investigated the contributions of sequence-intrinsic and chromosome-wide regulatory mechanisms to crossover control by examining the meiotic behavior of end-to-end chromosome fusions in hermaphrodites and males.

The genetic maps of mnT12 and eT6, two X:IV fusions that differ in the orientation of IV, have been completed. The map length of each of the fusion chromosomes is roughly 50 cM, the same length as a single wild-type chromosome. This surprising result indicates that the two fused chromosomes are now being perceived as a single chromosome by the organism and further implies that exchanges are limited by a chromosome-wide interference mechanism. The distribution of exchanges along the fusion chromosomes relative to wild-type suggests that the autosomal gene clusters are largely, but not entirely, due to local sequence-intrinsic effects. We have also characterized the meiotic behavior of a "megasome", a fusion of three complete chromosomes; in strains homozygous for this fusion, almost half of the genome is contained within a single chromosome. The partially completed genetic map reveals that the three-chromosome fusion has an apparent genetic length of 50-75 cM, indicating that the forces that exert crossover regulation are capable of acting over half the genome.

Examination of crossing over and chromosome segregation during meiosis in males heterozygous for mnT12 suggests that crossover control is exerted only along regions capable of homologous synapsis. The genetic length of the chromosome IV portion of mnT12 in heterozygous males is approximately 50 cM (similar to the genetic length of wild-type IV), suggesting that the partnerless X chromosome portion of mnT12 in these crosses does not influence the genetic length of the synapsis-competent chromosome IV portion. This in turn suggests that chromosomal regions incapable of homologous synapsis are not recognized as "chromosome" by the chromosome-wide mechanism that regulates crossover frequency during meiosis.

Our results have demonstrated the existence of both chromosome-wide and sequence-intrinsic forms of crossover control in *C. elegans*. While crossover distribution seems to be largely under the control of sequence-intrinsic regulation, a robust, chromosome-wide mechanism, capable of acting over half the genome, regulates the frequency of crossing over. Our results also suggest that only chromosomal regions capable of homologous synapsis influence crossover frequency, lending credence to models wherein the control of crossover frequency is exerted along regions of homologous synapsis, and perhaps along the synaptonemal complex itself.

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290. AIR-2 and CeGLC-7 Function in Chromosome Cohesion in Meiosis

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Chromosome cohesion is important for the faithful segregation of chromosomes during mitosis and meiosis. Chromosomes are initially held together by the cohesin complex and later pulled apart when the complex is destroyed in anaphase. How cohesion is regulated in a cell-cycle-dependent fashion is largely unknown. We report here the aurora-/Ipl1p related kinase AIR-2 and opposing phosphatase CeGLC-7 function in the regulation of cohesion in meiosis.

In *air-2(RNAi)* animals, both meiosis and mitosis are defective. These defects can be suppressed when the opposing phosphatase *Ceglc-7*α or *Ceglc-7*β is also removed by RNAi, suggesting that the balance of the phosphorylation state of a shared substrate(s) is essential for mitosis and meiosis. One such substrate is histone H3. Phosphorylated H3 (H3P) is not detected in *air-2(RNAi)* animals but is present in the *Ceglc-7*α-suppressed animals. Histone H3 phosphorylation has been implicated in chromosome condensation during mitosis and meiosis in other organisms. Mitotic chromosome condensation is drastically affected in *air-2(RNAi)* animals. However, meiotic chromosome condensation appears normal in *air-2(RNAi)* animals, suggesting that AIR-2 and H3P have distinct functions during meiosis.

To address the function of AIR-2 in meiosis, we performed 4-D imaging of *air-2(RNAi)* embryos using a reporter strain carrying histone H2B-GFP. We observed that the 6 bivalents are aligned properly in metaphase but fail to separate during anaphase in *air-2(RNAi)* embryos. After two attempts at separation, the 6 bivalents decondense and the oocyte nucleus fuses with the sperm pronucleus, resulting in polyploid embryos. An opposite result was obtained when we imaged embryos from *Ceglc-7*α*(RNAi);Ceglc-7*β*(RNAi);H2B::GFP* animals. The *Ceglc-7*α*(RNAi); Ceglc-7*β*(RNAi)* embryos are normal during metaphase at which time 6 bivalents are clearly observed. However, the 6 bivalents rapidly fall apart into 24 individual chromatids at the onset of anaphase. These results together suggest that AIR-2 and CeGLC-*7* regulate a common substrate(s) functioning in anaphase for chromosome separation.

The notion that AIR-2 phosphorylates a substrate required for chromosome separation in anaphase is supported by its sub-chromosomal localization. We show that AIR-2 localizes to the site between each pair of homologs in metaphase I and to the site between each pair of sister chromatids in metaphase II. Identical localization was observed with an antibody to BIR-1, a protein previously shown to be required for AIR-2 chromosomal localization. Interestingly, H3P staining is also focused intensely at these same sites in both MI and MII. When chromosomes are separated in anaphase, H3P staining remains on the inside faces of the separating chromosomes. Efforts are underway to investigate whether a mutation in *spo-11*, which results in chromosome non-disjunction during meiosis, can suppress the *air-2(RNAi)* meiotic defect specifically in meiosis I but not meiosis II.

The chromosomal localization of AIR-2 and BIR-1 are first detected in the -1 oocyte, later than H3P which first becomes detectable in the -4 oocyte. In oocytes prior to final chromosomal condensation and shortening, it is observed that H3P staining is concentrated at sub-chromosomal foci corresponding to the short arms of each bivalent. These sub-chromosomal foci are indeed the sites between each pair of homologs as the chromosomes condense and shorten. Using mutations that produce non-disjoined univalents, such as *him-5, him-8, her-1, spo-11,* and *him-14*, we showed that the sub-chromosomal foci observed with H3P, AIR-2 and BIR-1 antibodies are specific to bivalents and not univalents when only one or two univalents are present. The staining is, however, detected to a lesser extent on univalents in those strains where most or all chromosomes are univalents. We propose that H3 phosphorylation on diakinetic bivalents helps recruit BIR-1 and AIR-2 to the same sub-chromosomal location in the -1 oocyte. The localization of AIR-2 at the cohesion sites results in the phosphorylation of a yet unidentified substrate leading to chromosome separation at anaphase. The identity of the

substrate(s) is currently under investigation. One possible candidate substrate is a component of the cohesin complex.

291. Molecules underlying meiotic nuclear reorganization and the homolog pairing process.

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To segregate to opposite poles of the meiosis I spindle, homologous chromosomes must locate, recognize, and form stable associations with their appropriate partners. Using cytological tools to screen for mutants defective in homolog pairing, we identified two classes of genes that define separable components of the chromosome pairing process during meiosis.

One class of genes (*chk-2*, *hal-2*) is required for the initial establishment of homolog pairing. Genes of this class are also required for the dramatic spatial reorganization of meiotic nuclei that normally accompanies initial pairing, revealing a genetic/molecular link between these conserved early prophase events. *chk-2* encodes a *C. elegans* member of the Cds1/Chk2 family of checkpoint protein kinases, which are known to function in signaling pathways that safeguard genomic integrity, particularly during S phase. Germline nuclei in *chk-2* mutants retain function of checkpoint responses to ionizing radiation and respond normally to replication inhibition, but are defective in triggering a checkpoint response to an intermediate block in the meiotic recombination pathway. Despite failures in pairing, nuclear reorganization and crossover recombination, *chk-2* mutants undergo many other aspects of meiotic chromosome morphogenesis and complete gametogenesis. We propose that *chk-2* functions during premeiotic S phase to couple premeiotic replication with meiotic prophase nuclear reorganization and homolog pairing.

A second class of genes, defined by *syp-1* and *syp-2*, is required to stabilize homolog associations subsequent to initial pairing. SYP proteins contain extended coiled-coil domains and localize at the interface between lengthwise-aligned, synapsed chromosomes during the pachytene stage of meiotic prophase. These features suggest that SYP proteins are structural components of the synaptonemal

complex (SC), a structural hallmark of synapsed chromosomes. Crossover formation is severely reduced in *syp-1* mutants, indicating that initial pairing between homologs is not sufficient to allow exchange, and implicating a role for the SC in promoting and/or regulating crossover recombination. Opposite ends of a single chromosome exhibited different pairing dynamics throughout prophase in *syp-1* mutants, bolstering prior notions that synapsis along a chromosome in *C. elegans* initiates at the "pairing center" end. Further, the polarized nuclear organization characteristic of the onset of meiotic prophase persists in *syp-1* mutants, consistent with the idea that polymerization of the SC provides either a direct motive force or a signal that drives redispersal of chromosomes within the nucleus as synapsis is completed.

Our characterization of mutants defective in meiotic chromosome pairing has revealed genes required for two distinct phases of the pairing process: the initial establishment of homologous associations between chromosomes, and the maintenance of homolog associations in a context supportive to crossover recombination. These analyses led to the discovery of an unanticipated role for a Cds1/Chk2 protein in promoting early meiotic nuclear events, and have begun to reveal information about the functional importance of the SC as well as the mechanics of synapsis between meiotic chromosomes in *C. elegans*.

292. Mitotic Chromosome Condensation and Segregation by a Conserved Protein Complex

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The ordered compaction of chromosomes into two condensed and paired sister chromatids is a structural problem of all mitotic cells. If chromosomes fail to establish cohesion or to condense, they can become entangled, and break or missegregate at anaphase. The highly conserved SMC (structural maintenance of chromosomes) protein family directs these critical aspects of chromosome segregation. SMC proteins generally associate with an SMC partner and non-SMC proteins to form large complexes. An SMC complex called condensin was described in other organisms for its role in condensing mitotic chromosomes. In *C. elegans*, a homologous complex directs X dosage compensation. We are studying the composition and function of SMC complexes, and have found individual SMC proteins that participate in more than one chromosomal process. For example, MIX-1 is a condensin SMC homolog essential for both mitosis and dosage compensation. How does MIX-1 achieve its dual function within a single cell? MIX-1 requires the SMC protein DPY-27 for its role in dosage compensation and its X localization, but DPY-27 plays no role in mitosis. Thus, it seemed likely that MIX-1 would have a different SMC partner for mitosis. Searching the genome revealed another DPY-27-like SMC protein, CeSMC-4.

The RNAi phenotype and protein localization of CeSMC-4 indicate its involvement in mitosis. Like MIX-1, CeSMC-4 RNAi produces dead embryos with defects such as anaphase chromatin bridges and extremely large nuclei. In support of the idea that CeSMC-4 and MIX-1 are SMC partners for mitosis, MIX-1 protein is undetectable in CeSMC-4 RNAi embryos. Using histone::GFP and time-lapse microscopy, we see that chromosomes are poorly organized as they condense at prophase and metaphase, remain entangled at anaphase, and eventually become pulled into one cell which continues

more cycles of apparent replication without division. Meiotic chromosome segregation defects are also observed. In contrast to the expectation from studies in *Xenopus*, loss of *C. elegans* condensin causes chromosomes to be poorly organized but still substantially compacted, and the more dramatic phenotype is instead the chromatin bridges at anaphase.

CeSMC-4 and MIX-1 associate with chromosomes only during stages of the cell cycle when chromosomes are condensed. The mitotic kinase AIR-2, responsible for the mitosis-specific phosphorylation of histone H3, is required for CeSMC-4 and MIX-1 localization. CeSMC-4 and MIX-1 proteins do not coat the entire metaphase plate, but rather appear on the poleward face in a punctate pattern coincident with the centromere protein HCP-3, and internal to the kinetochore protein HIM-10. However, localization of these proteins is not interdependent.

Immunoprecipitation (IP) experiments substantiate that MIX-1 partners with CeSMC-4 for mitosis, but DPY-27 for dosage compensation. Co-IP from embryonic extracts is observed between CeSMC-4 and MIX-1, but not between CeSMC-4 and DPY-27. Moreover, IPs show that CeSMC-4 and MIX-1 are part of a large protein complex whose additional components are being investigated by mass spectrometry. One subunit called CeCAP-D2 (see R. Chan et al.) shares homology with both a conserved non-SMC component of the mitotic complex in other organisms, and a component of the *C. elegans* dosage compensation complex. In collaboration with the Cozzarelli lab, the mitotic complex was shown to drive ATP-dependent positive DNA supercoiling *in vitro*, a conserved activity thought to reflect its role in chromosome condensation. It will be interesting to learn if the mitotic and dosage compensation complexes share additional components, and to what extent the biochemical activities of these two condensin-like complexes are related.

293. Chromosome Counting to Determine Sex: X and Autosomal Signals

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In *C. elegans*, sex is determined by a chromosome counting mechanism that senses the ratio of X chromosomes to sets of autosomes. The low X:A ratio in XO diploid worms permits high expression of the sex switch gene *xol-1* and hence male development. Conversely, a high X:A ratio in XX animals results in low *xol-1* activity and hermaphrodite development. Although the autosomal component of the sex signal is uncharacterized, the X portion is comprised of several X-signal elements (XSE) that act cumulatively to repress *xol-1* function in a dose-dependent manner. A mere two-fold difference in the dose of XSEs is translated to a greater than ten-fold difference in *xol-1* expression between the sexes. The molecular identities of two XSEs have been determined. SEX-1 is a nuclear hormone receptor that represses *xol-1* transcriptionally, and FOX-1 is an RNA-binding protein that represses *xol-1* post-transcriptionally. We report the molecular mechanism by which FOX-1 represses *xol-1* and the identification of two new putative XSEs. Additionally, we report the initial characterization of the autosomal sex signal.

Our transgenic studies have shown that FOX-1 regulates *xol-1* expression through *xol-1*s sixth intron, which is both necessary and sufficient for repression. Overexpression of FOX-1 prevents removal of the sixth intron *in vivo*. The sixth intron can confer FOX-1-mediated repression of a reporter gene. Recombinant FOX-1 binds directly to the sixth intron *in vitro*. The region required for FOX-1 binding has been localized to two separate 37-nucleotide regions with extensive sequence overlap. The contribution of these binding sites to *xol-1* regulation is being analyzed *in vivo*. Together, these data indicate that FOX-1 represses *xol-1* directly by preventing proper splicing of its sixth intron.

Two new putative XSEs have also been identified. An XSE duplication strain that is 100% XO-lethal was screened by feeding double-stranded RNA from half of the ~140 ORFs in a region of X known to contain at least one XSE. RNAi of *ceh-39*, a member of the evolutionarily conserved ONECUT homeodomain family, suppresses the male lethality of this duplication and strongly enhances the sex determination and dosage compensation phenotypes of *fox-1* and *sex-1*. A genetic screen of the XSE duplication strain for suppressors of XO lethality identified three suppressors, *y323*, *y324*, and *y326*. These alleles have synergistic sex determination and dosage compensation defects in combination with *fox-1* or *sex-1* and map to a small region of X not previously known to contain an XSE.

The sex signal also consists of an autosomal component whose precise nature is unknown but presumably serves to increase the activity of *xol-1*. Discrete autosomal signal elements (ASE) that are analogous to, but act in opposition to, the XSEs may exist. If true, ASE loss-of-function mutations should suppress the complete XX lethality caused by mutating the two XSEs *fox-1* and *sex-1*. To test this hypothesis, we screened 4500 mutagenized haploid genomes for suppressors of the hermaphrodite lethality of *fox-1 sex-1* double mutants. Of the 47 mutations isolated, including dominant and recessive X-linked and autosomal suppressors, seven autosomal alleles with strong suppression phenotypes are under analysis to determine their role in chromosome counting.

294. A novel Goloco motif protein is required for the asymmetric division of one cell stage embryos

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In one cell stage embryos, the spindle sets up in the cell center, but is displaced towards the posterior during anaphase. This results in an asymmetric first cell division that generates a larger anterior blastomere and a smaller posterior one. Posterior spindle displacement occurs in response to polarity cues set up along the antero-posterior (AP) axis by the PAR proteins. We have shown that stronger pulling forces act on the posterior spindle pole than on the anterior one, thus explaining the overall posterior spindle displacement during anaphase (1; see also abstract by Grill et al.). However, the components that allow such differential pulling forces to be exerted in response to polarity cues remain to be identified.

During a large-scale RNAi-based screen (2), we identified two genes that may play a role in this process: *ags-3.2* (F22B7.13) and *ags-3.3* (C38C10.4). Since *ags-3.2* and *ags-3.3* are nearly identical at the nucleotide level, both genes are likely to be inactivated following injection of either dsRNA species. In *ags-3.2* (RNAi) and/or *ags-3.3* (RNAi) embryos, there is only minimal posterior displacement of the spindle during anaphase, and the first division is almost symmetric. *In vivo* laser cutting experiments demonstrate that pulling forces acting on the two spindle poles are weak and essentially equal, thus explaining the lack of overall posterior displacement. AP polarity cues do not appear to be affected, since P granules seem to be correctly segregated to the posterior of these RNAi embryos. Therefore, *ags-3.2* and/or *ags-3.3* are required downstream of polarity cues to generate differential pulling forces on the anterior and posterior spindle poles.

ags-3.2 and *ags-3.3* encode essentially identical proteins with weak homology to mammalian AGS3 (activator of G protein signaling 3-hence the gene names) and a Goloco motif characteristic of GTPase activating proteins for heterotrimeric G protein alpha subunits. Interestingly, RNAi of two G protein alpha subunits in combination also results in a essentially symmetric first division (3). Our preliminary observations indicate that AGS-3.3 distribution is polarized during anaphase, suggesting that differential activation of G protein alpha subunits along the AP axis translates into the generation of differential pulling forces on the anterior and posterior spindle poles.

References:

1 Grill et al. Nature 409: 630-633 (2001)

2 Gönczy et al. Nature 408: 331-336 (2000)

3 Gotta and Ahringer Nature Cell Biol 3:297-300 (2001)

295. Forces responsible for asymmetric spindle positioning in single cell stage *C. elegans* embryos

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Cell divisions creating daughters of different sizes are crucial for the generation of cell diversity during animal development. In such asymmetric divisions, the mitotic spindle must be asymmetrically positioned at the end of anaphase. The mechanisms by which cell polarity translates into asymmetric spindle positioning remain poorly understood.

We examined the nature of the forces governing asymmetric spindle positioning in the single cell stage *C. elegans* embryo. In order to reveal the forces acting on each spindle pole, we removed the central spindle in living embryos either physically with a UV laserbeam, or genetically by RNA mediated interference (RNAi) of a kinesin. We show that pulling forces external to the spindle act on the two spindle poles. A stronger net force acts on the posterior pole, thus explaining the overall posterior displacement observed in wild-type embryos. Importantly, we further show that the net force acting on each spindle pole is under control of the *par* genes required for cell polarity along the anterior-posterior (AP) embryonic axis. In addition, we demonstrate that inactivation of heterotrimeric G protein alpha subunits results in a loss of external forces (see abstract by Colombo et al.), thus explaining the symmetric phenotype observed in these cases. In summary, our work suggests a mechanism for generating asymmetry in spindle positioning by varying the net pulling force acting on each spindle pole.

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296. *C. elegans* MUTANTS IN THE *muc-1* GENE PROVIDE A MODEL FOR THE HUMAN LYSOSOMAL STORAGE DISEASE MUCOLIPIDOSIS TYPE IV

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In a genetic screen seeking suppressors of the *ced-9*(*n1950gf*) allele, which blocks all programmed cell deaths, we identified a single mutation, *n3194*, that resulted in the increased accumulation of refractile corpses and caused recessive maternal-effect lethality. Two additional alleles, *n3264* and *zu223*, were isolated in screens for cell-corpse engulfment-defective mutants and maternal-effect lethal mutants, respectively (Z. Zhou and J. Priess, personal communications).

We found that the predicted gene R13A5.1 rescued the maternal-effect lethal phenotype of *n3194* mutants. R13A5.1 encodes a protein similar to that of the recently identified human mucolipidosis type IV (ML-IV) disease gene, and therefore we have named the gene *muc-1*. ML-IV is a human neurological disorder caused by accumulation of lipid and polysaccharide material in lysosomes. Unlike other lysosomal storage diseases, such as Tay-Sachs disease, in which either catabolic degradative enzymes or activator proteins are defective, ML-IV cells have normal catabolic activity. Instead, ML-IV cells appear to have a defect in the trafficking or sorting of macromolecules, leading to the observed accumulation.

We expressed the human gene (kindly provided by G. Borsani) in *C. elegans* and were able to rescue the maternal-effect lethal phenotype of *muc-1(n3194)*. Using the acidophilic dye Lysotracker Red, we found that *muc-1* mutant embryos accumulate excess lysosomes, just as is observed in the human disease. In addition, electron microscopy indicates that *muc-1* mutant animals contain enlarged vacuoles and lamellar bodies characteristic of the human disease.

Blocking programmed cell death, either by using mutations in the *ced-3* caspase or *ced-4* caspase activator or by overexpressing the p35 viral caspase inhibitor, did not fully block the maternal-effect lethality of *muc-1* mutants but did slightly increase viability. *muc-1* mutants contain an increased number of TUNEL-positive cells, suggesting an increase in programmed cell death, and *ced-3* and *ced-4* mutations block this increase in TUNEL-positive cells. However, as blocking programmed cell death has only a mild effect on the viability of *muc-1* mutants, we suggest that the observed increase in cell death in these mutants is a secondary defect, possibly triggered by the increased accumulation of lysosomal materials. Alternatively, mutations in *muc-1* may activate programmed cell death, or aspects of the cell death process, downstream of *ced-3* and *ced-4*.

A MUC-1::GFP translational fusion capable of rescuing *muc-1(n3194)* was localized to the excretory canal cell and several neurons in the head. Human ML-IV cells have defects in secretion, and the *C. elegans* cells in which MUC-1 is expressed may all have secretory functions for which MUC-1 is required. Proper function of the excretory canal cell is required for viability, so a defect in excretory cell function might be responsible for the lethality of *muc-1* mutants.

We have initiated a screen for suppressors of *muc-1(n3194)* lethality. Genes identified in this screen may define counterparts of the as yet unidentified pathway involved in the human ML-IV disease. We have thus far identified a single recessive suppressor.

We believe that we have developed an excellent *C. elegans* model for the human lysosomal storage disorder mucolipidosis type IV. The etiology of this disease is not yet understood at the molecular level, and we hope that our characterization of the *muc-1* pathway in *C. elegans* will provide a greater understanding of both the normal function of *muc-1* and how the perturbation of its human counterpart results in disease.

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297. Mutations in the *C elegans pqe-1* gene enhance polyglutamine-mediated ASH neurodegeneration

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At least eight hereditary neurodegenerative disorders, including Huntington's Disease, have been identified in which the disease locus expresses a protein that contains an expanded glutamine tract. The mechanism by which these polyglutamine (polyQ) repeats cause neurodegeneration and cell death is unknown. We have established a *Caenorhabditis elegans* model system to identify proteins involved in polyglutamine (polyQ) neurotoxicity. (PNAS 96, 179-184, 1999). N-terminal fragments of the Huntington's Disease protein huntingtin (Htn) containing polyQ tracts of 2, 23, 95 or 150 residues are expressed in the ASH sensory neurons. Expression of Htn-Q150, but not Htn-Q2, Htn-Q23 or Htn-Q95, cause age dependent ASH degeneration without ASH cell death in aged (8-day-old) animals. ASH neurodegeneration is determined by the ability of the exposed sensory endings of ASH neurons to uptake lipophilic dyes. ASH cell death is assessed by immunohistochemical techniques or GFP expression. PolyQ-mediated ASH neurodegeneration is partially dependent on *ced-3* caspase function suggesting the involvement of the apoptotic cell death pathway. To identify genes that normally protect neurons from polyQ-mediated neurodegeneration, we performed an F2 EMS screen for mutations in genes that exacerbate polyQ-mediated ASH neurodegeneration. After screening approximately 30,000 mutagenized animals, 7 mutant strains were identified that carried mutations in the *polyQ enhancer-1* (*pqe-1*) gene. Overt phenotypes have not been observed in *pqe-1* mutant animals other than enhancement of polyQ neurotoxicity. In *pqe-1* mutant animals, ASH neuronal cell death is dependent upon the presence of expanded polyQ tracts (Htn-Q95, Htn-Q150), as ASH neurons expressing N-terminal fragments of huntingtin with shorter polyQ tracts (Htn-Q2, Htn-Q23) are unaffected. In addition, *pqe-1* mutant animals are wild-type in two ASH mediated behavioral assays (nose-touch and osmotic avoidance). Differential splicing of *pqe-1* results in three nuclear localized PQE-1 proteins. PQE-1A and PQE-1B contain a putative exonuclease domain. PQE-1A and PQE-1C contain a large glutamine/proline-rich domain which is essential for *pqe-1* function. PQE-1 proteins may interact with expanded polyQ tracts or their pathological targets via the glutamine/proline domain to protect neurons from polyQ insults. Molecular and biochemical experiments are underway to address the connection between PQE-1 function and polyQ neurotoxicity. To identify genes that are required for *pqe-1* function or poly-Q mediated neurodegeneration, we have initiated a genetic screen to isolate mutations that suppress this polyQ-mediated ASH neurodegeneration. Identification and characterization of genes isolated from these genetic screens will provide insight into pathogenic mechanisms underlying polyQ-induced neurodegeneration and cell death.

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298. UNC-83 and UNC-84 function at the nuclear envelope where they are required for proper nuclear migration

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Nuclear migration plays an essential role in the growth and development of a wide variety of eukaryotes. Mutations in *unc-83* and *unc-84* disrupt nuclear migration in three *C. elegans* tissues, larval P cells, the embryonic hypodermis, and the developing intestinal primordium. P cell nuclei normally migrate during L1 from a lateral position to the ventral cord where they eventually give rise to the vulva and neurons. In embryos, nuclei in precursors of the dorsal hyp7 migrate across the dorsal mid-line, and during polarization of the intestinal primordium nuclei normally migrate towards the mid-line. Mutations in *unc-83* and *unc-84* disrupt hyp7 nuclear migrations at all temperatures. P cell nuclear migrations are effected only at higher temperatures, while intestinal nuclear migrations in are more severe at lower temperatures.

unc-84 encodes for a novel protein with a predicted trans-membrane domain. UNC-84 also has a C-terminal domain (termed SUN domain) similar to the *S. pombe* spindle pole body component Sad1p and uncharacterized proteins from human and *Drosophila*. UNC-84::GFP localizes to the nuclear envelope of most or all somatic cells. Mutations in *unc-84* also disrupt nuclear anchorage.

We have mapped and cloned *unc-83* and find that it encodes for a novel protein with a predicted C-terminal trans-membrane domain. We identified three *unc-83* transcripts that are expressed in a tissue-specific manner as confirmed by the identification of DNA lesions in 16 *unc-83* alleles and transcript specific RNAi experiments.

Monoclonal antibodies against UNC-83 co-localize to the nuclear envelope with lamin and UNC-84. Unlike UNC-84, UNC-83 localizes to only certain nuclei. UNC-83 was first observed to localize to the nuclear envelope of migratory hyp7 precursors in pre-bean embryos. At the bean stage UNC-83 was also observed in migratory intestinal cells and P cells. Later, UNC-83 was detected in a number of other cells including some pharynx and hypodermal cells. UNC-83 fails to localize to the nuclear envelope in *unc-84* mutants with lesions in the conserved SUN domain of UNC-84, suggesting that the role of UNC-84 in nuclear migration is to recruit UNC-83 to the nuclear envelope.

We have shown that centrosomes remain properly associated with nuclei despite the *unc-83* or *unc-84* nuclear migration defects. Additionally, mutations in *unc-83* or *unc-84* do not disrupt the gross structure of the nuclear matrix in migrating cells. We favor a model in which UNC-84 recruits UNC-83 to the nuclear envelope where they function to help transfer force between the cytoskeleton and the nucleus. We are currently attempting to immunolocalize UNC-83 by electron microscopy to further refine our model.

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299. THE ACTIN-BINDING PROTEIN UNC-115 ACTS DOWNSTREAM OF RAC-2 IN NEURONAL MORPHOGENESIS

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Previous studies indicate that three *rac* genes, *ced-10, mig-2* and *rac-2*, act redundantly in axon pathfinding and some cell migrations and that different Rac molecules have distinct roles in different morphogenetic events (CED-10 alone is required for apoptotic cell corpse phagocytosis whereas MIG-2 and RAC-2 are not (see abstract by Reddien, Lundquist, *et al.*)). Further, we have evidence that differential use of upstream Rac regulators controls distinct downstream Rac effects: the UNC-73/Trio GTP exchange factor acts with all three Racs in axon pathfinding and cell migration but has no role in phagocytosis, whereas CED-5/DOCK180 acts with CED-10 in phagocytosis but with MIG-2 in axon pathfinding. These data indicate that Racs can act in different signaling complexes to mediate distinct morphogenetic events and that Rac redundancy in axon pathfinding involves three redundant Rac pathways rather than a simple substitutional redundancy of the Racs themselves.

We have begun to investigate the mechanisms that act downstream of Rac signaling in axon pathfinding. UNC-115 is an actin-binding binding protein and *unc-115* mutants show defective pathfinding of some but not all axons. Indeed, *unc-115* acts redundantly with *rac* genes in axon pathfinding: *unc-115; ced-10* and *unc-115; mig-2* double mutants display synthetic CAN and PDE axon defects but *unc-115; rac-2(RNAi)* do not, indicating that *unc-115* acts specifically in the *rac-2* branch of the tripartite redundant *rac* cascade. To further test this hypothesis, we constructed transgenes of *ced-10* (courtesy of P. Reddien), *mig-2* and *rac-2* harboring the G12V mutation which leads to constitutive Rac activation. When driven by neuron-specific promoters, these mutant transgenes caused dominant defects in neuronal morphogenesis: axons and dendrites displayed extensive ectopic branching, and the cell bodies and processes often displayed lamellipodial-like membrane ruffling, reminiscent of activated Rac in fibroblasts. Strikingly, loss of *unc-115* function completely suppressed the effects of activated RAC-2, including branching and membrane ruffling, but did not suppress activated CED-10 or MIG-2. These data indicate that UNC-115 acts downstream of RAC-2 but not MIG-2 or CED-10 and that UNC-115 might directly modulate the actin cytoskeleton in response to RAC-2.

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300. Cloning and characterization of *ced-12*/*elmo*, a novel member of the CrkII/Dock180/Rac1 pathway, which is required for phagocytosis and cell migrations

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Programmed cell death (apoptosis) plays a crucial role in animal development as well as in the elimination of damaged cells. The pathway underlying this process is evolutionarily conserved from worms to humans. An important step in apoptosis is the removal of dying cells from the organism via phagocytosis. In *C. elegans*, removal of dying cells proceeds via two partially redundant pathways. The first pathway is composed of two proteins at the cell membrane, CED-1/SREC and CED-7/ABC1, which may function in the recognition of the dying cell, and CED-6, a putative adapter protein. The second pathway is comprised of CED-2/CrkII, CED-5/Dock180, and CED-10/Rac1, thought to be involved in reorganization of actin filaments, which is required to extend the engulfing cell's membrane around the dying cell.

We genetically and molecularly characterized a novel member of the *ced-2*/*ced-5*/*ced-10* pathway, *ced-12*. In *C. elegans*, CED-12 is required for the engulfment of dying cells and for various cell migrations. Positional cloning of *ced-12* revealed that this gene encodes a protein of 731 amino acids, the molecular function of which is unclear from the sequence analysis. However, *ced-12* is clearly conserved through evolution, possessing at least one homologue in Drosophila, and two in mice and humans. We have named the mammalian *ced-12* orthologues *elmo* (genes involved in *e*ngu*l*fment and *mo*tility), and refer to the products of these two *ced-12* orthologues as ELMO1 and ELMO2.

In mammalian cells, ELMO-1 functions together with CrkII and Dock180 upstream of Rac1 during phagocytosis. ELMO-1 physically interacts with Dock180 and forms a ternary complex with CrkII, which appears necessary for the functional synergy between the two proteins during phagocytosis. ELMO-1 also regulates Rac-1 dependent cytoskeletal changes and localizes to membrane ruffles. Expression of ELMO-1 or ELMO-2 in CED-12 deficient worms significantly rescues the gonadal migration defect.

These studies show that *ced-12*/*elmo* is an evolutionarily conserved upstream regulator of *ced-10*/Rac1 that affects engulfment and cell migration in both *C. elegans* and mammalian cells.

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301. CED-12 functions in the CED-10 RAC-mediated pathway to control cell migration and cell-corpse engulfment

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The conserved GTPase signaling pathway mediated by CED-2/CrkII, CED-5/DOCK180 and CED-10/Rac controls cytoskeletal reorganization in multicellular organisms. We have identified and characterized a new gene, *ced-12*, which functions in this pathway to control the engulfment of apoptotic cells and the migration of distal tip cells (DTCs) in *C. elegans*.

We isolated a *ced-12*(*tp2*) mutant defective in DTC migration and the engulfment of apoptotic cells. Previous genetic studies suggest that the engulfment process is controlled by two partially redundant pathways: *ced-1, ced-6* and *ced-7* in one and *ced-2, ced-5* and *ced-10* in the other (1). Mutants defective in *ced-2, ced-5* or *ced-10* in the latter group also exhibit a defect in DTC migration. Our genetic analyses suggest that *ced-12* acts in the pathway defined by *ced-2, ced-5* and *ced-10* to control the phagocytosis of cell corpses.

We cloned *ced-12* and found that it encodes a novel and evolutionarily conserved protein. To examine the expression pattern of *ced-12*, we made a *ced-12*::gfp fusion construct using the endogenous *ced-12* promoter and microinjected it into *ced-12* mutants. We found that the construct rescues the Ced-12 engulfment defect in transgenic animals and that CED-12::GFP is widely expressed and is localized to the cytosol. We further explored if *ced-12* acts in engulfing cells or cell corpses during the phagocytosis of cell corpses by ectopic expression of *ced-12*. We found that overexpression of *ced-12* in engulfing cells but not in cell corpses can induce the engulfment of persisting cell corpses in *ced-12* mutants, suggesting that *ced-12* functions in the engulfing cells but not in cell corpses during the engulfment process. Using a similar strategy, we found that overexpression of *ced-12* in DTCs but not in body musculature can rescue the DTC-migration defect in *ced-12* mutants, suggesting that *ced-12* acts in migration DTCs during DTC migration along the defined path.

To determine the genetic relationship of *ced-12* with *ced-2*, *ced-5* and *ced-10*, we performed bypass experiments. We found that overexpression of *ced-10* but not *ced-2* or *ced-5* rescues the Ced-12 engulfment defect and that overexpression of *ced-12* fails to rescue the engulfment defect in *ced-2*, *ced-5* or *ced-10* mutants. In addition, overexpression of *ced-10* also rescues the DTC-migration defect in *ced-12* mutants. These results together suggest that *ced-12* functions upstream of *ced-10* in the genetic pathway that controls cell-corpse engulfment and DTC migration.

We then tested if CED-12 can interact with CED-2, CED-5 or CED-10. We found that CED-12 physically interacts with CED-5 but not with CED-2 or CED-10 in the GST pulldown assay and the yeast two-hybrid system. Because CED-5 has been shown to interact with CED-2 *in vitro* (2), we further explored if CED-5 can interact with CED-2 and CED-12 simultaneously using the yeast three-hybrid system. We found that CED-2, CED-5 and CED-12 can form a complex, with CED-5 bridging CED-2 and CED-12. Based on these results, we propose that the formation and localization of a CED-2-CED-5-CED-12 ternary complex to the plasma membrane activates CED-10, leading to the cytoskeletal reorganization that occurs in the polarized extension of cell surfaces in engulfing cells and migrating DTCs.

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302. Execution of necrotic-like cell death in *C. elegans* requires the activity of specific aspartyl proteases

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Distinct types of cell death underlie the pathology of many human diseases, yet very little is understood of non-apoptotic cell death mechanisms. Analysis of non-apopotic death mechanisms in *C. elegans* is likely to provide mechanistic insights relevant to critical problems in neuronal injury and pathology. Gain-of-function mutations in specific *C. elegans* ion channel genes encode hyperactive channels that induce necrotic-like death of the neurons that express these mutant genes (Chalfie and Wolinsky, 1990; Driscoll and Chalfie, 1991). For example, dominant mutations affecting the *mec-4* degenerin channel induce degeneration of six touch receptor neurons. *mec-4(d*)-induced cell death occurs independently of all known regulators and executors of programmed cell death in *C. elegans*, and is necrotic-like in that it is characterized by dramatic cellular swelling. We are investigating the requirements for the progression and execution of degenerative cell death inflicted by *mec-4(d)* and other insults in *C. elegans*. Essentially nothing is known of the molecules that execute necrotic cell death, although it is clear that, like in apoptosis, proteolysis is likely to be a major component of the death mechanism. We have found that specific aspartyl proteases of the Cathepsin D family are required for the efficient execution of necrotic-like cell death in *C. elegans*. Several mutant nematode strains have been found to exhibit up to 90% reduced Cathepsin D protease activity (Jacobson et al., 1988). We assessed the requirement for Cathepsin D activity in necrotic-like cell death induced by *mec-4(d)* by testing *cad-1* alleles *j1* and *j14* for the ability to block cell death. Necrotic-like cell death induced by *mec-4(d)* was significantly reduced in these genetic backgrounds. In addition, several mutations that have been reported to cause 80-90% reduced cathepsinD activity (*daf-4(e1364), mec-8(e398)* and *unc-52(su250)* also suppress *mec-4(d)*-induced degeneration. Starvation is another condition that leads to significant reduction of Cathepsin D activity in *C. elegans* (Sarkis et al. 1988). We have observed that starvation also interferes with necrotic-like cell death induced by *mec-4(d)*. To further demonstrate the involvement of Cathepsin D proteolytic activity in neurodegeneration, we assayed the effects of Pepstatin A, a potent and specific inhibitor of Cathepsin D on *mec-4(d)* induced necrotic-like cell death. Pepstatin A significantly blocked neurodegeneration. Thus, one or more cathepsin D proteases play a significant role in *mec-4(d)*-induced degeneration. There are several genes in the *C. elegans* genome that encode putative Cathepsin D proteases. The most highly similar to biochemically characterized mammalian aspartyl proteases has been designated *asp-4* (Tcherepanova et al., 2000). We employed dsRNA interference to quench *asp-4* expression and to determine the effects on neurodegeneration. Results indicate that ASP-4 activity is required for necrotic-like cell death induced by *mec-4(d*). Conversely, overexpression of *asp-4* in a *mec-4(d);cad-1(j1)* mutant completely reversed the suppressing effects of *cad-1(j1)* on *mec-4(d)*induced neurodegeneration. We tested all other Cathepsin D proteases in the C. elegans genome for similar effects on death when inactivated via RNAi or when over-expressed. Our analysis revealed that ASP-3 is the only other aspartyl protease required for necrotic-like cell death. This is the first clear genetic demonstration of a requirement for a protease in the execution of necrotic-like cell death in *C. elegans*. Aspartyl proteases such as Cathepsin D are mostly lysosomal enzymes but they have also been found to function in the cytoplasm. Interestingly, Cathepsin D has been implicated in endocytic degradation of proteins and *mec-4(d)*-induced cell death involves extensive plasma membrane internalization that resembles endocytosis. Such a process may require the function of Cathepsin D. Our results suggest that Cathepsin D activity plays a pivotal role in the destruction of the cellular contents during *mec-4(d)*-induced neurodegeneration. Preliminary results suggest that cathepsin D may also play a role in necrotic-like cell death induced by insults other than *mec-4(d)* in the nematode. Establishment of a central role for cathepsin D in the execution of degenerative cell death would make cathepsin proteases attractive targets for therapeutic intervention in human injury and disease.

303. The *C. elegans* Autosomal Dominant Polycystic Kidney Disease Genes *lov-1* and *pkd-2* act in the same pathway

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Autosomal Dominant Polycystic Kidney Disease (ADPKD) strikes 1 in 1000 individuals, often resulting in end-stage renal failure. Mutations in either PKD1 or PKD2 account for 95% of all cases. Recently, polycystin-1 and polycystin-2 (encoded by PKD1 and PKD2, respectively) have been shown to form a cation channel *in vitro* (1). Here we show that the *C. elegans* homologues of PKD1 and PKD2, *lov-1* and *pkd-2* (2), act in the same pathway *in vivo*. Mutations in either *lov-1* or *pkd-2* result in identical male sensory behavioral defects. Furthermore, *pkd-2; lov-1* double mutants are phenotypically indistinguishable from *lov-1* and *pkd-2* single mutants, indicating that *lov-1* and *pkd-2* act together. LOV-1 and PKD-2 protein colocalize to male-specific chemosensory neurons and concentrate in cilia. Consistent with a role in sensory signal transduction, basodendritic localization of PKD-2 is essential for function. In contrast to defects in the *C. elegans* Autosomal Recessive PKD gene *osm-5* (3), the cilia of *C. elegans* ADPKD mutants are wild type as judged by electron microscopy. This system provides a genetically tractable model for determining molecular mechanisms underlying PKD.

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304. A worm model for polycystic kidney disease

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Autosomal dominant polycystic kidney disease (ADPKD) is a genetic disorder that occurs with a frequency of 1 in 1000. Affected individuals typically develop large cystic kidneys ultimatively resulting in renal insufficiency in 50% of the patients. ADPKD is caused by mutations in the two human PKD genes (HSPKD1 accounts for ~85% and HSPKD2 for ~10% of the patients). Recently it was shown that mutations in lov-1 (worm pkd-1) cause aberrant male mating behavior1. We have isolated mutations in pkd-2 and found that males similar to lov-1 fail to respond to hermaphrodites and are merely able to locate the vulva. Although mating is impaired it is still possible, since spicule insertion and sperm transfer remain unaffected. We made a full-length pkd-2 -GFP fusion construct and found expression in a subset of male specific sensory neurons and the HOB neuron, which are required for proper vulva location. We have also found GFP-expression in another set of male specific head neurons, the CEM cells, which explains why pkd-2 males fail to recognize a hermaphrodite. The phenotypic data suggest that lov-1 and pkd-2 are both required for mating behavior and may define also in C. elegans a single pathway. Recent studies suggest for HSPKD1 a role in focal adhesion, JNK-signaling and Ca-transport. Since the C-terminal domain of HSPKD1, which is truncated in many ADPKD patients, plays an important role we expressed the C-terminal domain of CEPKD1 in body-wall muscle of C. elegans. We could show that the C-terminal domain co-localises to dense bodies, which contain the integrin complex. We propose that the C-terminal domain of PKD1 of both organisms share important structural features needed for integrin-signaling or integrin-complex association.

1Barr MM and Sternberg PW, Nature 401: 386-389 1999

305. CATION DIFFUSION FACILITATOR PROTEINS AND ZINC IONS REGULATE RAS-MEDIATED SIGNALING

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A conserved receptor tyrosine kinase-Ras-MAP kinase signal transduction cascade is required for specification of the *C. elegans* vulva. In a screen for suppressors of the multivulva (Muv) phenotype caused by a constitutively active *let-60 ras* gene, we identified the strong loss-of-function mutation *n2527*. The *n2527* mutation causes a weak vulvaless phenotype; epistasis experiments suggest that it functions downstream of *let-60* and upstream of *lin-1* if the genes act in a linear pathway. To positionally clone the gene affected by *n2527*, we created a local, high-density, single-nucleotide polymorphism map and positioned *n2527* within a 9.6-kb interval. The molecular lesion was identified by sequencing, and the candidate gene was confirmed by transformation rescue. The *n2527* mutation creates a premature STOP codon in a previously uncharacterized gene that we named *cdf-1*, since it encodes a predicted protein with significant similarity to members of the cation diffusion facilitator (CDF) family. CDF-1 is similar to mammalian ZnT-1 which reduces the intracellular concentration of Zn^{2+} ions.

CDF proteins have not been previously shown to be involved in signal transduction. We investigated if regulation of Ras signaling is an evolutionarily conserved property of CDF proteins. When expressed under the control of the *cdf-1* promoter, rat *ZnT-1* rescues the *cdf-1(n2527)* suppression of *let-60(gf) ras* Muv phenotype. Thus a vertebrate CDF protein can function in worms to promote Ras signaling. We also tested if CDF-1 could function in vertebrate signal transduction using *Xenopus laevis* oocytes. In a sensitized background, overexpression of CDF-1 induces MAP kinase phosphorylation and *Xenopus* oocyte meiotic maturation, suggesting that worm CDF-1 can stimulate Ras signaling in a vertebrate.

To determine if *cdf-1* affects the transport of a heavy metal ion, we grew worms in the presence of additional metal ion. *cdf-1* mutants were hypersensitive to Zn^{2+}, as indicated by a reduced growth rate, increased larval lethality and increased sterility compared with wild-type worms. This phenotype is specific to zinc ions, since the growth of *cdf-1* mutants on Co^{2+}, Cd^{2+}, or Cu^{2+} was similar to wild-type. Thus, *cdf-1* is likely to play a widespread role in Zn^{2+} metabolism and the analysis of this locus is likely to illuminate how *C. elegans* regulates Zn^{2+}.

Because CDF-1 is likely to reduce the intracellular concentration of zinc ions and positively modulate Ras signaling, we hypothesized that zinc ions inhibit Ras signaling. To test this hypothesis, we analyzed the phenotype of Ras pathway mutants exposed to increased levels of Zn^{2+} in the media. Extra zinc reduces the penetrance of the Muv phenotype caused by gain-of-function mutations in the *let-60 ras* and *let-23* genes while the *lin-1(lf)* Muv phenotype was unaffected. The effect of increased zinc ions on these mutants phenocopies that of *cdf-1* mutants and suggests that there is a zinc-sensitive pathway component downstream of *let-60 ras* and upstream of *lin-1*. We propose a model in which zinc ions inhibit signal transduction and that CDF proteins promote Ras signaling by reducing the intracellular concentration of this negative regulator. This regulatory circuit is likely to be conserved.

306. Genetics and biochemistry of RNAi in *C. elegans*

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RNA interference (RNAi) is the experimental silencing of endogenous gene expression of a given gene by the administration of homologous double stranded RNA. Recent work using *Drosophila* extracts indicates that the RNAi mechanism can be divided in two distinct steps: breakdown of the dsRNA molecules into discrete 21-23 nucleotide guide RNAs, that then subsequently target the endogenous mRNA for degradation. We investigated whether we could uncouple these events *in vivo* by injecting RNA oligonucleotides into the gonad of *C. elegans*. We found that single stranded RNA oligomers of antisense polarity are more potent inducers of RNAi than molecules in the double stranded conformation, while sense oligomers do not trigger RNAi. Injections into various mutants shows that the action of these single stranded RNA molecules is downstream of the *rde-1* and *rde-4* genes, but still depend on *mut-7* (a putative RnaseD) and *mut-14*. Subsequent positional cloning identified *mut-14* encoding a DEAD box RNA helicase, a likely component of the mRNA degrading complex.

In addition to these genetic studies, we have developed an *in vitro* system for RNAi using *C. elegans* extracts. We have been able to reconstitute the first step of the reaction, the conversion of dsRNA into guide RNA molecules. Using this assay we could show that the guide RNA's produced by *C. elegans* are two basepairs larger than those produced in *Drosophila*. We also found that the dsRNA is broken down into discrete products, suggesting that guide RNAs are not produced randomly from the dsRNA, but rather seem to be produced in a processive fashion, starting from the ends of the dsRNA. Several mutants were

tested for their ability to produce guide RNAs *in vitro*.

Based upon the combination of genetic and biochemical analysis we will propose a pathway for RNAi in *C. elegans*.

307. Target dependent accumulation of small RNAs during RNAi in *C.elegans*

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Homology-dependent gene silencing in diverse organisms occurs when extra copies of particular sequence are introduced either in a form of transgenes, viruses or dsRNA. The latter form of post-transcriptional gene silencing (PTGS) induced by dsRNA is known as RNA interference. Small 21-25nt RNA molecules have been detected in plants undergoing PTGS as well as in *Drosophila* RNAi. More recently, formation of a small amount of ~22 nt RNA derived from injected labeled dsRNA has been reported for RNAi in *C.elegans*. These small RNAs associated with PTGS are thought to guide an RNAse complex to the target mRNA providing for sequence specific targeting. In this study we analyzed by Northern analysis the accumulation of small RNAs in wild type worms or different *rde* mutants exposed to dsRNA. In strains expressing *unc-22* dsRNA from a transgene we detected small ~22 nt RNAs both in wild type worms as well as in *rde-1* and *rde-4* mutants. Thus, when dsRNA is abundantly expressed *in vivo* it can be processed into small RNAs even when RNAi and mRNA destruction are absent. A different result was observed when we used feeding to administer dsRNA targeting the maternal *pos-1* mRNA. In these experiments small RNAs derived from *pos-1* sequences were detected in wild type but not in the *rde* deficient strains tested, including *rde-1*, *rde-4*, *rde-2* and *mut-7* strains. One interpretation of these seemingly contradictory results is that small RNAs can be derived directly from the trigger RNA but are also formed or stabilized as a result of mRNA targeting. Thus, in the *unc-22* experiment abundantly expressed dsRNA made *in vivo* is sufficient for significant small RNA accumulation even in the absence of mRNA targeting, while in the *pos-1* dsRNA feeding assay, the small RNA detected could be formed in part from the ingested trigger RNA and in

part from a target dependent mechanism that requires *rde(+)* activities. In order to examine the relative contributions of these two mechanisms we decided to administer a dsRNA that has no corresponding homologous target sequence in *C. elegans*. To do this we fed *C. elegans* strains on *E. coli* expressing dsRNA targeting the jelly fish green fluorescent protein mRNA (GFP). In these experiments, wild type worms that lack a GFP transgene or GFP transgenic worms that lack *rde-1* (+) or *rde-4* (+) activities failed to accumulate detectable amounts of small RNAs corresponding to the GFP sequences. Strikingly however, wild type GFP transgenic worms fed on GFP dsRNA bacteria produced a dramatic quantity of small antisense RNA in the 22-24 nt range. Thus, the accumulation of small RNAs during RNAi in *C. elegans* exhibits target dependence and requires activities of all of the *rde* genes we have tested. In contrast, the initial formation of small RNAs presumably processed from the trigger dsRNA does not appear to require even the most upstream *rde* genes, *rde-1* and *rde-4*. In other systems small RNA species detectable by Northern analysis contained both sense and antisense molecules. We are now asking if the target dependent accumulation of small RNAs includes both sense and antisense in our experiments. These findings are consistent with a model in which small RNAs become stabilized or are amplified when they successfully engage and destroy a target mRNA. Selection of only the successful RNAi active molecules would insure that the capacity of the system to respond would not be compromised by competition from unsuccessful RNAs. A similar logic underlies the clonal expansion of successful antibody expressing cells in the vertebrate immune response.

308. A connection between RNAi and development in *C.elegans*

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In numerous organisms the introduction dsRNA can induce sequence specific post-transcriptional silencing (PTGS) of a corresponding gene. The experimental application of dsRNA to induce gene silencing has been termed RNA interference or RNAi. Although RNAi has gained wide acceptance in *C. elegans*, *Drosophila* and several other organisms as a tool for silencing genes, there is, as yet, relatively little known about the mechanism and the underlying physiological role of RNAi in the organism. Importantly, it is not yet known if mechanisms related to RNAi have a role in the natural developmental regulation of gene expression. One of the genes necessary for RNAi in *C.elegans, rde-1*, belongs to an ancient gene family with relatives in numerous eukaryotic organisms including plants, *Neurospora*, *Drosophila* and mammals. Although *rde-1* mutants do not have obvious developmental defects, its relative, *ago1*, which is involved in PTGS in *Arabidopsis* was initially found as a gene important for growth and proper morphogenesis of the plant. The *rde-1* family in *C.elegans* contains more than 20 members with two predicted genes that are very similar to the plant gene *ago1*. Members of a second gene family including the plant gene *carpel factory* (*caf1*) and the fly gene *dicer* encode multifunctional proteins that share several domains including a motif found in RDE-1 as well as RNase III, RNA helicase and dsRNA binding domains. Mutations in *caf1* cause developmental abnormalities not unlike those seen in *ago1* mutants. Drosophila *dicer*, has recently been shown to play role in processing a trigger dsRNA into small 22 nucleotide (nt) interfering RNAs important for RNAi. We used RNAi to target the *ago1* related genes which we call *alg-1* and *alg-2* (*a*rgonaute *l*ike *g*enes) as well as the *C. elegans* homolog of *caf1/dicer*

which we call *dcr-1*. Our analysis revealed developmental phenotypes reminiscent of the heterochronic mutant phenotypes displayed by *lin-4* and *let-7* loss of function mutants. *lin-4* and *let-7* encode small ~22 nt RNAs that regulate stage specific gene expression by pairing with complementary sequences in the 3' UTRs of their target genes *lin-14* and *lin-41*. Genetic analysis suggests that defects associated with *alg-1* and *dcr-1(RNAi)* are caused at least in part by misregulation of *lin-14* and *lin-41*. The RNA products of *lin-4* and *let-7* are predicted to be transcribed as ~70 nt precursor RNAs that fold into comparable stem-loop structures. Such RNA precursors are detected by Northern analyses of *C. elegans* RNA. Additionally, *let-7* homologues exist in numerous metazoan species and *let-7* precursor RNAs are observed in *Drosophila* and humans, supporting the conservation of a common secondary structure as well as the major ~22 nt *let-7* RNA sequence. In order to examine *lin-4* and *let-7* RNA expression we prepared RNA from L3/L4 staged progeny of *alg-1* and *dcr-1(RNAi)* animals. In *dcr-1(RNAi)* animals we observed a significant accumulation of the 70 nucleotide forms of *lin-4* and *let-7* transcripts. A similar accumulation of the large form of *lin-4* but not *let-7* was observed in *alg-1(RNAi)*. These findings would be consistent with a model in which the longer forms of *lin-4* and *let-7* are processed by DCR-1 into ~22 nt products. Interestingly, we find that *dcr-1(RNAi)* also appears to cause a significant reduction in RNAi itself. Thus, DCR-1 may have similar roles in processing dsRNA into small RNAs that mediate both developmental regulation and RNA interference. In contrast, RDE-1 and its homologues ALG-1 and ALG-2 appear to be dedicated to distinct functions. In the future it will be fascinating to learn more about the shared features of these mechanisms as well as how RDE-1 homologues and other factors specify apparent differences in the outcomes of these pathways: RNA destruction versus translation inhibition.

309. The maternal-effect gene *clk-2* affects developmental timing, is essential for embryonic development, encodes a protein homologous to yeast Tel2p, and is required for telomere length regulation in *C. elegans*.

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The *C. elegans* maternal-effect *clk* genes are involved in the temporal control of development and behavior, and participate in life-span determination [1,2]. We will present our detailed genetic and molecular characterization of *clk-2*. A temperature-sensitive mutation of *clk-2* affects embryonic and post-embryonic development, reproduction, adult behaviors, and life span, yet virtually all phenotypes can be fully maternally rescued. Genetic interactions of *clk-2* with other genes that affect life span indicate that *clk-2* acts independently of the genetic pathway that regulates dauer formation, and that *clk-2* increases life span by a mechanism that resembles caloric restriction. While *clk-2* is required throughout the life cycle, including for fertility, embryonic development strictly requires the activity of maternal *clk-2* during a narrow time window between oocyte maturation and the 2 to 4-cell stage. Positional cloning of *clk-2* reveals that it encodes a protein homologous to *S. cerevisiae* Tel2p. In yeast, the gene *TEL2* positively regulates telomere length, participates in gene silencing at subtelomeric regions, and Tel2p binds to telomeric repeats in vitro [3,4]. The gene *clk-2* is required for telomere length regulation in *C. elegans*, since *clk-2* mutants have elongated telomeres, and *clk-2* overexpression can lead to telomere shortening. CLK-2 is expressed throughout development and high *clk-2* transcript levels are present in the germline. We are currently exploring the relationships between the variety of *clk-2* phenotypes and whether the maternal effect is the result of epigenetic mechanisms comparable to subtelomeric silencing.

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310. The *C. elegans clk-2* gene encodes a novel regulator of telomere length and DNA damage response.

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Our laboratory has been working on elucidating the mechanisms of cellular senescence in mammals, and understanding its contribution to organismal aging. It is generally accepted that telomere shortening is a crucial contributor of aging in mammalian cells, giving rise to the "telomere hypothesis of aging". This hypothesis is currently being tested in mammals, but recent data suggest telomere structure, and possibly telomere organization, may influence cellular, and possibly organismal, phenotypes.

We investigated whether mechanisms of telomere length regulation is conserved in the metazoan nematode *Caenorhabditis elegans* in connection with aging. We examined seven mutants that have been shown to have an lengthened life span for changes in telomere length.

Among the seven mutants examined, the long-lived *clk-2* mutant showed a shortened telomere length compared with wild type N2 worms. Moreover, *clk-2* mutants had longer 3’-telomeric overhangs relative to wild type. These findings suggest that *clk-2* might be involved in telomere length regulation, and particularly may encode a single strand telomere binding protein. To explore this idea, we examined the *C. elegans* genome database for ORF’s encoding potential regulators of telomere length. From our database search, we found that the ORF C07H6.6 shares some similarity with single strand telomere binding proteins in budding yeast, such as EST1, Cdc13p and TEL2.

To determine whether C07H6.6 is the *clk-2* gene, we prepared a genomic region spanning the C07H6.6 by PCR and injected the fragment into *clk-2* (*qm37*) animals. We found that C07H6.6 rescues *clk-2* mutant phenotype. We also used RNAi to confirm that C07H6.6

inhibition produces a phenotype similar to the *clk-2* mutation. From both DNA transformation rescue and RNAi, we conclude that the ORF C07H6.6 is the *clk-2* gene.

To characterize the CLK-2 protein, we expressed the *clk-2* cDNA in *Drosophila* S2 cells. Currently, we are studying the biochemistry of CLK-2, and refining the phenotypes of *clk-2* mutants via adult life span, DNA damage response, apoptosis, and telomere clustering measurements. We will present our findings at the meeting.

311. DAF-9, a cytochrome P450 that influences diapause, reproductive development and longevity

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During early larval development the decision between reproductive growth and dauer formation is determined by sensory cues. TGF-beta, insulin-like and cGMP pathways integrate this sensory information, and relay signals throughout the organism by an endocrine mechanism. These inputs converge on the orphan nuclear hormone receptor DAF-12 in cellular targets.

To better understand the coupling between these pathways, we investigated the mutant *mig-8(rh50)*, which has *daf-12*-like phenotypes. We isolated more *mig-8* alleles and found two phenotypic classes. Class 1 mutants, which include the *rh50* allele, show delayed migration of the gonadal leader cells. Class 2 mutants arrest as partial dauer larvae, which slowly recover to infertile adults. The Daf-c locus *daf-9* mapped near *mig-8*. By complementation tests, we found that *mig-8* and *daf-9* are the same gene.

How do *daf-9* and *daf-12* act together? Epistasis experiments place *daf-9* downstream of TGF-beta and insulin-like signaling and upstream of *daf-12*, showing that *daf-9* couples these inputs to *daf-12*. We cloned *daf-9* and found that it encodes a member of the cytochrome P450 family related to vertebrate steroidogenic and fatty acid hydroxylases. This molecular identity suggests that DAF-9 could synthesize or degrade a DAF-12 ligand. Consistent with this, *daf-9* has phenotypes that resemble *daf-12* mutants with lesions in predicted ligand contact residues. *daf-9* and *daf-12* mutants also have similar effects on ageing, further lengthening the long life of insulin-like receptor mutants *daf-2(e1370)*, and abolishing the longevity of gonad ablated animals. The expression of *daf-9* in sensory neurons, hypodermis, and somatic gonad suggests potential endocrine tissues. We postulate that the DAF-9 substrate/DAF-12

ligand may be a sterol derivative, because cholesterol privation results in phenotypes similar to *daf-9* and *daf-12* mutants. Together these findings provide substantial evidence that lipophilic hormones influence reproductive development and longevity.

312. Steroid hormone regulation of dauer formation and adult longevity

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The *daf-9* gene functions to integrate TGF-beta and insulin-like signaling pathways to regulate larval development. Mutations in the *daf-9* gene result in transient dauer-like larval arrest, abnormal reproductive development, molting defects and increased adult longevity. The phenotype is sterol-dependent, and dependent on the activity of DAF-12, a nuclear hormone receptor. *daf-9* encodes a cytochrome P450 related to those involved in biosynthesis of steroid hormones in mammals. We propose that it specifies a step in the biosynthetic pathway for a steroid ligand for DAF-12. The *daf-9* promoter is mainly active in two ventral IL1 or URA neurons. Destruction of the two neurons eliminated cDNA rescue of a weak (homozygous viable) *daf-9* mutant, and it resulted in sterile adults similar to those of the severe *daf-9* alleles. We believe *daf-9* provides new insight into a neuroendocrine mechanism regulating dauer formation, adult fecundity and longevity.

313. Two Pathways for the regulation of lifespan by metabolic genes in C. elegans

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Using the technique of RNA inhibition (RNAi), we have found that two types of metabolic genes influence the lifespan of C. elegans in two separate screens of genes located on chromosome I. Interestingly, perturbations of these metabolic genes produce profoundly different phenotypes, indicating two different classes of genes. Inhibition of class 1 metabolic genes causes a Clk (clock) phenotype, including slow growth to adulthood, slow movement, and decreased pumping and defecation rates. In contrast, inhibition of class 2 extends lifespan without producing a clk phenotype. The DAF-16 transcription factor is required for the lifespan extension produced by the inhibition of class 2, suggesting that this lifespan extension is not merely a passive consequence of decreased metabolism, but instead requires a specific program of gene expression. In contrast, DAF-16 activity is not required for the lifespan extension by class 1 inhibition. Together these findings indicate that at least two distinct pathways can regulate lifespan in response to changes in metabolic gene activties in this animal.

314. *C. elegans* life span is extended by a diet of *E. coli* lacking coenzyme Q

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As originally described, the *clk-1* mutants of *C. elegans* develop slowly and exhibit an extended life span. Recently, the *clk-1* mutants have been shown to have a defect in the biosynthesis of coenzyme Q_9, the normal isoform synthesized by *C. elegans* where 9 designates the number of isoprene units in the polyisoprenoid tail. Coenzyme Q is an essential component of the mitochondrial respiratory electron transport chain, as it is required for the function of complexes I, II, and III. The *clk-1* mutants lack coenzyme Q_9 and instead rely on the coenzyme Q_8 supplied by their standard diet of *E. coli*. These results implied that a reduction in the levels of coenzyme Q may be responsible for the slowed development and increased life span reported for the *clk-1* mutants maintained on the standard Q-replete *E. coli* diet. Wild type worms appear to partially rely on dietary Q, as evidenced by a one-day delay in development (egg to adult) on Q-less food. To test whether a decrease in coenzyme Q could alter life span of wild type animals, wild-type L4 larvae were switched from a standard diet of Q_8-replete *E. coli* to a diet lacking coenzyme Q, and were subsequently maintained on this Q-less diet throughout adulthood. Three distinct *E. coli* mutant strains were provided as Q-less diets, including strains lacking either the *O*- or *C*-methyltransferase enzymes of Q biosynthesis, and an *E. coli* mutant lacking the 4-HB:polyprenyldiphosphate transferase. A 60% increase in nematode life span was observed for the first two of these Q-less *E. coli* strains, suggesting that the distinct Q-biosynthetic intermediate accumulating in each of these Q-less *E. coli* mutants was not responsible for producing the observed life span extension. Thus at face value, consumption of Q_8 shortens wild type life span and it is this short life span that is considered normal. Alternatively, the diet/environment interaction that changes longevity could be due to different metabolic products in the Q-replete and Q-less bacteria.

Mutations in two loci, *daf-12* and *daf-16*, suppress the life span extension of a number of previously isolated Age mutations. However, neither *daf-12* nor *daf-16* suppress the life span extension generated by the Q-less *E. coli* diet. Assuming that the life span extension resulting from the Q-less *E. coli* diet is a phenocopy of the *clk-1* mutation, then the lack of suppression by *daf-16* is consistent with previous reports. The Age mutants *clk-1(qm30, qm51,* and *e2519*) and *daf-2(e1370* and *m41*) at the restrictive temperature, were transferred to the Q-less *E. coli* diet as L4 larvae, and for each of the mutants tested, further increases in life span were observed. For *clk-1* mutants, the expectation had been premature death, since its larval development depends on a supply of dietary Q. However, it is likely that the *clk-1* mutants cultured on Q-replete *E. coli* during development accumulate coenzyme Q_8, and the life span extension observed for the adult animals is presumably due to the declining levels of Q_8 from lack of a dietary source. The increased life span extension in Age mutants fed diets lacking coenzyme Q suggest that these longevity mechanisms are additive.

315. INCREASED DOSAGE OF A *sir-2* GENE EXTENDS LIFE SPAN IN *C. elegans*

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To identify new genes that control life span, we performed a genetic screen for genes that extend life span when increased in copy number. We reasoned that an increase in dosage of a gene whose activity was rate limiting in the aging process would extend life span. To increase gene dosage, we obtained 35 duplication strains that cover approximately 50% of the genome and measured the life spans of animals carrying the duplications. From this screen, we identified a region on chromosome IV that increased both the mean and maximum life span significantly. This region contained the gene *sir-2.1*, a *C. elegans* SIR2 homologue (1). Remarkably, in yeast, the dosage of SIR2 alone can determine the life span of yeast mother cells (2). Additionally, yeast SIR2p functions to mediate chromatin silencing due to its NAD-dependent histone deacetylase enzymatic activity (3). The *C. elegans* genome has four genes with similarity to yeast Sir2p (named *sir-2.1*, *sir-2.2*, *sir-2.3, sir-2.4)*. Of the four genes, the most related to yeast Sir2p is *sir-2.1* (31% identity in the core domain).

To test if *sir-2.1* was the gene responsible for the life span extension of animals with the chromosome IV duplication, we made transgenic animals with extra copies of *sir-2.1* on an extrachromosomal array. The life span of the *sir-2.1* transgenic animals was increased by as much as 50% (1). To determine if *sir-2.1* extended life span through one of the known aging pathways, we made strains containing the *sir-2.1* array (either extrachromosomal or integrated) in combination with a *daf-16(mg50*), *daf-2(e1370), daf-4(m63),* or a *daf-1(m40)* mutation. In combination with a *daf-16(mg50)* mutation, the life span of the *sir-2.1* array animals was almost identical to the *daf-16(mg50)* single mutants. This is similar to *daf-2* or *age-1* mutants where life span extension is completely suppressed by a mutation in *daf-16* . In combination with a *daf-2(e1370)* mutation, the life span of the

sir-2.1 array animals was similar to the *daf-2(e1370)* single mutants. Therefore, there was no synergy between the *sir-2.1* array and a mutation in *daf-2* for life span. Finally, similar to other mutations in the insulin-like signaling pathway such as *daf-2* , the *sir-2.1* array in combination with a mutation in either *daf-1* or *daf-4* (TGF-β receptors) synergized for dauer formation. Therefore, the *sir-2.1* array extends life span through the insulin-like signaling pathway (1).

We are interested in determining where *sir-2.1* functions in the insulin-like signaling pathway. To address this issue, we are performing RNAi of *sir-2.1* in the wild type and in various mutant backgrounds and measuring the life span of these worms. Additionally, we are trying to determine the expression pattern of *sir-2.1*. Our data suggests that *sir-2.1* may act to couple nutrient availability and developmental decisions, i.e. whether to form dauer larvae and, in animals that proceed to adulthood, how long to live. The demonstration of *sir-2.1*'s ability to extend life span may be the first conserved molecular mechanism of aging.

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316. Dramatic changes in chromatin composition associated with dauer formation, caloric restriction, and increased longevity.

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Caloric restriction from yeast to vertebrates increases longevity. Increased levels of SIR2, a histone deacetylase, extend yeast and C. elegans lifespan [1]. SIR2 is required for extended longevity in calorie restricted yeast [2], and may act within an insulin-like pathway that extends worm lifespan [reviewed in 3]. Of interest is whether the insulin-like signaling pathway [4,5] regulates chromatin composition and whether such changes are necessary or suffcient for extended adult lifespan. To detect changes in chromatin composition during development, we fused GFP to the C-terminus of 7 H1 histone family members in C. elegans, and surveyed their expression patterns. Each H1 shows a distinct pattern in well-fed animals, and 6 of 7 are down-regulated during starvation and dauer formation. In contrast, one H1 (C30G7.1), while present at low levels in the nuclei of some head neurons and the somatic gonad of well-fed animals, accumulates more than 100-fold in starved animals at all post-embryonic stages (larvae and adults). The H1 isoform increase occurs in essentially all somatic nuclei in starved animals, and to higher levels in dauers. Using Daf-c mutants, we show that excess food cannot block C30G7.1 induction in newly formed dauers. The accumulation of C30G7.1 in wild type animals is efficiently reversed by addition of food or exit from dauer arrest. Dauer-defective mutations in daf-16, but not daf-3, suppress starvation-induced C30G7.1 accumulation. Thus, accumulation of a specific H1 histone (C30G7.1 is more similar to a specific human H1 isoform than to other worm H1s), shows a nutrient level response that depends on a transcription factor near the bottom of the insulin-like signaling pathway. Given the regulation of C30G7.1, we asked whether C30G7.1 is upregulated in adults that carry mutations known to extend lifespan. All mutants tested give rise to distinct, tissue-restricted C30G7.1 induction patterns. The most notable pattern is that of daf-2 adults

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which show a strong neuronal bias in C30G7.1 accumulation. The accumulation pattern of C30G7.1 in long-lived adults differs from the completely starved or dauer-induced patterns in that the intestine seems unresponsive in the long-lived strains. One exception is a daf-2;clk-1 double mutant (a very long-lived strain) that shows an adult C30G7.1 accumulation pattern that is not only a unison of the individual patterns, but also shows strong induction in the intestine; similar to that seen in completely starved wildtype or dauer larvae. The increased accumulation of C30G7.1 in neurons of daf-2 adults is suppressed by mutations in daf-16. Quantitative RT-PCR on wild-type and mutant animals reveals at least a 10-fold upregulation in C30G7.1 mRNA level in daf-2 adults, and the induction is suppressed by daf-16. In contrast, eat-2 long-lived adults, while showing a strong-tissue restricted C30G7.1 induction pattern when compared to well-fed wild type adults, do not detectably upregulate C30G7.1 mRNA levels, suggesting that the increased accumulation of C30G7.1 may occur by a post-transcriptional mechanism. The non-coding region of C30G7.1 that is required for the unique regulation reveals an element shared by a small set of genes that may permit extended longevity. One of these, the antioxidant enzyme encoded by sod-3, is known to be upregulated in a manner similar to that of C30G7.1 [6]. We analyzed an additional gene in this set and its enzymatic activity is induced in nutrient deprived wild type animals and in long-lived daf-2 mutants; induction of the latter is dependent on daf-16. The remaining set of genes potentially co-regulated with C30G7.1 encodes gene products essential for the utilization of fat as an energy source, repair of oxidative damage to DNA, prevention of neurodegeneration, and clearance of damaged mRNAs resulting from genotoxic stressors. Current efforts are directed at determining whether C30G7.1 and/or the above genes are necessary or sufficient for the changes in metabolism and longevity in C. elegans.

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317. Aging at the cellular level is tissue specific: the nervous system does not degenerate while muscle and epithelia dramatically decline

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With the identification of several single gene mutations that dramatically extend lifespan, *C. elegans* has become an increasingly popular model system for the investigation of genes that influence aging and lifespan. However, although the detailed subcellular characterization of *C. elegans* anatomy is unparalleled among metazoans, we know little of the cellular and subcellular changes that accompany *C. elegans* aging. With a goal of better understanding aging and mechanisms of lifespan extension *in C. elegans*, we characterized cellular aging in *C. elegans*, an analysis that revealed several unexpected findings.

To lay the groundwork for in-depth study of cellular changes that accompany aging in *C. elegans*, we first characterized the general appearance and the locomotory behavior of wild type adults over time. We scored age-synchronized individuals for spontaneous movement and for the response to prodding and found that late in the lifespan, we could distinguish three classes of behavioral types in a same-age population: A (vigorous), B (compromised) and C (impaired). By following individual animals throughout their lifespan, we confirmed that decline is progressive (A to B to C). However, we also established that even though the animals followed were essentially genetically identical and were raised in the same environment, both the individual time of onset and the rate of decline are strikingly variable among members of a same-age population. This suggests that at least one significant factor in the behavioral aging of *C. elegans* has a stochastic component.

To characterize the cellular changes that accompany aging in wildtype *C. elegans*, we used GFP reporters to visualize specific cells and tissues and over adult life and coupled/confirmed observations with data from electron micrographic examination of the same tissues in comparably aged nematodes. We identified GFP reporter fusions that were expressed throughout much of the lifespan with the intent of visualizing specific cells (rather than a goal of characterizing changes in reporter gene expression) over time. Our first major conclusion is that aging is tissue-specific. The nervous system remains remarkably intact in old animalswe see no evidence for cell loss or death in the nervous system over time. Moreover, in assays that probe probable function, we found that amphid dye filling capacity remained unchanged over time and numbers of *snb-1::GFP*-tagged synapses remained relatively constant over the adult lifespan. At the cellular level, the *C. elegans* nervous system does not appear to age. In striking contrast, bodywall and pharyngeal muscle undergo a dramatic age-related decline. For example, in bodywall muscle, nuclei change in shape and appearance and later appear to disintegrate; sarcomere contact with hypodermis and cuticle become disrupted and sarcomeres fray. Hypodermis and intestine also undergo dramatic decline. A second general impression is that large-scale mis-regulation of biosynthetic processes occur late in life. Lipids appear to accumulate in several tissues, the cuticle dramatically thickens and large quantities of yolk protein diffuse throughout the body cavity despite the fact that oocyte maturation ceased days several days earlier. These findings suggest that in post-reproductive animals (a population not subjected to natural selection pressures) gene expression and/or protein production is not efficiently regulated.

Overall, our analysis establishes that cellular aging in nematodes occurs at different rates in different tissues. Cellular aging in worms also bears striking parallels to human aging. In humans, in the absence of disease, there is little age-related cell loss in the nervous system. Like humans, nematodes suffer from sarcopenia, a progressive loss of muscle mass. These studies set the stage for studies of how lifespan-extending mutations affect tissue-specific aging, and for testing how tissue-specific signals known to influence lifespan affect cellular aging.

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318. Evolution of germ-line signals that regulate growth and ageing in nematodes

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One of the least understood aspects of animal development is how growth and body size are regulated[1,2]. Here we show that a signal from the germ-line represses growth in the nematode *Caenorhabditis elegans*. Laser-microbeam ablation of cells that give rise to the germ-line causes adults to become giant. Ablation of these cells in self-sterile mutant worms also causes gigantism suggesting that the germ-line represses growth because it is the source of a growth-antagonizing signal rather than a sink of resources required for reproduction. The *C. elegans* germ-line also emits a signal that represses longevity[3]. This longevity-repressing signal requires the activity of DAF-16, a forkhead/winged-helix transcription factor[3], but we find that that the growth-repressing signal does not. The growth-repressing signal also does not require the activity of DBL-1, a TGF-beta-related protein that promotes growth in worms[4,5]. By ablating the germ-line precursors of other species of free-living nematodes we found that both the growth-repressing and longevity-repressing signals are evolutionarily variable. Some species have both, others have just one or the other. We suggest that variation in germ-line signaling contributes to body size and life-history diversity in the nematodes.

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319. Multiple genetic loci determine sensitivity of *C. elegans* to the deformation-causing pathogen *M. nematophilum*

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The coryneform bacterium *Microbacterium nematophilum* is the causative agent of an unusual Dar (Deformed Anal Region) phenotype in *C. elegans.* These bacteria can adhere to the surface of worms in the rectum and post-anal region, causing substantial swelling of the underlying tissue. Infected worms become severely constipated and exhibit substantially slower growth rates. The mechanism of infection and induced swelling has been examined by isolating resistant and hypersensitive mutants.

Resistant mutants are easily obtained by selecting for normal growth rate and absence of swelling in the presence of the pathogen. Over 100 independent Bus (Bacterially UnSwollen) mutants have been recovered after mutagenesis with EMS or from mutator strains. These define at least 18 complementation groups, of which a few are known genes that had been previously found to affect sensitivity (*srf-2, srf-3, srf-5).* Remarkably, some mutations in the Hox gene *egl-5* also confer resistance. However, most resistance loci are novel (fourteen genes, *bus-1* to *bus-14).* These new genes have been mapped to locations on LGI, IV, V and X. Some of the mutants exhibit pleiotropic defects in movement, egg-laying or viability. Examination of *bus* mutants exposed to *M. nematophilum* indicates that resistance can arise in several ways, allowing genetic dissection of the processes of bacterial attachment, host response to infection and morphological change.

Mutations in nine of the *bus* and *srf* genes have been recovered from *mut-7* mutator strains and are therefore probably transposon-induced. Progress in the molecular identification of these genes will be reported.

Clonal screens for hypersensitive mutants have also been carried out, searching for strains that are severely growth-impaired or inviable in the presence of the pathogen. A number of *gmp* (Growth iMPaired by pathogen) mutants have been recovered and are being further analysed.

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320. Identifying universal *Serratia marcescens* virulence factors using *C. elegans*.

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Tan and Ausubel, working with the enterobacterium *Pseudomonas aeruginosa*, have established *C. elegans* as a model for the study of pathogenesis and host defences. A second opportunistic human pathogen, *Serratia marcescens*, is also capable of infecting *C. elegans*. Like *P. aeruginosa*, *S. marcescens* is able to infect a broad range of plant and animal hosts. Using a strain of *S. marcescens* that expresses GFP, we have been able to follow the infection process. The bacteria are able to survive within the usually hostile environment of the nematode intestine, proliferate and kill the host. Under standard assay conditions, the progression of the infection is highly reproducible.

We have used a transposon mutagenesis system to create a library of insertion mutants of *S. marcescens*. We are currently screening these mutant bacterial clones individually for those showing reduced virulence. Of the first 2000 mutants screened, 9 showing markedly reduced virulence have been retained for further study. The molecular characterization of these mutants has revealed novel virulence factors. In order to determine whether these virulence factors are specific to the infection of the nematode, we have also tested them in a Drosophila infection model and found that 4 of them are attenuated for their virulence. Tests in a mammalian model will reveal whether we have identified virulence factors that are important irrespective of the host.

Tan MW, Ausubel FM. (2000) *Caenorhabditis elegans*: a model genetic host to study *Pseudomonas aeruginosa* pathogenesis. Curr Opin Microbiol. 3: 29-34.

321. Identification of inducible innate immune defences in *C. elegans.*

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Until now, it has not been clear whether *C. elegans* possesses an inducible system of defence or not. We have used high-density cDNA arrays to address this issue. We have found that following infection with the Gram-negative bacterium *Serratia marcescens* (see abstract by Kurz *et al.*) a number of genes are induced. We found 14 genes that showed a greater than 2-fold induction after both 24 h and 48 h of infection. Among them, other than genes for which no known homologues exist, we have identified genes encoding lectins and lysozymes, known to be involved in immune responses in other organisms. By in situ hybridisation, we have shown that the majority of these are expressed in the intestinal cells. Studies using invertebrates have significantly contributed to our current understanding of vertebrate innate immunity. Our findings thus open a new avenue for the investigation of evolutionary conserved mechanisms of innate immunity.

322. Automatic cell lineage acquisition system and analysis of early embryogenesis

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We have developed a system that automatically acquires cell lineages of *C. elegans* from the 1-cell stage up to approximately the 25-cell stage. The system utilizes a set of 4D Nomarski DIC microscope images of *C. elegans* embryo consisting of more than 50 focal plane images at each minute for about 2 hours. An image-processing algorithm, utilizing the image entropy, detects the region of cell nucleus in each image, and 3D nucleus regions, each of which is a complete set of nucleus regions that represent the same nucleus at the same time point, are made. Each pair of 3D nucleus regions is then connected, if they represent the same nucleus and their time points are consecutive, and the cell lineage is created based on these connections. The resulting cell lineage consists of the three-dimensional positions of nuclei at each time point and their lineage. The system utilizes our Beowulf PC cluster, made up of 32 PC, to execute all the above processes and can deduce the cell lineage within 9 hours. We also developed a software package that three-dimensionally visualizes the resulting lineage data, which may help three-dimensional understanding of nucleus movement and division. Moreover, with this package, lineages of two different individuals - e.g. wild-type and mutant - can be visualized on the same screen. The cell lineages of 10 individual N2 worms were deduced, which were quite similar to each other and to the Sulston's cell lineage. We are establishing a standard

wild-type cell lineage, which describes the mean value of nucleus position at each time point together with some statistical data, such as the variance, error distribution, etc. The lineages of *par-1* (b274) mutants were analyzed and the difference from wild-type was recognized as reported previously. Encouraged by the performance of our system, we have started systematic cell lineage analysis of knock-out animals. Studies of nucleus movement will also be presented.

323. New Methods for Imaging Endogenous Protein Structures in Live Cells and Tissues: High-Resolution Second Harmonic and Dual-Mode Non-Linear Microscopy

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Extensive processing fixation, staining, sectioning is often required to image the fine three-dimensional structure of complex biological specimens. Second harmonic imaging microscopy (SHIM), however, proves to be a powerful and unique tool for high-resolution, high-contrast, 3-dimensional studies of live cell and tissue architecture. We find that several key endogenous protein structures give rise to intense second harmonic generation (SHG) non-absorptive frequency doubling of an excitation laser line. Unlike fluorescence, SHG suffers no inherent photobleaching or toxicity and does not require exogenous labels. Unlike polarization microscopy, SHIM provides intrinsic confocality and deep sectioning in complex tissues. Furthermore, SHIM is fundamentally compatible with spatially and temporally synchronous two-photon excited epifluorescence (TPEF) microscopy.

In this study we demonstrate the clarity of SHIM optical sectioning and three-dimensional reconstruction within live, unstained, thick specimens: whole *C. elegans* animals and fresh samples of tissue from mouse. We have also combined SHIM and TPEF in dual-mode non-linear microscopy (DMNLM) to elucidate the molecular sources of SHG in live cells and tissues. SHG arises not only from coiled-coil

complexes within connective tissues and muscle thick filaments, but also from microtubule arrays within interphase and mitotic cells. Effects of both molecular orientation and symmetry upon SHG allow the signal from species generating the second harmonic to be decoded, by ratiometric correlation with TPEF, to yield information on local structure below optical resolution. Thus SHIM and DMNLM should be valuable tools for studies of cellular and structural biology both in *C. elegans* and less GFP-friendly nematodes, as well as vital histology and pathology in larger, more complex phyla.

Figure: Mitiotic spindle in an one-cell embryo expressing beta-tubuin::GFP presented in four separate representations of DMNLM.

324. Intrinsically Thermotolerant Substrains Of *C. elegans* Maintain Their IT State, Show Increased Longevity, And Elevated Hsp16 Levels.

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The response to external stresses has been studied in a number of organisms including C. elegans with the heat shock response being a classic example. We reported at the 12th International C. elegans Meeting (Smith et al., page 787) the isolation of substrains of N2 which demonstrated an intrinsic thermotolerance (defined as a significantly increased survival probability when taken from either 16 or 25 ^{o}C to 37 ^{o}C for two hours as compared to controls). These substrains were designated N2IT# (intrinsically thermotolerant) to distinguish these from EMS induced mutations which also can confer intrinsic thermotolerance. We report here that over two years after these N2ITs were isolated, that they are still maintaining their intrinsic thermolerance. In our most recent experiment (March, 2001), using N2IT6, we observed a survival probability (SP) = 0.33 as compared to a SP = .029 for the controls. Because of continual concerns that perhaps it was microenvironment that was inducing this intrinsically thermotolerant substrain of N2s, we tested three different "lots" of N2s obtained from the CGC. When tested, these three different lots of N2s also demonstrated SPs < 0.1, which implies that it is not the microenvironment of the petri dish that is producing these IT substrains.

We have examined one of our substrains, designated N2IT6, for evidence of heat shock protein induction. Western blot analysis indicated significantly elevated hsp16 levels when compared to unshocked controls (thanks to Peter Candido for the anti-hsp16 antibody). Coomassie stained SDS gels do not show any elevated hsp70 levels. Because of the poor

quality of many commercial antibodies against heat shock proteins, we have initiated RT-PCR studies on total RNA preparations from both N2IT6 and N2 controls. At this time, we are analyzing levels of hsp 70 and hsp 16 mRNA in these worms. Studies will also be initiated to look at hsp60 and hsp90 mRNA levels in N2IT6.

The stress response is designed to help the organism survive a stress that it otherwise would not, and it has been noted in other systems that the response to stress often comes at a price for the organism such as a shortened lifespan. Therefore, we decided to see what impact the IT state would have on the lifespan of these intrinsically thermotolerant worms. We performed several longevity studies comparing N2IT6 to N2 controls. Four separate experiments, involving approximately 40 worms/experiment, were conducted at 25 ^{o}C. Average lifespan for N2 Controls was 11.5 + 0.5 days. Average lifespan for N2IT6 was 13.5 + 0.7 days, which represents a 17% increase in average lifespan for the N2IT6 substrain. It was noted in every experiment that at least 2-3 worms lived significantly longer than the mean (18 - 19 days). The F1s from these extremely long lived worms were saved and are now maintained as our N2IT6 substrain. We are currently examining N2IT6 for its longevity at 16 ^{o}C versus 25 ^{o}C.

In summary, we have been able to maintain our N2IT substrains for over 2 years now. In addition to their intrinsic thermotolerance, they show increased longevity and elevated hsp16 levels. At the meeting, we will also be reporting on our RT-PCR studies examining mRNA levels for the various hsp genes.

325. Genetics of body fat and fat droplets in *C. elegans*

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In mice and humans, a complex neuroendocrine pathway functions to balance energy intake with energy expenditure, ultimately to determine body weight. Energy is mostly stored as triglycerides in specialized fat droplets, the biogenesis and cell biology of which are poorly understood.

Little is known about the determinants of body weight in *C. elegans.* As in mammals, a neuroendrocine system, including intact insulin and serotonergic signaling pathways, couple metabolism with food intake and behavior in worms.

We have used Nile Red to visualize fat droplets in the intestinal and hypodermal cells of living *C. elegans*. Nile Red is colorless in aqueous environments but becomes brightly fluorescent in hydrophobic environments. We previously showed that Nile Red staining largely recapitulates the known patterns of staining in various *C. elegans* mutants (e.g. *daf-2, daf-2:daf-16*), and conditions where body fat is known to be altered (e.g. dauer). We have performed screens on EMS-mutagenized worms to identify genetic components that may determine total body fat content, and/or fat droplet formation, size, and localization. In addition to screening mutagenized wild type (N2) worms, the screens were also conducted on *daf-16* (a forkhead transcription factor which is a key target of insulin signaling pathway), and *tph-1*(a mutation in tryptophan hydroxylase resulting in lack of serotonin biosynthesis) animals. These latter screens were aimed at identifying genetic components which may only be revealed in the absence of daf-16 mediated insulin signaling or serotonin.

A total of 12 mutants from the three screens have been selected for further analysis (8 from the mutagenized N2, 2 from *daf-16*, and 2 from *tph-1* animals). Both of the mutants recovered in the *daf-16* background and one of the mutants recovered from the *tph-1* background have been back-crossed into otherwise wild type animals (N2). The 12 mutants fall into several general categories. They include: (i) increased Nile Red Staining, (ii) decreased staining, (iii) small, diffuse droplets, (iv) enlarged droplets, and (v) animals with altered color of stain (e.g. green instead of red).

The Nile Red patterns of staining of each of these mutants were fully recapitulated by feeding fluorescently labeled C12:0 and C5:0 fatty acids to the animals (Bodipy C12:0, and Bodipy C5:0). Similarly, most of the staining patterns were mimicked by Sudan Black B staining of the animals (a method of fat staining that requires fixation of the animals).

None of the mutants are defective in dauer formation or form dauers constitutively at 25°C or 27°C. Two of the mutants confer longer lifespans (up to 2.5 fold over wild type). We are currently mapping the mutations for molecular analysis.

326. Age-related decline spares the nervous system: detailed characterization of cellular changes that accompany aging in wildtype and long-lived nematodes

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As nematodes age, they exhibit visible changes in their behavior and physiology. Older worms feed, move and defecate more slowly than their younger counterparts and have an appearance that is rough and lumpy with a generally distorted morphology. However, little is actually known about what happens at the cellular level as nematodes age. To characterize in detail the cellular changes that accompany aging in wild type *C. elegans* we coupled Nomarski microscopy, Transmission Electron Microscopy and light microscopic analysis of specific cells labeled with GFP (see abstract by Herndon et al.). Unexpectedly, this analysis indicates that aging is a tissue-specific process. We found that in the nervous system, the major structures remain present and clearly distinguishable throughout the lifespan. GFP observations using *mec-4* and *unc-129* driven expression showed that at least two specific neuron systems, the touch and VA and VB motor neurons, remain intact even in very old animals. To assay whether these neurons might be capable of functioning, we counted the numbers of apparent synapses by observing a GFP fusion to the synaptic vesicle associated membrane protein synaptobrevin expressed in both the touch neurons and the VA motorneurons. We found that in both sensory and motor neurons, similar numbers of labeled synapses are present in the processes of young and old nematodes. In a second survey, we examined the amphid neurons for their ability to take up dye present in culture. Even in very old nematodes, we observed dye-filling by the amphid neuron cluster. Overall, in our survey of the nervous system, we found no evidence of cell death, cell degradation or cell dysfunction, even in animals very close to death.

While the nervous system appears to remain remarkably intact in old nematodes, we found that other tissues such as the musculature and the hypodermis undergo a severe decline with aging. Both GFP and EM analysis revealed that these tissues deteriorate with time, with extensive changes noted in nuclei morphology and loss of cytoplasmic components. We also note that accumulation of several macromolecules in old animals, suggesting that protein synthesis may be unregulated in post-reproductive animals. For example, we find that old animals accumulate large amounts of lipid not only in the intestine where it is usually found, but to a lesser degree also in hypodermal and muscle cells. Additionally, EM studies have shown that the cuticle layer becomes profoundly thickened and wrinkled in old nematodes, suggestive of excessive collagen synthesis. Most strikingly, the body cavity of older nematodes is filled with yolk protein, which in younger animals is secreted by the intestine and efficiently taken up by developing oocytes. These examples of seemingly unregulated macromolecular synthesis might reflect the absence of selective pressure to maintain efficient body economy.

Our studies have revealed that several different tissues undergo extensive deterioration and remodeling with age. However, we do not yet know whether maintenance of one of these tissues might in fact be critical to the survival of the animal. We are now testing whether representative long-lived mutants have different kinetics of tissue-specific decline. For this analysis, we are following GFP reporters expressed in muscle (*myo-3*) and hypodermis (*col-12*) and coupled to yolk protein (*vit-2*) in long-lived mutant backgrounds. We will present our results of comparisons of these GFP constructs in wildtype and *age-1* mutant backgrounds. These studies should help identify the tissues whose preservation and function are necessary for health and longevity.

327. Effect of Sterols on Life Span and Stress Resistance in *Caenorhabditis elegans*

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C. elegans is defective in *de novo* sterol biosynthetic pathway, although it requires sterols as essential nutrient. Supplemented cholesterol undergoes extensive enzymatic modification in *C.elegans* to form other sterols of unknown function. 7-Dehydrocholesterol reductase (Dhcr7) catalyzes reduction of Δ^7-double bond of sterols and suspected to be defective in *C. elegans*, in which major endogenous sterol is 7-dehydrocholesterol (7-DHC). We microinjected human DHCR7-expression vector into *C. elegans*, which was incorporated into chromosome I by γ-radiation. This transgenic *C. elegans* was named as *Cholegans* -Cholesterol producing *C.elegans*, because it was able to convert 7-DHC into cholesterol. We investigated the effects of changes in sterol composition change on longevity and stress resistance by examining brood size, mean life span, thermotolerance and UV-resistance. The brood size of *Cholegans* with cholesterol was substantially reduced by 40 % as compared to the control group *N2*, although the growth rate was not significantly changed. The Mean life span of *Cholegans* was increased by 10% with 7-DHC for dietary sterol and by 18 % without sterol as compared to *N2*. In changes, TS_{50}, a 50 % survival time at 35 ^{o}C, was increased by 11%, while dauer formation rate unaffected. UV-resistance under 20 J/min^2 was also increased by 8%. To examine the cause of extended life span, we measured both catalase and SOD activity of *Cholegans* and found these activities increased by 10% as compared to wild type *N2*. These data suggest that a new transgenic worm, *Cholegans*, which acquired DHCR7 activity and had cholesterol as major sterol, induced stress resistance and consequently extended the mean life span.

328. Role of sensory cilia and sensory neurons in the regulation of worm lifespan

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Worms with sensory defects live longer than normal worms, which suggests that the lifespan of *C. elegans* might be regulated by the perception of a signal(s) from the environment (Apfeld and Kenyon, (1999). *Nature* **402**, 804-809). One of the mutations isolated from an EMS lifespan screen performed by our lab is an allele of *daf-10*, which is required for the normal development of the sensory cilia of the worm. *daf-10*(*mu377*) has a temperature-sensitive defect in its sensory cilia, which correlates with its temperature-sensitive lifespan phenotype. At the restrictive temperature, *mu377* exhibits a defect in its sensory cilia and lives longer than wild-type worms, whereas at the permissive temperature, *mu377* exhibits normal sensory cilia and normal lifespan. Since the defect in the sensory cilia of *mu377* is reversible, we determined the temporal requirement for the gene product of *mu377* in regulating worm lifespan by performing temperature-shift experiments. Our data suggest that the gene product of *mu377* is required late in larval development for proper lifespan control. Furthermore, to gain insight into which of the sensory neurons of the worm are necessary to regulate its lifespan, we are ablating subsets of sensory neurons in wild-type or sensory mutant worms. We are also expressing wild-type sensory genes in subsets of mutant sensory neurons to determine which functional sensory neurons are sufficient to rescue the lifespan phenotype of sensory mutants. Since different sensory neurons sense different types of environmental cues, *e.g.*, attraction to or repulsion from volatile *vs*. soluble compounds, the identification of which sensory neurons control lifespan could provide insight into what type of environmental signal regulates animal lifespan.

329. *C. elegans* Sarcopenia: Characterization of Changing Muscle Protein Expression Patterns in Aging *C. elegans*

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C. elegans body wall muscles maintain a number of proteins unique to this cell type. Many of these muscle-specific proteins comprise or regulate the function of intracellular protein structures called sarcomeres, which are able to contract or relax, thus moving the animal. In aging worms, a number of changes are observed in the anatomy of the sarcomeres using light or electron microscopy (see abstract by Herndon et al. at this meeting). First, the sarcomeres begin to lose their higher order clustering. This is observed in increased distances between the individual sarcomeres and a decreased rigidity of the individual sarcomeres. Second, the individual sarcomeres variably exhibit kinking and fraying. Finally, the individual sarcomeres sometimes lose their directionality within the muscle cells. It is not known whether these changes arise from defects intrinsic to the sarcomere structures (i.e. - their assembly or integrity), or defects in sarcomere function. To better understand the changes in sarcomere anatomy observed in aging *C. elegans*, it is necessary to gain an understanding of changes that might be occurring in the expression levels and localization of muscle-specific proteins. We chose to use a microscopy approach, with GFP-tagged proteins expressed in worm muscles, to look for changes in candidate proteins (some suggested by microarray data) that may mediate or better delineate observed changes in sarcomere anatomy. Candidate proteins include both structural proteins for sarcomeres, as well as regulatory proteins that mediate or drive the contraction of the sarcomeres. Our long term goal is to identify markers of age-related muscle deterioration that can be used to genetically dissect the molecular bases of nematode sarcopenia (the term used to describe the progressive muscle loss that accompanies human aging).

330. Molecular analysis of aging in individual nematodes

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We are interested in the relative contributions of genetic and stochastic elements to the aging process. *C. elegans* provides an ideal system in which to study this issue, since single gene mutations have been identified that extend life span, indicating the existance of a genetic component to life span. However, a population of aging, genetically identical worms experiences a marked heterogeneity in life span, demonstrating the potential interaction of stochastic factors with gene expression in determining life span. We have therefore been developing methods to study the genetic variability that arises between genetically identical individuals with age.

Deletions of the mitochondrial DNA (mtDNA) have been observed to increase in incidence with age in several organisms, including humans (Melov et al. (1995) NAR 23:4122) and *C. elegans* (Melov et al. (1995) NAR 23:1419). The accumulation of such alterations in mtDNA is a potential stochastic influence on life span. We are using real-time quantitative PCR to determine the copy number of mtDNA in individual worms with age. In addition to N2, *mev-1* (a mutant sensitive to oxidative stress) and *daf-2* (a long-lived and stress-resistant mutant) have been studied. In conjunction with deletion mapping by long-extension PCR of the mtDNA and sequencing of breakpoints, quantitative PCR will allow us to determine the mutational load of these large scale deletions that individual worms are carrying.

To compare molecular characteristics of senescence between individual nematodes across their life span, we are investigating gene expression in single worms and characterizing worm to worm variation. Development and optimization of methods to use microarray analysis of gene expression profiling in individual worms will be presented.

331. GENE EXPRESSION ANALYSIS OF C. elegans LIFESPAN MUTANTS

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The discovery of long-lived mutants in various systems suggests that aging is a regulated process. C. elegans, a soil nematode whose lifespan is on the order of weeks and whose genome has been fully sequenced, is an ideal organism in which to study the genetic basis of aging. Mutants of the daf-2 insulin-like receptor pathway have been found to extend lifespan in C. elegans. This pathway is presumed to affect the output of an HNF/forkhead transcription factor, DAF-16; daf-16 mutants suppress the life extension of daf-2 and age-1 mutants. Signals from the sensory neurons and the reproductive system of the worm affect lifespan, acting through this signaling pathway. In an effort to monitor changes in gene expression during the aging process, we have built a full-genome C. elegans microarray containing PCR fragments of almost 20,000 predicted genes. We are using these microarrays to analyze the transcriptional output of aging worms and lifespan mutants to define the group of genes whose expression is necessary for long life.

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332. Effects of ubiquinone metabolism on *clk-1* biology

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In *C. elegans*, the *clk-1* gene product controls lifespan and many physiological rates, such as defecation, pharyngeal pumping, and movement, and the length of embryonic and post-embryonic development. *clk-1* encodes a 187 amino acid protein localized in mitochondria (Branicky *et al.*, 2000). Interestingly, the *clk-1* homolog in yeast, *coq7*, is implicated in the synthesis of ubiquinone (Q), an important electron transporter especially in mitochondria. Recently, the involvment of CLK-1 in Q biosynthesis in *C. elegans* has been demonstrated by the finding that *clk-1* mutants do not synthesize Q (Jonassen *et al.*, 2000, Miyadera *et al.*, 2001). Rather, they accumulate demethoxyubiquinone (DMQ), which is a Q synthesis intermediate able to sustain mitochondrial respiration *in vitro*, such that mutant mitochondria have almost wild-type levels of electron transport despite a complete absence of Q (Miyadera *et al.*, 2001). Only DMQ is present in all three *clk-1* alleles irrespective of the severity of their effect on physiological rates, which suggests that the lack of Q cannot solely account for the Clk phenotype. Recently it has also been found that *clk-1* mutants are unable to grow on a Q-deficient bacterial strain (Jonassen *et al.*, 2000). This seems to indicate that the endogenous Q manufactured by the bacteria on which the worms are grown, is capable of complementing some key cellular process that requires Q. Alternatively, it could be that *clk-1* mutants cannot grow on Q-deficient strains because of the imbalanced metabolism of these strains, which could result in an accumulation of compounds that are selectively detrimental to the *clk-1* mutants. In order to better understand the impact of Q metabolism on *clk-1*, we are systematically evaluating the behavior of Clk worms, when fed with bacteria deficient in each of the genes implicated in Q biosynthesis in *E. coli* (*ubi* genes). We will also evaluate the effect of exogenous Qs, fed to the live worms. Finally, we will investigate the status of *ubi* homologs in Clk mutants, at the mRNA and the protein level. This approach will help to understand how the ubiquinone biosynthesis defect relates to the Clk phenotype: whether it causes the pleiotropy of *clk-1* mutants, or whether it is one of their pleiotropic features.

References:

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333. Identification of two novel *clk* mutants

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It has previously been shown that mutations in the genes *clk-1*, *clk-2*, *clk-3*, and *gro-1* are highly pleiotropic, causing an increase in the average period of nematode development, a slowing of rhythmic behaviors such as defecation, swimming, and pharyngeal pumping, and a lengthening of life span.

Intuitively these genes may be positioned in the same genetic pathway because mutations in any one of them give rise to similar mutant phenotypes. However, it remains possible that they might also function independently of each other. We are particularly interested in the life span extension conferred by *clk* mutants.

To begin to understand how *clk* genes function in life span regulation, we performed five independent EMS mutagenesis experiments, screening 20, 000 mutagenized genomes in total. We identified two novel *clk* mutants. Both mutants complement each other, or the four known *clk* mutants, *clk-1*, *clk-2*, *clk-3*, and *gro-1*.

Currently, we are characterizing the phenotypes of the *clk-4* and *clk-5* mutants.

334. Developmental control of coenzyme Q levels - the L2 arrest in *clk-1* mutants coincides with an increased reliance on respiratory energy metabolism

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The developmental and long-lived phenotypes described for the *C. elegans clk-1* mutants depend critically upon a dietary source of coenzyme Q. Coenzyme Q functions in cells as an essential redox-active component of both mitochondrial and plasma membrane electron transport, as an essential lipid antioxidant, and plays a role in uridine synthesis and uncoupling activity in mitochondria. When provided a diet lacking coenzyme Q, the *clk-1* mutants display a growth arrest in early development from eggs, and sterility upon emerging from dauer larvae. As observed for the corresponding yeast *coq7* mutants, the *clk-1* mutants have a defect in coenzyme Q biosynthesis. Following growth on Q_8-replete *E. coli*, the standard diet for *C. elegans*, the *clk-1* mutants completely lack Q_9 and accumulate the biosynthetic intermediate demethoxy-Q_9. The subscripts 8 and 9 refer to the number of isoprene units in the tail of Q synthesized by *E. coli* and *C. elegans*, respectively. The *clk-1* mutants display the early developmental arrest when fed any of three distinct Q-less *E.coli* strains, including *ubiA*$^-$, *ubiE*$^-$, or *ubiG*$^-$ mutants. These results indicate that the *clk-1* arrest phenotype results from the lack of Q and not from the distinct Q-intermediates that accumulate in these Q-less *E. coli* strains.

Slow growing *C. elegans* mutants that map to genetic loci other than *clk-1*, were evaluated for Q biosynthesis. This was of particular interest due to the overlap of the physical map position of the homologs of the yeast genes *COQ2* and *COQ5* with the genetic interval containing *clk-2*. We find that *clk-2*, *clk-3*, and *mev-1* produce coenzyme Q, as detected by HPLC/ECD assays. We find that *clk-2*, *clk-3*, *gro-1* and *gro-2* all grow to adulthood on a diet of Q-less *E. coli*. The very slow developing and long-lived double mutant *daf-2 clk-1* is

Q-defective by both assays. These data indicate that slow growth phenotype can be accounted for by decreases in the level of Q_9 only for *clk-1*.

We have speculated that the *clk-1* mutant worms fed the standard Q-replete *E. coli* may compensate for their Q_9 deficiency by increasing synthesis of demethoxy- Q_9 and rhodoquinone-9 (RQ_9), and by increasing their uptake of Q_8 from their diet of *E. coli*. To investigate this in detail, we have quantified the levels of quinones in several developmental stages for wild type fed OP50 to establish the normal pattern. We then quantified the level demethoxy-Q_9 and other Q isoforms in *clk-1* mutant eggs, L1 larvae, arrested L2, L4, and young adult. These results show distinct changes in quinone content during the development of the animals. These data suggest that the arrest coincides with a period of development where there is increased demand for respiratory metabolism/nucleotide synthesis.

335. No reduction of energy metabolism in Clk mutants.

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The Clk genes *clk-1, clk-2, clk-3,* and *gro-1,* control the timing of a range of physiological processes in *C. elegans*. Mutation in these genes causes a mean increase of adult life span, cell cycle length, and a slowing down of development and behavioral activity (pharyngeal pumping, defecating, egg laying, and moving).

Is a reduced energy metabolism responsible for both the Slow and Age phenotype of the Clk mutants, as the rate-of-living hypothesis would suggest? We determined metabolic rate by monitoring several physiological and biochemical parameters in synchronously ageing cohorts: oxygen consumption, heat generation, ATP levels, metabolic potential (lucigenin assay), reductase activity, and autofluorescence. All measurements were normalised to protein content.

Surprisingly, oxygen consumption rate, heat output, ATP levels, metabolic potential, reductase activity, and oxidised flavin autofluorescence were generally higher in most Clk mutants compared to wild type. In contrast to the other Clk's, *clk-2(qm37)* showed a wild-type pattern for most parameters, but, at advanced ages, light production (lucigenin assay) was significantly lower than wild type. Autofluorescence of lipofuscin (and reduced nicotinamids as well) was slightly reduced in *clk-1(e2519)* and *clk-2(qm37)* mutants, whereas *clk-3(qm38)* and *gro-1(e2400)* showed wild type levels.

Contrary to the above speculation, we found that several parameters of energy metabolism are upregulated in *clk-1(e2519), clk-3(qm38),* and *gro-1(e2400)* mutants. We conclude that energy production is not lower, rather higher, in the Clk mutants. The enhanced reductase activity may confer increased resistance to oxidative stress

and prolonged life span. Unlike the other Clk's, *clk-2(qm37)* showed a wild-type like metabolism, but produced less superoxide in the lucigenin assay. Its low oxidised flavin autofluorescence may indicate an altered redox-balance.

336. Distinct mechanisms of Caenorhabditis elegans life extension by daf-2 mutation and by dietary restriction

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Dietary restriction (DR) can extend the life span of many animals including Caenorhabditis. Longevity also can result from activation of a dauer-larva formation (daf) signaling pathway, akin to insulin signaling, effected by mutations in daf-2 or age-1, but blocked by daf-16 defects. Using pharyngeal-defective eat mutants, Lakowski and Hekimi [1998] provided strong but indirect evidence that DR- and daf-mediated aging retardation are additive, suggesting mechanistic independence. We have shown 42% and 39% life extensions, respectively, of the normal-lived daf-16(m26); daf-2(e1370) and long-lived daf-2(e1370) strains, resulting from a 6.7-fold reduction in sedimented E. coli (var. OP50) density in unstirred shallow-liquid cultures, thus providing direct confirmation of the eat strain results.

Poly[A]+ RNA was prepared from such cultures and reverse-transcribed to generate fluoroprobes for C. elegans DNA microarray hybridization. Three arrays have been probed to date: (1) a survey of expression effects of 6.7-fold food reduction on daf-16; daf-2; (2) the same survey using daf-2; and (3) a comparison of these two strains at the higher OP50 density (270 million/cm2). Data exploration by cluster analysis and other statistical methods is in progress. Preliminary results suggest that DR affects expression of many of the same genes in the two strains, and that this set of genes shows only modest intersection with the set that differs

in expression between the two strains when both are provided with normal nutrition. If this holds true upon complete analysis, it is consistent with mechanisms for daf- and DR-mediated longevity that are distinct.

337. Optimising dietary restriction-mediated lifespan extension

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A number of reports have shown that dietary restriction (DR) results in increased lifespan in *C. elegans*, with the magnitude of increase depending upon the method used (1-4). How DR extends lifespan is unknown, but one possibility is that it down-regulates the insulin/IGF (IIS) signalling pathway which regulates ageing. Work to date is both consistent with (5) and contradictory to (4) this hypothesis.

We are testing four methods used previously to exert DR: diluted monoxenic liquid culture (1), reduced nutrient NGM (2), axenic culture (3), and the use of *eat* mutations (4). We are determining which method can be optimised to (a) give the greatest magnitude of lifespan extension; (b) can be made quantitative (so the degree of DR imposed can be varied); and (c) is easy to perform. We are also seeking to maximise DR-mediated lifespan extension such that further reduction of dietary intake would lead to malnutrition and premature death.

Our aim is to use an optimised DR protocol to understand the mode of action of DR, and its relationship to IIS, reproduction and oxidative damage.

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338. Caloric restriction activates stress resistance and confers life extension

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Caloric restriction increases life span of a wide range of animals including *C elegans*. The mechanisms underlying this effect are still unknown. We used axenic culture medium and *eat-2* mutants to study the effect of dietary restriction on metabolism and stress resistance. All empirical data were normalised to protein content to account for differences in body mass. Stress resistance was assessed by assaying survival at elevated temperature.

Wild-type worms grown in axenic culture medium consume more oxygen and produce more heat than worms grown on a bacterial diet and this effect is slightly higher in *eat-2* mutants (at advanced ages). ATP levels decline steeply with age in worms grown on *E. coli*. A much milder decrease is seen in axenically cultured worms. However, the light production potential (a measure of *maximum* metabolic, mainly mitochondrial, performance) decreases similarly in worms grown in either nutritional regime. Axenically cultured worms have enhanced catalase activities. Wild-type worms acquire an Itt (increased thermotolerance) phenotype in axenic culture medium. *eat-2* mutant worms are Itt, and this phenotype is strengthened in axenic medium.

We conclude that axenic culture medium confers life span extension by eliciting enhanced stress resistance, rather than lowering metabolic rate.

339. Molecular mechanisms underlying the effects of caloric restriction on aging in *C. elegans*

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Although low caloric intake (caloric restriction, CR) has been documented to significantly lengthen lifespan in a wide variety of organisms, very little is known about the molecular mechanisms that are responsible for this effect. In *C. elegans*, lowering food intake by reducing its availability in the environment prolongs animal lifespan, indicating that a CR-related mechanism of extending lifespan exists in the worm (Johnson et al., 1984). In addition, genetically reducing feeding efficiency by means of mutations that compromise the animals ability to ingest food has a similar effect on longevity (Lakowski and Hekimi, 1998).

Gene expression profiling studies in mice subjected to CR highlight several genes involved in protein turnover (synthesis and degradation), which have significantly altered expression levels (Lee et al. 1999). These findings suggest that a shift towards higher protein turnover rates is associated with CR. Increased protein turnover might consequently be one of the major causes of lifespan extension under CR, by facilitating the maintenance of a fresher pool of proteins with less accumulated damage. We are currently testing this hypothesis by overexpressing wild type and mutant variants of the eukaryotic elongation factor 2 (eEF-2), in *C. elegans*. eEF-2 is a key translation factor that is required for the translocation step of peptide chain elongation, which involves movement of the ribosome one codon at a time along the mRNA. When phosphorylated at threonine 56 and 58 this factor is rendered inactive and translation is blocked. Our preliminary work suggests that overexpression of wild type and non-phosporylatable eEF-2 variants can prolong lifespan. We hypothesize that this effect could reflect a postulated higher protein synthesis rate in the overexpressing lines. We will extend this study to include eEF-2 mutants that mimic its phosphorylated form and are expected to have the opposite effect on protein synthesis.

Furthermore, we will include other key protein turnover regulatory factors in our analysis in an effort to determine how general are the effects of protein turnover on aging. Taken together, our observations suggest that a study of the relation between CR and protein turnover should have an immediate impact on our understanding of aging.

Our overall plan is to investigate the link between caloric restriction, protein turnover and aging in the facile *C. elegans* model organism. We will focus our efforts in identifying the molecular requirements for CR-induced lifespan extension. To achieve this goal we propose to first, determine the role of protein turnover in CR-mediated lifespan extension in the nematode and second, to screen for and characterize nematode mutants incapable of a normal response to CR.

340. DOES DAF-4 ALTERNATIVE POLYADENYLATION GENERATE A RECEPTOR ANTAGONIST?

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DAF-4 is the only type II TGF-beta/BMP receptor encoded in the C. elegans genome. daf-4 mutants are Daf, Sma and Mab, indicating a role for this receptor kinase in continuous (non-dauer) development, normal body size and male tail development. There are two daf-4 transcripts. During continuous growth, a 2.0 kb message is between 1.2 and 1.9-fold more abundant than the full-length 2.9 kb mRNA. In dauer larvae this ratio increases to 5-fold. However, when dauer larvae recover in fresh medium and resume growth, the relative abundance of 2.0 kb/2.9 kb mRNA declines to that of well-fed populations. This correlation suggests that alternative processing of daf-4 may be environmentally regulated.

The 2.9 kb mRNA contains an SL1-spliced 11-exon open reading frame encoding the 744-amino acid DAF-4 receptor kinase. Using RT-PCR, we cloned and sequenced a cDNA of the 2.0 kb transcript, which includes an SL1 leader, all of exons 1-5, and intron 5 sequence upstream of an alternative poly(A) addition site. The open reading frame in the 2.0 kb cDNA encodes a putative 206-amino acid polypeptide that lacks transmembrane and kinase domains, but maintains 90% of the extracellular domain.

To measure the effect of overexpressing the short daf-4 transcript on dauer formation, a daf-4 genomic fragment containing upstream sequence through intron 5, was subcloned and microinjected with the rol-6 marker into a weak daf-4 mutant. In three transgenic lines, constitutive dauer formation was enhanced in Rol progeny compared to non-Rol siblings. In one line at 22.5°C, 70% of the Rol progeny formed dauer larvae, whereas only 2% of the non-Rol siblings entered diapause. These results indicate that overexpression of the short daf-4 transgene increases dauer formation. We are currently testing whether this may be due to antagonism of DAF-4 signaling by the truncated receptor. For example, the secreted extracellular domain may bind ligand in competition with

full-length receptor. Its abundance in the dauer stage may prevent dauer recovery in response to transient ligand expression.

341. A *C. elegans* CREB PROTEIN MODULATES TGF-BETA SIGNALING

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The cyclic AMP-response element binding protein CREB plays a central role in long-term memory in *Aplysia*, *Drosophila* and mice. Analysis of the genome sequence showed that *C. elegans* has a single *CREB*-homologous gene (*crh-1*). We characterized the *C. elegans* and *C. briggsae crh-1* genes and found that they have a similar gene structure and that their encoded proteins are 85% identical. The DNA-binding bZIP domain and cAMP-dependent kinase site, as defined in the mammalian and *Drosophila* CREB family members, are highly conserved in the nematode proteins. The *C. elegans crh-1* gene has several alternatively-spliced isoforms. A *crh-1::gfp* transgene is ubiquitously expressed during early embryogenesis and is specifically expressed in several neurons from the L1 stage to adulthood. CRH-1 can bind to cyclic AMP-response element (CRE) sites and can be phosophorylated by cAMP-dependent protein kinase (PKA) and Calmodulin-dependent protein kinase II (CaMKII) *in vitro*.

To determine the function of *crh-1*, we isolated three *crh-1* deletion alleles from a deletion library. Two deletions remove the second exon and are predicted to cause early truncations; the third deletes part of the bZIP domain. No CRH-1 protein is detected by western blot analyses of these three mutant strains, suggesting that all three are null alleles.

crh-1 mutants are viable and show no obvious abnormalities in brood size, locomotion, mechanosensation, chemotaxis or thermotaxis. We found that mutations in *crh-1* confer a dauer-constitutive phenotype (Daf-c). However, like *unc-3, unc-31*, and *unc-64*[1] mutants, *crh-1* mutants form dauers at 27C but not at 25C. In addition, like the Daf-c mutants *daf-1, daf-7, daf-8, and daf-14*, *crh-1* mutants tend to accumulate at the edge of a bacterial lawn (bordering) and form clumps of animals. Mutations in many genes that cause a Daf-c phenotype at 25C have been isolated.

Characterization of these mutants has shown that an insulin-like and a DAF-7 transforming growth factor (TGF)-beta signaling pathway act in parallel to regulate dauer formation. Dauer pheromone, temperature, and food signals modulate the dauer decision in part by regulating the expression of the TGF-beta homolog, DAF-7 in the ASI chemosensory neurons.

Although *unc-3, unc-31, unc-64* [1], and *crh-1* single mutants do not have strong defects in dauer formation at lower temperatures, double mutant combinations of *unc-3, unc-31,* and *unc-64* [1] are strongly Daf-c at 25C. Double mutants between *crh-1* and either *unc-31* or *unc-64*, but not *unc-3*, show a strongly enhanced Daf-c phenotype at 25C. This observation suggests that *crh-1* and *unc-3* affect similar aspects of dauer formation. *unc-3* encodes a transcription factor that is expressed in the ASI neurons and may regulate the expression of *daf-7* [2]. Consistent with this notion we found that the expression of a *daf-7::gfp* reporter is strongly reduced in *crh-1* mutants. Our data suggest that *crh-1* mutants incorrectly integrate environmental cues that induce dauer formation and that *crh-1* is part of a chemosensory cascade that regulates TGF-beta signaling.

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342. DIN-1, a putative DAF-12 cofactor that regulates the *C.elegans* dauer diapause

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The nuclear receptor *daf-12* acts as a control gene at the intersection of heterochronic and dauer pathways. To further understand the molecular mechanisms of DAF-12 regulated pathways, we screened for DAF-12 interacting proteins by the Yeast-Two Hybrid method. Three candidate interactors were identified. One of these, called DIN-1 (DAF-12 interacting Protein 1) is predicted to be a nuclear protein of 2328 amino acids, containing several nuclear localisation sites, an RNA recognition motif, and regions of homology to the mammalian Msx-2 interacting target protein (MINT). In the C-terminus, there are several motifs characteristic of nuclear receptor corepressors in the region that interacts with DAF-12. These structural data suggest that DIN-1 might be a DAF-12 cofactor.

din-1 was shown to have clear effects in functional knock out assays done by RNAi feeding experiments. *din-1* partly or completely suppressed the dauer constitutive (Daf-c) phenotypes of mutants that act upstream of *daf-12* in the dauer pathway, including insulin-like (*daf-2*), TGF-beta (*daf-7*), cGMP (*daf-11*) and hormonal (*daf-9*) signaling. Moreover, *din-1* dramatically suppressed the delayed heterochronic and Daf-c phenotypes of *daf-12* gain-of-function alleles, but had no effect on *daf-12* null mutants. Consistent with two-hybrid interaction, these genetic data strongly suggest that DAF-12 and DIN-1act at the same step. We think that DIN-1could function in a transcriptional complex with DAF-12, perhaps as a corepressor or coactivator controlling developmental arrest and dauer formation.

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343. A bin's-worth of bounty: SAGE data mining in dauer larvae and mixed-stage transcript profiles

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What is the constitution of a survivor? *Caenorhabditis elegans* can endure adverse environmental conditions by entering the developmentally-arrested dauer stage. Metabolic changes in the dauer larva reflect the fasting survival period, which may extend to 12 times the normal life span. We compared gene transcript profiles between dauer larvae and non-dauer (mixed-stage) populations to further characterize the dauer state. Serial Analysis of Gene Expression (SAGE) enabled the identification of extant gene transcripts and a quantitative estimate of their respective abundance by correlations between predicted genes and short sequences (tags) from dauer and mixed-stage cDNA libraries. The number of SAGE tags per tag-transcript correlate indicates the abundance of the transcript in the dauer or mixed-stage profile. The existence of more than one unique tag per gene can be due to alternate transcripts.

After exclusion of single occurrence tags, 9304 unique SAGE tags (7642 genes) constituted the dataset from which a dauer profile (7676 tag-transcript correlates, 1251 dauer specific) and mixed-stage profile (8053 correlates, 1628 mixed-stage specific) were derived. When tag-transcript correlates were grouped by transcript abundance classes (bins) in doubled increments, frequency distributions for the two transcript profiles were exponential. A negative log linear relationship between the transcript abundance bin and the number of transcripts in each bin was observed in both the dauer and mixed-stage profiles. A significantly greater variance in the dauer profile compared to the mixed-stage profile is explained, at least in part, by differing variances between pooled bins in the tails of the two distributions, where transcript abundance is high. Specific transcripts within bins can be ordered to show contrasts in the dauer versus mixed-stage profiles, by either abundance differences or ratios. The exponentially binned classes of transcript abundance not only provide manageable subdivisions for comparisons of dauer and mixed stages, but may reveal more global factors that govern the *C. elegans* transcriptome, such as gene location relative to condensed versus open chromatin regions.

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344. A Model of Human Niemann-Pick Type C disease in *C. elegans*

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Niemann-Pick type C disease (NPC) is a fatal childhood onset neurodegenerative disorder. The majority of NPC cases are caused by mutations in the NPC-1 gene. It has been proposed that NPC-1 protein acts as a pump to transport cholesterol and other lipids from the late endosomes/ lysosome compartment into ER, but very little is known about its exact function. A search of the *C. elegans* genome identified two genes, *npc-1* and *npc-2*, that are homologues of the human NPC-1 gene. Deletion alleles of both *npc* genes were obtained in a large scale PCR based screen. (Sym *et. al.*, 2000) Animals homozygous for deletion of either *npc* gene are viable and fertile. Animals mutated for both *npc-1* and *npc-2* appear and behave normal as larvae, but develop into adult worms that are thin and pale, have reduced fertility, and significantly shortened life span. These phenotypes of the double mutant animals might be due to defective cholesterol homeostasis. Currently, we are developing assays to determine whether cholesterol homoeostasis is disrupted in the *npc* mutant worms. In addition, we are going to examine neuronal morphology in staged animals using gfp marker to detect any neuronal degeneration.

Animals that are mutated for both *npc* genes also have an unusual transient temperature-insensitive dauer-constitutive (Daf-c) phenotype, suggesting overlapping functions of *npc-1* and *npc-2* in dauer formation. The formation of dauer, an alternative developmental larval stage in *C. elegans*, is controlled by parallel signal pathways that respond to various environmental stresses. Epistasis analysis indicates that the Daf-c phenotype does not depend on the function of any of the three known dauer formation pathways, but does depend on the nuclear hormone receptor DAF-12. We have conducted a genetic screen for suppressors of the Daf-c phenotype. Seven independent suppressors have been identified in a screen of 10,000 genomes. Many of the suppressors not only suppress the Daf-c phenotype of the double mutant worms, but also increase significantly their life span. Thus they may identify proteins that are functional partners of NPC proteins and are potential targets for the treatment of NPC disease. We are currently mapping and cloning these suppressors.

Reference: Sym, M. *et. al.* Current Biology 2000, 10(9): 527-30.

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345. Pheromone Regulation of daf-7expression

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A major regulator of the decision to enter the dauer stage is the level in the environment of the secreted dauer promoting pheromone. Dauer pheromone controls the expression of the TGF-β-like ligand DAF-7 in the ASI amphid neuron (Ren et al. 1996, Schackwitz et al. 1996). We are studying the molecular mechanism by which this extracellular pheromone signal regulates *daf-7* transcription. We have identified *daf-11* as an important regulator of *daf-7* expression. *daf-11* encodes a transmembrane guanylyl cyclase, an enzyme that converts GTP to the intracellular second messenger cGMP. In *daf-11* animals daf-7pGFP levels are dramatically reduced. We have also found that loss of function mutations in the α subunit of a cyclic nucloetide gated ion channel *tax-4* also cause a loss of DAF-7 expression. The dauer and low daf-7p::GFP phenotypes of *daf-11* but not *tax-4* mutants are reversed by exogenous addition of cGMP analogues. The cilia mutant *osm-1(p808)*, while capable of suppressing the DAF-c phenotype of *daf-11*, does not suppress the associated low daf-7p::GFP phenotype suggesting that cilia mutants do not suppress *daf-11* by activating the signaling pathway to *daf-7*. These results suggests that pheromone signaling *in C. elegans*, like mammalian photoreceptor signaling, involves the second messenger cGMP and a cGMP regulated ion channel.

To identify additional signaling molecules functioning between dauer pheromone and daf-7, we have undertaken two genetic screens. The first screen seeks to identify mutations that both suppress the *daf-11* Daf-c phenotype and lead to a reversion in daf-7p::GFP expression. From an initial screen of 250,000 haploid genomes we have identified two mutations: **p**heromone **s**ignaling **i**nhibitor: psi-1(*mg296)* and psi-2(*mg297)* that satisfy these criteria. psi-1(*mg296)* is a recessive mutant that blocks dauer pheromone downregulation of daf-7p::GFP. We are now mapping psi-1 and psi-2 for molecular analysis. Our second genetic screen seeks to identify mutations that both suppress the *tax-4* Daf-c phenotype and also lead to daf-7p::GFP expression. From an initial screen of 10,000 haploid genomes we have identified two mutations: psi-3(*mg318)* and psi-4(*mg319*) that satisfy these criteria. Progress on the characterization, mapping, and identification of these mutations will be reported.

346. Searching for new proteins involved in dauer formation by two-hybrid protein interaction mapping.

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This project aims to identify new proteins involved in dauer formation by large-scale yeast two-hybrid screens using all the known dauer gene products as baits. Open reading frames encoding each of 34 proteins implicated in dauer formation (daORFs) were cloned into yeast two-hybrid bait (DB-daORFs) and prey (AD-daORFs) vectors by Gateway recombinational cloning. First all pairwise DB-daORF/AD-daORF combinations were tested. This matrix experiment recapitulated an interaction between the DAF-3 (an ortholog of SMAD4) and DAF-5 (a relative of the SNO oncoprotein; Garth Patterson, personal communication) gene products. Individual yeast two-hybrid screens of a C. elegans AD-cDNA library were then carried out with each DB-daORF. This large-scale mapping experiment identified a total of 58 putative interactors. Among others, an interaction between AGE-1 (an ortholog of PI-3-kinase) and the worm ortholog of the p85 adaptor protein was detected. Most of the interactors identified correspond to previously uncharacterized gene products. They will be subjected to functional analysis by systematic RNA-mediated interference to test their involvement in dauer formation.

347. Natural variation in dauer formation genes

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Natural isolates of *C. elegans* show significant variation in their response to dauer pheromone. CB4507, a strain from the Anza Borrega Desert in California, is virtually nonresponsive to pheromone at temperatures below 25°. However, like N2, it forms dauers without pheromone at 27°. Thus, the dauer defect in this strain appears to be specific to pheromone response and not temperature response. We found that the pheromone-insensitive phenotype of CB4507 could be mapped to a single locus (*sa935*) on chromosome V. Additionally, *sa935* partially suppresses the Daf-c phenotype of *daf-7* and *daf-14* (in the TGF-β branch of the dauer pathway) but not *daf-11* or *daf-2* (in other branches). Mutations in the gene *scd-2* also map to chromosome V, suppress TGF-β branch mutations and are pheromone-insensitive. Complementation tests showed that *sa935* is an allele of *scd-2*. We cloned *scd-2* and found that it encodes a receptor tyrosine kinase most closely related to the human oncogenes ALK and LTK. *sa935* leads to substitution of arginine for glycine near the N-terminus of the kinase domain. This glycine is strongly conserved in protein kinases and is essential for kinase activity. Thus, *sa935* is predicted to result in a kinase-null form of the SCD-2 protein. The two EMS-induced *scd-2* alleles have mutations in the kinase domain and a splice-site. Thus, none of the alleles are predicted to be null. Consistent with the possibility that SCD-2 has a kinase-independent function, there are isoforms of human LTK lacking the kinase domain.

Like *scd-2*, mutations in *scd-1* suppress the Daf-c phenotype of TGF-β branch mutants. However, *scd-1* mutations do not affect pheromone response, but are stronger suppressors of the TGF-β branch mutants and suppress other pleiotropies of these mutants as well. We cloned *scd-1* and found that it encodes a novel protein with several regions that are extremely glutamine (Q) rich. Within these regions, there are long runs of Q interspersed with other amino acids, usually alanine. The

longest pure poly-Q run is 8 amino acids. The *C. briggsae scd-1* homolog has many differences from *C. elegans* within the Q-rich regions. A *scd-1::gfp* fusion is expressed in many tissues, most notably hypodermis and neurons. Expression of constructs carrying the Q-rich regions is cytoplasmic, with a distinctly speckled and stringy appearance. This localization pattern appears to depend on the presence of the Q-rich regions and may be a result of protein aggregation.

348. Variation in dauer formation in *C. elegans*

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Dauer formation in *C. elegans* is an example of phenotypic plasticity. We have searched for variation in this phenotypic plasticity by comparing dauer larva formation under a range of dauer inducing conditions in different wild isolates of *C. elegans*. There was substantial within- and between-assay variation in dauer formation for all isolates. In spite of the between-assay variation, by ANOVA we have detected reproducible, significant variation between some isolates in their dauer formation over a range of dauer inducing conditions. However, before genetic mapping of phenotypic plasticity of dauer formation in *C. elegans* is possible, this trait needs to be accurately and reliably determined in the offspring of a cross. Hence currently we are measuring the phenotypic plasticity of dauer formation in recombinant inbred lines of *C. elegans*.

349. DAF-7/ TGF-b expression required for larval development in *C. elegans* is controlled by presumed guanylyl cyclase DAF-11.

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In *C. elegans* development, unfavorable growth conditions lead a larva to an arrested and enduring form called a dauer. To elucidate components upstream of DAF-7/TGF-b in this control pathway, we isolated a mutant that was defective in *daf-7* promoter::gfp reporter expression and showed arrested (dauer-constitutive) phenotype. It has a new mutation in the *daf-11* gene encoding a transmembrane guanylyl cyclase. We show that *daf-11* gene and a related gene *daf-21* act upstream of *daf-7*, and cilium-related genes *che-2* and *che-3* act between *daf-11* and *daf-7*, to control dauer formation. Expression of *daf-11* cDNA by cell specific promoters suggests that *daf-11* acts cell autonomously in ASI chemosensory neurons and that ASI and ASJ neurons have different signaling pathways for dauer formation.

350. A novel role for LAG-2/LIN-12 signaling in dauer recovery

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lag-2 is a member of the Delta/Serrate/LAG-2 (DSL) family of ligands involved mainly in cell fate specification. LIN-12 and GLP-1 are the two Notch receptors identified in C. elegans capable of responding to the LAG-2 signal. During post-embryonic development, Notch signaling has been well characterized in numerous developmental processes while it has recently been implicated in thermotactic behavior. Up to now, no known neuronal function for GLP-1 or LIN-12 has been described in the worm. We noticed that a *LAG-2::GFP*translational fusion (kindly provided by Judith Kimble) was expressed in 3 pairs of IL-1 neurons in the head region exclusively during dauer and post-dauer development. This suggests that *lag-2* and Notch signaling may be involved in some aspect of dauer development. To test this possibility, we used a dauer constitutive mutation, *daf-7(e1372),* that allowed us to further investigate the function of Notch signaling during the dauer stage.

Using a strain that expresses *lag-2* from a transgene *(qIs56),*we found that dauer formation occurred normally at 25°C in a *daf-7 (e1372ts)* background (100%, n=1255). However, 55% of the *qIs56; daf-7 (e1372)* worms recover at restrictive temperature compared to 9% of *daf-7 (e1372)* after 24 hours (~ 6 fold increase).*daf-2,* a member of a parallel dauer pathway, is required for this*lag-2* induced recovery since none of the *qIs56; daf-2 (e1370ts)* animals recovered under the same conditions (n=451). Therefore, *lag-2*requires wild type *daf-2* gene product to cause this phenotype.

Since extra copies of *lag-2* likely cause this recovery by activating the Notch signaling pathway, we want to confirm if a *lin-12(d)* allele*(n302)* could phenocopy this effect independent of the *LAG-2::GFP*transgene. In a different set of experiments, worms carrying an integrated LIN-12*::GFP (arIs41)* transgene (kindly provided by Iva Greenwald) in a *daf-7(e1372)* background also recover to a significantly greater extent then the

*daf-7(e1372)*animals (63%, n=154 compare to 9%). Therefore, *lag-2*likely signals through *lin-12* in order to initiate dauer recovery.

We are currently performing antibody staining to determine in which neurons of the head LIN-12 is expressed to support our genetic results. We are also analyzing the promoter region of *lag-2* to determine elements required for its dauer-specific expression. Since this *lin-12*-mediated dauer recovery requires *daf-2* function, we will concentrate on how these two important pathways converge to affect dauer recovery in C. elegans.

351. IDENTIFICATION OF TWO GENES THAT CONTROL THE SURVIVAL OF THE MALE-SPECIFIC CEM NEURONS

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During wild-type hermaphrodite development, 131 somatic cells undergo programmed cell death. While many genes involved in the execution of cell death have been identified, the mechanisms that control the commitment of specific cells to undergo programmed cell death are poorly understood. To date, mutations in four genes, *ces-1*, *-2*, and *-3* (cell death specification), and *egl-1*, have been found to affect specifically the deaths of particular cells. *ces-1* and *ces-2* encode transcription factors. Mutations in a transcriptional regulatory element of *egl-1*, which encodes a protein required for all somatic cell deaths, cause inappropriate expression of *egl-1* in the HSNs in hermaphrodites, resulting in their deaths.

To identify additional genes that act in the specification of cell death, we have performed a genetic screen for hermaphrodites in which the male-specific CEM neurons fail to undergo programmed cell death. The CEM neurons die during normal hermaphrodite development but survive and differentiate in males. The reporter *pkd-2*::*gfp* (kindly provided by Maureen Barr and Paul Sternberg) expresses in the CEMs of males and in the CEMs of *ced-3(n717)* hermaphrodites, which are defective in essentially all programmed cell death. By using the *pkd-2::gfp* reporter as a marker for CEM survival, we were able to screen efficiently for survival of a single cell using a dissecting microscope equipped with fluorescence optics.

A screen of 60,000 mutagenized haploid genomes yielded at least 146 independent mutations that cause survival of the CEMs, including 40 alleles of known cell-death genes and at least 60 mutations that cause sexual transformation. Seven mutations, which define two genes, cause CEM survival but do not cause other obvious sexual transformation or a nonspecific defect in programmed cell death. Both genes act epistatically to null mutations in *fem-2* and *fem-3*, indicating that these genes

likely act downstream of the sex determination pathway to control the activation of programmed cell death in the CEM neurons. Fine mapping and rescue experiments are currently in progress.

352. *sue-1* and *sue-2* are required for HSN death

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Programmed cell death eliminates diverse types of cells during development in *C. elegans*. All of these deaths are regulated by the core apoptotic machinery which is encoded by the *egl-1*, *ced-9, ced-4* and *ced-3* genes. However very little is known about the genes that regulate the life vs. death decision of specific cells. A subset of cells that are fated to die (*e.g.*, the sisters of the NSM and I2 neurons) requires the function of the cell type-specific genes *ces-1* and *ces-2*. In addition, there are sex-specific cell deaths: in males, the hermaphrodite-specific neurons (HSNs) die by programmed cell death.

In hermaphrodites, the dominant gain-of-function mutation *egl-1(n1084)* causes the HSNs to die by programmed cell death resulting in adults that are defective in egg-laying. We took advantage of this strain background as a powerful tool with which to screen for novel mutants that are compromised in their ability to specify death of the HSN. We identified several classes of suppressors and have focused our efforts on two complementation groups, *sue-1* and *sue-2* (suppressor of egl).

Loss-of-function mutations in either *sue-1* or *sue-2* prevent HSN death and restore egg-laying capacity to *egl-1(n1084)* hermaphrodites. They also prevent the death of the HSNs in males. These gene products appear to function in a cell-type specific manner because other cell types that normally undergo programmed cell death, such as the pharyngeal cells and the ventral nerve cord precursors maintain the ability to die in *sue-1* and *sue-2* mutants. To better understand how *sue-1* and *sue-2* mediate HSN apoptosis, we are in the process of cloning these two genes using SNP mapping.

In addition to their effect on HSN death, *sue-1* and *sue-2* mutants also display similar pleiotropic phenotypes including uncoordinated movement, neuronal migration defects and incompletely penetrant rod-like larval lethality. Based on the pleiotropic phenotypes exhibited by these mutants, we speculate that *sue-1* and *sue-2* may play a broader role in development and function in the differentiation of multiple neuronal cell types.

353. Identification of Genes that Specify the Deaths of the Hemaphrodite-Specific Neurons

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In *C. elegans* 131 of the 1090 cells generated during development of the hermaphrodite adult animal undergo programmed cell death. We are interested in identifying new genes involved in the life vs. death decisions of cells which normally die. Specifically, we are interested in studying the signaling pathways that regulate the deaths of hermaphrodite specific neurons (HSNs). HSNs in *C. elegans* control egg-laying in hermaphrodite animals and normally undergo programmed cell death in males where they are not needed. The life vs. death decisions of the HSN neurons present an interesting paradigm for studying sex-specific cell death specification.

Mutations in several genes (*her-1, tra-2,* and *egl-1*) have been identified that cause inappropriate death of HSNs in hermaphrodites. *her-1* encodes a novel secreted molecule, which promotes male differentiation, while *tra-2* encodes a putative transmembrane receptor for the HER-I protein. The *egl-1* gene is generally required for cell-killing in nematodes but is not involved in sex determination. Thus *her-1* and *tra-2* mediate a novel signal transduction pathway that integrates into the cell-death pathway through the *egl-1* gene to control HSN cell death.

In order to identify new components in this signaling pathway, we have performed several genetic screens to isolate suppressors of inappropriate HSN cell death caused by loss-of-function mutations in the *tra-2* gene. We expected to isolate mutations in genes that control general sex determination, genes that control general programmed cell death, and genes that are involved in specifying the death of HSN neurons in males. So far, we have isolated more than forty mutations. Two of them are alleles of the *ced-3* and *ced-4* genes, both of which are general cell-death genes. Fourteen mutations result in *fem* phenotypes and are

likely to be alleles of *fem* genes. Five mutations seem to affect only HSN cell death. Among them, *rsd-1(sm9)* (*rsd*: **r**egulator of **s**ex-specific **d**eaths) is a dominant suppressor of HSN death in *tra-2* hermaphrodites. *rsd-4(sm124)* is a recessive suppressor of HSN death in *tra-2* hermaphrodites. Both genes were mapped to LGV, and we are currently doing fine mapping of these two genes to facilitate their cloning. We are working on mapping the other three mutations as well. Further genetic and molecular characterization of these *rsd* genes should help elucidate the mechanism that controls the life vs. death decision of HSN neurons.

354. Specification of the Sexually Dimorphic Programmed Cell Death of the CEM Neurons

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Sexual dimorphism in the nervous system of *C. elegans* is created by differential numbers of cell divisions, by differential cell fates or by programmed cell death (PCD). The hermaphrodite specific neurons (HSNs), two motoneurons necessary for egg laying, and the male-specific cephalic companion cells (CEMs), four sensory neurons of so far unknown function, are the only neurons that are sex-specific as a result of sexually dimorphic PCD. The HSNs and CEMs are born in both sexes; however, the HSNs are subsequently eliminated by PCD in males and the CEMs in hermaphrodites (1).

The survival of the HSNs in hermaphrodites is specified by a direct interaction between the terminal, global regulator of somatic sexual fate, the Zn finger DNA binding protein TRA-1A (*tra*, *tra*nsformer) (2), and the *egl-1* locus (*egl*, *egg-l*aying defective), which encodes the primary activator of PCD in somatic tissues (3). The TRA-1A protein, which acts to promote female development in somatic tissues, binds to a TRA-1A binding site in the *egl-1* locus thereby causing the repression of *egl-1* transcription in the HSNs and HSN survival in hermaphrodites (3). To determine the role of the *tra-1* and *egl-1* genes in the specification of the death of the CEMs in hermaphrodites, we analyzed the survival of the CEMs in hermaphrodites (XX) homozygous mutant for loss-of-function (lf) mutations in either *tra-1* or *egl-1*. We found that both genes are required for the death of the CEMs in hermaphrodites. Furthermore, an *egl-1*(lf) mutation blocks the ability of a *tra-1* gain-of-function (gf) mutation to induce the CEMs to undergo PCD in males (X0). This suggests that TRA-1A acts as an activator of *egl-1* in the CEMs. The TRA-1A binding site required for the ability of TRA-1A to repress *egl-1* transcription in the HSNs, is, however, not required for the ability of TRA-1A to induce the CEMs to die. This indicates that TRA-1A acts as an indirect activator of *egl-1*

transcription or through a different binding site within the *egl-1* locus.

To determine how TRA-1A might specify the death of the CEMs and to identify additional factors required for this process, we are using two approaches. First, we are determining the region of the *egl-1* locus that is required to rescue the death of the CEMs in *egl-1*(lf) hermaphrodites. So far, we have identified a 690 bp region 3 kb downstream of the *egl-1* transcription unit that is required to cause CEM death in hermaphrodites. Second, using a CEM-specific GFP reporter (*pkd-2*::*gfp*) (4), we performed a screen for mutations that cause the CEMs to survive in hermaphrodites and have identified at least two new loci that can mutate to cause CEM survival in hermaphrodites.

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355. Analysis of the Specification of the NSM Sister Cell Death: Is CES-1 a Transcriptional Regulator of *egl-1*?

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Gain-of-function (gf) mutations in the gene *ces-1* (*ces*, cell death specification) block the death of the NSM sister cells and the I2 sister cells, four cells that normally undergo programmed cell death (PCD) (1). Genetically, *ces-1* acts as a negative regulator of the cell-death activator gene *egl-1* (*egl*, egg laying defective), which is required for most if not all PCD events that occur during development (2). *ces-1*, hence, is a candidate cell-type specific regulator of the central PCD pathway in *C. elegans* (3). *ces-1* encodes a zinc finger DNA binding protein most similar to the Snail family of transcription factors (4) and *egl-1* is transcriptionally regulated, at least in some cells (2). It has therefore been proposed that the CES-1 protein specifies the cell death fate of the NSM and I2 sister cells by acting as a repressor of *egl-1* transcription (4).

The *egl-1* locus contains four Snail family consensus sites, which are conserved in the *egl-1* locus of *C. briggsae*. To test whether CES-1 can bind to these sites *in vitro*, we performed gel mobility shift assays with bacterially expressed GST-CES-1 fusion protein. Using this assay, we could show that GST-CES-1 binds to the four Snail-binding sites in a specific and cooperative manner. To investigate whether CES-1 protein can bind to the *egl-1* locus *in vivo*, we are using a methodology devised by Carmi *et al*., which will allow us to determine whether CES-1 protein localizes to extrachromosomal arrays containing multiple copies of the *egl-1* locus in nuclei of transgenic embryos (5).

Loss-of-function (lf) mutations in *egl-1* block most if not all PCD during development, including the death of the NSM sister cells. When injected into *egl-1*(lf) animals, an 8 kb fragment that includes the *egl-1* transcription unit, 1 kb of its upstream region and 5.6 kb of its downstream region (*egl-1* rescuing fragment) can rescue the cell death defect of the animals.

To determine whether the region that spans the four CES-1-binding sites (region B) is required to specify the NSM sister cell death *in vivo*, we are analyzing whether region B is necessary and sufficient for the ability of the rescuing fragment to rescue the death of the NSM sister cells in *egl-1*(lf) mutant animals. To score the survival of the NSM sister cells, we are using the *tph-1::gfp* reporter (*tph*, tryptophan hydroxylase gene), which is specifically expressed in serotonergic neurons including the NSMs and surviving NSM sister cells (6). Using this experimental approach, we found that region B is required for the specification of the NSM sister cell death *in vivo*.

Since lf mutations in *ces-1* have no obvious phenotype we suggest that its function is redundant. In order to identify additional factors that act with CES-1 to specify the cell death fate of the NSMs and their sister cells, we are therfore performing a screen for mutations that cause the NSMs to undergo PCD in a *ces-1*(lf) mutant background.

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356. pvl-5 prevents Pn.p cell death during C. elegans vulval development.

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During the L1 larval stage, the P blast cells divide to generate 12 Pn.p cells, of which six (P3.p - P8.p) become the Vulval Precursor Cells (VPCs) that go on to divide and form the vulva. *pvl-5(ga87*), a mutation which affects the generation of Pn.p cells, was isolated in a screen for mutations causing a Protruding vulva (*pvl*) phenotype. In *pvl-5(ga87)* mutants there are an average of 7.0 large Pn.p cells [N=172; Range 4-10] in the ventral midline compared to 11 (P1.p - P11.p) in wild-type animals. Consequently, there are fewer than 6 VPCs and usually fewer than 22 cells available to form the vulva. The average number of Pn.p cells in *pvl-5*(*ga87*)/ *mnDf39* animals is similar to that in *ga87* homozygous mutants [7.4 (N=48; range 4-11)], suggesting that *ga87* is likely a null allele. *de3*, a second allele isolated in an F1 non-complementation screen, exhibits a similar range and severity of phenotypes as *ga87* [average number of large Pn.p cells = 8.6 (N=145; range 4-11)]. The decrease in the number of Pn.p cells could be attributed to a loss of P cells or Pn.p cells by cell death, a failure of P cells to migrate into the ventral midline, or a Pn.p to a Pn.a cell fate transformation. Lineage analysis shows that the 12 Pn.p cells are born correctly in *pvl-5* mutants, but later undergo abnormal cell death around the L1 molt. The nuclei of dying cells appear swollen, pitted and stain brightly with Acridine Orange (AO) and SYTO-12, nucleic acid stains that have been used to identify dying cells (1). This indicates that the Pn.p cells are dying in *pvl-5* mutants. If the cells are undergoing apoptosis in *pvl-5* mutants, then perturbing the machinery responsible for apoptosis should cause the cells to live. In *pvl-5; ced-3(lf)* double mutants the average number of Pn.p cells is 10 (N=92; range 7-11). Further, in *pvl-5*; *ced-9(gf)* double mutants, the average number of Pn.p cells increases to 10.2 (N=87; range 7-11). This suggests that the Pn.p cells are undergoing apoptosis in *pvl-5* mutants and that PVL-5 might function to prevent Pn.p cell death in

wild-type animals. Curiously, *ced-4* does not appear to suppress the *pvl-5* phenotype. A single ORF, C56E6.6, rescues the Pn.p cell defect in *pvl-5* mutant animals, increasing the average number of Pn.p cells from 7.2 to 10.3 (N=95). C56E6.6 encodes a Leucine-Rich-Repeat (LRR) containing protein. The LRR is a protein-protein interaction motif and is present in some PCD regulating proteins. Experiments to determine the localization of PVL-5 protein are in progress and results will be reported.

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357. Inactivation of *hus-1*, a conserved checkpoint gene in *Caenorhabditis elegans*, results in impaired cell cycle arrest and apoptosis induced by genotoxic stress.

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Tumorigenesis is characterized by the accumulation of genetic alterations that drive the advancing transformation of normal cells into highly malignant derivatives. Therefore it is not surprising that sophisticated mechanisms exist to maintain the integrity of the genome. Damage to DNA triggers checkpoint controls that result in cell cycle arrest and repair of the lesion. In metazoans, when DNA damage is extensive, these potentially harmful cells are eliminated by apoptosis. Defects in the communications between DNA damage and the apoptotic program leads to the survival of cells with unstable genomes vulnerable to oncogene activation, ultimately leading to tumor development. Genetic work in yeast has greatly improved our understanding of the molecular mechanisms of DNA damage-induced checkpoint arrest and repair. On the other hand, DNA damage-induced apoptosis cannot be studied in yeasts, as the apoptotic program is missing in both *S. cerevisiae* and *S. pombe*. In *C. elegans*, gamma irradiation induces apoptotic cell death of meiotic germ cells as well as proliferation arrest of mitotic germ cells. DNA damage-mediated apoptosis is dependent on *ced-3*, *ced-4* and is negatively regulated by *ced-9*. The positive death regulator, *egl-1*, participates in, but is not essential for radiation-induced apoptosis. Recently, three mutants - *op241, rad-5(mn159)*, and *mrt-2(e2663)* - have been identified in *C. elegans* that block DNA damage induced apoptosis and cell cycle arrest. We have recently mapped *op241* to the left arm of *LGI* between

unc-11 and *stu-4*. Sequence analysis revealed a mutation in the homologue for the *S. pombe* *hus1*$^{+}$ checkpoint gene. In addition, we have isolated a deletion mutant of *hus-1(op244)* from a deletion library. This deletion mutant, as expected, fails to complement *op241* for both the cell cycle arrest and apoptotic defects induced by DNA damage. Furthermore, RNAi with *hus-1* sequence inhibits DNA damage induced apoptosis of germ cells. Preliminary characterization of *op244* reveals other phenotypes suggesting a role for *hus-1* in maintaining genome stability. Expression studies of *hus-1* suggest that it is expressed in the soma during embryogenisis and in the germline of larvae and adult hermaphrodites. Current studies are focused on the molecular biology of the HUS-1 protein and its role in regulating the apoptotic machinery.

358. Role of *C. elegans p53* in germ cell apoptosis and embryonic development.

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p53 is the most frequently mutated gene in human cancer and is a critical regulator of DNA damage-induced apoptosis. We have been characterizing the *C. elegans p53* ortholog, *cep-1*, and its role in DNA damage-induced germ cell apoptosis. Previously, we reported that a *cep-1* deletion mutant, isolated by TMP mutagenesis, was resistant to DNA damage-induced germ cell death and showed elevated levels of physiological germ cell death. However, we recently discovered that this mutation is a complex rearrangement in which the deleted *cep-1* gene has been translocated to another region of the genome, leaving the wild-type copy intact. Preliminary evidence suggests that this mutation acts in a dominant negative manner to confer radiation resistance in the germline. Using RNAi we are able to eliminate embryonic expression of a *cep-1::gfp* fusion reporter. We find that the resistance of germ cells to DNA damage-induced apoptosis observed in the mutant strain is phenocopied by RNAi, but physiological germ cell death appears normal. We are also characterizing the embryonic lethal phenotypes that occur at low frequency in the mutant strain and in the F1 generation of *cep-1(RNAi)* animals.

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359. *abl-1* Regulates *C. elegans* Germ-cell Apoptosis

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Mammalian c-Abl belongs to the family of non-receptor tyrosine kinases. It contains the src homology domains SH1 (kinase domain), SH2 and SH3, and a long carboxy-terminal regulatory domain. The structural complexity of c-Abl and its localization to both nucleus and cytoplasm suggest that c-Abl may participate in multiple cellular functions.

The genomic sequence of *C. elegans abl-1* is contained within the cosmid clone M79. By RACE cDNA amplification, we found that the *abl-1* locus generates four transcripts using different start sites which display high homology to mammalian *c-abl*. A deletion allele of *abl-1*, termed *abl-1(ok171)* was generated at the *C. elegans* Gene Knockout Consortium (Oklahoma). Sequence analysis of this mutant revealed that 2.5 kb of genomic DNA was deleted, which results in elimination of exons 8-12 of *abl-1*, including the kinase domain and most of the SH2 domain. *abl-1(ok171)* develops normally. However, it is hypersensitive to irradiation-induced germ cell apoptosis. At all time points examined, *abl-1(ok171)* displayed more apoptotic corpses compared with N2. With 120Gy radiation, the peak number of germ cell corpses was 4-fold that of N2. Transformation with cosmid M79 containing *C. elegans abl-1* partially rescued the *abl-1(ok171)* phenotype. Apoptotic cells were removed within one hour in both N2 and *abl-1(ok171)*, indicating that the increase in cell corpses in *abl-1(ok171)* is not due to a defect in engulfment.

To evaluate whether *C. elegans abl-1* interacts with the core machinery of the cell death pathway, we constructed double mutants containing *abl-1(ok171)* with either *egl-1(n1084n3082lf)*, *ced-3(n717lf)* or *ced-9(n1950gf)*. Our results showed that loss-of-function *ced-3* and gain-of-function *ced-9* prevent radiation-induced germ cell apoptosis in *abl-1(ok171)*, while loss-of-function *egl-1* only partially reduced this event.

We are interested in understanding the downstream pathway mediating the anti-apoptotic effect of *C. elegans* ABL. In *Drosophila*, Ena binds to Abl and is a substrate for the Abl kinase. We found that three mutants of the *C. elegans ena* homolog *unc-34* suppress the *abl-1(ok171)* phenotype. This result is consistent with the genetic evidence from *Drosophila* showing these proteins function in the same pathway to regulate nervous system development.

360. RNA interference, a way to check for inhibitors involved in germline cell death in *Caenorhabditis elegans*

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In addition to the role that programmed cell death plays during development in *C. elegans*, we have shown that germ cells undergo programmed cell death. Unlike somatic cells, germ cells in *C. elegans* do not have a fixed lineage in which each cell has a certain fate - in the germ line cell survival and cell death occurs stochastically. These cell deaths, like those in the soma, are dependent on the genes of the core apoptotic machinery: *ced-9*, *ced-4* and *ced-3*. Interestingly, the pro-apoptotic gene *egl-1* (egg-laying defective), which is upstream of *ced-9*, is required for cell death in the soma, but not in the germ line, suggesting that some other factor(s) may be involved in the commitment to die.

We started a screen to identify other genes that affect germ cell death. Genetic interference mediated by double-stranded RNA (RNAi) is a valuable tool for the analysis of gene function in *C. elegans*. By analysis of chromosome I we have found several genes involved in germ line cell death.

In the future we would like to test the whole genome for genes involved in germ cell death by RNAi. Prospective candidates will then be tested in different mutant backgrounds to characterize interaction with known members of the core apoptotic machinery.

361. A screen for genes that control programmed cell death in the germ line

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Although we know a great deal about the apoptotic program in *C. elegans*, we know very little about how the process is controlled in the germ line and why roughly half of all germ cells undergo programmed cell death during oogenesis.

In order to identify genes that may be involved in this process, we have developed a screen using the vital dye acridine orange, which specifically stains apoptotic cells in the germ line. Using this screen we have looked at approximately 45,000 genomes, identifying 22 mutants that have increased levels of germ cell apoptosis. We have termed these *gla* genes (germ line apoptosis). The thirteen mutants that have thus far been mapped represent ten complementation groups, only one of which has multiple alleles. Of these, two have been cloned *gla-1(op234)* and *gla-3(op212)*, both of which are predicted to encode zinc finger proteins.

In order to determine if these mutants are specifically defective in the regulation apoptosis, or if they are pathologically compromised, and thus have damage that causes the cells to undergo apoptosis, we made double mutations with *ced-3* and other apoptotic genes. Most of the mutations examined are suppressed in the *ced-3* background, suggesting that the damage is non-pathological (we would have expected necrosis or some other gross morphological consequence if this were the case).

362. What factors regulate programmed cell death in the germ line of *C. elegans*?

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In the nematode *C. elegans,* programmed cell death (PCD) is the fate of approximately 50% of all germ cells (Gumienny et al. 1999). The cellular content of dying cells was proposed to nurse the surviving germ cells. Only germ cells in which the *ras* signal transduction pathway is activated were found to be competent to die (Gumienny et al., 1999). The factor or factors that activate the *ras* pathway in the germ line as well as the signal or signals that trigger the *ced-9*, *ced-4* and *ced-3* (ced, cell death defective) dependent execution of programmed cell death in some but not all germ cells remain, however, unknown. To identify factors and signals involved in the regulation of germ cell PCD in *C. elegans*, we are using forward and also reverse genetic approaches.

Of the 19,099 *C. elegans* genes, 766 genes were recently found to be expressed specifically in the *C. elegans* germ line and in oocytes (Reinke et al. 2000) (there is no PCD in the male germ line). Using RNA mediated interference (RNAi), we are currently investigating the function of these genes in the regulation of PCD in the hermaphrodite germ line. In addition, we have initiated an unbiased genetic screen for mutants with reduced germ line PCD.

Another goal of our studies is to analyse whether environmental factors influence germ cell PCD. It has been shown that DNA damage (Gartner et al., 2000) or infection with Salmonella (Aballay and Ausubel, 2001) can induce additional PCD in the germ line. Our current focus is to analyse already characterized mutants with defects in the perception of the environment (e.g. chemotaxis mutants) or with reduced stress resistance with respect to a possible defect in germ cell homeostasis. In addition, the effect of environmental factors like starvation, elevated temperature or salt stress on germ cell homeostasis will be studied.

363. Analysis of Torsin Protein Function in *C. elegans*: Towards a Nematode Model for Early-Onset Dystonia

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Early-onset torsion dystonia is a human neurological movement disorder caused by a deletion of single codon in the *TOR1A (DYT-1)* gene encoding a protein termed torsin A. We have utilized the *C. elegans* genome sequence database to identify 3 predicted open-reading frames that share high amino acid sequence identity to the human torsin A protein. Our focus has been on two of these genes, that we have named *tor-2* and *tor-1*, which share the highest identity to torsin A and lie a putative operon on Chr. IV of *C. elegans*. We are examining the function of these proteins using a combination of reverse genetic and functional genomic methods.

As an initial step toward characterizing this protein family, we have generated a GFP reporter gene fusion to *C. elegans tor-2*and examined its expression *in vivo.* Like its human counterpart, the worm torsin A homolog is expressed in neurons. Fluorescence is detected in early larvae and persists through adulthood in neuronal processes of the head and tail. We are currently confirming this expression and determining the localization of this protein using affinity-purified antisera we have generated vs. worm TOR-2. We have also isolated a full-length cDNA for *C. elegans tor-2*, created a deletion of a corresponding codon designed to mimic the dominant human defect in this protein, and are studying the effect of overexpression of this construct in transgenic animals. Complementary phenotypic analyses by RNAi are also in progress.

Torsin proteins share distant similarity to members of the Hsp100/Clp family of heat-shock proteins that have exhibited an ability to prevent polyglutamine aggregation. We are also investigating the possibility that worm TOR-2 and TOR-1 may function in a similar capacity. Elegant studies from the

Morimoto and Kramer labs (PNAS, 97:5750-55) have shown that worms containing a transgenic array coding for 82 polyglutamine-repeats fused to GFP form aggregates when expressed in body wall muscle. We have placed the *C. elegans tor-2* gene under the control of the *unc-54*promoter and have co-expressed it with either Q82-GFP and Q19-GFP constructs. Preliminary results from this analysis indicate that TOR-2 may act as a molecular chaperone in that it exhibits an ability to reproducibly reduce polyglutamine-induced GFP aggregation. We are determining if this effect is either diminished by the substitution of wild-type TOR-2 with mutant TOR-2 or is further ameliorated by co-expression of TOR-1 in this assay. It is our hope that these studies will provide novel insights into torsin protein function towards gaining a more complete understanding of dystonia at the molecular level.

364. Characterization of Human Early Onset Torsion Dystonia-Related Genes in *C. elegans*

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Human Early Onset Torsion Dystonia is an autosomal dominant movement disorder characterized by sustained muscle contractions that cause involuntary twisting, repetitive movements, or abnormal postures. The disorder is caused by a three-base pair deletion in the coding region of the *TOR1A* gene that results in the loss of one of a pair of glutamic acid residues near the carboxy terminus of the protein. TorsinA shares functional domains with the AAA/HSP/Clp ATPase protein superfamily and is expressed in a variety of tissues and cell types, including neurons of the brain. However, neither the function of TorsinA, nor the mechanism by which deletions in TorsinA result in the disease state, is known.

We are using *C. elegans* as a model for exploring the function of torsin. The *C. elegans* genome contains three genes predicted to encode torsin-related proteins (TOR). One of these genes, F44G4.1, has been characterized by Basham *et. al.* (2000 West Coast Meeting abstract #55) and corresponds to *ooc-5*. *ooc-5* was identified in a screen for maternal-effect lethal mutants with altered embryonic cleavage patterns. *ooc-5* mutants produce embryos of reduced size that fail to undergo P1 nuclear rotation. It is speculated that OOC-5 functions in P1 to effect the asymmetric localization of other PAR proteins.

We are currently investigating the expression pattern and function of the two other torsin genes, *tor-1*(Y37A1B.12) and *tor-2*(Y37A1B.13). These genes lie together on linkage group IV (+12.3 map units), separated by only 400 bps. This suggests the possibility that they may either constitute an operon or may be coordinately regulated. Interestingly, two of the human torsin genes, *TOR1A* and *TOR1B*, have a similar organization. Through the use of GFP reporters we find that, as in humans, *C. elegans tor-1* and *tor-2* are expressed in a variety of cell types, including neurons. *tor-1*

expression is confined to neurons and muscles associated with feeding, defecation, and egg laying, namely M1, AVL, DVB, the anal depressor muscles, excretory canal and vulval muscles. *tor-2* is expressed in a subset of these cells. This represents a further similarity to humans, as *TOR1A* and *TOR1B* also appear to have overlapping expression patterns. To investigate the function of *tor-1* and *tor-2* we are currently performing dsRNAi. In addition, we are constructing potential dominant negative versions of these genes based on the human dominant alleles to determine whether or not they can induce a mutant phenotype.

365. A role of sarcoglycans in *C. elegans*

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In vertebrates, muscle-wasting disorders arise from mutation in dystrophin and dystrophin associated proteins such as the sarcoglycans. Sarcoglycans are trans-membrane proteins that are part of the dystroglycan complex that links dystrophin to the plasma membrane in muscle cells (1). In humans, limb girdle muscular dystrophy arises from mutation of sarcoglycan proteins, which leads to sarcolemma dysfunction and ultimately necrosis of the skeletal muscle cells (1).

In *C elegans* many of the components of the dystrophin/dystroglycan complex have been conserved, including dystrophin, dystroglycan and three sarcoglycans (alpha, beta, gamma) (2). Surprisingly, the phenotype of mutated dystrophin in *C. elegans* is mild; mutants display a hyperactive phenotype but no muscle degeneration (3), whether mutating other genes within the *C elegans* dystrophin/dystroglycan complex has a more severe effect remains to be determined. We are investigating the functional role of the sarcoglycans using RNAi in variety of different muscle mutant background. Moreover, antibody staining and/or GFP reporter gene analysis will examine the distribution and expression of all three sarcoglycans.

In addition to examining the functions of the *C elegans* homologs of classic vertebrate muscular dystrophy genes, we are interested in molecular characterization of genes in C elegans initially identified through a dystrophic paralytic phenotype. These genes include the *mua-1* (for *m*uscle *a*ttachment-1) gene. MUA-1 is a zinc finger protein of the EKLF family and is required for normal muscle attachment to hypodermis. Mutations in the *mua-1* results in postembryonic paralysis and progressive detachment of body wall muscles from the hypodermis. We are determming the molecular lesions of *mua-1* mutants alleles as well as using RNAi to help define the null phenotype of this gene.

1. Culligan et.al. International Journal of Molecular Medicine (1998) 2:639-648.
2. Hutter et.al. Science (2000) 287:989-994
3. Bessou et.al. Neurogenetics (1998) 2:61-72

366. Use of C. elegans to study tumour suppressor genes

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We are studying tumour suppressor genes in normal and neoplastic cells. These genes, whose function is altered in a large number of tumours, have a fundamental role in the cycle cycle, in cellular differentiation, cellular aging or in the response to genotoxic agents.

We are using C. elegans in order to better understand the function of these genes and their position within complex signalling pathways. We will more particularly study homologues in C. elegans of the genes BTG-1, BTG-2, FRG-1, FVT-1 and of known interacting proteins. The C. elegans homologue of BTG-1 and BTG-2 is fog-3. Preliminary results will be presented and discussed.

367. Characterization of a *C elegans* homolog of a human tumor biology target

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We are investigating the role of a *C. elegans* homolog of a novel human gene indicated to be involved in tumor formation. No mutant strains are currently known to exist for this gene, so characterization has been done using RNAi and overexpression constructs. The RNAi phenotypes include slow maturation (twice as long as wildtype), some larval arrest/tiny adults, and a failure for the germ line to proliferate resulting in adult sterility. DAPI staining of the gonad shows that the germ line cells arrest in the mitotic prometaphase. Because of this gene's involvement with cancer, the potential for a role in the *ras* pathway was examined. RNAi of this gene suppressed the multivulval phenotype in two *let-60* gain of function mutants, *let-60 (ga89)* and *let-60 (n1046*), though exhibited no genetic interactions with *let-23*, *sem-5*, *lin-45*, *mek-2*, or *lin-1*. In all these genetic backgrounds the RNAi phenotypes of slow growth and lack of germ line proliferation were as strong as in N2s; therefore, the effect on ras activity appears to be indirect.

We postulated one such indirect mechanism might be via an influence on cell cycle. To address this we analyzed the effect of RNAi in a *cul-1* mutant background. *cul-1* is required for cell cycle exit, and in a *cul-1* mutant most postembryonic blast cells, including the germ line, overproliferate. RNAi of this gene in a *cul-1* mutant did not effect the overproliferation of most blast cells. However the germ line showed the same extent of reduced proliferation as RNAi into wildtype animals. Therefore, it seems that wildtype cul-1 may act through this gene to regulate exit from the cell cycle in the germ line.

Human mutations have been identified in this cancer gene. We have engineered similar mutation in the *C. elegans* gene and have made transgenic lines overexpressing either the wildtype or mutant forms. Evaluation of these transgenic lines and further analysis of this gene will be reported.

368. RNAi screen of tumorigenesis-related genes: potential developmental roles

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We are performing an RNAi-based screen with *C. elegans* homologues of a variety of human tumor suppressors and oncogenes to characterize developmental processes that might illuminate mechanisms of tumorigenesis. We have observed an RNAi-induced phenotype for about half of the genes screened thus far. Two genes with particularly striking RNAi phenotypes are currently being characterized. The first gene encodes a cytoplasmic tyrosine kinase related to the human *fer/fes* oncogene. The predicted protein contains SH2 and tyrosine kinase domains, implying that it may interact with a receptor tyrosine kinase pathway. The RNAi-induced phenotype of this gene includes embryonic lethality with severe defects in morphogenesis, a markedly reduced rate of larval development, defects in germline differentiation, and clear (Clr) phenotypes. Collectively, these phenotypes suggest that this kinase acts in many events throughout development. A second gene encodes a protein with a RING-finger domain that is homologous to that of the human gene *ret*. This gene belongs to the RBCC gene family, one member of which, LIN-41, acts to regulate developmental timing in worms (Slack et al., 2000). RNAi of this gene causes some larvae to arrest at the L1/L2 stage and others to exhibit a delayed development into adulthood. In both cases, a blockage of the gut is seen as bacteria gradually accumulate within the gut lumen. A more in-depth characterization of each gene mentioned above will be presented focusing on expression patterns and possible cellular pathways in which the proteins may act.

Slack, F.J., Basson, M., Liu, Z., Ambros, V., Horvitz, H.R., Ruvkun, G. (2000). Mol. Cell 5, 659-669.

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369. NEURONAL SENSITIVITY TO POLYGLUTAMINE-PROTEIN AGGREGATES IN *CAENORHABDITIS ELEGANS* .

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A number of human neurodegenerative diseases such as Huntington's, Parkinson's, and ALS, are characterized by protein aggregates and often associated with aging. The biochemical consequence of misfolded or mutant proteins that self-associate to form protein aggregates should, in principle, be independent of cell type. However, the Huntingtin's gene, for example, is expressed widely in the body, yet protein aggregates and cell death are restricted to specific subsets of neuronal cells. The expression of polyglutamine (polyQ or CAG) expansions, as occurs in Huntington's, results in the appearance of size-dependent protein aggregates in yeast, Drosophila, and *Caenorhabditis elegans*, thus providing alternate model systems for genetic, biochemical, and cell biological investigations.

Of these, *C. elegans* is a particularly interesting model, as subsets of the 302 neurons in the adult hermaphrodite can be differentially targeted to express aggregation-prone proteins. We have already shown that the expression of polyQ-containing proteins in *C. elegans* body wall muscle cells causes the size-dependent appearance of protein aggregates. The transition to aggregate formation expansions occurs at approximately 40 polyQ repeats. To investigate the sensitivity of neurons to polyQ induced aggregation, we have made constructs containing polyQ-YFP (Q0, 19, 40, and 82) under the control of the pan-neuronal unc-119 promoter for the generation of transgenic animals. Our preliminary data reveals that Q19-YFP expressing animals exhibit a number of deficiencies including an egg laying defect and uncoordinated phenotype. In contrast, animals expressing Q40-YFP yield transgenic animals with low viability while no progeny expressing Q82-YFP were obtained. Future analysis will take advantage of the both forward and reverse genetics to screen for genes involved in the pan-neuronal, polyQ-induced phenotypes. We will also examine the role of neuronal sub-groups such as sensory or dopaminergic neurons, by generating constructs with subset-specific promoters expressing a series of polyQ-YFPs. This will enable us to determine whether neuronal subsets have differential susceptibility to polyQ induced protein aggregation. The use of subset-specific neuronal promoters will also provide a mechanism for investigating whether polyQ-induced phenotypes differ depending on the neuronal sub-group expressing polyQ.

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370. MOLECULAR REGULATORS OF POLYGLUTAMINE AGGREGATE FORMATION IN *C. ELEGANS<i/>*

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Eight human neurodegenerative disorders are characterized by the misfolding and aggregation of different proteins that have in common expanded polyglutamine tracts. The expression of an extrachromosomal array of polyglutamine repeats as GFP fusion proteins in body wall muscle cells in C. elegans results in the appearance of variable sized cytoplasmic protein aggregates that occurs in a length dependent manner. The threshold for appearance of protein aggregates is associated with the expression of q40-gfp, which suggests the presence of "molecular buffers" involved in cellular homeostasis including chaperones and proteases. Overexpression of molecular chaperones such as Hsp104 have previously been shown to partially suppress the appearance of protein aggregates. We have extended this candidate gene approach by expression of other well characterized chaperones including the yeast ssa1 (hsp 70) and ydj1 (hsp40) proteins in body wall muscle cells. Expression of either chaperone reduces the appearance of polyglutamine aggregates while increasing the number of diffuse GFP ’patches’. Taken together, our data demonstrates that co-expression of individual molecular chaperones results in a phenotypic change in the aggregation phenotype. To determine the components involved in the soluble/aggregate phenotypic change, we have identified the corresponding C. elegans orthologs and initiated studies to determine functional combinations of C. elegans chaperones that alters the appearance of polyglutamine aggregates. In parallel, we have also initiated integration of the different chimeric polyglutamine-YFP arrays. We will use these lines to conduct a forward genetic screen for the identification of novel components involved in protein homeostasis.

371. The C. elegans Homologue of Human Huntingtin Interacting Protein 1 has Multiple Roles

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The HIP1/SlA2 family of genes have been ascribed numerous functions including maintenance of the cortical actin cytoskeleton, endocytosis, vesicle trafficking, RNA stability and apoptosis. Human HIP1 is reported to have a pro-aptotic function and may contribute to the etiology of Huntington disease. The majority of the work with this family of genes has come from mammalian biochemistry and yeast genetics. Analysis of this gene family in a metazoan animal system has not been fully explored. We have used C. elegans to study the role of the nematode homologue, CeHIP1, in the development of an animal system.

CeHIP1 is found in several tissues of adult animals including the germline, somatic gonad and pharynx. RNAi results in reduced fecundity; treated animals have a decreased brood size and lay a large number of unfertilized oocytes. Also, the pharynxes of treated adult animals are deformed. We have evidence that the locus is dose sensitive, with overexpression being toxic to the animal. We beleive the study of CeHIP1 may provide a good model to understand the function of human HIP1. Both genes show restricted expression (HIP1 in the central nervous system and testes, CeHIP1 as described) and have dose sensitive toxic effects. This is the first description of this gene family in animal system.

372. Transgenic *C. elegans* overexpressing human alpha-synuclein as a model of Parkinson's disease.

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Parkinson's disease is a major neurodegenerative disease, presenting extrapyramidal motor symptoms, characterized pathologically by loss of monoaminergic neurons in the brainstem, formation of filamentous intraneuronal inclusions termed Lewy bodies. Two rare mutations in the alpha-synuclein gene (A53T, A30P) have been identified in some pedigrees of familial Parkinson's disease and alpha-synuclein is a major component of Lewy bodies and Lewy neurites in sporadic as well as familial forms of Parkinson's disease. Alpha-synuclein is an abundant presynaptic neuronal protein of unknown function.

Recent studies have shown that overexpression of human alpha-synuclein in neurons of transgenic animals, including mouse and *Drosophila*, leads to formation of cytoplasmic inclusions similar to Lewy bodies, as well as some defects in neuronal function, although the mechanism whereby alpha-synuclein aggregates and forms inclusions, as well as the pathogenic significance of alpha-synuclein aggregates in neuronal degeneration is still unclear.

To understand the molecular pathogenesis of Parkinson's disease, we have generated transgenic *C. elegans* overexpressing human alpha-synuclein in neurons.

We expressed both normal and familial Parkinson's disease mutant forms of human alpha-synuclein, with or without EGFP fused at the carboxy terminus, in *C. elegans* neurons

using *unc-51* promoter for panneuronal expression. We have used additional promoters, i.e. *mec-7* or *sra-6,* for expression in specific subsets of neurons.

These transgenic animals expressed high levels of human alpha-synuclein in neurons. Neuronal cell bodies and neurites were extensively EGFP positive, or labeled by anti-human alpha-synuclein antibody LB509. Western blot analysis of lysates of transgenic animals showed alpha-synuclein positive 48 or 17 kDa bands, corresponding to human alpha-synuclein with or without EGFP fusion. We are currently studying the effects of alpha-synuclein toxicity in relation to aging, as well as those of the missense mutations linked to Parkinson's disease, by behavioral assays.

Our goal is to generate a *C. elegans* model of Parkinson's disease by overexpressing alpha-synuclein, which recapitulates features of Parkinson's disease, i.e. formation of alpha-synuclein inclusions and neuronal death. Such models should contribute to the understanding of the molecular mechanism whereby alpha-synuclein is aggregated and leads to neuronal death in Parkinson's disease.

373. Identification and characterization of the *C elegans* orthologue of the mammalian molecular adaptor Fe65, a cytosolic ligand of Alzheimer's beta-amyloid precursor protein

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Mammalian fe65 genes encode three highly related proteins, Fe65, Fe65-L1 and Fe65-L2. The common modular structure of the Fe65s, composed of a WW domain and two independent phosphotyrosine binding domains, PTB1 and PTB2, suggests a potential role of molecular adaptors for these proteins. The PTB2 domains of the three proteins interact, at the level of the corresponding cytodomains, with the products of another gene family, which has the Alzheimer's beta-amyloid precursor protein gene (APP) as the most studied member. The Fe65s may interact with other cellular proteins; in the case of Fe65, its WW domain binds to several proteins, including Mena and the non-receptor tyrosine kinase Abl. The PTB1 domain may interact, at the membrane level, with the low-density lipoprotein receptor-related protein LRP, and in the nucleus with the transcription factor CP2/LSF/LBP1.

APP is cleaved by alternative pathways mediated by alpha- or beta-secretase; in this latter case, the subsequent action of gamma-secretase determines the release, under pathological conditions, of the beta-amyloid peptide, the major component of the senile plaques typical to Alzheimer's disease. Gamma-secretase activity requires, or is coincident with presenilins. Most of the proteins taking part in these complex molecular machineries have been identified and, in some instances, characterized in the nematode. In *C elegans*, apl-1 is the nematode orthologue of APP; the sequence conservation at the C-terminal, cytosolic domain, between APL-1

and the mammalian proteins is strikingly elevated and the structural properties of the encoded protein suggest that APL-1 adopts the same topology of APP and of related proteins. Based on these evidences, we attempted to identify, in the *C elegans* genome, putative Fe65-like genes. We demonstrated that a single gene encodes a protein structurally and functionally related to mammalian Fe65s. In fact, the nematode protein has the same modular organization of the Fe65s, and is capable of binding both APL-1 and APP. Studies with reporters and with specific antibodies indicate that the corresponding gene is expressed in the nervous system and in the pharynx, starting in the embryo, through larval stages and in adults. By chemical mutagenesis and PCR screening we generated mutant worms bearing a null mutation of the *C elegans* fe65 orthologue. The mutant is homozygous lethal and its phenotype suggests that the encoded protein provides functions essential for embryogenesis and larval development. We are currently characterizing in more details the phenotype of these mutant worms. The nematode constitutes a simple, non redundant system in which to study the basic biology of the complex network of interacting proteins centred to APP and Fe65 proteins, and will hopefully give insights into the mechanisms leading to the alterations typical to Alzheimer's disease pathogenesis.

374. The Role of CaM Kinase II and GABA in preventing seizures in *C. elegans*

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Mutations in a few *C. elegans* genes can result in whole body convulsions caused by the simultaneous contraction of dorsal and ventral body-wall muscles. *unc-43* encodes the only CaM Kinase II homologue in *C. elegans*. *unc-43(lf)* worms have rare spontaneous convulsions that are intensified in frequency and severity by exposure to neurostimulants (e.g. pentylenetetrazol [PTZ] and pilocarpine) and elevated temperature. Mutants affecting GABA function (*unc-25*, *unc-46* and *unc-49*) can have pilocarpine induced convulsions as well, though weaker.

To investigate the underlying molecular mechanisms of these convulsions, we are looking in detail at the specific neuronal requirement for UNC-43. We have established that transgenic expression of a wild-type *unc-43* cDNA solely in neurons can alleviate drug-induced convulsions in *unc-43(lf)* animals. Ongoing experiments have been designed to identify the specific subset of neurons involved and to establish when during worm development CaMKII must be expressed to rescue convulsions.

In addition, we are continuing screens to discover other genes able to confer convulsion susceptibilities in worms. We have conducted mutagenesis screens for other mutants that convulse in response to neurostimulants and mutations that enhance or suppress *unc-43(lf)* convulsions. Nine independent PTZ-sensitive alleles have been isolated. By complementation testing, three are *unc-43(lf)* alleles and two are not. Additionally, sixteen independent *unc-43(lf)* convulsion suppressors and two convulsion enhancers were isolated. Using *unc-43(lf)*;enhancer double mutants that convulse in the absence of drug, we are testing the potential ameliorating effects of clinically used antiepileptic drugs.

Taken together, these experiments establish *C. elegans* as a model for mechanisms underlying simultaneous excitation of neuronal networks. Similar types of neuronal synchrony may be causal for human epilepsies, since CaMKIIa knockout and GABA pathway deficient mice are susceptible to epileptic seizures and are hypersensitive to neurostimulant-induced convulsions. Our hope is to characterize key molecular components controlling seizures and perhaps to identify new drug targets for treatment.

375. Genes which regulate social feeding behaviour in *C.elegans*

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npr-1 encodes a putative G-protein coupled seven transmembrane receptor homologous to neuropeptide Y receptors. In wild isolates of *C. elegans* a single residue change in this receptor determines whether these strains exhibit social or solitary feeding behaviour. In order to delineate the molecular pathways and neuronal networks involved in this behaviour, a screen was previously carried out to identify mutations which convert social foragers into solitary feeders.

Using Snp-Snip mapping and the generation of recombinants I have mapped six suppressors of social behaviour to within 500 kb. Non-complementation experiments and approximate map positions suggest that these suppressors are mutated in previously uncharacterised genes. Furthermore, all the mutants appear wild type and behaviourally normal. We are currently carrying out further behavioural characterisation and transgenic rescue experiments for some of these mutants.

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376. FOOD-DEPRIVATION AND MODULATION OF BEHAVIOR: *mod-6* AND A SCREEN FOR NEW GENES

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Upon entering a bacterial lawn, well-fed hermaphrodites exhibit a basal slowing response, while acutely food-deprived animals exhibit enhanced slowing. The basal and enhanced slowing responses are genetically separable and involve different neurotransmitters. A number of cloned genes define a molecular genetic pathway in which serotonin signaling is critical for the enhanced slowing response. Food-deprived animals are no more sensitive to exogenous serotonin than well-fed animals, suggesting that food-deprivation induces a physiological change that modulates serotonin release rather than altering sensitivity to endogenous serotonin.

mod-6(n3076) (MODulation defective) mutants are defective in the enhanced slowing response, serotonin positive, slightly hypersensitive to exogenous serotonin, and fluoxetine (Prozac) resistant. These characteristics suggest that *mod-6* may be involved in modulating serotonin release in response to food deprivation. We mapped *mod-6* to a small interval on chromosome I and rescued the serotonin hypersensitivity phenotype of these mutants with cosmid F18C12. F18C12.1 corresponds to the cloned gene *che-3* (CHEmotaxis defective). We are presently determining the sequence of F18C12.1 in *n3076* mutants. *che-3* encodes a cytosolic dynein heavy chain required for the growth and maintenance of the ciliated processes of a subset of sensory neurons in *C. elegans*. Thus, *mod-6* mutants may be defective in the enhanced slowing response because they cannot detect a bacterial lawn by chemosensation.

We are also performing a *mod-5(n3314)* enhancer screen for mutants that display a constitutive hyperenhanced slowing response when entering a bacterial lawn. *mod-5* encodes a SErotonin Reuptake Transporter (SERT). SERTs have been shown to clear serotonin from the synapse after pre-synaptic release of the neurotransmitter, thus attenuating the serotonin signal. *mod-5(n3314)* mutants maintain a normal rate of locomotion in the absence of bacteria and exhibit a normal basal slowing response. However, upon food-deprivation these mutants become nearly immobilized upon entering a bacterial lawn. We have termed this more pronounced enhanced slowing response the hyperenhanced slowing response. We have isolated a number of *mod-5(n3314)* enhancers that cause the mutants to become immobilized when entering a bacterial lawn in the well-fed state while maintaining a nearly wild-type locomotory rate in the absence of bacteria. In this screen, we hope to isolate mutants that are defective in establishing and signaling the well-fed state but remain normal for the ingestion and absorption of food.

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377. ACM-2, a neuronal muscarinic acetylcholine receptor, contributes to the regulation of feeding

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The pharynx of *C. elegans* is a neuromuscular pump, whose coordinated contraction and relaxation allows the worm to efficiently ingest bacteria. In wild type worms, pharyngeal muscle contraction is triggered by the motor neuron MC, which releases acetylcholine to nicotinic receptors on the pharyngeal muscle. Pharyngeal pumping can respond to changes in the worm's surroundings, suggesting additional regulatory influences on pharyngeal muscle. Muscarinic acetycholine receptors are likely to play a role in such regulation; three genes that appear to encode muscarinic receptors are expressed in pharyngeal tissue, and investigations into the G_o / G_q signaling network have revealed a muscarinic component.

acm-2 encodes a G-protein coupled muscarinic acetylcholine receptor that is expressed in neurons of the extra-pharyngeal nerve ring and in pharyngeal neurons I1, I2 and M4. A deletion mutant, *acm-2(by124)*, was generated by Stefan Eimer and Ralf Baumeister (Ludwig-Maximilians-Universitaet, Munich). We have previously reported that *acm-2(by124)* confers a feeding phenotype: pharynxes of mutant worms display an increased pumping rate and an increased sensitivity to nicotine. We had also shown that gap junctions are necessary for the full expression of the mutant phenotypes. A gap junction links the extra-pharyngeal neuron RIP to the pharyngeal neuron I1, and this connection may be important for ACM-2 action. However, gap junctions also link neurons within the pharynx.

We have since determined that the pumping phenotypes of *acm-2(by124)* can be rescued by the injection of wild type copies of the *acm-2* genomic region. We have also noted that the phenotype of increased nicotine sensitivity is dominant. Because the *by124* mutation removes two exons without inducing a frame shift or premature stop, we hypothesize that *acm-2(by124)* encodes an antimorph.

We have continued to examine the effects of the *by124* mutation, and found additional effects on worm behavior. The mutation alters the worm's ability to respond to different types of food: while wild type worms increase their pump rate when presented with certain types of bacteria, *acm-2(by124)* worms do not. We hypothesize that ACM-2 acts in a pathway that allows environmental cues to influence pharyngeal pumping rate. We suspect that MC is the ultimate target of this pathway, and that information from the extra-pharyngeal nervous system alters pumping rate by adjusting the frequency of MC firing. ACM-2 is an inhibitory element of the pathway, perhaps providing negative feedback at stimulatory cholinergic synapses.

We have also found that *acm-2(by124)* mutants change direction while crawling more often than do wild type worms. This observation suggests that ACM-2 plays a similar negative regulatory role in several behavioral pathways. We are currently attempting to identify the site of action of ACM-2 within each behavioral pathway, and to further characterize the dominant nature of the *by124* mutation.

378. Genetic analysis of the neuromodulatory control of search behavior in *C. elegans*

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Following removal from food, many animals engage in an area-restricted search, a stereotypic search strategy characterized by a concentrated local search followed by a more directed global search. To understand the neuromodulatory control of the plasticity underlying area-restricted search behavior, we have quantified this search phenotype in *C. elegans*. Using a computer-assisted motion analysis system, search paths for a small population of worms can be analyzed for dynamic changes in turning angle, speed, and other statistical properties. The sensitivity of this system to subtle behavioral changes allows for the general construction of a library of quantified phenotypic effects of genetic mutations on locomotion. Our preliminary results suggest that the area-restricted search behavior is generated by a two-state neural circuit controlling forward and backward motion that is tuned by dopamine via a possible kinetic modulation of glutamate receptors. A separate but not independent pathway controls locomotory rate and appears to be mediated by serotonin and glutamate. We are currently engineering worms with dopaminergic neurons that are either prematurely apoptotic, constitutively depolarized, or constitutively hyperpolarized. By crossing these worms into various genetic backgrounds and using specific pharmacological agents, we will test the hypotheses that dopamine and serotonin act presynaptically to glutamate and that serotonin inhibits the effects of dopamine in the generation of this ecologically ubiquitous search behavior.

379. Isolation of mutants which exhibit social feeding behaviour

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Natural isolates of *C.elegans* exhibit either solitary or social feeding behaviour (Cassada, wbg 9(3):29; Davis & Avery wbg 11(15):69; Hodgkin & Doniah, 1997; de Bono & Bargmann, 1998). Solitary animals move slowly on a bacterial lawn and disperse across it, while social animals move rapidly on bacteria and aggregate together at the edge of the lawn (the clumping and bordering phenotype). This natural polymorphism has been shown to be generated by two alternative isoforms of NPR-1, NPR-1 215F and NPR-1 215V, a putative neuropeptide receptor. A loss-of-function mutation in the *npr-1* gene causes a solitary strain to take on social behaviour. From phylogenetic comparisons the NPR-1 215V isoform, which confers solitary behaviour, is likely to have been the result of a gain-of-function mutation. Since solitary and social strains are found to coexist in at least some locations, this suggests that both behavioural strategies provide selective advantages under different environmental conditions.

We are interested in isolating other members of the NPR-1 pathway. I have conducted an EMS mutagenesis and selectively screened 4900 genomes for mutations which convert the solitary N2 strain to social behaviour. As *npr-1* activity dominantly either represses social behaviour or activates solitary behaviour, such mutations are expected to identify positive regulators of the NPR-1 pathway or an unidentified parallel pathway. I have isolated 8 such mutants from this screen. One mutant is X-linked and fails to complement a null allele of *npr-1*. I am currently mapping the other mutants by the snip-SNP method. In order to take advantage of this mapping method, it was necessary to construct a solitary CB4856 strain as this strain exhibits social behaviour. I did this by replacing the NPR-1 locus of this strain with that from the N2 strain by picking recombinants and repeated outcrossing. Curiously, the CB4856 *npr-1(N2)* strain retains some level of social behaviour suggesting multiple loci contribute to this behaviour in the CB4856

strain. I intend to investigate this finding further.

380. Genes regulating social feeding behaviour in *C elegans*.

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Natural isolates of *C. elegans* exhibit either social or solitary feeding behaviour. Social strains aggregate and feed together on a bacterial lawn; solitary strains show no aggregation and feed in isolation. This variation is due to a single amino acid change in NPR-1, a seven transmembrane receptor related to mammalian neuropeptide Y receptors. Null mutations in *npr-1* transform solitary wild strains into strongly social animals.

We wish to elucidate the molecular pathways controlling social versus solitary feeding. Two EMS mutagenesis screens have been performed, starting with strongly social *npr-1*(null) animals in both N2 and CB4856 (a snip-SNP-bearing strain) backgrounds, and selecting for mutants that feed alone. About sixty mutations have been isolated thus far, which show varying degrees of suppression of social behaviour. Several known suppressors (e.g. *odr-8*, *osm-9* etc.), which function in other sensory pathways, have been pulled out from these two screens. Fifteen strong, novel suppressors have been chosen, mapped and characterised further. We hope these suppressors will identify molecules that either regulate the choice between social and solitary feeding, or are essential signal transduction components of the sensory inputs leading to social behaviour.

Davis M.W. and Avery L.. Social Behavior in *bor-1* Mutants Depends on Bacterial Smell. Worm Breeder’s Gazette 11(5): 69.

Cassada R.. Burrowing, Spontaneous Mutants, etc. with Another Wildtype Strain. Worm Breeder’s Gazette 9(3): 29.

Hodgkin J. and Doniach T. (1997). Natural variation and copulatory plug formation in *Caenorhabditis elegans*. Genetics 146: 149-164.

de Bono M. and Bargmann C.I. (1998). Natural variation in a neuropeptide Y receptor homolog modifies social behavior and food response in *C. elegans*. Cell 94: 679-689.

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381. Neurons regulating social feeding behaviour in *C. elegans*

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The C. elegans *npr-1* gene encodes a seven transmembrane receptor related to mammalian neuropeptide Y receptors. In mammals, neuropeptide Y signalling regulates food intake and alcohol tolerance. In *C. elegans*, natural variation at the *npr-1* locus determines whether worms behave in a social or a solitary manner in the presence of food.

We seek to identify the neuronal circuits regulating this foraging behaviour. To begin to address this question, we identified the neurons that express *npr-1*. A functional NPR-1::GFP fusion protein under the control of the *npr-1* promoter is expressed in about 20 types of neurons. These include paired and unpaired neurons in the head and tail, and also a subset of ventral nerve cord motorneurons.

We then asked when NPR-1 expression is required to suppress social behaviour. Transient expression of NPR-1::GFP in adult worms using the heat shock promoter suppresses social behaviour. This suggests that *npr-1* is required acutely rather than for development of the nervous system. In addition, expression of the solitary form of NPR-1 in just four of the 20 types of neurons that express NPR-1 can suppress the social behaviour of *npr-1(null)* worms strongly. Expression of NPR-1 in subsets of these neurons gives partial suppression. The data suggest that some neurons may act redundantly within a network, whereas other neurons have an additive effect on suppressing social behaviour.

Loss-of-function mutations in the*eat-4* glutamate transporter (1) also suppress social behaviour, suggesting that the glutamatergic activity of certain *eat-4*-expressing neurons may be required for worms to be social. We are trying to discover which neurons are required for this effect by adding back wild type *eat-4* cDNA to subsets of neurons in *eat-4; npr-1* mutants and looking for a rescue of social behaviour in these animals.

1. Lee RYN, Sawin ER, Chalfie M, Horvitz HR, Avery L; J. Neurosci 19: 159-167 (1999)

382. Isolation and analysis of mutants abnormal in orientation to food.

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E. coli, food of *C. elegans* in the laboratory, is a special attractive source for *C. elegans*. When well-fed wild type animals are put on a bacterial lawn, most animals are kept in the lawn for several hours. To investigate the mechanisms of this food orientation in *C. elegans*, about $1x10^5$ genomes were screened, and five mutant strains were isolated. In these mutant strains, about 2-8% of animals moved to an area more than 1.5cm far from a bacterial lawn within three hours, while that fraction of the wild type animals was less than 0.1%. Dye filling to chemosensory neurons and chemotaxis to NaCl were examined in these mutant strains. FK247 *(ks68)* and FK263 *(ks69)* mutant animals showed Dyf (dye filling defective) phenotype, and FK247 *(ks68)*, FK263 *(ks69)* and FK252 *(ks70)* mutant animals showed Che (chemotaxis defective) phenotype. Two of these five mutants: *ks68* and *ks70* are allelic to *che-2* and *eat-4* genes, respectively. Two other mutations, *ks69* and *ks72*, were mapped by using single nucleotide polymorphisms to about 2.7 map unit region on chromosome II, and to about 1.7 map unit region on chromosome IV, respectively. The pheonotype of *ks69* was rescued by introduction of a YAC Y74E4.

383. A Genetic Screen for Components of the G Protein Signaling Pathways that Control Egg-Laying Behavior in *C. elegans*

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Neurotransmitters can mediate neural communication by activating heterotrimeric G proteins that signal through poorly understood pathways. In *C. elegans*, homologs of Go (GOA-1) and Gq (EGL-30) G protein alpha subunits are expressed throughout the nervous system. These proteins act in opposition to each other to tightly regulate egg-laying behavior. Mutants in components of these G protein regulated pathways exhibit altered egg-laying behavior. Screens for egg-laying defective (Egl) mutants have been performed, but many elements of these pathways remain unidentified, including receptors, ligands and likely downstream effectors. To uncover these components, we screened 39,000 mutagenized haploid genomes for mutations that confer a hyperactive egg-laying phenotype similar to that caused by loss-of-function mutations in the G protein signaling components *goa-1, eat-16,* and *dgk-1*.

We isolated mutants from this screen with the desired hyperactive egg-laying phenotype, including one allele of *eat-16*. and four alleles of *dgk-1*. Twelve additional isolates, representing at least 5 genes, may encode new components of the GOA-1 or EGL-30 signaling pathways. Many of these fall into a new class of mutants that are hyperactive for their egg-laying but not their locomotion behavior. We are currently pursuing fine mapping and cloning of genes identified in this screen. Studying these genes should help us understand how G proteins regulate behavior at the molecular level.

The screen also identified an apparent null allele of the homeodomain transcription factor *unc-4*, previously studied for its role in determining the synaptic input of certain motor neurons involved in locomotion. However, *unc-4* is also expressed in the VC neurons, which synapse onto the egg-laying muscles and whose role in egg-laying behavior remains obscure. Expressing the *unc-4* cDNA in the VC neurons rescues the hyperactive egg-laying phenotype of

unc-4 mutants, suggesting that the VC neurons normally play an important role in regulating egg-laying behavior and that UNC-4 is necessary in the VC neurons for their proper function.

384. Mapping the site of action of G protein signaling components in *C. elegans* egg-laying system

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C. elegans egg-laying behavior is regulated by signaling through the heterotrimeric G protein alpha subunits GOA-1 (ortholog of mammalian Goα) and EGL-30 (ortholog of mammalian Gqα). GOA-1 and EGL-30 have opposing effects on egg-laying behavior; egg laying is inhibited by GOA-1 while stimulated by EGL-30. GOA-1 and EGL-30, along with other known components of the two G protein signaling pathways, are widely expressed in the nervous system and may be present in the egg-laying muscles as well. Exactly in which cells GOA-1 and EGL-30 act to control egg laying is unknown. Determining the action sites of G protein signaling components will allow us to test alternative models of how G protein signaling controls egg laying. For example, one model places GOA-1 function in egg-laying muscles, a second model places EGL-30 function in the neurons controlling egg-laying muscles, yet a third model places GOA-1 upstream of EGL-30. These models can not all be correct.

We are expressing cDNAs for G protein signaling components from cell-specific promoters to test their ability to function in the various cells of the egg-laying system. Our approach to mapping the action sites is illustrated by the following example. The *goa-1* cDNA, for instance, under the control of a neural promoter is introduced into *goa-1 loss-of-function* mutants. If the GOA-1 protein function is required in neurons, the transgene should rescue the egg-laying defect of *goa-1* mutants. We have generated a collection of cell-specific promoters to test possible action sites. This includes promoters that drive gene expression in all neurons or in either of the two types of neurons that directly synapse to egg-laying muscles, *i.e.* HSN neurons and VC neurons. We also obtained promoters that were specific for all egg-laying muscles or subtypes of those muscles. Our preliminary results indicate that GOA-1 and EGL-30 function in the

neurons but not in the muscles to control the egg-laying behavior.

385. Serotonin modulates locomotory behavior and coordinates egg-laying and movement in *C. elegans*

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In order to understand the neural and molecular mechanisms by which the decision to execute a given motor program is influenced by the activity of other motor pathways, we have analyzed the correlation between egg-laying and locomotory behavior in *C. elegans*. More specifically, we have characterized the long-term pattern of locomotory behavior and identified neurons that correlate these patterns with the activity of the egg-laying motor program.

We found that two aspects of locomotor function, velocity and directional reversals, are random processes with a lognormal probability density. In addition, their occurrence is temporally coordinated with the onset of egg-laying events: there is an increase in velocity prior to an egg-laying event, and an increase in reversal frequency following an egg-laying event.

To investigate the genetic and neural requirements for the correlation between these two behaviors, we have analyzed the locomotor and egg-laying behavior of mutant and cell-ablated animals. Animals lacking HSNs had approximately normal locomotion behavior but lacked the velocity and reversal bursts during periods of active egg-laying. We observed that serotonin-deficient mutants had a decrease in locomotive velocity and a lack of the velocity burst prior to egg-laying, suggesting that serotonin functions generally to promote forward locomotion and is specifically required for the velocity burst prior to egg-laying events.

We also identified a potential target of serotonin, the AVF interneurons, which receive synaptic input from the HSNs. Ablation of these neurons causes an effect similar to that of serotonin-deficient mutants: the velocity burst prior to egg-laying was reduced if not eliminated, and the average velocity overall was significantly reduced.

Through these studies, we have gained important insights into how the egg-laying circuitry back-modulates the brain to affect locomotion.

386. Electrophysiologic characterization of cultured *C. elegans* mechanosensory neurons

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C. elegans has provided a powerful model system for defining the molecular mechanisms by which organisms feel and respond to mechanical force. Gentle body touch is sensed by specialized touch neurons. Genetic studies have demonstrated that degenerin-type cation channels, cytoskeletal proteins and extracellular matrix proteins are required for touch neuron mechanotransduction (Tavernarakis and Driscoll, *Ann. Rev. Physiol.* 59:659-689, 1997).

Direct electrophysiological testing of touch neuron mechanotransduction models is technically demanding. *C. elegans* neurons are small and a tough pressurized cuticle limits access for patch clamp studies. We have developed robust methods that allow the *in vitro* culture of differentiated embryonic cells. Cultured cells can be readily patch clamped and studied by quantitative imaging methods. We have begun to use culture methods to characterize the electrophysiological and functional properties of *C. elegans* touch neurons.

mec-7 encodes a β-tubulin and is predominantly expressed in touch neurons (Hamelin et al., *EMBO J.* 11:2885-2893, 1992). Four touch neurons (ALML/R and PLML/R) develop during embryogenesis and two (AVM and PVM) arise during larval development. We cultured embryonic cells isolated from *mec-7*::GFP-expressing worms. GFP expression was detected in 0.2% of the cells 2 h after isolation and plating. Freshly plated cells exhibit little morphological differentiation. Within 24 h after isolation, differentiation is largely complete. Intense GFP expression was detected in 1.5-2.0% of cells in culture 1-3 days after plating. All cells expressing bright GFP fluorescence had extensive processes. These processes are considerably longer than those we have observed in other classes of neurons expressing cell-specific GFP reporters. The mean process length in *mec-7*::GFP-expressing

neurons is 25 ± 4 μm.

mec-7::GFP-expressing cultured neurons were readily patch clamped in the cell-attached and cell-detached membrane patch modes as well as in the conventional whole-cell configuration. When cells were patch clamped in the whole-cell mode using bath and pipette solutions identical to those described by Goodman et al. (*Neuron* 20:763-72, 1998), membrane depolarization (+20 mV to +100 mV) activated a strongly outwardly rectifying current (n=7). The current activated and inactivated rapidly. Mean peak current was 490 ± 39 pA at +100 mV. Steady state current was 177 ± 33 pA at +100 mV (n= 7). This current was not decreased by replacement of bath Cl^- with gluconate. However, replacement of K^+ in the pipette solution with Cs^+ blocked the majority of outward current indicating that it is due to the activity of K^+ channels.

We are presently carrying out current isolation and pharmacological studies in an effort to identify the various current and channel types expressed in touch neurons. In addition, we are attempting to identify mechanosensitive ion channels in cell-detached and cell-attached membrane patch recordings.

387. Functional characterization of cultured *C. elegans* body muscle cells and cholinergic motor neurons

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Recently, we established methods in the laboratory that allow primary culture of differentiated cell types derived from isolated *C. elegans* blastomeres. The availability of robust cell culture models provides experimental opportunities heretofore unavailable in the field. We have begun to characterize the physiological properties of cultured body muscles and cholinergic neurons.

myo-3 and *unc-54* encode specific myosin heavy chain isoforms that are co-expressed in body muscles and muscle precursor cells. The PD4251 worm strain expresses two GFPs with peptide signals that target them to either the nucleus or mitochondria of all body wall and vulval muscles. Cultures derived from PD4251 worms exhibit bright GFP fluorescence *in vitro*. GFP expression is detected in 8-10% of undifferentiated, freshly isolated blastomeres. Twenty-four hours after isolation, GFP fluorescence was detected exclusively in spindle-shaped cells with 1 or 2 well-formed processes. Approximately 20% of cells in culture express *myo-3*::GFP, which is similar to that observed in the newly hatched L1 larva. GFP was localized to both the nucleus and elongated intracellular structures that are likely mitochondria. All *myo-3*::GFP-positive muscle cells in culture also exhibited immunofluorescence localization of UNC-54 myosin.

unc-4 encodes a homeodomain transcription factor that is expressed in the 13 embryonic cholinergic motor neurons, which represent 2.4% of the 550 cells that comprise the newly hatched L1 larva. GFP-positive cells are present at a frequency of 2-4% in cultures produced from *unc-4*::GFP transgenic worms. All GFP-expressing cells in culture have well-developed, neuron-like morphology. Cholinergic motor neurons form

neuromuscular junctions with striated body wall muscles *in vivo*. A specific synaptic vesicle protein, synaptotagmin (SNT-1), that functions at these neuromuscular synapses co-localized to *unc-4*::GFP neurons *in vitro*.

In *unc-4*::GFP-expressing cultures, we observed rare examples of cholinergic motor neurons sending out processes that made physical contact with spindle-shaped muscle cells. Furthermore, we observed muscle cells undergoing what appeared to be rhythmic contractions. Removal of bath Cl^-, which may depolarize the muscle cell membrane, increased the number of cells exhibiting contractile-like activity and the frequency of apparent contractions. These intriguing observations suggest that co-cultures enriched in muscle cells and motor neurons may be useful for investigating neuromuscular synapse formation and physiological processes associated with muscle contraction.

Muscle cells were readily patch clamped in the whole-cell mode. When dialyzed with a high K^+ pipette solution, slowly inactivating, strongly outwardly rectifying K^+ currents were detected. These currents were inhibited ~40% by 20 mM TEA and ~85% by 20 mM TEA plus 3 mM 4-aminopyridine (4-AP). Recently, Richmond and Jorgensen reported elegant *in situ* patch clamp studies of *C. elegans* body wall muscles (*Nat. Neurosci.* 2: 791-797, 1999). The electrophysiological properties of *C. elegans* muscle cells in culture are remarkably similar to those that they described. The similarity between *in vivo* and *in vitro* patch clamp recordings argues that primary cultures of body wall muscles recapitulate at least some of their native functional properties.

388. Basic electrophysiological properties of body wall muscle from the Nematode *C elegans*.

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Electrophysiological properties of striated muscle cells were investigated with the patch clamp technique in the Nematode *C elegans*. Worms were immobilised with cyanoacrylate glue and longitudinally incised using a tungsten rod sharpened by electrolysis. Recording pipettes were sealed on GFP-expressing body wall muscle cells. In the whole cell configuration, under current clamp conditions, in the presence of Ascaris medium in the bath and K-rich solution in the pipette, no action potential could be induced in response to current injection. Under voltage clamp control and in the same ionic conditions, depolarizations above -30 mV from a holding potential of -70 mV gave rise to outward K currents. Outward K currents resulted from two components, one fast inactivating component blocked by 4-aminopyridine, one delayed sustained component blocked by tetraethylammonium. In the presence of both blockers, an inward Ca current was revealed and inhibited by cadmium. Single channel recording using the inside-out configuration revealed the existence of a Ca-activated Cl channel and a Ca-activated K channel. Single channel experiments are currently performed to characterise voltage-gated conductances at the unitary level.

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389. Engineering pharyngeal pumping behaviors in *C. elegans*

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The *C. elegans* pharynx is a neuromuscular pump that undergoes a series of synchronized contractions and relaxations, which allows the worm to feed efficiently. Several ion channels that regulate pharyngeal pumping behaviors have been identified. EXP-2, a voltage-gated potassium channel, is expressed in pharyngeal muscle and is responsible for the rapid repolarization of the pharynx at the end of each action potential. We believe that a human cardiac potassium channel, HERG (human *ether-a-go-go*), may perform a similar role in the human heart. Based on kinetic studies in Xenopus oocytes, EXP-2 and HERG appear to have functionally similar gating mechanisms, although the two proteins are structurally unrelated.

To determine whether HERG can replace the function of EXP-2 in *C. elegans*, and thus to attempt to engineer a predicted pharyngeal pumping behavior, we have expressed a functional HERG::GFP fusion protein[1] in the pharynxes of *exp-2* and *eat-4; exp-2* mutant worms. *exp-2* mutants lack repolarization spikes in electropharyngeograms (EPG), but otherwise have no visible feeding defects. *eat-4; exp-2* appears to be slightly starved on HB101 bacteria, but is visibly starved and slow-growing on DA837 bacteria. These mutants lack both M3-mediated IPSPs and the repolarization spike. HERG-expressing transgenic progeny show no rapid inward current by EPG, which preliminarily suggests that HERG cannot functionally replace EXP-2 in *C. elegans*. We have also expressed a mutant form of HERG (HERG N629D) in the pharynxes of the *exp-2* and *eat-4; exp-2* mutants. Lees-Miller *et al.* (2000) reported that this mutation blocks inactivation of HERG channels; and, in addition, the channels are no longer potassium-selective[2]. Again, we do not see any striking alterations in the EPG. However, worms that express the mutant form of HERG appear to grow more slowly on DA837 bacteria than worms expressing wild-type HERG or their non-transgenic sibs.

Finally, 5/6 of our HERG(N629D)-expressing transgenic lines appear to produce very few viable transgenic progeny. To determine whether this is due to a partial lethal phenotype, we examined worms expressing the HERG(N629D) transgene in body wall muscle, where paralysis or severely uncoordinated contractions of the muscle would not necessarily lead to lethality. In a small subset of these transgenic worms, body wall contractions do appear to be severely uncoordinated, and several of these adults appear to develop an egg-laying defect over time. This phenotype has not been observed in worms that express wild-type HERG or GFP alone. To test whether these are specific effects of HERG function, we are currently testing whether HERG-blocking drugs can reverse these effects.

[1] *Petrecca K. Atanasiu R. Akhavan A. Shrier A.* N-linked glycosylation sites determine HERG channel surface membrane expression. *Journal of Physiology.* 515 (Pt 1):41-8, 1999 Feb 15.

[2] *Lees-Miller JP. Duan Y. Teng GQ. Thorstad K. Duff HJ.* Novel gain-of-function mechanism in K(+) channel-related long-QT syndrome: altered gating and selectivity in the HERG1 N629D mutant. *Circulation Research.* 86(5):507-13, 2000 Mar 17.

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390. Ionic channels recorded from patches excised from the soma of the chemosensory neurons AWA and AWC

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Nematodes probably use ionic channels in novel ways. While the genome of *Caenorhabditis elegans* contains representatives of most of the channel families found in both vertebrate and invertebrate nervous systems, it lacks the ubiquitous Hodgkin-Huxley Na+ channel that is integral to long-distance signaling in other animals. Nematode neurons are presumed to communicate by electrotonic conduction and graded depolarizations. This fundamental difference in operating principle requires different channel properties to regulate transmission and transmitter release.

We have recorded single ionic channels in cell-attached and excised patches from the somata of two chemosensory neurons (AWA and AWC). With the cytoplasmic face of the patch exposed to low-calcium solution (calcium chelated by 5 mM EGTA), two classes of channel are common. An outwardly rectifying K+ channel with a unit conductance averaging 53 pS is active at membrane potentials more positive than -20 mV. An inwardly rectifying channel is active at potentials more negative than -50 mV. The inward channel is notably flickery even in the absence of divalent cations; this prevented determination of its conductance and reversal potential. Both of these common channels were inactive over a range of membrane potentials near the likely cell resting potential; this would account for the region of very high membrane resistance observed in whole-cell recordings. Other channels were seen in only a few patches, but may have important functional roles. A small-conductance, rapidly inactivating channel was active at positive membrane potentials; this channel may account for inactivating currents seen in whole-cell recordings. A very large-conductance (>100 pS), hyperpolarization-activated, inwardly rectifying channel may account for step conductance changes seen in whole-cell recordings. Exposure of the cytoplasmic face of the patch to high (100 micromolar) calcium activates a number of high-conductance channels.

391. ELECTROPHYSIOLOGICAL ANALYSIS OF *RIC-3*

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ric-3 is thought to be a protein specifically required for the assembly or trafficking of acetylcholine receptors. *ric-3* mutants were originally isolated because they were resistant to the effects of aldicarb, an inhibitor of acetylcholinesterase. Subsequent studies showed that *ric-3* is also resistant to levamisole, an agonist of acetylcholine receptors. These data suggested that *ric-3* is needed for the function of the levamisole-sensitive class of acetylcholine receptors. *ric-3* alleles were also identified as suppressors of the dominant *deg-3(u662)* allele, which encodes a neuronal nicotinic acetylcholine receptor subunit. These observations suggest that *ric-3* might be required postsynaptically for the localization or function of both the levamisole receptor and other classes of nicotinic acetylcholine receptors.

In collaboration with Millet Treinin's lab, we are determining whether *ric-3* mutants show a reduction of functional acetylcholine receptors at the body wall muscle. It has previously been shown that the *C. elegans* body wall muscle has one GABA receptor and two acetylcholine receptors. The two acetylcholine receptors can be distinguished because one is sensitive to nicotine and the other to levamisole. If *ric-3* is indeed required for the function of all acetylcholine receptors then we would expect a reduced response to levamisole, acetylcholine, and nicotine in *ric-3* mutants while the response to GABA would be unaffected. Our experiments show that the levamisole response is indeed dramatically reduced in *ric-3* mutants, although interestingly not absent. This is in agreement with the incomplete suppression of *deg-3(u662)* in *ric-3* mutants. Further experiments will address both the specificity of *ric-3* for cholinergic transmission and the generality of *ric-3* for different classes of acetylcholine receptors.

392. Olfactory Coding in *C. elegans* Requires Modulatory G_{alpha} proteins.

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C. elegans senses at least five attractive odors with one pair of olfactory neurons, AWC, yet distinguishes among these odors in behavioral assays. The two AWC neurons are structurally and functionally similar, but the G protein-coupled receptor STR-2 is expressed in only one of the two AWC neurons (AWC^{ON}), never in both. Discrimination between benzaldehyde, butanone and 2,3-pentanedione is achieved by segregating the ability to sense these odors into unique combinations of AWC neurons: Benzaldehyde is detected by both AWC neurons, whereas butanone and 2,3-penatanedione are detected solely by AWC^{ON} and AWC^{OFF}, respectively[1]. However, since five odors are distinguished by the two AWC olfactory neurons, some odors must be detected by the same cell, necessitating additional mechanisms for odor discrimination. Odor recognition in the AWC neurons depends mainly on the ODR-3 G_{α} protein, but the AWC neurons also express several other G_{α} proteins that affect olfaction only subtly. We found that mutations in the gene encoding the G_{α} protein, *gpa-5*, abrogated discrimination between butanone and isoamyl alcohol, but did not affect odor recognition. Furthermore, double mutant combinations between *gpa-5* and another G_{α} protein, *gpa-6*, exhibited additional odor discrimination defects. Intracellular G protein signaling networks may therefore mediate some forms of odor discrimination in *C. elegans*.

[1]Wes, P.D. and Bargmann, C.I. (2001). *C. elegans* odour discrimination requires asymmetric diversity in olfactory neurons. *Nature* **410**, 698-701.

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393. Analysis of octanol avoidance mutants and serotonin modulation of the ASH sensory circuit

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We are interested in how neurons can detect and distinguish diverse stimuli, and how neuronal circuit function can be modulated. As a model, we have chosen the *C. elegans* ASH neurons, which are primarily responsible for detecting high osmolarity, touch to the nose, and volatile repellents including 1-octanol.

To address the molecular pathways involved in octanol avoidance, we have conducted EMS screens in various genetic backgrounds for mutants which fail to respond to octanol. From these screens, we expect to identify genes involved in stimulus detection, signal transduction, or modulation of the ASH circuit. More than 7,500 F2 progeny have been screened, and more than 15 mutant strains have been isolated. All the mutant strains are normal for dye-filling, indicating that the gross morphology of the ASH neurons is normal.

Two mutant alleles, *rt97* and *rt98*, are severely defective in octanol response. Nose touch response is normal in *rt97* animals, but response to volatile attractants sensed by the AWA and AWC neurons is perturbed. *rt98* animals are mildly defective for osmotic avoidance and nose touch response, but are normal in their response to AWC- or AWA-mediated stimuli. *rt97* maps on chromosome IIIL, and *rt98* is on IVR. Further characterization of these two mutants and mapping of the corresponding genes is underway.

The remaining mutant strains have less severe and more transient defects in octanol response. The animals are defective in octanol response when starved for 10 minutes, but they do respond to octanol when tested on food. Some of these mutant strains also recover their ability to respond after 60 minutes of starvation. Serotonin has been implicated in modulating food-dependent behavioral changes in *C. elegans*, so we tested if serotonin modulates octanol response in these mutants. Indeed, exogenous serotonin rescued the defective behavior of these mutant strains. Genes corresponding to these mutations may be involved in the modulation of synaptic coding or octanol detection pathway.

How is the response of animals modulated by their feeding status? To answer this question, we are currently testing normal and mutant strains for their response to octanol under variety of conditions. So far, our results suggest that the animals' sensitivity to octanol is dependent on their feeding status, and that some compensatory mechanism is activated upon starvation. Response to octanol seems to be a dynamic process, despite the apparent unchanging ability of the normal animals to respond irrespectively of starvation. Modulation of sensory circuits by serotonin is a common phenomenon observed in a variety of organisms from *Aplysia* to vertebrates. The analysis of octanol avoidance in *C. elegans* will help us elucidate the stimulus detection pathway and mechanisms underlying circuit modulation.

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394. Mechanisms involved in regulating olfactory receptor expression

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Each sensory neuron is uniquely identified by its sensory properties. One mechanism responsible for generating the specificity and diversity of sensory cells is the regulated expression of receptors and signaling components in a cell-type specific fashion. We are interested in understanding how the expression of these components is regulated, both during development and in response to environmental cues, using *C. elegans* as a model system. Unlike rodents, in which only one olfactory receptor is expressed in a neuron, in *C. elegans*, diversity of sensory cell function is generated in part by the expression of multiple, distinct subsets of receptors in each chemosensory neuron type. Our laboratory has identified SNS-8, a homolog of MARK/PAR-1/SAD-1 kinases, as a key component of efficient olfactory receptor gene expression in *C. elegans* (see abstract by Lanjuin and Sengupta). Animals lacking SNS-8 activity exhibit reduced expression of *str-1*::GFP in the AWB neurons and *sra-6*::GFP specifically in ASH neurons. In addition, *sns-8* mutants are small and are developmentally delayed. We have conducted a genetic modifier screen of the *sns-8* phenotype of reduced *str-1*::GFP expression and have identified at least four distinct loci which rescue the reduced expression phenotypes of *str-1*::GFP in the *sns-8* mutant background. Progress towards characterizing the phenotypes of *sns-8* suppressors will be presented. Furthermore, we have found a link between olfactory receptor expression and the TGF-beta arm of the dauer pathway (Group II mutants), suggesting that expression of olfactory receptors is dynamic and modulated by environmental signals. Little is known about the molecular mechanisms governing expression of olfactory receptors; therefore, characterizing the mechanisms through which SNS-8 and TGF-beta signaling function to control olfactory receptor expression may also elucidate how the diverse functions of the neurvous system are achieved in *C. elegans* as well as in higher organisms.

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395. Characterizing the Neural Circuitry of Chemotaxis to Volatile Odorants

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The *C. elegans* chemotaxis circuit is an experimentally tractable neural circuit that allows worms to interpret gradients of various molecular cues. Anatomically, the circuit consists of three layers: sensory neurons, interneurons, and motor neurons. The sensory input to the chemotaxis circuit is relatively well-understood. As a first step in characterizing the remainder of the circuit, we are using laser ablations to identify functionally important interneurons and motor neurons.

The original chemotaxis assay asks whether worms on an agar plate are able to travel to a point source of attractant. Although this type of assay proved useful for identifying the sensory neurons that detect odorants, it may be less useful in identifying interneuron and motor neuron functions, since some of these defects may affect particular aspects of odor responses without entirely destroying chemotaxis. To help characterize defects in animals lacking particular neurons, we have developed a behavioral assay that allows us to control circuit input more specifically and to assay circuit output more directly. In this assay, a stream of air flows over a plate of worms. By introducing odorants into the air stream, we can create temporal gradients of volatile odorants. We use a video camera and computer to track and analyze worm movement in response to these gradients. One advantage of this approach is that we are able to distinguish between animals whose movement is generally defective and those that have specific defects in odorant responses.

Our measurements of odorant-modulated changes in turning frequency in wild-type worms are consistent with the pirouette model of chemotaxis developed for water-soluble attractants (1). According to this model, chemotaxis is accomplished by a biased random walk, in which sharp turning is less frequent when attractant concentration is increasing and more frequent when attractant concentration is decreasing. Sharp turns, or pirouettes, are composed of omega bends and reversals. Consistent with this theory, we find that both omega bends and reversals are modulated by temporal gradients of volatile odorants. We are using this assay to characterize mutant and laser-ablated animals, and we are also characterizing other aspects of odorant responses.

1. Pierce-Shimomura et al. (1999) *J Neurosci*. **19(21)**, 9557-69

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396. Sniffing out the mechanisms of ODR-7 function in AWA neurons

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The AWA olfactory neurons mediate chemoattraction to a subset of volatile odorants. The orphan nuclear receptor gene *odr-7* is required for the sensory functions of the AWA neurons. We are interested in investigating the specification of AWA fate by ODR-7 and other factors.

In *odr-7* null mutants, the AWA neurons fail to express the diacetyl receptor gene *odr-10*, the G-alpha protein subunit *gpa-5*, and the *trp*/*trpl* channel related gene *osm-9*. In addition, ODR-7 is required to maintain its expression by autoregulation. It is not known if ODR-7 regulates gene expression directly or indirectly. Many nuclear receptors function as heterodimers. ODR-7 may have a partner or multiple partners for regulation of different genes. To understand how *odr-7* functions, we are currently defining the promoter elements required for ODR-7 regulated gene expression in the AWA neurons. These regions will be examined for common sequence features that might represent a cognate DNA binding sequence for ODR-7 or other transcription factors. We are also examining the promoter of ODR-7 to determine what regions are important for early and late expression.

To further explore *odr-7* function, we are examining the effects of misexpression of *odr-7* in other chemosensory neurons. We misexpressed *odr-7* in the AWB and AWC olfactory neurons using the *odr-1* promoter. Misexpression of *odr-7* is not sufficient to result in misexpression of *odr-10*::GFP. However, expression of ODR-7 from this construct leads to the repression of the AWC-specific marker *str-2*::GFP. These worms still chemotax to the AWC-sensed attractant isoamyl alcohol, indicating that the sensory function of AWC is not grossly altered. We are currently examining this in further detail and also examining the affects of this construct on the function of the AWB neurons. To define the domains of ODR-7 required for its gene regulatory functions, we have created an *odr-7* minigene. We are creating point mutations in the DNA binding domain and other regions of *odr-7* to examine their effects.

Finally, we are currently mapping *sns-1* (sensory neuron specification). *sns-1* mutants fail to chemoattract to diacetyl and there is reduced expression of *odr-10*::GFP in *sns-1* mutants. However, ODR-7 is expressed, suggesting that *sns-1* could be a binding partner or a downstream target of *odr-7*.

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397. Characterization of the *E. coli*-induced suppression of olfactory habituation

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Food is an important motivator for animals. In *C. elegans*, the presence of *E. coli* causes changes in locomotion, egg laying, pharyngeal pumping and thermotaxis. We have been investigating the effect that food has on olfactory behavior and found that the presence of *E. coli* suppresses both the approach to and the habituation to 100% benzaldehyde. Control experiments demonstrated that this suppression is not caused by an inability to sense odorants in the presence of *E. coli*. Both of these activities (the suppression of chemotaxis and the suppression of habituation) are inactivated by heating the *E. coli* to 95°C for 15 minutes. As with other food-induced behavioral modulations in *C. elegans*, these effects can be mimicked by the addition of exogenous serotonin and are dependent on the *tph-1* and *cat-4* gene products. We will present a further genetic characterization of this behavior.

We are also exploring the behavioral characteristics of olfactory habituation. Associative learning involves the association between a conditioned stimulus (CS) and an unconditioned stimulus (US). With food acting as a US while benzaldehyde represents the CS, exposures to benzaldehyde in the absence of food during habituation training would result in the worms learning that benzaldehyde predicts the *absence* of food, and they no longer approach benzaldehyde on subsequent tests. The lack of habituation observed in the *presence* of *E. coli* therefore would occur because these conditions indicate that benzaldehyde is a good predictor of food. Experiments which increase the exposure to benzaldehyde while varying the exposure to food indicate that the degree of habituation is not determined simply by the time of exposure to benzaldehyde, as non-associative (single-stimulus learning) accounts of habituation would predict. Rather, the amount of habituation is dependent on both the duration of the benzaldehyde exposure and the amount of time that food is present during that exposure, revealing an associative component of benzaldehyde habituation. Supported by NSERC.

398. Isolation and analysis of chemotaxis mutants in *C. elegans*

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C. elegans shows chemotactic behavior to water-soluble chemicals, such as NaCl. This behavior is thought to be important for seeking food. Our goal is to reveal the molecular mechanism for this behavior. About 40 genes have been identified genetically, mutations of which cause defects in chemotaxis to water-soluble attractants . However, the majority of them seem to be more related to structure than to function of neurons. Many genes involved in the neuronal function for chemotaxis remain to be uncovered.

We screened EMS-mutagenized worm populaions representing about 150,000 haploid genomes for new mutants. 126 independent strains abnormal in chemotaxis to NaAc were isolated. 47 of them were defective in dye-filling in sensory neurons (Dyf), and the other, 79 strains were non-Dyf. 60 strains among the Che non-Dyf strains were back crossed to N2. Genetic mapping is in progress.

399. A screen for suppressors of the *let-60* chemotaxis defect

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We have previously shown that the ras-MAPK pathway plays an important role in olfaction in *C. elegans*. Both loss-of-function and gain-of-function mutants of *let-60 ras* showed reduced chemotaxis to odorants. We also showed that the ras-MAPK pathway is involved in plasticity of chemotaxis to odorants as well: wild type worms pre-exposed to an odorant fail to show chemotaxis to that odorant, but the *let-60(lf)* mutant still show positive chemotaxis after such treatment. Loss-of-function mutants of *mek-2* and *mpk-1* suppress the chemotaxis defect of *let-60(n1046gf)*, indicating that the MAPK pathway acts downstream of *let-60*. However, a loss-of-function mutant of *lin-1*, a gene that encodes a MAPK target transcription factor in the vulval induction pathway, does not show chemotaxis defects, suggesting that other molecules act downstream of the MPK-1 MAPK in chemotaxis.

To identify the components downstream of *mpk-1*, we conducted a screen for suppressors of the *let-60(n1046gf)* chemotaxis defect. Thirteen mutants that show improved chemotaxis to isoamylalcohol were isolated after mutagenesis of *let-60(gf)*. During the course of the screen, we observed that the *let-60(n1046)* mutant also has a lethargic phenotype. When left undisturbed on bacteria-rich plates, the *let-60(gf)* mutant show little spontaneous locomotion compared to the wild-type. The suppressor mutants fall in several categories. Apart from suppressing the chemotaxis defect, only some of them suppressed the multivulval phenotype of *let-60(gf)*; some suppressed the lethargic phenotype; some showed a hyperactive phenotype; and some showed abnormal locomotion patterns. These phenotypes apparently behaved independently, suggesting that the suppressors may each represent partially non-overlapping functions. Through analysis of these new mutants, we hope to gain further understanding of the biological processes that the ras-MAPK pathway regulates in the nervous system of *C. elegans*.

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400. THE A, B, C s OF CHEMOSENSORY NEURON SPECIFICATION

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Many inputs contribute to an animal's perception of its environment. These inputs are transduced by different receptor cells. Each receptor cell shares similarities with other cells of its type in terms of the general sensory functions, however, the specificity of different cells of the same type varies. How are the common features, as well as the diversity brought about? We are using the chemosensory system of *C. elegans* as a model to study these processes. The current model proposes that all olfactory neurons (AWA, AWB and AWC) share a common default fate which is then overridden by cell-fate specific transcription factors. These transcription factors are the LIM homeodomain gene*lin-11* and the nuclear hormone receptor gene*odr-7* in the AWA neurons and the LIM homeodomain gene*lim-4* in the AWB neurons. When expression of any of these transcription factors is lost, the cell develops along the default route, losing its own identity and function. What regulates the expression of these factors?

In order to identify genes contributing to the formation and specification of the AWA neurons, we conducted a screen looking for mutants showing abnormal expression of *odr-7*. In this screen we isolated *sns-10*. This mutant shows various pleiotropies suggesting that *sns-10* is involved in the formation and/or function of a number of cells. With respect to AWA development, in *sns-10* mutants, there is a variable loss in *odr-7* expression and rarely, ectopic expression. Behavioral studies indicate that AWA-mediated chemosensation is defective suggesting that its functional specification is compromised. *sns-10* encodes a paired-like homeodomain transcription factor with high homology to the *Drosophila* gene *aristaless*. Interestingly, *aristaless* is involved in the formation of the aristae sensory organs (Schneitz et al. 1993). Previous studies in our lab identified other factors that regulate *odr-7* expression and hence AWA specification. Lineage restriction of *odr-7* to the AWA neurons is regulated by the forkhead transcription factor *unc-130* (Sarafi-Reinach et al. 2000), whereas the initiation of *odr-7* expression involves the LIM-homeodomain transcription factor *lin-11* (Sarafi-Reinach et al. submitted). Investigation of the interaction of *sns-10* with these genes is underway. This analysis should elucidate the mechanisms underlying the specification of this olfactory neuron and possibly identify a mechanism that is common to sensory organ specification in different species.

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401. Identification and Characterization of Genes Required in a Cell-Cell Signaling Event that Results in Asymmetric Odorant Receptor Expression in *C. elegans*

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We are studying the role of cell-cell signaling in the development of the AWC neurons, a bilaterally symmetric pair of olfactory neurons. The candidate odorant receptor *str-2* is expressed asymmetrically in only one AWC cell (termed the AWC^{ON} cell) because of a stochastic, coordinated decision that requires normal axon guidance and probably direct cell-cell signaling between the two AWC cells. Studies of *nsy* (neuronal symmetry) mutants in which both cells express *str-2::*GFP have demonstrated that a calcium to JNK/p38 signaling pathway regulates asymmetry. The upstream signaling molecules involved in this asymmetry have not yet been determined, but epistasis experiments suggest that *nsy-3* and *nsy-4* act upstream of the calcium signaling pathway. Therefore, these genes may directly mediate signaling between the AWC neurons. We have mapped *nsy-3* and *nsy-4* to small genetic intervals and are attempting to get cosmid rescue of *nsy-4*.

In *nsy-4* mutants, both AWC cells fail to express *str-2::*GFP. The two AWC neurons have partly overlapping olfactory functions; AWC^{ON} senses butanone, AWC^{OFF} senses 2,3-pentanedione, and both sense benzaldehyde. Behavioral experiments indicate that in *nsy-4* mutants, the AWC^{ON} neuron adopts an AWC^{OFF} fate rather than both AWC neurons adopting a non-AWC fate. To isolate additional mutants with a similar phenotype to *nsy-4*, we performed a screen for animals that fail to express *str-2::*GFP in the AWC neurons. We mutagenized *str-2::*GFP expressing animals with EMS and enriched for mutants defective in chemotaxis to the AWC^{ON} sensed odorant butanone. Chemotaxis defective mutants were examined for absence of *str-2::*GFP expression. Sixteen mutants were isolated and are being further characterized.

The mutants with high penetrance GFP phenotypes fall into two classes on the basis of their ability to chemotax toward AWC^{ON} or AWC^{OFF} sensed odorants. At least five mutants are defective in chemotaxis to an AWC^{ON} sensed odorant, but not to an AWC^{OFF} sensed odorant. These mutants may have two AWC^{OFF} neurons. At least five mutants are defective in chemotaxis to both AWC^{ON} and AWC^{OFF} sensed odorants. These mutants may have more severe defects that disrupt both AWC neurons. Based on previous analysis of AWC development, the mutants could have defects in AWC fate, AWC axon guidance, or cGMP-mediated olfactory signaling. Preliminary dye-fill experiments indicate that only one mutant has defects in axon guidance. Further analysis of these mutants will be presented at the meeting.

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402. The regulation of sensory cilia specific genes in *C. elegans*

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Cilia are an important cellular differentiation of sensory neurons for receiving information from the environment. 60 of the 302 *C. elegans* neurons have ciliated endings, accounting for the bulk of informational input into the worm. Mutations in a large number of genes have been identified that affect the structure and function of these sensory cilia. One of these genes, *daf-19*, encodes an RFX-type transcription factor. By acting through its target site in promoter regions, the x-box, DAF-19 appears to be the main regulator of a number of effector genes involved in sensory cilium formation. These effector genes, including *che-2, osm-1, osm-5, osm-6* and *daf-19* itself, are expressed in essentially all ciliated sensory neurons and, when mutated, cause morphological defects in sensory cilia. DAF-19 is also involved in the regulation of x-box containing genes that are expressed only in subsets of ciliated sensory neurons (e.g. *odr-4*). *odr-4* is implicated in localizing odorant receptors to sensory cilia. Our results strongly suggest that DAF-19 regulates the structural and functional differentiation of sensory cilia by activating the transcription of a battery of genes whose products form the cilium and endow this structure with sensory functions.

We initiated the identification of many more ciliary components by analyzing genes that have an appropriately spaced x-box promoter element, which we call *xbx* genes. In repeated searches through the *C. elegans* genome sequence (with Kerry Bubb, Genetics, UW), using an algorithm designed to find promoter elements, we uncovered hundreds of candidate *xbx* genes, several of which had *C. briggsae* and *Drosophila* homologues that also had an appropriately positioned x-box promoter element. Initial expression analyses of a subgroup of these *xbx* genes showed that some of them were specifically expressed in ciliated sensory neurons in a *daf-19* dependent manner. Presently we are attempting to generate cilium specific phenotypes for some of the *xbx* genes using gene specific RNA interference techniques. To further characterize the set of genes that comprise the cilium formation module we are searching through promoter sequences of selected subsets of cilium specific and *xbx* genes for other conserved promoter elements (with Martin Tompa, Computer Sciences and Engineering, UW). In combination with previous data about sensory cilia, these approaches have the potential to reveal all the genes required for sensory cilium structure and function.

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403. The application of novel positional cloning strategies for forward genetics: the case of dyf-8.

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The dye-filling (*dyf*) defective gene, *dyf-8*, was first isolated in a screen for animals that fail to take up a lipophillic dye into amphid and phasmid neurons (Starich *et al*, 1995, Genetics 139: 171-188). We cloned this gene using two novel strategies: SNP mapping (Wicks et al. in press) and Mos transposon-insertion mutagenesis.

The original *m509* allele of *dyf-8* was isolated from a mutator strain, and therefore possibly arose as a consequence of a Tc1-insertion event. A transposon-insertion display (Wicks *et al*, 2000. Dev. Biol, 221: 295-307) was used to identify all inserts that co-segregated with the mutant phenotype. We identified genomic flanks for six Tc1 inserts near the centre of LGX, ranging in position from 3.42 mu to +1.16 mu. We then crossed *dyf-8* into a CB4856 background. Bulked segregant analysis was used to verify the approximate map position and identify snip-SNPs that flank the mutation. Recombination events between these flanking markers were mapped with a high density SNP map, and the recombinants phenotyped. We were able to map the mutant phenotype with the progeny from a single cross to a 40 kb interval that included one of the six known transposon inserts in the *dyf-8* mutant. This insert was in sixth exon of the predicted ORF C43C3.3.

In a parallel series of experiments, we used the Mos1 transposon from *Drosophila mauritiana* to identify mutants (Bessereau,J.-L. *et al*. 2000, European WM abstract) defective for the detection of high osmolarity (Osm). We determined whether the mutants were defective for the uptake of a lipophillic dye into amphid and phasmid neurons. Seven of the ten mutants were Dyf. Of these, three contained transposon insertions suggesting that the defects were possibly caused by the insertion event. We used inverse single worm PCR (ISWP) to clone genomic sequences flanking the transposons. One of the mutants *(ox195)* contained an insert in the tenth exon of C43C3.3.

m509 animals, transgenic for the C43C3 cosmid, were completely rescued with respect to the Dyf phenotype. C43C3.3 is predicted to encode a peptide with three suggestive features: First, the N-terminus consists of a signal sequence, suggesting that the peptide is secreted. Second, a single predicted transmembrane domain is found near the C-terminus. In between, on what would be the extracellular domain of this membrane-bound protein, is a Zona Pellucida domain, which has been shown in other proteins to mediate some forms of protein-protein interactions on the extracellular surface of cells. Our working hypothesis is that *dyf-8* directly, or indirectly, mediates the stabilisation of the cilia/support cell interactions. We are testing this hypothesis through further genetic and biochemical analysis of the gene.

404. Olfactory G-protein signalling in *C. elegans*

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We study signal transduction via heterotrimeric G-proteins. *C. elegans* has 21 G alpha, 2 G beta and 2 G gamma subunits. At least 14 of the G alpha-subunits are involved in sensory signalling in the amphid neurons. With these neurons, *C. elegans* can sense chemicals from its environment. Two pairs of these neurons, AWA and AWC, can detect and discriminate among many attractive odorants. Some odorants are detected by AWA or AWC only, others can be detected by both.

Analysis of GFP-fusion expression patterns and mutant phenotypes indicated that six G alpha subunits play a role in AWA and AWC. These are ODR-3, GPA-2,-3,-5,-6 and -13. ODR-3 provides the main stimulatory signal. The other subunits have redundant stimulatory or inhibitory roles, depending on the odorant. The interactions between the G alpha subunits probably contribute to the ability of the animal to discriminate among odorants. We intend to determine how these different G alpha subunits can function in one cell.

We use a forward genetic screen to identify novel components of the GPA-5 signalling cascade. To this end, we make use of the fact that *odr-3* mutants are defective in the detection of the odorant diacetyl. This defect, however, is suppressed upon inactivation of the negative regulator GPA-5. Following mutagenization of *odr-3* mutants, we can therefore screen for animals that have regained their ability to detect diacetyl. In this way we expect to identify novel components of the GPA-5 signalling cascade. To increase the efficiency of our screen, we use 2,3-pentanedion. *odr-3* mutants favour this odorant over diacetyl, while *gpa-5,odr-3* double mutants do not. In this way we enrich our mutant population significantly.

405. A reverse genetic approach to neuropeptide function in the ASH sensory circuit

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Backward locomotion in response to nose touch, 1-octanol, benzaldehyde, and high osmolarity is mediated by the ASH sensory neurons (CGC 1705, 2314). Glutamate neurotransmission between ASH and the interneurons of the ASH circuit appears to be absolutely required, since the response to all stimuli is abolished by a mutation in EAT-4, a sodium-dependent P_i cotransporter proposed to load glutamate into synaptic vesicles (CGC 3349, PMID 11001057). However, a mutation in the GLR-1 glutamate receptor subunit eliminates the response to only nose touch (PMID 7477294). GLR-1 functions post-synaptically, in the interneurons of the ASH circuit, not in detection in ASH itself, indicating that glutamatergic neurotransmission is modulated in a stimulus-specific manner (PMID 7477294). That is, glutamatergic neurotransmission is necessary but not sufficient for the response to volatile odorants and high osmolarity.

To understand how the ASH circuit transmits stimulus-specific information across the ASH-interneuron synapse, we are investigating the function of novel neuropeptides in the ASH circuit. There are at least 32 neuropeptide-like preprotein (*nlp*) genes in the genome, and GFP-reporter studies show at least two of these are expressed in ASH (ECWM 2000ab56). Additionally, electron microscopy has shown dense-core vesicles, presumably containing peptidergic transmitters or modulators, at the ASH-interneuron synapse (PMID 8806). These observations raise the possibility that NLP peptides could modulate glutamate neurotransmission in a stimulus-specific manner. To address the function of putative ASH-specific neuropeptides, we generated a frozen deletion library of 2.4×10^6 genomes, and are currently screening for deletions in the putative neuropeptide genes. Results of the deletion screening and preliminary phenotypic analysis will be presented.

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406. Activity-dependent transcription in the AWA sensory neuron: the roles of *osm-9*, *ky440*, and other transduction molecules

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osm-9 encodes a predicted cation channel that is involved in multiple *C. elegans* sensory modalities. Loss of *osm-9* function eliminates chemotaxis to the AWA-sensed odorant diacetyl, compromises adaptation to a subset of AWC-sensed odorants, and diminishes avoidance of ASH-sensed noxious stimuli. *osm-9* mutants also exhibit reduced AWA expression of a Green Fluorescent Protein (GFP) transgene driven by the promoter of the diacetyl receptor, ODR-10. OSM-9 is similar to sensory channels in other species, including the TRP channels and the mammalian capsaicin receptor VR-1. We are characterizing *osm-9* and related genes to learn more about the regulation and function of this novel channel family.

In a forward genetic screen, we used the activity-dependent regulation of *odr-10*::GFP as an assay to identify additional genes that affect OSM-9 signaling. *ky440* was isolated in a screen for dominant suppressors of the reduced *odr-10::*GFP expression in *osm-9* mutants. *ky440* restores *odr-10::*GFP expression in an *osm-9 (n2743)* background (where the channel is present, but mislocalized) or in an *osm-9* null mutant. OSM-9 belongs to a family of *C. elegans* channels, and both OSM-9 and the related channel OCR-2 are expressed in AWA neurons. The *osm-9 ky440 ocr-2* triple mutant does not exhibit expression of *odr-10::*GFP. These results suggest a model whereby *ky440* enhances a low level of channel activity to allow for *odr-10*::GFP expression, but that some level of activity through this channel family is required for this suppression. Recent progress in snip-SNP mapping of *ky440* and another mutant, *ky456*, will be presented.

We have also taken a candidate approach to studying *osm-9* signaling, using existing *C. elegans* signaling mutants. The involvement of *osm-9* in olfaction suggests that it is activated by a G-protein signaling pathway. Animals doubly mutant for the olfactory G protein genes *odr-3* and *gpa-3* have a defect in *odr-10*::GFP expression similar to that of *osm-9* mutants. Our results suggest that the G_{alpha} proteins *odr-3* and *gpa-3* play redundant roles in the regulation of *odr-10::* GFP.

We suggest that the activation of G-protein coupled receptors stimulates ODR-3 and GPA-3, which in turn activate OSM-9/OCR-2. Channel activity impinges upon transcriptional control of *odr-10*, as well as the behavior of the animal. We hope to clone *ky440* and *ky456*, and understand their roles within the AWA transcriptional and behavioral pathways.

407. Transient disruption of IP3 receptor function in *C. elegans*.

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Inositol 1,4,5 trisphosphate (IP3) controls Ca2+ release from intracellular stores via IP3 receptors (IP3Rs) and is central to a wide range of cellular responses. However the specific functions of this signalling pathway in animals remain poorly understood. ITR-1, the *C. elegans* IP3R is widely expressed(1,2,3) and known to participate in the control of ovulation(4) and defecation(2). To determine the functional significance of IP3 signalling more fully, we have disrupted IP3 signalling in whole, live animals using two transient approaches: inducible, high level expression of the IP3 binding domain of ITR-1 and RNA mediated interference by feeding(5). While confirming the roles of IP3 signalling in regulation of defecation and in ovulation, these approaches identify important, previously unidentified functions. IP3-mediated signalling is important in multiple events during embryogenesis, especially gastrulation, and in the behavioural regulation of pharyngeal pumping in response to the presence of food. To understand the cellular basis of these functions more fully, we are now preturbing IP3 signalling in a tissue specific manner utilising cell specific promoters.

1. Baylis *et al.* (1999) J. Mol. Biol. 294, 467-476 2. Dal Santo *et al.* (1999) Cell 98, 757-767 3. Gower *et al.* (2001) J. Mol. Biol. 306, 145-157 4. Clandinin *et al.* (1998) Cell 92, 523-533 5. Timmons & Fire (1998) Nature 395, 854

408. Characterization of rcn-1, a calcipressin homologue in *C. elegans*

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Calcipressins are a family of calcineurin binding proteins conserved from fungi to yeast to humans. They have shown to be negative feedback regulators of the calcium/calmodulin phosphatase calcineurin, inihibit cardiac hypertrophy in mammals, and also may play a role in Down Syndrome in humans.

We identified rcn-1, a calcipressin homologue, in *C. elegans* on chromosome III in cosmid F54E7 and cloned the gene from a cDNA library. GFP expression of promoter regions of rcn-1 was seen mainly in pharyngeal muscle, excretory cells, vulval epithelial cells, ventral and dorsal nerve cords and commissures, neurons, hypodermal cells and intestine. Whole-mount immunostaining patterns with DS-24 polyclonal antibody showed similar expression patterns. DS-24 antibody was raised against a 24 bp oligonucleotide of the most conserved region of DSCR-1, a human calcipressin. This expression not only confirms our previous GFP expression results, but also shows the conservation of calcipressins from humans to *C. elegans*. Preliminary data of calcineurin GFP and antibody expression patterns from our laboratory has shown much similarity with rcn-1 suggesting a relationship between the two proteins. This relationship was further confirmed by GST *in vitro* binding assay. GST-fused rcn-1 bound calcineurin A in a calcium-dependent manner suggesting that the activity of calcineurin may be important for the binding of the two proteins. Furthermore, we are interested in testing the effect of rcn-1 on calcineurin activity by phosphatase assay, and are also currently raising antibodies against rcn-1 for further protein analysis.

Northern blot analysis has confirmed a low-level of expression of a 1.0 kb mRNA transcript. In addition, we are currently studying the effect of calcineurin on the transcriptional

expression of rcn-1 through GFP analysis with calcineurin mutants. We are planning to conduct RNAi of rcn-1 and will attempt to obtain deletion mutants by UV-TMP mutagenesis to observe loss-of-function phenotypes.

409. Calcineurin Functions in C. elegans: growth and memory

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Calcineurin (CN), a protein phosphatase 2B (PP2B), is a serine/threonine phosphatase under the control of Ca^{2+}/calmodulin. Calcineurin is a heterodimer of a catalytic and CaM-binding subunit, Calcineurin A, tightly bound to a Ca^{2+}-binding regulatory subunit, Calcineurin B and two-subunit structure is conserved from yeast to human. It is well known that CN is a signaling molecule involved in various pathways in several types of tissues with a plethora of functions including T cell activation, cardiac hypertrophy, LTP, muscle remodeling, and development. The genome database predicted the genes encoding for both the catalytic and regulatory subunits in *C. elegans* on C02F4 and F55C10 (LG IV and V), respectively.

We identified and characterized the two essential subunits for the CN homologue in *C. elegans*, we termed *cna-1* (calcineurin A) and *cnb-1* (calcineurin B). We found that *cna-1::gfp* is expressed in neurons and muscles whereas *cnb-1::gfp* shows more neuronal expression. Even though the expression of both genes was expected to co-localize, they showed slightly different expression patterns. Immunostaining using each polyclonal antibody show similar expression patterns of the proteins, which is in ventral nerve cord, gonad, spermatheca, and sperm. CNB-1 in *C. elegans* also has high capacity of Ca^{2+} binding confirmed by a calcium overlay experiment. We performed a yeast two hybrid experiment and *in vitro* phosphatase assay in order to understand the interaction and role of the proteins *in vivo* and *in vitro*. These experiments showed that CNB-1 is necessary for the phosphatase activity, and the interaction between *cna-1* and *cnb-1* and the enzyme activity are Ca^{2+} dependent. We have isolated and characterized a deletion mutant for *cnb-1* that displayed several phenotypic defects related with growth, body size, movement, brood size and sperm function. The various

phenotypes seen in *cnb-1* mutants are consistent with the diverse functions that calcineurin as a signaling molecule plays roles in. In addition, since *tax-6* mutants that have a mutation in the calcineurin A gene show thermotaxic defects, we are testing whether the *cnb-1* deletion mutant also has any thermotaxic defects.

410. Characterization of synaptic defects in mau mutants

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mau genes are defined by maternal effect unc genes. mau-7, mau-8 and mau-9 display the same pattern of phenotypes: they are lethargic, present a discontinuous ratchet-like movement when moving backward and they show defects in the defecation cycle. These phenotypes are not due to defects in neuronal development since the overall structure of the nervous system appears normal.

To determine if mau mutations affected the distribution of presynaptic components, we examined the expression of synaptotagmin by immunocytochemistry. mau-8 and mau-9 mutations caused diffuse localization of synaptotagmin in the dorsal cord. In addition, frequent defasciculation of the dorsal cord and abnormal synaptic branches were observed in mau-8 and mau-9 respectively.

To assess neurotransmission, we quantified the resistance of mau mutants to aldicarb (acetylcholinesterase inhibitor), and levamisole (acetylcholine receptor agonist). mau mutants and wild type animals showed comparable sensitivity to the drugs suggesting that cholinergic synaptic transmission is not affected in mau. mau mutations show defects in the defecation cycle with frequent absence of expulsion contractions, a behaviour known to require the normal fonction of GABAergic neurotransmission. Preliminary results suggest that postsynaptic GABA receptors are abnormally distributed along the dorsal and ventral cord in mau-8 mutants.

mau-7, mau-8 and mau-9 are all defined by a single allele. mau-7 is close to daf-1 on chromosome IV, mau-8 is between unc-26 and dpy-4 on chromosome IV and mau-9 is to the right of rol-1 on chromosome II. Further mapping and transformation rescue experiments to clone the genes are in progress.

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411. Isolating redundant pathways that regulate synapse formation in *C. elegans*

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Nervous system maturation occurs when neurons establish synaptic connections with their targets. The molecular pathways that regulate the initiation and stabilization of synapses remain to be fully elucidated. We are using GABAergic neuromuscular junctions in *C. elegans* as a model system to genetically dissect the molecular pathways that regulate synapse formation. Using a GFP marker, P_{unc-25}-SNT-1::GFP that is specifically localized to the presynaptic termini of 19 GABAergic motorneurons, Zhen and Jin isolated a number of new genes that affect presynaptic morphology. One of these new genes, *syd-2* encodes a member of the Liprin (LAR-type-receptor-tyrosine phosphatase-interacting proteins) protein family. SYD-2 is localized at or near the presynaptic active zone regions. In *syd-2* mutants, the complete loss of SYD-2 function resulted in moderately diffuse localization of the vesicle marker, an elongated active zone region and slightly rigid backward movement. (Zhen and Jin. Nature 401. 371-375. 1999)

syd-2 is the only *C. elegans* liprin gene that is expressed in the nervous system. The moderate behavioral and anatomical defects of *syd-2* null mutants suggest the presence of functionally redundant pathways that regulate synaptogenesis. To identify these redundant pathways, we performed a genetic screen for mutations that enhance *syd-2* phenotypes. Out of this screen we have isolated 8 mutants that have mild or no behavioral defects on their own, but become paralyzed in a *syd-2* null background.

At least three of these mutants represent new alleles of *rpm-1*, a putative GEF gene that regulates synaptic growth, and is known to function in parallel with *syd-2* in synapse and muscle development (See abstract by Zhen, Xun and Jin, this meeting). We identified at least one new gene defined by a single allele, *hp1*, that is likely to function in parallel with *syd-2*, but is unrelated to *rpm-1*. Although both *rpm-1*;*syd-2* and *hp1*;*syd-2* animals show similar behavioral defects, they display different phenotypes in vesicle protein expression. While the expression of synaptic vesicle proteins is almost diminished in *rpm-1*;*syd-2* double mutants, *hp1*;*syd-2* animals have relatively normal levels of vesicle protein expression. However, the distribution of these proteins is completely diffuse at presynaptic termini.

We are continuing our analysis of the *syd-2* enhancer mutants and are pursuing the molecular analysis of these genes.

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412. *syd-5* may play a role in regulating synapse size in *C. elegans*

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syd-5 (ju89) was identified in a screen for mutations that alter synapse morphology in *C. elegans* using a presynaptic vesicle marker, *Punc-25::SNB-1-GFP* (1,2). This marker is expressed specifically by the GABAergic DD and VD motor neurons which form inhibitory synapses onto the body wall muscles. The sinusoidal movement of *C. elegans* is generated by the coordinated action of excitatory cholinergic motor neurons and the inhibitory GABAergic motor neurons. In wild-type adult worms, *Punc-25-SNB-1-GFP* is expressed as puncta of uniform size and spacing along the dorsal and ventral nerve cords. *syd-5(ju89)* mutant worms move with reduced sinusoidal amplitude and exhibit abnormal SNB-1-GFP puncta of various sizes in an irregular pattern along the nerve cords. Consistent with a change in synapse morphology, we also observe similar defects in the size, shape and spacing of the post-synaptic GABA receptor marker *UNC-49B-GFP* (3) in *syd-5* animals.

The SNB-1-GFP phenotype of *syd-5* mutants resembles mutants of *rpm-1*. *rpm-1* encodes a protein with GEF and ring finger domains. Ultrastructural analysis of GABAergic synapses in *rpm-1* mutants revealed multiple active zones within a single presynaptic site (4). To determine if *syd-5ju89)* and *rpm-1* mutants exhibit similar defects in synapse morphology, we examined worms co-stained with antibodies to GFP and to the active zone marker, SYD-2. SYD-2 localizes to synapses independent of neurotransmitter vesicles and is required to restrict the size of active zones1 . We found a pattern of enlarged SYD-2 puncta at synapses with increased SNB-1-GFP and smaller SYD-2 puncta at sites of reduced SNB-1-GFP in the *syd-5* mutants. This phenotype appears to be distinct from the *rpm-1* phenotype, suggesting that *syd*-5 plays a role in regulating synapse size.

syd-5(ju89) behaves genetically as a weak gain-of-function mutation. Twenty-four suppressors of *syd-5(ju89)* were identified previously from a screen of 30,000 F1 worms. Four suppressors are linked to *ju89* and may represent loss-of-function *syd-5* alleles. Mapping and characterization of the suppressors will be presented, along with progress in the cloning and molecular characterization of *syd-5*.

1Zhen & Jin. 1999. Nature

2 Nonet, M. 1999. Neurosci. Methods

3Bamber et al., 1999. J. Neurosci.

4Zhen et al. 2000. Neuron

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413. *syd-2* and *rpm-1* function synergistically in synapse and muscle development

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During synaptogenesis, specialized subcellular structures are developed in both pre- and postsynaptic termini to accommodate synaptic transmission. Using a synaptobrevin::GFP marker that is expressed in GABAergic motoneurons of *C.elegans*, we conducted a screen to isolate genes that affect presynaptic morphology (Zhen and Jin, in preparation). We identified SYD-2, a Liprin (LAR-receptor tyrosine phosphatases-interacting protein) family protein, that is localized near or at the active zone region, and restricts the size of activity zones (Zhen and Jin, Nature 401, 371-375, 1999). We also identified RPM-1, a putative GEF-domain containing protein that appears localized in 'peri-active zone' subdomain in presynaptic termini, but excluded from the active zone and vesicle regions. In *rpm-1* mutants, some GABAergic NMJs have multiple presynaptic active zones, supporting that RPM-1 negatively regulates synaptic development in these GABAergic synapses (Zhen et al., Neuron 26, 331-343, 2000).

While *syd-2* and *rpm-1* mutant animals display only mild behavioral defects, *rpm-1;syd-2* double mutant animals are severely uncoordinated. They hypercontract and nearly paralyze, and the body size of these animals is significantly shortened. In *rpm-1;syd-2* animals, the expression of the synaptobrevin::GFP marker in GABAergic NMJs is almost diminished, and that of the endogenous vesicle protein SNT-1 (synaptotagmin) is greatly reduced. Ultrastructural analysis of an adult double mutant animal revealed that presynaptic differentiation was disrupted in motoneurons. In addition, we observed defects in body wall muscle structures, which had not been noticed in either single mutant. Some dense bodies are missing, and the thin filaments are scattered among the thick filaments. We are further examining defects of the nervous system and muscle structures in *syd-2;rpm-1* mutants using other synaptic markers and antibodies against dense body proteins. We are also performing mosaic analysis to determine if *syd-2* and *rpm-1* regulate presynaptic development and muscle independently, or if the muscle defects we had observed in the double mutants is caused by defective presynaptic differentiation. These results suggest that *syd-2* and *rpm-1* may function in parallel pathways in synapse formation, and possibly in muscle development.

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414. Optical characterization of the vesicle pool size in ventral cord synapses

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Regulation of synaptic transmission underlies the nervous system's capacity to process and store information. One of the major targets of neuromodulation and use-dependent plasticity is the presynaptic release apparatus. Within a presynaptic terminal, neurotransmitter-containing vesicles are thought to be organized into distinct functional pools, and modulation of vesicle pool size can alter synaptic strength. We are interested in understanding how presynaptic modulatory pathways act to regulate vesicle pool size in *C. elegans*, and how this aspect of synaptic strength is affected in a variety of mutations that affect transmitter release. Using a synaptobrevin::GFP fusion protein (SNB-1::GFP) as an optical marker for synaptic vesicles, we quantified the pattern of fluorescence in ventral cord synapses of wild type and mutant animals. Epifluorescence images were captured with a CCD camera and analyzed using custom-made software that locates "punctate" regions of fluorescence and quantifies their widths and amplitudes. Loss of the DAG-binding protein UNC-13 severely impairs vesicle fusion, resulting in accumulation of synaptic vesicles as shown by electron microscopy [1]. We found that both the average widths and amplitudes of fluorescent puncta were increased by ~40% in *unc-13* mutant animals. Our lab (and others) have previously shown that *dgk-1* DAG kinase *and goa-1* $G_{\alpha o}$ act to inhibit acetylcholine release in motor neurons and that release is enhanced in loss of function mutants [2,3,4]. We analyzed the size distribution of fluorescent puncta in *dgk-1* and *goa-1* mutants and found that the mutations had opposite effects on puncta size: *dgk-1* animals had about a 30% *decrease* in average punctum size while *goa-1* animals had a 35% increase in average punctum size relative to wild type animals. This observation suggests that although both proteins function to inhibit release, they may have distinct effects on the release machinery. Future experiments in other synaptic transmitter release mutants will help to identify the molecules involved in regulation of vesicle pool size. This optical approach may prove to be a useful tool in characterizing the presynaptic effects of neuromodulation and plasticity in *C. elegans*.

1. Richmond and Jorgensen, Nat Neurosci 2: 791-797 1999.

2. Nurrish et al., Neuron 24: 231-242 1999.

3. Miller et al., Neuron 24: 323-333 1999.

4. Hajdu-Cronin et al., Genes Dev. 13:1780-1793 1999.

415. A screen to identify regulators of DGK-1

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Diacylglycerol (DAG) is a critical regulator of the release of acetylcholine (ACh) by motorneurons onto the body wall muscles [1-4]. Increases in DAG levels result in the recruitment of the DAG binding protein UNC-13, which is essential for neurotransmitter release [5], to release sites and this correlates with increases in ACh release. ACh release is increased by activation of Gq alpha (EGL-30) which in turn activates a Phospholipase C beta (EGL-8) which hydrolyzes PIP2 to produce DAG. Such Gq alpha/ PLC beta signaling pathways have been extensively characterized in many systems. Levels of DAG, and thus ACh release, are lowered by the action of the DAG kinase DGK-1 which phosphorylates DAG to phosphatidic acid to which UNC-13 cannot bind. In contrast to the activation of PLC beta little is known of how DAG kinases are regulated, although our previous work has suggested that DGK-1 may be a component of a Go alpha (GOA-1) signaling pathway. We aim to identify regulators of DGK-1 using a genetic approach. Animals overexpressing DGK-1 [dgk-1(xs)] have reduced rates of locomotion and to identify DGK-1 regulators we have performed an EMS screen for suppressors of the dgk-1(xs) reduced locomotion phenotype. We have screened 3906 haploid genomes and have identified 6 suppressors. We are currently mapping these suppressors using SNIP-SNPs.

1. Nurrish et al.,(1999) Neuron:24 231-242

2. Lackner et al.,(1999) Neuron:24 335-346

3. Hajdu-Cronin et al.,(1999) Genes Dev 13: 1780-1793

4. Miller et al.,(1999) Neuron 24: 323-333

5. Richmond et al., (1999) Nat.Neurosci 2: 959-964

416. Identifying regulators of DGK-1.

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We (and others) have previously described two competing G-protein pathways acting in motor neurons to either facilitate or inhibit synaptic transmission at neuromuscular junctions (NMJs) [1-4]. Facilitation of release occurs via a pathway composed of a Gq alpha (EGL-30), a phospholipase C beta (EGL-8), and the diacylglycerol (DAG) binding protein UNC-13 [2,4]. The Gq alpha pathway can be activated by muscarinic agonists leading to the formation of the membrane bound second messenger DAG by EGL-8 and the subsequent recruitment of UNC-13 to sites of Acetylcholine release [1,2]. UNC-13 is essential for neurotransmitter release [5] and it's enrichment at release sites correlates with an increase in neurotransmitter release. In contrast to the well characterized Gq alpha pathway, inhibition of release via Go alpha signaling is poorly understood. We believe that Go alpha is activated by serotonin as addition of serotonin agonists inhibits acetylcholine release at NMJs in a GOA-1 dependent manner. In addition, inhibition of ACh release by serotonin and by expression of constitutively signaling GOA-1 mutant protein is substantially blocked by mutations in a diacylglycerol kinase (DGK-1) which phosphorylates DAG to Phosphatididic Acid which is unable to bind UNC-13. However, the relationship between serotonin, GOA-1, and DGK-1 has not been established. The simplest model to explain these results is that Go alpha is coupled to a serotonin receptor and that activation of the Go alpha activates DAG kinase which removes DAG. Thus the Gq and Go pathways converge to regulate UNC-13 localization by regulating levels of DAG [1,2]. Evidence from mammalian DAG kinases suggests that they can be regulated by changes in localization and/or kinase activity but the signaling pathways involved have not been identified. To understand DGK-1 regulation we are identifying other proteins that bind DGK-1 using two methods. Firstly we are using a yeast two hybrid screen. In the second approach we have generated a rescuing MYC tagged GFP::DGK-1 construct. The myc-DGK-1 protein can be

immunoprecipitated from adult animals and possesses DAG kinase activity. We are currently testing whether other proteins are co-immunoprecipitated with DGK-1.

1. Nurrish et al.,(1999) Neuron:24 231-242

2. Lackner et al.,(1999) Neuron:24 335-346

3. Hajdu-Cronin et al.,(1999) Genes Dev 13: 1780-1793

4. Miller et al.,(1999) Neuron 24: 323-33

5. Richmond et al., (1999) Nat.Neurosci 2: 959-964

417. A motoneuron-derived signal is required for differentiation of post-synaptic domains at GABAergic neuromuscular junctions in *C. elegans*

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Chemical synapses are formed by the apposition of two highly specialized structures *i.e.* the pre- and post-synaptic differentiations. In vertebrates, genes that are required for coordinate differentiation of these structures have been identified such as agrin at cholinergic neuromuscular junctions or neurexin / neuroligin and cadherin at glutamatergic synapses. However, no such genes have been identified for GABAergic synaptogenesis. We would like to identify the genes that participate in the formation of GABAergic neuromuscular junction in *C. elegans* using a genetic approach.

Body wall muscle cells are innervated by cholinergic excitatory motoneurons and GABAergic inhibitory motoneurons. In order to analyze the relationship between pre- and post-synaptic domains, we expressed a fusion between synaptobrevin (a synaptic vesicle protein) and CFP in GABAergic motoneurons and a fusion between the GABA receptor UNC-49 and YFP in body wall muscle cells. In wild-type adults, there was a strict correlation between accumulation of pre-synaptic vesicles and clusters of GABA receptor in discrete post-synaptic domains. Furthermore, during development, no GABA receptor clusters were detected dorsally before rewiring of the DD motoneurons that innervate dorsal muscles after the L1 stage. These results suggested that a signal provided by motoneurons was triggering post-synaptic differentiation.

To test this hypothesis, we expressed *unc-47::snb-1-CFP*; *unc-49-YFP* in *unc-104(e1265)*. In this kinesin mutant, synaptic vesicles are no more transported in neurites and remain concentrated in cell bodies (Hall and Hedgecock, 1991). No SNB-1-CFP nor UNC-49-YFP could be detected dorsally. Ventrally, GABA receptor clusters were observed in contact with motoneuron cell

bodies. Therefore, at least one neural factor seems to be transported by UNC-104 up to pre-synaptic sites and to cause formation of GABA receptor post-synaptic clusters.

To characterize the role of GABA in this process, we examined UNC-49 distribution in *unc-25* mutants that do not synthesize GABA. We could not see any difference between *unc-25* and wild-type worms. Together with the results of Jin et al. (J Neurosc., 1999) who demonstrated that pre-synaptic differentiation is normal in *unc-25* background, we concluded that GABA transmission *per se* is not required for GABAergic synaptogenesis. We then analyzed GABAergic synaptogenesis in *unc-30* mutants. *unc-30* encodes a transcription factor that controls most GABAergic features of D neurons. The number of pre-synaptic vesicle clusters was decreased much more dramatically in the ventral cord than in the dorsal cord. These results are in agreement with electron microscopy reconstruction data (John White, unpublished results). Despite the drastic reduction of synapse number in the ventral cord, we observed that UNC-49 concentrated near the cord ventrally but did not form high density aggregates. This suggests that either the few remaining GABAergic synapses or the cholinergic innervation which is not affected by *unc-30* mutations are sufficient to polarize muscle cells and generate a subcellular compartment where the GABA receptors are addressed.

To identify the motoneuron factors responsible for GABAergic post-synaptic differentiation, we have undertaken a genetic screen for mutants with altered UNC-49-GFP distribution.

418. Characterization of the *shn-1*, shank homologue in *C. elegans*

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Shank is a novel family of the PSD (post-synaptic density) protein complex. It was found in rat brain and contains multiple sites for protein interaction including a PDZ (PSD-95, Disk-Large, ZO-1) domain that mediates binding to GKAP, ankyrin repeats, a SH3 domain, a SAM domain that mediates multimerization, and a proline-rich domain that binds cortactin. It was reported that these multiple protein-interactions cause shank to function as a scaffold protein in the PSD, cross-linking receptor/PSD-95 complexes and coupling them to regulators of the actin cytoskeleton.

A *shn-1*(C33B4.3), *C. elegans* homologue of shank, was found in the *C. elegans* genome database and shows about 40% identity over 1,000 amino acids. A *shn-1* shows relatively high sequence identities in the regions of ankyrin repeats and the PDZ domain. GFP expression of promoter regions of *shn-1* was seen mainly in pharyngeal muscle, head sensory neurons, nerve cords and the tail region. Whole-mount immunostaining patterns with shank-1 polyclonal antibodies showed similar expression patterns. shank-1 polyclonal antibodies were raised against ankyrin repeats-containing region of *rat* shank-1. This expression not only confirms our previous GFP expression results, but also shows the conservation of shank protein. We cloned and sequenced the full cDNA from a cDNA library and a EST clone from Yuji Kohara. Currently we are conducting RNAi experiments to observe loss-of function phenotypes and elucidate its biological role in *C.elegans*. Screening for deletion mutant by UV-TMP mutagenesis is also under way.

419. Characterization of Candidate Genes for Postsynaptic Proteins in *C. elegans* Nervous System.

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GFP-labeling of synaptic vesicles using SNB-1::GFP enabled visualization of presynaptic specializations in living worms [Nonet, ML, J. Neuroscience Methods 89: 33-40, 1999]. These worms have been proved to be powerful tools for isolation and description of mutants in which the synapse formation is affected. On the other hand, studies using a GFP-labeled glutamate receptor subunit, GLR-1, revealed that the neurotransmitter receptor is targeted to postysynaptic structures and that the targeting depends on PDZ proteins such as LIN-10 [Rongo, C. et al., Cell 94: 751-759, 1998]. In order to obtain genetic tools to study further the formation of pre- and postsynaptic structures during development as well as the mechanisms of targeting postsynaptic proteins to synapses, we are searching for a panneuronal postsynaptic marker protein in *C. elegans*. We amplified cDNA fragments of homologous genes of postsynaptic proteins recently identified in mammalian postsynaptic structures. The proteins whose putative homologues we are studying are: *Drosophila* Discs Large and mammalian PSD-95/SAP90 family proteins; S-SCAM/SLIPR/MAGI family, proteins associating with various kinds of receptor proteins; MAGUIN, a scaffolding protein interacting with PSD-95 and S-SCAM; synamon/shank, proteins coupling NMDAR/PSD-95 complex and mGluR/Homer complex; CRIPT, comprising complex with PSD-95 and tubulin. Sequencing of the cDNA fragments revealed genomic structures and complete amino acid sequences. Domain structures are well conserved between worm and mammals for these genes. We are now studying the expression pattern of these genes using GFP reporter constructs. We are also analyzing the subcellular localization of these proteins in neurons by expressing GFP-fusion proteins.

420. Characterization of UNC-31 (CAPS) in C. elegans neurotransmission

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The *unc-31* gene encodes the *C. elegans* homolog of CAPS (calcium activated protein for secretion)[1]. CAPS has been shown to be required for the calcium dependent exocytosis of large dense core vesicles which contain peptides, biogenic amines, and hormones. However, it is not known whether CAPS plays a role in the exocytosis of small clear synaptic vesicles which contain classical neurotransmitters such as acetylcholine, GABA, and glutamate. We have undertaken a genetic and an electrophysiological approach to better define the function of CAPS in *C. elegans*neurotransmission.

unc-31 mutants have a number of behavioral defects. They are uncoordinated and are defective in both egg laying and pharyngeal pumping. The behavioral phenotypes observed in *unc-31* mutants are not due to developmental defects. We demonstrated that axon morphology and synapse distribution are normal in *unc-31* mutants. In addition, the uncoordinated phenotype of *unc-31* animals was rescued by expressing UNC-31 in adults using a heat shock construct, suggesting that the UNC-31 protein is required for neuronal function rather than for neuronal development.

unc-31 mutants exhibited a decrease in neurotransmission at the neuromuscular junction. *unc-31* mutants exhibited a 50% reduction in evoked release of neurotransmitter and a 50% reduction in the frequency of miniature postsynaptic events. These data suggest that the uncoordinated phenotype observed in *unc-31* mutants is due to a decrease in small clear synaptic vesicle release at the neuromuscular junction. If the requirement for CAPS at motor neurons acts via peptide regulation of motorneurons then we can make

two predictions: (1) that if it is a polysynaptic effect, the uncoordinated phenotype of *unc-31* should be rescued by CAPS expression in cells other than in motorneurons, and (2) the Unc-31 phenotype should be rescued by mutations in G protein signaling in motorneurons. We are currently expressing UNC-31 in different tissues and in different subsets of neurons in *unc-31* mutants and looking for rescue of the mutant phenotype.

To determine if *unc-31* function is acting via a peptide signaling mechanism we conducted a suppressor screen. In this screen a single allele of *egl-30*, which encodes Gqα, was identified. This allele is a gain-of-function activated form of Gqα. In addition, we identified multiple loss-of-function alleles of *goa-1*, which encodes Goα. *egl-30* is required for a signaling cascade that leads to localization of the UNC-13 protein to nerve terminal membranes. This localization of UNC-13 leads to an increase in acetylcholine released at the neuromuscular junction. GOA-1 antagonizes Gqα and hence the amount of UNC-13 localized at the plasma membrane. These data suggest that CAPS is acting via 7-pass transmembrane receptors and G proteins to alter the basal levels of motorneuron function. Thus, it is likely that CAPS facilitates the release of large dense core vesicles that modulate release of classical neurotransmitters rather than directly facilitating the release of small clear core vesicles.

1 Livingstone, D. 1991, Thesis.

421. TOWARDS IDENTIFICATION OF *TRANS*-FACTORS REGULATING NEURON-SPECIFIC EXPRESSION OF THE *UNC-18* GENE

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The *unc-18* gene encodes a protein that modulates synaptic vesicles at the presynaptic terminal and is expressed in a highly specific pattern in the *C. elegans* nervous system. We coupled the *unc-18* gene to the reporter gene *lacZ* or GFP and examined the expression pattern of the reporter protein in the developing nervous system of transgenic organisms. We identified at least three sites are present determining the neuron specific expression between nucleotides -486 and +268 of the gene. Of these three sites we are especially interested in the 19-bp sequence element TGACAAGTCCGAAATTGCT (positions -359 to -341) in the upstream region of the *unc-18* gene that drives the expression of the gene primarily in neurons. Site-specific mutations in this 19-bp sequence result in decreased in neural expression of the reporter gene. Within this neural specific promoter element, sequences are also conserved in the *unc-18* gene of the related nematode *C. briggsae*. Electrophoretic mobility shift assays reveal the presence of a sequence specific binding protein in nuclear extracts isolated from animals at an early larval stage.

We therefore started screening the λgt11 cDNA expression library provided from Fire Lab with the concatenated potential *cis* elements as probe.

422. The role of unc-86 in the development of serotonergic neurons

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We are interested in the genetic network that regulates serotonin synthesis. In wild-type worms, NSM, AIM, RIH, HSN, and ADF are serotinergic: they are detected by anti-serotonin antibody. Our previous studies indicate that the tryptophan hydroxylase gene tph-1 is expressed in the serotonergic neurons and is essential for the biosynthesis of serotonin. We have been using tph-1::gfp as a reporter to identify genes that regulate tph-1 expression.

The POU-homeodomain transcription factor UNC-86 is necessary for tph-1 expression in NSM, AIM, RIH, and HSN. In the unc-86-null mutants n846 and e1416, these neurons are generated but do not express tph-1::gfp, whereas expression in ADF is not affected. unc-86 is not expressed in ADF. Interestingly, the unc-86-null mutations do not affect the expression of cat-1::gfp (provided by Steven Nurrish and Josh Kaplan), encoding the transporter that loads serotonin into the synaptic vesicle (Duerr et al., 1999; Nurrish et al, 1999), and the putative genes encoding GTP cyclohydrolase I, the tryptophan hydroxylase cofactor. We suggest that UNC-86 has a restricted role in the development of the serotonergic phenotype in NSM, AIM, RIH, and HSN, including the activation of tph-1 expression. Analysis of the tph-1 promoter has identified three cis-regulatory regions. Two regions coordinately regulate the expression in ADF, and one region is essential for the expression of NSM, AIM, RIH, and HSN. We are testing whether UNC-86 directly regulates tph-1 expression via the site.

423. Identification of target genes of a transcription factor UNC-86.

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UNC-86 is a POU-domain transcription factor which controls development and function of a variety of nerve cells in *C. elegans*. The mutant shows many interesting phenotypes including abnormality in cell division, cell migration, cell-type determination, cell maturation etc, resulting in a number of behavioral disorders. Because the protein is a transcription factor, it seems to act by regulating downstream genes. Thus, identification of downstream genes may be useful to understand the roles of those genes which play a critical role in neuronal development and function.

We produced a transgene which encodes an UNC-86/GFP fusion protein and expressed by a native unc-86 cis-element in the *unc-86* mutant background. The plasmid rescued *unc-86* mutant phenotypes, including serotonin expression and touch sensitivity, suggesting that the fusion protein is functional. We cross-linked protein-DNA complex *in vivo*, then immunoprecipitated the UNC-86/GFP fusion protein together with its binding DNA fragments. A known target gene of UNC-86, *mec-3* was specifically recovered when examined by PCR. We made a library by DNA fragments purified this way. By sequencing each clone and searching *C. elegans* genome sequence database for the identical sequences, we examined what kind of DNA sequences are bound by UNC-86/GFP *in vivo*. Most common sequences found are autoregulatory elements of unc-86 gene itself (about 5% of total immunoprecipitated DNA sequences). Others were located all over the chromosomes. Of about 1,000 candidate DNA sequences cloned by immunoprecipitation, we found about 50 genes had more than 2 independent putative UNC-86/GFP immunoprecipitated DNA fragments very close to each other. We

examined expression patterns of four genes among the candidates and found that two showed expression patterns related to UNC-86. The gene F47E1.1 which is of unknown nature was expressed in a pair of head neurons, HSN neurons and a pair of tail neurons in wild-type animals. The expression was colocalized with *unc-86* as examined by double staining with *f47E1.1:: egfp* and *unc-86:: rfp*. The expression of F47E1.1 was absent in the *unc-86* mutant background. Thus, the gene appeared to be activated by UNC-86. The gene F25E2.1 which has homology with G-protein coupled receptor was expressed around pharynx in wild-type animals. Ectopic expression was observed in a number of head neurons in the *unc-86* mutant background. Thus, the gene appeared to be repressed in head neurons by UNC-86 in a cell-type specific manner. To get insight into the function of these genes, we are currently screening for the deletion mutants of these genes by the TMP/UV method.

424. Cloning of a collagen which functions specifically during sensory organ morphogenesis in *C. elegans*.

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Extracellular matrix assembly is an important component for cell signaling and morphogenesis during animal development. Mutations in genes encoding the extracellular matrix components could result in failure of cellular communication, differentiation and thus mutant phenotypes. Collagen is one of the most abundant ECM proteins. It is an important structural component of the extracellular matrix in the animals that it has been shown to play significant role in body morphogenesis.

Cloning of *ram-4* gene reveals that it encodes a cuticular collagen. *ram-4* mutants show a male specific phenotype: abnormal morphology of the 9 pairs of male sensory rays in the tail. Each ray shows tapered shape in wild-type male but has amorphous shape in *ram-4* males (lumpy ray). A ray is composed of two neuronal cells, a hypodermis and a structural cell. All these ray cell components gave abnormal morphology in a lumpy ray, which suggest that the expression of this molecule has major impact on all ray cells during the retraction stage in late L4 larvae. Expression analysis revealed that RAM-4 was highly expressed specifically in the male hypodermis at the onset of ray retraction.

The molecular features of this cuticular collagen is similar to other collagen protein with Gly-X-Y tripeptide repeat. It consists of three domains: amino and carboxyl non-Gly-X-Y domains flanking the central Gly-X-Y domain. Domain-deletion analysis has been performed to address the function of these domains in terms of rescue activity. Preliminary results suggesting that replacement of amino non-Gly-X-Y domain with synthetic signal sequence, which was used to ensure proper secretion of modified RAM-4 to the extracellular matrix, resulted in dominant negative effect. Interestingly, expression of amino non-Gly-X-Y domain alone also produced lumpy ray in WT background. This observation suggests a novel function of this domain for molecular interaction. The detail of

this analysis will be discussed in the poster.

425. Development of Left/Right Asymmetry in the Nervous System

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The nervous system of nearly every animal displays morphological bilateral symmetries that are usually paralleled by symmetric patterns of gene expression and function. While a wealth of information exists regarding dorsoventral and antero-posterior development, very little is known with respect to the induction of left/right gene expression in the nervous system and how it is integrated into morphologically bilaterally symmetric structures in the adult brain. We have been using *C. elegans* as a model system to study the genetic basis of how left/right asymmetry is established within the nervous system. Specifically, we have been studying the ASE neurons, which are the main chemosensory neurons in the worm. The ASE neurons are a group of two neurons, ASEL and ASER, that are located in a bilaterally symmetric left and right position in the main head ganglion. In addition to their bilaterally symmetric position, they share similar axonal and dendritic morphology, similar gene expression profiles and identical patterns of neural connectivity. However, these two cells are intrinsically left/right asymmetric in that they were found to express different types of putative sensory receptors of the guanylyl cylcase receptor family (Yu et al., 1997). Also, the LIM homeobox gene, *lim-6*, is normally expressed asymmetrically and exclusively in ASEL only (Hobert et el., 1999). Unlike the stochastic and primarily activity-dependent expression of the odorant receptor gene *str-2* (Troemel et al., 1999), asymmetric expression of *gcy-5,6,7* and *lim-6* is stereotyped and developmentally predetermined. These observations pose the intriguing question of how patterns of left/right asymmetric gene expression are established in development.

We describe here a genetic approach to investigate how the left/right asymmetric gene expression patterns in ASEL/R are initially established and maintained. Using both a candidate gene and genetic screening approach, we have revealed multiple components that are involved in the asymmetric expression of *lim-6,*

gcy-5, gcy-6, and gcy-7. In each of these approaches, we found that the *tax-2* and *tax-4* genes, whose predicted products are similar to the cyclic nucleotide-gated channel subunits used in vertebrate vision and olfaction, are required at specific times during development for the proper expression of *lim-6* and *gcy-7*, but not *gcy-5*. Surprisingly, *tax-2* and *tax-4* display embryonic effects on asymmetric gene expression. In addition to these genes, *let-60*/Ras is also required early in development for *lim-6* expression. Double mutants reveal that *tax-2* may in fact signal via ras in order to establish the asymmetric expression of *lim-6* that is seen in wild-type. Genetic evidence suggests that ras does not signal via the canonical MAPK pathway in order to establish this asymmetry. In addition to these genes, we have also shown that *che-1*, a putative transcription factor that encodes a zinc-finger protein similar to Drosophila *glass* (Nakano et al., 2000 Japanese Worm Meeting abstract 73), acts to positively regulate *lim-6* expression in ASEL and presumably every other cell fate marker in ASEL/R. We are currently mapping various mutants retrieved from a genetic screen that show symmetrization of *lim-6* expression such that *lim-6* is either expressed in both ASEL and ASER, it is off in both, or it shows a randomization of on/off. We ultimately hope to gain insight into the molecular patterning events that generate asymmetry in the brain.

426. THE FAX-1 NUCLEAR HORMONE RECEPTOR FUNCTIONS DOWNSTREAM OF OTHER TRANSCRIPTIONAL REGULATORS TO SPECIFY NEURON IDENTITY

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The *fax-1* nuclear hormone receptor is required for specifying aspects of AVK neuron identity. Mutations in *fax-1* cause defects in axon pathfinding and expression of the FMRFamide-related neuropeptide precursor gene *flp-1*. *fax-1* mutants also exhibit movement, chemotaxis and foraging behavior defects, suggesting that the *fax-1* gene also functions in the development of other neurons. *fax-1* encodes the *C. elegans* NR2E3 receptor, an ortholog of human PNR, which is expressed exclusively in the retina. This group of NHR's is closely related to *Drosophila* Tailless and its vertebrate and *C. elegans* orthologs. Mutations in human PNR are the likely cause of an inherited progressive human blindness called enhanced S-cone syndrome. Individuals carrying mutations in PNR have night blindness, enhanced sensitivity to blue light, and often suffer progressive retinal degeneration that eventually leads to blindness. Although direct evidence of the developmental events leading to these phenotypes is lacking, there has been speculation that the disease results from an identity defect in the specification of photoreceptors; rods and red and green cones may be converted into blue (S-) cones. Given the apparent role for *fax-1* in specifying neuron identity in *C. elegans*, the study of the mechanism of *fax-1* function serves as a relevant model for understanding the human disease. We are testing whether human PNR can function for *fax-1* in nematodes. A full-length PNR clone was not able to rescue a *fax-1* mutant, however the ligand-binding domains (LBD) of the two proteins show little relationship, suggesting that a chimeric protein consisting of the PNR DNA-binding domain

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(DBD) fused to the FAX-1 LBD may be functional.

We determined the expression pattern of *fax-1* by generating antibodies to FAX-1 in mice. FAX-1 protein is observed in nuclei of mid-stage embryos, just after neurogenesis but before most axon outgrowth occurs, and in all larval stages and adults. We observe FAX-1 protein in 18 neurons consistently at all stages, including the AVK's, AIY's, RIC's, MI, and DVA. The additional 5 pairs of neurons (all in anterior ganglia) have not been definitively identified, but likely include AVA, AVE, and another interneuron pair that is either AVB, AVH or AVJ. The expression of FAX-1 in a neuron pair involved in chemotaxis (AIY) and in neuron pairs involved in coordination of movement (AVA, AVB, AVE) provide potential sites for *fax-1* function in these behaviors. FAX-1 is also expressed transiently in the dtc's in L2-L4 larvae and the transverse (T) vulval cells of the 2° lineages (P5.ppal/r and P7.papl/r) in L4 larvae. The timed expression of FAX-1 in non-neuronal cells that are undergoing movement (migration and morphogenesis, respectively) is tantalizing, however we observe no gonad or vulval phenotypes in *fax-1* mutants.

Given that *fax-1* is a likely transcriptional regulator and its apparent role in specifying neuron identity, we are studying the regulatory circuits in which *fax-1* participates. Previous analysis showed that *flp-1* expression requires *fax-1*. Because *unc-42*, a homeobox gene, is also expressed in AVK's we examined the regulatory relationships among these genes. Expression of *flp-1* is more severely compromised in *unc-42* mutants than *fax-1* mutants. Furthermore, four pairs of anterior FAX-1-positive neurons fail to accumulate FAX-1 in *unc-42* mutants, suggesting a regulatory hierarchy in which *unc-42* regulates *fax-1* and other genes, which in turn regulate *flp-1*. FAX-1 also does not accumulate in AIY's of *ttx-3* mutants, suggesting that *fax-1* also functions downstream of the *ttx-3* LIM homeobox gene. We are also attempting to determine the position of *fax-1* in regulatory hierarchies in other neurons. Our efforts to identify downstream targets of *fax-1* regulation have focused on one-hybrid strategies and candidate sequence gel-shift studies.

Finally, we have also begun studying other NHR's that are closely-related to *fax-1*. Among these is *nhr-111* (F44G3.9), which shows relationship to *fax-1* in both the DBD and LBD. *nhr-111::gfp* fusions are expressed in a single pair of anterior ganglion neurons in embryos and a second pair of cells in the mid-body.

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427. Isolation of mutants defective in AFD thermosensory neuron development

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The AFD sensory neurons play a critical role in thermosensation of *C. elegans*. Like other sensory neurons, AFD neurons extend dendrites to the tip of the head and project axons to the nerve ring, where synaptic connections with other neurons occur. The sensory ending structure of AFD has a characteristic shape. All the head sensory neurons except AFD have conspicuous ciliated structures, whereas AFD neurons have a degenerated cilium and elaborated microvillus-like structure at the sensory ending. How are AFD neurons with complex sensory ending structure generated during development? To answer this question, we have screened mutants that are defective in AFD development. We co-introduced AFD specific marker *H13::GFP* and AIY specific marker *ttx-3::GFP* to NL917(*mut-7*)strain, in which insertions of transposons are frequently occurring. AIY neurons are downstream interneurons that are also critical for thermotactic behavior. We have screened 500,000 *mut-7* animals and found over a dozen mutants that are categorized into the following groups.

Group1-*nj11*: In *nj11* mutant, AFD specific GFP markers *H13::GFP* and *gcy-8::GFP* are both ectopically expressed in ASI chemosensory neurons. Ciliated structure at the sensory ending of ASI is transformed to be microvillus-like structure, which is reminiscent of the sensory ending of AFD. Also, we cann't detect DiI-uptake by ASI neurons in *nj11* mutant. These results suggest that ASI chemosensory neurons are transformed to be AFD thermosensory neurons. To see whether ASI neurons are functionally changed to be thermosensory, we conducted laser ablation experiments, by killing AFD neurons of *nj11* mutant. These animals responded to temperature on a temperature gradient, despite of the absence of AFD neurons. Thus, ASI neurons seem to function as thermosensory neurons in *nj11* mutant. Altogether, our results suggest that a gene responsible for *nj11* mutation is normally required for ASI cell fate decision by inhibiting to become AFD.

Group2-*nj12* and other two mutants: Axon extensions are defective in these mutants. After entering the nerve ring, pre-mature termination of axons occurs within the ring. Movement of *nj12* is normal, but other two mutants show UNC phenotype.

Group3-*nj13* and other eight mutants: Cell body position of AFD is abnormal in these mutants. Although the normal position of AFD cell body is posterior to the nerve ring, AFD cell bodies are mislocalized anterior to the nerve ring.

We are now cloning these genes by transposon display method developed by Plasterk Laboratory.

We thank T. Ishihara for *H13::GFP*, O. Hobert for *ttx-3::GFP*, H. van Luenen, S. Wicks and R. Plasterk for *mut-7* strain and transposon display method.

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428. MEMBRANE LOCALIZATION OF THE ACTIN-BINDING PROTEIN UNC-115 CAUSES NEURONAL MORPHOGENESIS DEFECTS

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UNC-115 is an actin-binding protein that acts in axon pathfinding: UNC-115 has three N-terminal LIM domains and a C-terminal actin-binding villin headpiece domain, and *unc-115* mutants display axon pathfinding defects. Loss of function studies and epistasis experiments indicate that UNC-115 acts downstream of the RAC-2 Rac GTPase in axon pathfinding and neuronal morphogenesis (see abstract by Struckhoff, *et al.*).

We have begun to characterize the mechanism of UNC-115 regulation by RAC-2. RAC-2, like all Rac GTPases, contains a membrane-targeting CAAX motif and is likely tethered to the plasma membrane. An UNC-115::GFP fusion protein is evenly distributed in the cytoplasm and processes of neurons and displays no detectable plasma membrane localization. Possibly, UNC-115 is activated by translocation to the plasma membrane in response to RAC-2. To test this idea, we studied the consequences of constitutive localization of UNC-115 to the cell membranes of neurons. We constructed an *unc-115* transgene that can encode a full-length UNC-115 with a membrane-targeting myristylation (myr) sequence at the N terminus. In contrast to UNC-115 alone, MYR::UNC-115::GFP was localized to cell membranes, including the plasma membranes of neurons. Furthermore, MYR::UNC-115::GFP caused striking dominant defects in neuronal morphogenesis when expressed from neuron-specific promoters, including ectopic axon and dendrite branching and lamellipodia-like membrane ruffling. Occasionally, defective cells with extensively-ruffled cells bodies and multiple filopodia-like projections were observed. Neither MYR::GFP nor wild-type UNC-115 expression caused severe defects like MYR::UNC-115; however, high-copy expression of wild-type UNC-115 led to low penetrance axon branching and ruffling. These results indicate that UNC-115 is involved in neuronal morphogenesis and suggest that UNC-115 activity is regulated by localization to cell membranes. The effects of MYR::UNC-115 resembled the effects of constitutively-active RAC-2, consistent with the idea that RAC-2 signaling recruits UNC-115 to the plasma membrane where UNC-115 is then activated. Experiments in progress include testing the effects of UNC-115 tagged with an alternate plasma membrane-specific localization signal (the CAAX motif) and identifying factors that regulate UNC-115 at the plasma membrane.

429. Two-hybrid Screen for UNC-44 AO13 Interacting Proteins

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Mutations in *unc-44* result in axon outgrowth and guidance defects. The cloning of *unc-44* demonstrated that it encodes a series of ankyrins, including a novel ankyrin called AO13 ankyrin [Otsuka, A. J. *et al. J. Cell Biol. 129*, 1081-1092 (1995)]. AO13 ankyrin is unusual in that it is much larger than other ankyrins (6994 aa compared to 4377 aa for the largest vertebrate ankyrin, ankyrin$_G$). AO13 ankyrin is also unusual because the carboxyl end of the protein contains 7 potential transmembrane domains and Ser/Thr/Glu/Pro-rich (STEP) repeats. We hypothesize that UNC-44 AO13 ankyrin may be part of a signal transduction complex involved in axon guidance. To test this hypothesis, two-hybrid screens [Fields, S. and Song, O. *Nature 340*, 245-247 (1989)] were performed with AO13-specific baits. Baits were constructed from a STEP repeat region (pDD3) and from the carboxyl terminus of AO13 ankyrin (pDD4 and pDD5). These baits were introduced into either yeast strain Y190 (*HIS3* and *lacZ* reporters) or AH109 (*HIS3*, *lacZ*, and *ADE2* reporters). These strains were transformed with R. Barstead's *C. elegans* cDNA clone libraries. A number of positive clones were obtained and are currently being analyzed. In addition to the two-hybrid approach, the UNC-44 ankyrins are being examined for changes in phosphorylation. Finally, dsRNAi is being used in an attempt to knock out AO13 ankyrin expression.

430. A neuronal-specific classical cadherin encoded by the *hmr-1* locus is involved in axon patterning.

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The classical cadherin/catenin adhesion system regulates a variety of morphogenetic events in metazoan cells. Our recent survey of the *C. elegans* cadherin superfamily (1) has determined that the *C. elegans* genome contains a single classical cadherin locus, *hmr-1*. This locus can encode an epithelial cadherin, HMR-1, which is essential for epidermal morphogenesis during embryonic development (2). In contrast *D. melanogaster* possesses two classical cadherins, *D*E- and *D*N-cadherin. *D*E-cadherin is expressed in epithelial tissues and is required for the maintenance of adherens junctions, whereas *D*N-cadherin is expressed in neuronal tissues and is required for axon patterning (3,4,5).

We have uncovered an unusual mechanism by which the *C. elegans hmr-1* locus generates an additional isoform using alternative splicing and an alternative promoter. We propose that the novel isoform be named HMR-1B, and the previously characterised isoform be renamed HMR-1A. Sequence analysis reveals that HMR-1B and *D*N-cadherin encode the same number of cadherin repeats, and the sequences of individual cadherin repeats are highly conserved between these two proteins. Promoter::GFP fusions have shown that the HMR-1A isoform is expressed at high levels in epithelial tissues, whereas HMR-1B is localised exclusively in the nervous system. Animals rescued for HMR-1A but lacking HMR-1B display minor defects in ventral nerve cord fasciculation, and also show variable defects in the trajectories of commissures. In addition, animals homozygous for a weak viable allele of the α-catenin gene *hmp-1* also display fasciculation and commissural defects, suggesting a role for the classical cadherin/catenin complex in axon patterning. Other members of the *C. elegans* cadherin superfamily also display neuronal expression patterns, and we are currently investigating how each of these cadherins contributes to the

development of the *C. elegans* nervous system.

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431. *ast-1* and *ast-2*, novel genes important for fasciculation of axons in the ventral cord

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One of the major problems of developmental biology is how axons build their specific pattern of synaptic connections, which is crucial for the function of every nervous system. The mechanisms through which axons respond to guidance cues are not well understood. In the final stage of axon guidance the selection of synaptic partners within the target area takes place. Since synapses in *C. elegans* are made with neighbouring axons in an axon bundle the correct fasciculation of axons even within a bundle is crucial for the correct wiring of the nervous system. To study the molecular basis of this recognition mechanism between axons, a screen for mutants exhibiting fasciculation defects was designed. Animals that express GFP under the control of the *glr-1* (glutamate receptor) promoter were used to label interneuron axons that are part of the motor circuit. In wildtype animals GFP labelled axons form a tightly fasciculated subbundle within the right ventral cord. After mutagenesis with EMS F2 progeny were scored under the fluorescence microscope for defects in fasciculation. This direct screen for axonal outgrowth defects is very sensitive for obtaining mutants with minor defects that do not lead to gross behavioural defects.

Two novel genes identified in these screens are currently studied in more detail. *ast-1* and *ast-2* mutants are characterized by defasciculated axons in the ventral cord, where axons cross from the right to the left bundle. In a small number of animals one single interneuron axon is misguided and does not reach the ventral cord, but runs in lateral position. The penetrance of these defects is about 45% and the mutations are slightly temperature sensitive. There are no obvious defects in the outgrowth of other axons as judged by various GFP markers. Other tissues and cell migrations appear normal. The axonal

phenotypes of both mutants are similar, but *ast-2* mutants are somewhat lethargic and slightly Egl, whereas *ast-1* mutants only show subtle changes in exploratory behaviour.

Two alleles of *ast-1* called *rh300* and *hd1* were isolated independently. The phenotypes are very similar and the mutants do not complement. One allele of *ast-2* was isolated so far. *ast-2* maps close to *unc-13* on chromosome I. *ast-1* maps to chromosome II four map units left of *dpy-10* and near *vab-1*. *ast-1(hd1)* complements *vab-1(dx31)*. Fine mapping was based on single nucleotide polymorphisms that can be detected by RFLP as a consequence of the modification of a restriction enzyme recognition site. The gene locus has been narrowed to a 400 kb region enclosed by cosmids ZK430 and C33F10.

432. RNAi MEDIATED DISRUPTION OF *VEM-1*, A NOVEL MEMBRANE-ASSOCIATED PROTEIN, PERTURBS THE PATTERNING OF A SUBSET OF AXONS IN THE VENTRAL NERVE CORD

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A critical phase in the development of the nervous system is the formation of connections between axons and their synaptic targets. These axon pathfinding events are controlled by both long- and short-range guidance cues that are expressed by intermediate targets which exist at various points along a given axonal trajectory. We have previously identified *VEMA* as a novel marker of two vertebrate midline structures that are important intermediate targets for midline-crossing axons: the floor plate of the ventral spinal cord and the optic chiasm of the ventral diencephalon. Interestingly, *VEMA* is also expressed in specific early-developing neuronal populations as they initiate axongenesis.

The deduced amino acid sequence of *VEMA* contains a single transmembrane domain and several distinct sorting motifs that are thought to mediate trafficking of proteins between intracellular compartments and the plasma membrane. The *C. elegans* genome encodes for a single ortholog of *VEMA* that is 36% identical to the amino acid sequence of vertebrate *VEMA*. We refer to the *C. elegans* ortholog of *VEMA* as *vem-1*. To elucidate the distribution of *vem-1* in embryonic and larval stage *C. elegans* we utilized a *vem-1::GFP* transcriptional reporter line. Prior to the comma stage, *vem-1::GFP* was expressed in distinct neurons of the head ganglia during the period of axonal outgrowth. During later stages of embryogenesis (1.5 fold to 3 fold stage), *vem-1::GFP* was expressed in pioneer axons of the developing ventral nerve cord. Throughout the larval stages and into adulthood, expression of the *vem-1::GFP* transgene was maintained on a specific subset of neurons located within a variety of head and tail ganglia. Interestingly, expression in the AVG and several of the PV neurons suggests a possible role for *vem-1* in regulating the outgrowth

and/or guidance of axons which pioneer the ventral nerve cord. To test this hypothesis, we employed dsRNAi to disrupt *VEMA* function in a variety of GFP reporter lines. We utilized both the *unc-119::GFP* reporter which is a pan-neural marker, and the *glr-1::GFP* reporter, which labels interneurons and their axons (particularly the AV class) in the right ventral nerve cord to identify potential defects in ventral nerve cord structure. Importantly, these perturbations resulted in no observable change in cell body position, implying that *vem-1* has no major role in the migration of neuronal precursors. Strikingly, similar types of axonal defects were observed in the both the *glr-1::GFP* and the *unc-119::GFP* reporter lines (23% and 17%, respectively), thereby suggesting *vem-1* plays a role in the correct formation of the right ventral nerve cord. The defects most frequently observed were longitudinal breaks, a defasciculation phenotype in which an axon left the ventral nerve cord bundle for a short distance then eventually rejoined the fascicle, or lateral axons, which represent inappropriate projections from the ventral nerve cord into more lateral regions. The premature termination of an axon or the abnormal movement of an axon from the right fascicle across the midline to the left fascicle represent examples of less frequently observed defects. Most of the axonal defects occurred in the posterior ventral cord between the vulva and the tail. However, a few lateral axons were observed in anterior regions where an axon never joined the ventral nerve cord after extending through the nerve ring and instead traveled laterally above the retrovesicular ganglion. The use of more specific GFP reporter lines will be required in order to unambiguously identify the specific axons which are affected in these perturbations. Whether these perturbed axons are from neurons that express *vem-1* remains undetermined. Taken together, these RNAi results suggest that *vem-1* may play a critical role in regulating axon guidance and/or fascicle formation in the *C. elegans* ventral nerve cord. Furthermore, the restricted developmental expression pattern in nematodes and vertebrates suggests a possible evolutionarily-conserved role for *VEMA/vem-1* in the developing central nervous system.

433. Isolation and Characterization of Genes Involved in Motor Axon Sprouting

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Disruption of neuron-target communication has been shown to cause axonal sprouting in vertebrates and more recently in *C. elegans* (Holland and Brown, 1980; Nonet et al., 2000; Peckol et al., 1999). The neuronal sprouting defect may represent an attempt to reestablish a functional synapse. Activity dependent remodeling is presumably caused by retrograde signaling from the target to the neuron leading to cytoskeletal rearrangement. However, the nature of retrograde signals remains elusive. In an attempt to identify a potential retrograde signal at the neuromuscular junction, we have decided to study the DVB motor neuron. DVB is a postembryonically born GABAergic neuron that mediates the expulsion step of the defecation motor program by supplying synaptic output onto the enteric and anal depressor muscles. A number of known mutants involved in neuron differentiation or synaptic activity have been tested for DVB sprouting. A modification in the transcriptional control program for motor neuron differentiation due to a mutation in the Lim homeobox transcription factor, *lim-6*, reveals DVB neurite sprouting (Hobert et al., 1999). Disruption of anterograde signaling by the kinesin gene *unc-104* or by the GABA synthesizing enzyme *unc-25* also displays DVB sprouting. Axogenesis mutations in the ankyrin gene *unc-44*, the septin gene *unc-61*, or the *enabled* homologue *unc-34* cause DVB sprouting. Blockade of enteric muscle activity by hyperactivation of the *eag*-like K^+ channel *egl-2* (gf) or loss of enteric muscle formation in *hlh-8* mutants manifests DVB motor neuron sprouting. Silencing or absence of the target muscle reveals that the DVB neuron must have an intrinsic propensity to sprout. Having established that neural activity dependent communication is required to suppress neurite sprouting, we have conducted a screen for DVB motor neuron sprouting with the goal of identifying genes that are

specifically involved in retrograde signaling. Such a screen may also yield anterograde signaling and *lim-6* interacting components.

A transgenic strain expressing *unc-47*::GFP (*oxIs12*) (McIntire et al., 1997) was mutagenized using ethyl methanesulfate (EMS) and the progeny of almost 3000 mutagenized F_1 animals were screened using a compound microscope for DVB motor neuron sprouting. Twelve mutants, *ot1-ot9*, *ot32*, *ot40*, and *ot42* have been isolated and placed into at least 5 different complementation groups. All of these mutant animals show a sprouting defect of at least 30%. No obvious pleiotropies such as body morphology and locomotion are affected suggesting that these mutants may represent different or even novel genes than known synaptic activity mutants which tend to display an uncoordinated phenotype. Visualization of ventral and dorsal nerve cords and D-type motor neurons using *unc-47*::GFP, the AIY interneuron using *ttx-3*::GFP, and sensory neurons using DiI filling reveals that *ot1-ot9* appear to specifically affect DVB. *Ot42*, however, displays pleiotropic neuron sprouting defects comparable to sensory axon defective (sax) mutants and has shown to complement *sax-1*, *sax-2*, and *sax-6* (Zallen et al., 1999). The presence of the enteric muscle using the transgenic strain *ceh-24Nde*::GFP (*nuIs63*) (Madison and Kaplan, pers. comm.) has been confirmed for *ot1-ot9*. I have started cloning and characterizing *ot1*, *ot6*, and *ot42*. Three factor mapping has placed *ot1* between *lin-31* and *rol-6* on chromosome II. Further mapping of single nucleotide polymorphisms (SNPs) between the N2 and the Hawaiin strains will narrow down a region for rescue. Also by three factor mapping, *ot6* has been placed between *bp1* and *sma-1* on chromosome V. Candidate genes are being amplified and sequenced and cosmids will be injected for rescue. Further cloning and characterization of these three mutants will elucidate potential roles in motor neuron differentiation, anterograde activity, and retrograde signaling.

434. GENETIC ANALYSIS OF AXON BRANCHING IN C. ELEGANS

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Neurons innervate multiple targets by sprouting secondary axon collaterals from a primary axon and/or by bifurcation of a primary axon during process outgrowth or on reaching post-synaptic targets (arborization). Little is known about the molecular identity of molecules that are involved in the formation, stabilization, and elongation of axon branches. We have undertaken a genetic screen in C. elegans to try to identify some of these components. Among the C. elegans neurons that display branching morphologies are the six VC (VC1-6) and two HSN motor neurons that synapse onto vulval muscles to control egg laying behavior. The VC neurons are evenly spaced along the ventral nerve cord (VNC) and send axons toward the developing vulva (situated between VC4 and VC5) where they sprout dorsally extending branches that innervate the vulval muscles. The HSNs, situated on the lateral body wall near the vulva, each send an axon ventrally which then turns and extends anteriorly along the VNC. At the vulva the HSN frequently (but not always) extends a dorsally directed branch. In both VC and HSN neurons branching has been shown to require external cues from vulval cells as branches are not observed in Lin (lineage defective) mutants that do not have vulvae (Li and Chalfie,1990; Garriga et al.,1993). To identify new genes involved in axon branching we have primarily focused on VC4 and VC5 as they display the most branched axon morphologies. VC4 and VC5 were visualized using a GFP transcriptional fusion to the vesicular monoamine transporter (VMAT) encoded by the cat-1 gene (Duerr et al.,1999). We have performed a visual genetic screen for mutants that display defects in VC4/5 branching, including reduced or excessive branching as well as elongation or guidance defects. In an initial screen of approximately 2,000 EMS mutagenized genomes we have identified several mutants that display branching defects. One of these, bam-1 (Branching AbnorMal) displays a highly penetrant excess branching or arborization defect in VC4 and

VC5. We have cloned bam-1 and shown that it encodes a peptide:N-glycanase (PNGase).

435. DNA arrays and mutations that reverse the direction of the ALM cell migrations

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We are interested in how different types of neurons are specified and are focusing on the cell divisions that give rise to ALM touch receptor neurons and BDU interneurons. Touch neuron-specific genes in the ALMs are activated by a MEC-3/UNC-86 heterodimer. UNC-86 is present in both the ALMs and the BDUs while MEC-3 is only present in the ALMs. PAG-3 represses *mec-3* in the BDUs and activates *mec-3* in the ALMs. After the ALM/BDU cell division, the ALMs migrate posteriorly and the BDUs migrate anteriorly. Mutations in *pag-3* cause the BDUs to express touch neuron specific genes but the ALMs and BDUs still migrate to their normal positions, suggesting that the migration and differentiation are controlled by separate mechanisms. We are interested in how the ALM and BDU cell migrations are controlled. We have identified a 104 bp DNA sequence from the *mec-3* upstream control region that, when present in the ALM touch receptor neurons in 50-100 copies, causes ALM migration defects.

In animals transformed with high copy extrachromosomal arrays containing the *mec-3* upstream sequence, the ALM touch receptor neurons failed to migrate to their normal positions and sometimes migrated anteriorly. Furthermore, the PLM touch receptor neurons showed a number of axonal defects. Their axons were often short, misplaced, and some ended in a bulge that may have been a stalled growth cone. The ALM migration defect did not result from RNA interference (RNAi) because double stranded RNA that matched the *mec-3* upstream sequence did not induce ALM migration defects or PLM axonal defects. These defects did not result from nonspecific effects of carrying a transgenic array because most arrays did not cause either ALM migration defects or PLM axonal defects. In this study, the ALMs and PLMs were being visualized by GFP fluorescence. Arrays that did not contain the active sequence but had very bright GFP

fluorescence did not cause the ALM or PLM defects, which shows that the defects did not result from GFP expression. Instead, the ALM migration and PLM axonal defects resulted from transgenic arrays containing many copies of a specific 104 bp DNA sequence. Transgenic arrays containing this sequence did not affect all cell migrations.

The *mec-3* upstream sequence appears to be sequestering (titrating out) a specific DNA-binding factor that is required for the ALMs to migrate correctly. Because titration of this factor reversed the direction of ALM migrations, it may be part of a program that specifies both the direction and extent of ALM migrations. The titrating sequence is from the *mec-3* gene, which is a master regulator of touch receptor neuron genes, so the factor or factors that bind this sequence may also be involved in specifying the fate of the ALM touch receptor neurons. Perhaps the ALM and BDU migrations are controlled by a factor that is preferentially segregated into the ALMs and away from the BDUs during the ALM/BDU cell divisions. Cells receiving more of this factor, the ALMs, might migrate posteriorly while cells receiving less of this factor, the BDUs, might migrate anteriorly. If this hypothesis is correct, mutations in genes that produce this factor, or produce proteins that interact with this factor, should cause the ALMs to migrate anteriorly rather than posteriorly. We have isolated mutations that phenocopy the ALM migration defect and will use these mutations to identify genes and proteins involved in controlling the direction and extent of the ALM migrations.

436. An expression pattern study of *mig-10*, a gene required for nervous system development in *C. elegans*

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The gene *mig-10* was originally identified as being required for the embryonic migration of the neurons CAN, ALM, and HSN, as well as development of posterior excretory canals (Manser and Wood, 1990). *mig-10* was subsequently cloned, and was found to have homology to the Grb family of cytoplasmic signal transduction proteins (Manser et al., 1997). Surprisingly, mosaic analysis revealed that *mig-10* acts cell nonautonomously during excretory canal development. This suggests either that *mig-10* is not acting in signal transduction, or that the signaling system is fairly complex. For example, *mig-10* might transduce signals within migratory path or target cells rather than within migratory cells themselves.

Recently, we observed that *mig-10* is also required in the formation or maintenance of the axons of IL2 neurons. These are six symmetrically positioned head sensory neurons that form a topographic map onto the nerve ring. In particular, IL2 axons in *mig-10(ct41)* mutants often stop before reaching the nerve ring or simply branch at the nerve ring rather than first extending posteriorly past it as in wild-type animals (Burket et al., ECWM 1998). A number of genes have been found to have effects on both cell migration and axon guidance. We are interested in understanding whether *mig-10* is a gene in this class, or whether the axon defect is secondary to a migration defect (for example, a target of the IL2 neurons might be mispositioned in *mig-10* mutants). We would also like to know whether *mig-10* is acting cell autonomously in the IL2s.

To better understand the role of *mig-10* in both cell migration and axon outgrowth or maintenance, we are looking at the expression pattern of *mig-10*. A *mig-10* promotor::GFP fusion construct was successfully microinjected into worms using *dpy-20* as a co-transformation marker. The extrachromosomal array has been integrated, and we are currently in the process of characterizing the expression pattern. The construct is expressed in many cells embryonically, first coming on at about 270 minutes. In L1 animals, no expression is observed in the head, while in older larvae, we see expression in four cells in the head that we are currently attempting to identify. We are just beginning to analyze the expression pattern in the body. Using a PCR technique called SOEing (Splice by Overlap Extension), we have fused GFP to a *mig-10* rescuing construct. Work on generating a transgenic strain containing this construct is in progress. EFR is supported by a NSF CAREER Award (IBN-9984662).

437. *C. elegans* EMAP-like protein (ELP-1) is a microtubule-binding protein.

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Our lab is interested in developmental events dependent upon microtubule-associated proteins (MAPs). In sea urchins, the most abundant MAP in the first cleavage mitotic apparatus is the 75 kDa echinoderm microtubule-associated protein (EMAP). Sea urchin EMAP binds microtubules (MTs) in a concentration-dependent manner and promotes MT dynamics by suppressing rescue (the transition from a shortening to a growing MT). EMAP is a member of a unique family of WD repeat proteins identified in a variety of organisms including protozoans and mammals. In *C. elegans*, there appears to be a single EMAP-like protein (*elp-1*) gene located on the right arm of chromosome V. Ce ELP-1 is predicted to be a 98 kDa WD repeat protein, that is 35% identical and 53% similar to sea urchin EMAP.

To characterize worm MT-binding proteins, Taxol-stabilized MTs were purified from mixed-stage hermaphrodites and analyzed by SDS-PAGE. Several proteins ranging in Mr from 14 kDa to 465 kDa copurify with worm MTs. The most abundant MT-binding protein has an approximate molecular mass of 100 kDa, consistent with the predicted molecular mass of ELP-1 (98 kDa). Antibodies raised against bacterially-expressed ELP-1 recognize a single band of approximately 95-98 kDa in these MT preparations suggesting that the major 100 kDa MT-binding protein is ELP-1. To confirm this prediction, the 100 kDa polypeptide was trypsinized and analyzed by MALDI-TOF mass spectrometry. Surprisingly, the 100 kDa polypeptide was identified by 23 peptide mass matches as a kinesin heavy chain, identical to the gene product of unc-116. A different profile of MT-binding proteins was obtained when worm extracts were supplemented with excess bovine brain tubulin. The kinesin-like protein, KLP-3, rather than UNC-116, was the most abundant MT-binding protein in these preparations. Thus, UNC-116 may preferentially

associate with endogenous worm MTs. These studies demonstrate that like EMAP, ELP-1 is a MT-binding protein and the association of worm MT-binding proteins may be dependent upon MT composition and lattice structure.

438. Molecular characterization of a novel microtubule binding protein

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The echinoderm microtubule-associated protein (EMAP) is a WD-repeat protein that localizes both to the mitotic spindle apparatus and to interphase microtubule arrays in sea urchin eggs. Recently, EMAP-like proteins (ELPs) were identified in various organisms including protozoans and humans. A single EMAP-like protein gene (*elp-1*), with 16 exons, is predicted by the *C. elegans* genome project. RT-PCR has revealed that there are at least two *elp-1* transcripts: a full-length transcript with all 16 exons, and a shorter transcript that is missing exon 5, an 81bp fragment. The structure of the shorter transcript was confirmed by sequencing the *elp-1* cDNA clone yk209e10 (provided by Dr. Kohara, NIG, Japan). The 81bp alternatively-spliced exon can encode an amino acid sequence that contains over 44% serine and threonine. We speculate that translational modification of these amino acids may contribute to the regulation of ELP-1's function during development.

To begin to identify ELP-1 function in the worm, we have shown that ELP-1 cosediments with Taxol-stabilized worm microtubules purified *in vitro*. To examine the time and place of ELP-1 expression, we fused a genomic fragment including the endogenous promoter and the ELP-1 coding sequence to the green fluorescent protein (GFP) reporter gene. ELP-1::GFP is located in body wall muscles, male-specific sex muscles, the vulva, spermatheca, sensory neurons and intestinal cells. Adjacent to the hypodermis of the body wall muscles, ELP-1::GFP is expressed in regular discrete spots similar to the location of dense bodies. Also, there is localization to microtubule-like filaments that traverse the cytoplasm of body wall muscles in a crisscross pattern outside of the myofilament lattice. The sensory neurons that express the GFP fusion protein include the microtubule-rich touch-sensitive neurons (ALM, AVM, PLM, PVM), the ciliated phasmid neurons (PHB &

PHA), and ciliated neurons in the head that are possibly the inner and/or outer labial sensory neurons.

439. A *C.elegans* JSAP/JIP3 homolog, UNC-16, interacts with kinesin light chain, KLC-2

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The c-Jun N-terminal kinase (JNK) of MAP kinase (MAPK) superfamily is involved in the various stress responses and apoptosis in mammal. The components of JNK MAPK cascade are well conserved from worm to human. The *C. elegans* JNK cascade is mediated by JNK-1 (MAPK) and JKK-1 (MAPKK), and functions in type D GABAergic motor neurons to modulate coordinated locomotion. In mammalian JNK MAPK pathways, JIP3/JSAP acts as a scaffolding protein. *C. elegans* has a JIP3/JSAP homolog encoded by the *unc-16* gene. To identify additional components involved in the *C. elegans* JNK pathway, we screened for UNC-16-binding proteins by using a yeast two-hybrid system. One of the isolated genes is *klc-2* encoding kinesin right chain. Co-immunoprecipitation experiments reveal that KLC-2 associates with UNC-16 and UNC-116 kinesin heavy chain when they are co-expressed in mammalian cells. To investigate the *klc-2* function in *C. elegans*, we isolated a *klc-2* mutant by TMP/UV method. The *klc-2(km11)* mutation produces a truncated form of the KLC-2 protein lacking its C-terminal portion. The mutant exhibits Unc and weak Egl phenotypes. We will discuss the connection between conventional kinesin, scaffolding protein and JNK signaling in the neuronal system.

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440. Microtubule based conventional kinesin activity is coupled to the actin-myosin system via an atypical kinesin

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Kinesins are microtubule based molecular motor proteins, which mediate axonal transport in neurons, and diverse intracellular transport such as chromosomes and organelles movement in cell. Mutant in conventional kinesin unc-116 is defective in locomotion, coiler, poor backing, and embryonic lethality etc. (Patel et al., 1993). Inactivation of unc-116 gene function using dsRNAi, results embryonic lethality in the 1st cell stage is consistent with the mutant phenotype. Using several lacZ fusion gene constructs, we show that unc-116 gene expression is not only observed in neurons, axonal processes and motor neurons, but also in muscle cells. We also show using post embryonic in situ hybridization experiment that the mRNA is expressed in the muscle cells and neurons. VAB-8 is an atypical kinesin, with merely 12% homology with UNC-116. It has a motor domain that harbors some but not all residues critical for the ATP and microtubule binding. Interestingly, VAB-8 is co-localized with both actin and microtubule as revealed from our immunocytochemistry experiment. A punctative staining was observed in muscle cell as seen by anti-VAB-8 (Wolf et al., 1998). Our Western blot experiment suggests that the actin protein is molecularly bind to the VAB-8 protein. Using different kinesin mutants including vab-8, we examined whether in vivo activity of the KHC requires the expression of other kinesin like proteins (KLPs). Behavioural analysis on mutant of unc-116 and different other kinesin like proteins reveal genetic interactions between unc-116 and vab-8, and we also show that the vab-8 is required for the expression of unc-116. Based on these observations, we suggest that VAB-8 is an atypical kinesin, which interacts with UNC-116 and playing a critical role in communicating between the actin based myosin and the microtubule based kinesin system. The unc-116 cDNA was fused to GST expression vector and expressed in E. coli and then purified the UNC-116 protein. We are in the process of looking for different biochemical aspects of UNC-116 and its interaction with various proteins including VAB-8.

441. Characterization of FERM-domain protein homologs in C. elegans

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We are interested in the role of cytoskeletal linker proteins in growth regulation and morphogenesis and have started to analyze FERM (for "4.1-ezrin-radixin-moesin"; also called protein 4.1 or band 4.1) domain containing proteins. Members of this class of proteins have been implicated in the pathogenesis of several human diseases, including hereditary elliptocytosis (caused by defects in the "primary" or archetypal band 4.1 domain protein), cancer susceptibility syndromes (nfm/Neurofibromatosis 2) and cancers including lung, breast cancer, and central and peripheral nervous system tumors.

We have previously characterized two FERM domain proteins (*erm* and *nfm*) and a search of the *C. elegans* genome reveals 17 additional genes whose protein products are predicted to contain FERM-like motifs. We have begun to analyze the function of these genes by evaluating their loss-of-function phenotypes using RNAi. We are attempting to generate germline deletions in some of these genes. We have selected one of the genes (tentatively called *frm-3*) for further characterization and have generated transgenic lines carrying a lacZ promoter construct. In contrast to the widespread and predominantly membranous expression patterns of *nfm* and *erm*, *frm-3* expression is confined to a few cells in the head. A germline knockout of *frm-3* is in progress. We will further characterize the subcellular expression pattern of *frm-3* and examine its function and possible role in cytoskeletal membrane integrity and growth regulation.

442. *vab-21* functions in epidermal morphogenesis and may encode intermediate filament protein IF-B1

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vab-21(ju71) mutants display defects in epidermal morphogenesis and a total embryonic and larval lethality of about 50%. Examination of the embryonic development of *vab-21* mutant with time-lapse Nomarski microscopy showed that the head morphological defect and embryonic arrest occurred during embryonic elongation at two-fold and three-fold stages respectively.

vab-21(ju71) maps to chromosome II and fails to complement *maDf4*. *ju71/maDf4* heterozygotes display a higher larval lethality than *ju71* homozygotes, suggesting *ju71* is not a null allele. The phenotypes of *vab-21* mutants are rescued by a subclone that contains a single predicted gene, F10C1.2, which encodes an intermediate filament gene denoted IF-b1 (Dodemont, et. al. EMBO J. 13: 2625-2638). This gene encodes two isoforms, differing at the N-termini due to alternative 5’ exons. A 6kb genomic subclone of the shorter variant of IF-b1 fully rescues *vab-21* mutants. We are currently determining the lesion associated with the *vab-21(ju71)* mutation.

A GFP fusion transgene of VAB-21/IF-b1 shows localization to epidermal fibrous organelles (FOs). To study the role of *vab-21* in the establishment of FOs and the coordination of the cytoskeletons in epidermal morphogenesis, we are examining the expression patterns of other components of FOs and of the epidermal cytoskeletal components in *vab-21* mutants.

To ask whether *vab-21* functions redundantly with other intermediate filaments (IFs) in epidermal morphogenesis, we are currently constructing double mutants with *mua-6*, which encodes another epdiermal intermediate filament protein, IF-a2 (Hresko, et. al. 2000 Midwest worm meeting). To explore the role of *vab-21* in embryonic morphogenesis, we have

constructed double mutants of *vab-21* with elongation defective mutants, *sma-1(e30)* and *vab-9(ju7)* respectively. Both double mutants display reduced embryonic lethality although they show additive morphological phenotypes. The mechanism of the suppression of embryonic lethality is under investigation.

443. Functions of intermediate filaments and their regulatory proteins during embryonic tissue formation

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Cytoskeletal rearrangement is indispensable for tissue formation. Function of intermediate filaments, one of the major cytoskeletal components, in tissue is remained to be clarified due to their difficulty in biochemical and cell biological handling. In this study, we try to examine dynamic function and regulation of intermediate filaments in *C. elegans* embryonic tissue formation.

From *C. elegans* Genome Sequencing database, we found 11 cytoplasmic intermediate filament genes except one nuclear lamin. Eight genes are reported by Dodemont *et al.* (1994) and three genes are novel. From sequencing homology, these 11 intermediate gene products were revealed to be prototype of many types of mammalian cytoplasmic intermediate filaments. We also performed characterization of MH antibodies reported to be recognized intermediate filaments in *C. elegans*. Using these MH antibodies, we found that intermediate filaments are expressed from mid phase of embryogenesis, suggesting that intermediate filaments are involved in also embryonic tissue organization.

We also found a possible regulatory molecule of intermediate filaments, GEI-4. We previously identified *gex-2* and *gex-3* genes as essential factors for tissue formation. We performed yeast two hybrid screening of interacting molecules with GEX-2 (*gei* genes, gex interacting molecule), and obtained 6 genes including GEI-4. GEI-4 shared weak homology to trichohyalin, a mammalian intermediate

filament interacting protein. We found interaction between GEI-4 and *C. elegans* intermediate filaments by yeast two-hybrid method. RNAi of *gei-4* caused an embryonic lethality with disorganization of tissue morphology. Consistent with the interaction of GEI-4 with GEX-2, immunostaining analysis revealed that GEI-4 is colocalized with GEX-2, GEX-3, and many intermediate filaments at the peripheral region of almost all the cells. Terminal phenotypes of *gei-4*, *gex-2*, and *gex-3* showed disorganization of intermediate filaments. Thus, these results suggest that dynamics of intermediate filaments, regulated by GEX-2/GEX-3/GEI-4 protein complex, is essential for embryonic tissue formation.

444. The cytoskeletal linker ERM is required for the integrity of epithelial luminal surfaces of internal organs in *C. elegans*

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We are interested in the role of submembraneous cytoskeletal linker proteins of the protein 4.1 family in growth regulation and morphogenesis and have previously reported the identification of two *C. elegans* members of the ezrin-radixin-moesin-merlin family, *erm* and *nfm* (previously called *nf2*). The highly conserved *C. elegans* homolog of ezrin-radixin-moesin, *erm*, is predominantly expressed in apical epithelial membranes of the digestive tract, excretory system and somatic gonad. Its loss during embryonic development induces early larval lethality with extensive morphogenesis defects in the digestive and excretory organs. Death occurs at L1 subsequent to the appearance of cysts that were found to be diverticula of the lumina of the intestine and excretory system. The intestinal cysts can run the full length of the gut, and the excretory cysts can fill the head, pressing the pharynx or anterior intestine against the bodywall.

Confocal analysis of the intestine using antibodies against proteins localized to adherens junctions, to the apical and baso-lateral membranes and to actin, indicate that the polarity of the epithelium is preserved, but that cell shapes can be grossly distorted by the cysts or by defects of the epithelial tube formation. Ultrastructural analysis of L1s by TEM demonstrates loss of microvilli in the intestinal lumen in the cystic region, but adherens junctions are still intact. Judging by TEM, posterior intestinal cells appear to be less well organized into an epithelial tube. By TEM, the excretory canals are found to be missing, shortened and/or to contain large cysts, and

connections between the canal, duct and pore cells are often missing.

ERM is also expressed within the somatic gonad in the spermatheca, the uterus and the vulval opening. Loss of *erm* during larval development induces severe defects in gonad morphogenesis. The uterine wall (and perhaps the spermatheca) may fail to form a proper epithelium or may fail during use, so that developing embryos and adult sperm leak into the body cavity. Thus all three organ systems show defects in luminal epithelia.

We propose that *erm* is required for the integrity of luminal surfaces of internal organs in *C. elegans*.

445. *mir-1* and *mir-2* exhibit defects in pronuclear migration and spindle orientation in the P_0 stage *C. elegans* embryo

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The position of the first mitotic spindle in the *C. elegans* embryo is crucial for establishing the cellular asymmetry necessary for proper patterning . Events such as pronuclear migration, centration, and rotation of the centrosome/nuclear complex are important precursors to proper spindle orientation, but remarkably little is known about the machinery which enacts these processes. To investigate the genes responsible for these and other early events, we

screened for temperature sensitive embryonic lethal mutations with defects in P_0 spindle orientation. We isolated alleles of *mel-26, zyg-9, rot-2* (see abstract by Greg Ellis), and *dnc-1* (see abstract by John Willis), in addition to two new loci we have named *mir-1* and *mir-2* , for pronuclear migration and P_0 rotation defective.

In *mir-1* and *2* mutant embryos, the maternal pronucleus often fails to migrate to the posterior of the embryo before the first mitotic spindle sets up. In these embryos, the maternal DNA may not be integrated into the mitotic apparatus, and instead seems to be randomly segregated to one of the daughter cells, appearing as an extra nucleus. The pronuclei do not centrate in these mutants, and the centrosome/nuclear complex does not rotate, resulting in a posteriorly positioned, transversely oriented spindle and a cleavage plane through the long axis of the embryo. Initial polarity appears normal, but cytoplasmic determinants are subsequently mislocalized due to the aberrant cleavage plane.

I am working to characterize these migration and rotation defects in detail, using time lapse video microscopy, immunocytochemistry, and GFP fusion lines in which we can visualize tubulin and histone in living embryos. *mir-1* and *2* map to chromosomes I and II,

respectively. I am continuing to map each mutant to small intervals on these linkage groups, and will be employing RNAi and transgenic rescue to clone *mir-1* and *mir-2* .

446. Spindle Dynamics and the Role of Gamma-tubulin in Early *C. elegans* Embryos

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Gamma-tubulin is a ubiquitous and highly conserved component of centrosomes in eukaryotic cells. Genetic and biochemical studies have demonstrated that gamma-tubulin functions as part of a complex to nucleate microtubule polymerization from centrosomes. We show that, as in other organisms, *C. elegans* gamma-tubulin is concentrated in centrosomes. To study centrosome dynamics and chromosome segregation in embryos, we generated transgenic worms that express GFP::gamma-tubulin or GFP::beta-tubulin in the maternal germline and early embryos. Multiphoton microscopy of embryos produced by these worms revealed the time course of daughter centrosome appearance and growth, and the differential behavior of centrosomes destined for germline and somatic blastomeres. To study the role of gamma-tubulin in nucleation and organization of spindle microtubules, we used RNA interference (RNAi) to deplete *C. elegans* embryos of gamma-tubulin. Gamma-tubulin(RNAi) embryos failed in chromosome segregation, but surprisingly, they contained extensive microtubule arrays. Moderately affected embryos contained bipolar spindles with dense and long astral microtubule arrays, but with poorly organized kinetochore and interpolar microtubules. Severely affected embryos contained collapsed spindles with numerous, long astral microtubules. Our results suggest that gamma-tubulin is not absolutely required for microtubule nucleation in *C. elegans*, but is required for the normal organization and function of kinetochore and interpolar

microtubules. (This work will appear in the June, 2001 issue of Molec. Biol. Cell.)

447. Spindle rotation in the early *C. elegans* embryo

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We have isolated a genetic mutant that fails to undergo spindle rotations required for correct cell division orientation in the *C. elegans* embryo. Spindle rotations normally occur in the P0, P1, EMS, P2, and P3 blastomeres in the early embryo and act to ensure that the subsequent cell division results in segregated cell fate determinants being separated to the correct cells. This process is thought to occur by the capture of astral microtubules radiating from the centrosome by a protein complex associated with the cell membrane. A motor protein in the complex then pulls the centrosome towards the cell cortex causing a rotation of the centrosome/nuclear complex (Hyman, 1989). PAR proteins, which are asymmetrically localized and confer A-P polarity to the early embryo, may act upstream to control the rotation decision.

The mutant we isolated, *qt1*, fails to undergo spindle rotation events in P0, P1, and EMS. Rotations in P2 and P3 have not been completely evaluated. Tubulin antibody staining shows that, excepting their orientation, spindles are wildtype. P granule, PAR-2, and PAR-3 antibody staining is wildtype indicating that polarity is correctly specified in *qt1* mutant embryos. Thus the defect does not result from gross abnormalities in spindle formation or a lack of upstream polarity establishment. Perhaps the protein affected by the *qt1* mutation is a component of the machinery required to generate the spindle rotation. Mapping of the mutation has localized the affected gene to a 0.6mu region on the left arm of linkage group III. We will present the results of experiments we have done to determine the effect of the *qt1* mutation on the *C. elegans* embryo as well as our mapping progress.

Hyman, A. A. (1989). J. of Cell Biology. 105:2123-2135

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448. *spn-3*: A GENE INVOLVED IN SPINDLE POSITIONING

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The timing and orientation of cell divisions is critical for establishing cell contacts and partitioning asymmetrically localized cytoplasmic factors required for proper development. In *C. elegans* embryos, the first spindle sets up along the anterior-posterior (AP) axis which is also polarized for a number of developmental determinants. This first division is asymmetric, leading to the generation of daughter cells that differ with respect to size, localized factors, and developmental fates. Following fertilization, the two parental pronuclei meet in the posterior of the cell, move to the center, and rotate 90^{o}. This movement of the nuclear-centrosome complex serves to place the centrosomes on the AP axis and enables the spindle to set up on this axis. During spindle elongation the spindle is displaced towards the posterior. The movement and rotation of the pronuclei is known to be dependent on astral microtubules, and the microtubule motor dynein and its associated dynactin complex. It has been shown that differences in the forces exerted on the two halves of the spindle account for the net movement to the posterior. Dynein is not asymmetrically localized but it, or other proteins, must be functioning asymmetrically to produce the anterior movement of the nuclear-centrosome complex, its rotation, and the posterior movement of the spindle.

We are studying a gene, *spn-3*, that functions in spindle positioning in many cells of the early embryo. As a first step in the characterization of *spn-3*, we have used time-lapse video microscopy to examine spindle orientation defects in the mutant background. Initial analysis of a maternal effect lethal allele, *spn-3(it150)*, identified a role in rotation and spindle stabilization during the second and third cleavages. During the past year, complementation tests with other genes mapping to the same region identified stronger alleles of this gene that are homozygous larval lethal (a.k.a. *let-711*). *it150/let-711(s2587)* worms produce a stronger maternal effect lethal phenotype than *it150/it150*, which reveals a role for this gene in spindle positioning during the first cleavage. Mutant embryos often fail to align their spindle along the correct axis due to a failure of nuclear-centrosome rotation. In addition, in cases where rotation occurs, as the spindle elongates it moves abnormally far into the posterior region of the cell. Analysis of polarity markers in *it150/let-711(s2587)* and *it150*/deletion embryos suggests that other aspects of polarity are normal. These data, combined with analysis of *it150/it150* embryos, suggest *spn-3* plays a role in spindle orientation and stabilization during the first three divisions after fertilization.

Based on these observations we hypothesize that *spn-3* is part of a mechanism that ensures proper spindle orientation. We propose that in the 1-cell, the product of this gene is functioning at the anterior and is regulating interactions between the cortex and microtubules that are necessary for proper spindle placement. In order to test this hypothesis, and begin to elucidate the relationship between *spn-3* and other genes known to be involved in spindle positioning, we are isolating this gene using a positional cloning approach. Deletion mapping narrowed down the region containing *spn-3* to an overlapping set of 19 cosmids. Transformation rescue experiments are currently being used to identify a cosmid that rescues the lethal phenotype. Examination of the *spn-3* gene, combined with the analysis of other genes in our lab, will provide a basis for understanding the mechanism behind spindle orientation and stabilization in all blastomeres of the early embryo.

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449. *or358ts* is involved in mitotic spindle orientation and positioning in the one-cell stage *Caenorhabditis elegans* embryo

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The first mitotic division of the 1-cell stage *C. elegans* embryo is asymmetric, producing a smaller posterior daughter (P1), and a larger anterior daughter (AB), two cells born committed to distinct fates. During this first division, the mitotic spindle aligns along the longer anterior-posterior (a-p) axis of the zygote, and moves posteriorly during anaphase. This posterior displacement results in the first division being unequal. To understand how spindle orientation and positioning are regulated in the early worm embryo, we are characterizing the role or *or358*ts, a temperature sensitive maternal-effect embryonic lethal mutant in which spindle positioning and orientation at the first division are defective.

*or358*ts was identified in a screen for temperature-sensitive embryonic lethal mutations. In this mutant, the first mitotic spindle sometimes fails to rotate fully and therefore does not always align with the a-p axis (its range is between 0-86 degrees relative to the a-p axis). The first spindle of these mutants then either remains transverse, or eventually flips to orient with the a-p axis. If the spindle of *or358*ts mutant embryos aligns a-p, it is hyper-displaced posteriorly, resulting in larger than usual AB, and smaller than usual P1 daughter cells. Earlier defects of *or358*ts can also be detected. These include reduced pseudocleavage, posteriorly hyper-displaced pro-nuclear meeting position, and failure of pro-nuclei to centrate after meeting. Preliminary data suggest that some *or358*ts mutant embryos at the one-, two- and four-cell stages have microtubule nucleation defects, and in some embryonic cells microtubule nucleating centers appear dissociated from the nucleus. Mapping analyses of *or358*ts positions it to linkage group (LG) I, near or to the left of *lin-17* (-6.4 map units), and we are presently positionally cloning the wild-type gene. Although asymmetric cell divisions occur in a number of developmental contexts, the mechanisms regulating spindle positioning and orientation are not well understood. We think *or358*ts should provide insights into the mechanisms that control these processes.

450. Genetic and Phenotypic Analysis of *spd-3* : A Mitotic Spindle Defective Mutant in *Caenorhabditis elegans*

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In order to develop a complex organism, cells must have the ability to divide asymmetrically to produce two daughter cells of different developmental potential. A cell accomplishes this by segregating cytoplasmic components to opposite ends such that, upon cleavage, each daughter inherits distinct components. The cleavage plane is specified by the alignment of the mitotic spindle. To understand the molecular mechanism of spindle alignment we are studying a temperature sensitive, maternal effect, lethal mutation in the *spd-3* gene of *C. elegans*. Cytological analysis reveals that the *spd-3(oj35)* mutant is defective in nuclear and spindle positioning. During pronuclear migration the egg pronucleus is retarded, and the sperm pronucleus often breaks down prior to their meeting. During the first mitosis the spindle fails to align along the anterior/posterior axis leading to abnormal cleavage configurations. We are currently investigating localization of polarity markers such as PAR2 and PAR3 in *spd-3(oj35)* embryos. *spd-3(oj35)* mutants also exhibit a failure to extrude polar bodies, which could be attributed to a misaligned meiotic spindle. Post-embryonic defects, including uncoordination and failure to mature to adulthood, are consistent with cell division defects indicating that SPD3 may be involved in spindle alignment in all tissues. Cloning efforts have narrowed the position of *spd-3* to the right arm of chromosome IV, between 2.51 and 2.91, a region consisting of five cosmids. Rescue attempts and RNAi injections of candidate genes are in progress. It is possible that SPD3 is a component of the dynactin complex since *spd-3(oj35)* and dynactin RNAi embryos show similar phenotypes. In studying genes such as *spd-3* we hope to reach a better understanding of factors involved in spindle alignment which is essential for defining the plane of cytokinesis and ensuring proper asymmetric cell division.

451. Three-dimensional reconstruction of the early C. elegans mitotic spindle

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C. elegans is an exciting model system for studying the molecular mechanisms of cell division. It is well suited for microscopic analysis, because each hermaphrodite contains a "row" of early embryos of various stages. We apply a method which allows us to analyze high pressure frozen, plastic embedded embryos in whole mounted worms by transmission electron microscopy. We have started to cut serial sections through entire C. elegans embryos to reconstruct the early C. elegans mitotic spindle . Using three-dimensional reconstruction we have started to model the spatial organization of microtubules, chromatin, centrosomes and the nuclear envelope. We are analyzing embryos which contain about 20-30 cells, because these embryos are much smaller compared to one-cell stage embryos. We have also started to look at the structure of the C. elegans kinetochore. In embryos we found a ribosome-free zone which extends along the length of the chromosome. We will present preliminary three-dimensional models.

452. CeMCAK, a *C. elegans* kinesin with functions in meiosis and mitosis.

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Completion of each cell cycle requires faithful chromosome segregation, which relies on proper control of microtubule dynamics. Xenopus XKCM1, which is a homolog of the internal-motor-domain kinesin MCAK, is known to depolymerize microtubules from their ends. We have identified a *C.elegans* homolog (*klp-7*, K11D9.1) that we call CeMCAK. The CeMCAK protein expressed in insect Sf-9 cells causes microtubule destabilization. Immunolocalization in oocytes and early embryos suggests that CeMCAK is concentrated at spindle poles, the anaphase spindle midzone, and all along the condensed, mitotic chromosomes. To test the effects of CeMCAK depletion, we used RNA-mediated interference (RNAi) followed by time-lapse movies or immunostaining. Nomarski optics revealed large polar bodies, cortical instability, and mispositioning and/or failed rotation of the first mitotic spindle. In anaphase B the normally rigid pole-to-pole bundle of microtubules appears weak at the center. This suggests poor interactions between the two sets of overlapping microtubules that extend from the spindle poles. Anti-tubulin staining of fixed CeMCAK(RNAi) embryos suggests overgrowth of mitotic astral microtubules and depletion of microtubules in the spindle midzone. So far, immunostaining with antibodies to other spindle proteins has shown normal localization patterns. Movies of GFP::histone in RNAi embryos show the occasional presence of chromosome bridges during anaphase. Our results suggest that CeMCAK functions to destabilize microtubules in vitro and in vivo. The weakening of the midzone microtubules in RNAi embryos may reflect a depletion of soluble tubulin driven by incorporation into the astral microtubules. As the anaphase spindle elongates, the interzone microtubules cannot, so they slide apart.

453. Cytoplasmic Dynein in the Early Embryo

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To study dynein's different functions, we are using dominant temperature-sensitive (dom-ts) alleles of the *let-354* complementation group. The evidence that *let-354* encodes dynein heavy chain (DHC-1) includes: 1) *let-354* has been mapped genetically to the same region of LGI as *dhc-1*, 2) the mutant phenotype of *let-354*(dom-ts) embryos is similar to the phenotype observed in *dhc-1(RNAi)* embryos, 3) injection of two overlapping cosmids (T21E12 and ZK973), which together contain the *dhc-1* gene, rescued the recessive larval-lethal phenotype of *let-354*.

let-354(dom-ts)/+ worms or their embryos were cultured at permissive temperature (15°C) and shifted to non-permissive temperature (25°C). At 15°C, mutant embryos undergo apparently normal development. At 25°C, mutant embryos show severe defects in meiotic chromosome separation, mitotic centrosome separation, mitotic spindle formation, spindle rotation, chromosome segregation during mitosis, cortical membrane integrity and cytokinesis. The dom-ts alleles respond rapidly to non-permissive temperature. With a thermal stage controlled by Peltier devices, embryos can be shifted to 25°C in ~ 1 min. and immediately show defects. Embryos shifted to 25°C after normal pronuclear migration display defects in mitotic spindle formation, cortical membrane integrity, and cytokinesis. This dominant ts effect is reversible. For example, failure to form a mitotic spindle and undergo cytokinesis at the restrictive temperature is followed, after a shift down to the permissive temperature, by spindle formation and cytokinesis during the next cell cycle. Using different temperature shift regimes, we are now attempting to define dyneins's role in various stages of pronuclear migration, spindle positioning and mitosis.

(Thanks to Paul Mains and Ann Rose for *let-354* alleles.)

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454. The dynactin subunit, *dnc-1* (*or404ts*), is required for mitotic spindle orientation during early*C. elegans* embryogenesis

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To understand the mechanisms governing the cell division processes of the early embryo, we have conducted genetic screens to identify temperature sensitive, maternal effect, embryonic lethal mutations. From these screens we have identified a class of mutants with spindle orientation defects at the one-cell stage P0 blastomere (see posters by Jen Phillips, Greg Ellis and Sandra Encalada). One of these mutations, *or404ts*, was positioned by meiotic mapping near the *dnc-1* locus. *dnc-1*, the orthologue of the vertebrate gene p150Glued, is a component of the multi-subunit dynactin complex, which is required for all known functions of the microtubule-associated dynein motor protein. Sequence analysis of *dnc-1* from *or404ts* animals identified a missense mutation in the C terminus of the protein. To our knowledge, *or404ts* is the only known mutant allele of *dnc-1* in C.elegans.

Roles for dynein and dynactin in the early embryo were revealed by studies in which known components, including *dnc-1*, were inhibited by *RNAi* (1,2). These studies show that dynein and dynactin are required for multiple microtubule dependent events in the early embryo; these include pronuclear migration, centrosome migration, and rotation of the centrosomal/nuclear complex. In wild type embryos, the sperm contributed centrosome duplicate and migrate to opposite poles of the male pronucleus, transverse to the anterior-posterior (a-p) axis of the embryo. Meanwhile, the female pronucleus migrates to meet the male pronucleus at the posterior of the embryo. This centrosomal/nuclear complex then rotates 900 to lie along the polarized a-p axis of the embryo before the completion of the cell cycle. In *dnc-1* (*or404ts*) mutant embryos pronuclear migration and centrosome migration occur as in wild-type, but centrosomal/nuclear complex rotation does not occur, resulting in a mitotic spindle which aligns transverse to the a-p axis.

Since dynein and dynactin *RNAi* mutant embryos exhibit early defects in embryogenesis, a role for dynein and dynactin in spindle orientation past the one cell stage has not been addressed. In both the P1 blastomere and the EMS blastomere at the 2 and 4 cell stage, respectively, the mitotic spindle rotates to align along the a-p axis. It is not known if dynein or dynactin are required for these rotations. To address this we are performing temperature up-shift experiments with *dnc-1* (*or404ts*) to determine its role in these rotations. Preliminary experiments show that P1 spindle orientation is aberrant when *dnc-1* (*or404ts*) embryos are shifted to the restrictive temperature after a wild-type first division (1/3 embryos). This suggests that dynactin and dynein may function in P1 spindle orientation. Further characterization of this function and a possible requirement for *dnc-1* (*or404ts*) in EMS spindle orientation will be covered in this poster.

1. Skop A. and White J. (1998) Current Biology 8: 1110-1116. 2. Gönczy et al., (1999) JCB,14(1) : 135-150.

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455. Characterization of *rot-2*, a gene required for proper P_0 Spindle Orientation

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or362 is a temperature sensitive allele isolated in a screen for embryonic lethal ts mutants. Mutant embryos show defects in centration and rotation of the first mitotic spindle. Using complementation tests and snip-snp mapping, we have found that or362 is an allele to *rot-2*, a gene previously identified in a screen for non-conditional maternal effect mutants (Gonczy *et al.* 1999).

In wild-type one-cell stage embryos, shortly following fertilization, the maternal pronucleus migrates from the anterior of the zygote towards the posterior where it meets with the paternal pronucleus. The pronuclei then move back towards the center of the embryo as the centrosome pronuclear complex rotates 90 degrees such that the first mitotic spindle sets up along the longitudinal axis of the one-cell stage embryo called P_0. The mitotic spindle then moves posteriorly during anaphase, resulting in the anterior blastomere, AB, being larger than the posterior blastomere, P_1.

In *rot-2* (or362ts) mutants, the P_0 spindle fails to rotate and remains orientated transversely in the posterior of the embryo. This causes an ectopic cleavage furrow along the a-p axis that bisects the transverse spindle. Furthermore, in *rot-2* (or362ts) mutant embryos, the maternal pronucleus frequently fails to migrate posteriorly. In these embryos, the sperm pronucleus-associated centrosomes nevertheless form a transversely oriented spindle. Thus, *rot-2* is required for two microtubule-dependent events: migration of the maternal pronuclei, and rotation of the first mitotic spindle. Further studies will focus on the determination of the molecular identity and additional phenotypic characterization of *rot-2*.

Gonczy *et al*. (1999). *JCB* **144**: 927-946.

456. Analysis of *spd-1*, a gene required for mitotic spindle integrity and cytokinesis in *C elegans*

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Several mutants, such as *zen-4* and *air-2*, have illustrated that the midzone of the mitotic spindle is important for the completion of cytokinesis. When these mutant embryos are viewed with Nomarski, the spindle appears to break and the cleavage furrow ingresses to near completion before regressing and forming a multinucleated cell. *spd-1 (oj5)* is a temperature sensitive mutant in which the spindle also appears to break, yet the first division completes normally. The embryo continues to divide normally until the EMS fails to divide at the four-cell stage. Live imaging of *spd-1* embryos using an integrated ß-tubulin::GFP construct shows that microtubules are absent or much decreased in number and organization at the spindle midzone compared to wild type embryos. Some evidence however suggests that a few microtubules remain and these may be sufficient to allow cytokinesis to complete in the early divisions. We are currently looking at other markers such as ZEN4 and histone 2B to further characterize the defects in this mutant.

We are attempting to clone this gene using positional cloning and have mapped this mutation to a region containing 1 MB at the left arm of chromosome I. There are several possible candidate genes in this region, which we are analyzing further using RNAi.

457. A gain-of- function mutation in *cdc25.1* phosphatase causes intestinal-specific cell cycle aberrations.

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The functional analysis of *cki-1,* the *C.elegans* p27KIP cyclin-dependent kinase inhibitor homologue, has demonstrated a major role for this cell cycle regulator in the developmental regulation of the G1/S progression throughout postembryonic development. The removal of *cki-1* function through dsRNA-mediated interference causes extra cell divisions in multiple postembryonic lineages. In order to further understand how cell division is coordinated with developmental processes, and to identify upstream regulators of *cki-1*, we have been performing screens to identify mutants which phenocopy *cki-1(RNAi)* animals in specific lineages.

Joel Rothmans group has previously shown that deficiencies that remove *cki-1* result in significantly more intestinal cells in embryos. Similarly, following *cki-1(RNAi)* the average number of intestinal nuclei in *cki-1(RNAi)* animals is 50 (+/-3), as compared to 32-34 in wildtype animals. The extra nuclei mostly arise from an additional cell division during embryogenesis. In a screen for mutants which phenocopy the extra intestinal cell proliferation, we have found a mutant, *rr31*, with an average number of 57(+/-4) intestinal nuclei. As in *cki-1(RNAi)* animals, the additional cells arise during embryogenesis, although at an earlier stage. The loss of *cki-1* activity in *rr31* mutants does not enhance the total number of intestinal nuclei suggesting that these two proteins might cooperate to regulate divisions of the E lineage.

Interestingly, the *rr31* mutation was shown to be a fully dominant, maternal-effect, gain-of-function allele. The *rr31* mutation was mapped using a combination of STS-polymorphism, snip-SNP mapping and three-factor crosses to a region between 0.97 and 1.23 on LGI. This region was analysed for the existence of known cell cycle regulators and candidates were tested using PCR amplification of the corresponding genes from mutant animals

followed by injection into wild type animals. A 7.3kb PCR product amplified from the *rr31* mutant that corresponded to the *cdc25.1* cell-cycle phosphatase (corresponding cosmid region K06A5.7a) was injected into N2. This resulted in transgenic animals with extra intestinal nuclei, albeit at low frequency, consistent with maternal expression of transgenes. Furthermore, *cdc25.1(RNAi)* in *rr31* animals fully suppressed the extra intestinal cell defect. Finally, a GC to AT transition was found at the splice site between the first and second exon in the mutant *cdc25.1 (rr31)*. We are currently investigating the effect of the gain-of-function allele in postembyonic lineages through ectopic expression studies. We are also trying to understand the basis of the intestinal specificity of this mutant cell cycle regulator through the characterization of extragenic suppressors and two-hybrid interactions. Biochemical and structural methods will be undertaken to compare the mutant and wild type forms of CDC25.1.

458. GENETIC SCREEN FOR FZY-1 ALLELE

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Accurate chromosome segregation at the metaphase to anaphase transition is ensured by a highly regulated process. This transition is triggered by activation of the anaphase promoting complex (APC), which functions to target key proteins such as cyclin B or anaphase inhibitors, for ubiquitin mediated proteolysis (reviewed in Page and Hieter 1999). The APC is activated by WD-repeat proteins, which have been identified in all eukaryotes studied and fall into two distinct groups: fizzy and fizzy-related. In *Saccharomyces cerevisiae*, a fizzy protein, Cdc20, is known to activate APC to target an anaphase inhibitor, PdsI, and its degradation is essential to trigger sister chromatid separation. In addition, the spindle assembly checkpoint arrests cell cycle at metaphase before anaphase in response to spindle damage or kinetochore defects by inhibiting the APC activity via Cdc20. Thus, Cdc20 is a key component to regulate the timing of metaphase-anaphase transition during mitotic cell division in eukaryotes.

We have been studying the mechanism of regulation of chromosome segregation in mitotically dividing cells during animal development using *C. elegans*. Recently we have identified a *C. elegans* homolog of budding yeast CDC20. The gene ZK177.6, tentatively named *fzy-1*, is located at about -1.8 map position on chromosome II. By yeast two-hybrid system, FZY-1 has been shown to interact with a spindle assembly checkpoint component, MDF-2. Loss of function by RNAi for *fzy-1* showed one-cell embryonic arrest phenotype, which is similar to "*mat*" (metaphase anaphase transition defective) phenotypes observed in *emb-27* (Golden et al 2000) *or emb-30* (Furuta et al 2000) mutants at restrictive temperature, suggesting that *fzy-1* is involved in APC activation.

To address the question of how chromosome segregation is regulated by *fzy-1* gene product during animal development, we set out to isolate *fzy-1* deletion mutant strains by screening UV-TMP mutagenized worms. In the primary screen, we screened 9942 chromosomes by using the RNAi phenotype as a guideline, and obtained 201 candidates. One of these candidates, *h2066*, which displays the embryonic arrest phenotype, has been shown to have a deletion at the *fzy-1* locus by genomic PCR test. Using this strain, we are analyzing the FZY-1 function during development. The roles of FZY-1 in regulation of APC activation and MDF dependent checkpoint will be discussed.

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459. Investigation of the function of Ran by RNAi

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Ran is a small GTPase implicated in multiple cellular processes throughout the cell cycle. During interphase Ran was first shown to regulate transport of macromolecules in and out of the cell nucleus. Ran probably does so by serving as a molecular switch that signals the subcellular localization to transport receptors. Later Ran was also demonstrated to have a strong effect on microtubule dynamics in mitotic cell extracts where it is required for formation of a bipolar spindle. Closing the cycle, Ran is finally required for formation of a nuclear envelope around chromatin in *Xenopus* extracts, most likely reflecting a role for Ran in regeneration of the nuclei after cell division. Strikingly, Ran presumably carries out all three functions by creating protein gradients around chromatin.

While the function of Ran in nucleocytoplasmic trafficking is well-characterized from numerous biochemical and *in vivo* studies it remains to be precisely determined if and how Ran regulates the mitotic spindle apparatus and nuclear envelope assembly in living cells. To address these important issues we have investigated the effects of RNAi-mediated suppression of Ran expression in several transgenic *C. elegans* lines. We have generated *C. elegans* lines that express various GFP-tagged cellular reporter proteins in the germline enabling us to make detailed time lapse microscopy recordings of early embryos. From these studies we can now evaluate the effects of Ran depletion on the dynamics of chromatin, microtubules and the nuclear envelope during early embryogenesis.

Embryos in which Ran expression is inhibited show strong abnormalities in pronuclear and nuclear appearance and in severe cases no nuclei can be detected. This strongly supports an essential role for Ran in generation of a closed nuclear envelope. Secondly, targeting Ran by RNAi prevents formation of a mitotic spindle

while astral microtubules are unaffected, which again provides evidence in favor of recent *in vitro* observations.

We are continuously generating suitable markers to study the effects of Ran on the cell cycle and are currently also disrupting the expression of a broad range of *C. elegans* genes whose homologues in other systems are known to interact with Ran.

460. Regulation of the exit from mitosis in C.elegans.

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During eukaryotic cell division the correct segregation of the genetic material is paramount for controlled development and growth. Therefore it is essential that the physical separation of the two nascent cells (cytokinesis) happens *after* the chromosomes have been successfully segregated. How is the co-ordination of these events achieved? In the budding yeast *Saccharomyces cerevisiae* a network of proteins, comprising several kinases, a small GTPase and a protein phosphatase, controls the initiation of the exit from mitosis. In metazoa, however, this process is not well understood. We have started analysing the regulation of the exit from mitosis in *Caenorhabditis elegans.* As a first step the putative *C. elegans* homologues of yeast *mitotic exit network* (MEN) genes were screened for involvement in the exit from mitosis or meiosis by RNA-mediated interference. Our preliminary results indicate that the *C. elegans* homologues of yeast MEN genes are not essential for embryonic divisions but might be important for the proper development of the germline and gametogenesis.

461. Screen for mutants defective in membrane deposit during cytokinesis

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At the end of the cell cyle, a new plasma membrane has to be formed in order to separate the two daughter cells to which the chromosomes have been distributed. This process occurs at the end of M-phase and is termed cytokinesis. Although a number of cytoskeletal elements involved in cytokinesis have been identified, the origin of the membrane and how the membrane fusion event is concerted within the process of cytokinesis remains elusive.

We intend to perform a screen for mutants defective in cytokinesis, with special emphasis to mutants that are unable to deposit membranes in the division plane. In parallel screens we are checking for conditional (temperature-sensitive) as well as recessive mutations. The screen is based upon the survival of egg-laying defective hermaphrodites after mutagenesis. The hermaphrodites can only survive if they are unable to produce any viable offspring. The secondary screen of the mutants involves the use of a GFP-marker protein, which localizes in the division plane. We are looking for mutants where the GFP-marker is mislocalized in the developing egg. In addition, we are currently using RNAi to check the involvement of proteins required for intracellular protein and membrane traffic in cytokinesis.

462. Analysis of single-cell cytokinesis-defective mutants in *C. elegans*

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Mitosis culminates in the equal segregation of sister chromatids to each of two newly- formed cells. Genetic instability can be caused not only by defects in the segregation process itself, but also by failures in cytokinesis that can lead to the formation of polyploid cells. Our studies are focused on two kinases that are directly involved in each of these events. The *C. elegans* AIR-1 protein is required for proper assembly and function of the mitotic spindle, whereas the highly related AIR-2 protein is required for the chromosome segregation and cytokinesis. In an attempt to isolate mutations in the *air-2* locus as well as identify new components in the *air-2* pathway, we have recovered temperature-sensitive maternal-effect lethal mutants that display a similar phenotype to *air-2(RNAi)* embryos. Three of these mutants fail to complement one another and map very close to *dpy-5* on Chr. I. Complementation tests with other embryonic lethal mutants in the same region have shown that this locus is novel. Further phenotypic characterization of these alleles and analysis of candidate genes within the relevant interval will be presented.

463. Investigating the possibe role of calmodulin in cytokinesis

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Cytokinesis, the physical separation of a cell during cell division, is fundamentally important to all cell types and must be tightly regulated. Both spatial and temporal regulation are required to ensure proper segregation of chromosomes and molecules involved in processes such as development and differentiation. Calmodulin is a good candidate for a regulator of cytokinesis. It has been localized to the cleavage furrow and/or mitotic apparatus in different organisms and is a known regulator in other calcium dependent pathways, through its calcium sensing properties.

Our work has focused on determining the role of calmodulin in regulating cytokinesis in *C. elegans* using a combination of standard and novel techniques. There are several candidate calmodulins in the worm database, the most conserved of which is CMD-1. Surprisingly, RNAi experiments using *cmd-1* did not show cytokinetic defects, although a dramatic embryonic arrest at morphogensis was observed. Other candidate calmodulins are currently being examined with RNAi. Localization studies are also underway. Additionally, we are attempting to phenocopy the RNAi results and to study calmodulin in a more temporally defined manner using a caged calmodulin inhibitor.

464. Assessing the role of microtubules in the establishment of the first asymmetric cell division of the *C. elegans* embryo

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We are using the *C. elegans* 1-cell embryo as a model to understand how cells divide asymmetrically. The first embryonic division of *C. elegans* gives rise to a larger, anterior cell, termed AB, and a smaller, posterior cell, P1. This asymmetry is the result of the position of the first mitotic spindle, which moves from the center to the posterior of the 1-cell embryo during metaphase. Several mutations, including alleles of the *par* genes, disrupt embryonic polarity and affect the posterior displacement of the spindle, suggesting that components localized at the cell cortex can influence spindle positioning (1). Recent experiments by Grill *et al*. (2) demonstrated that severing the spindle at the start of anaphase B results in both the anterior and posterior centrosomes migrating toward their respective poles at a faster rate than normal, with the posterior centrosome moving faster than the anterior one. In order to address the role of astral microtubules in spindle positioning, we ablated either the anterior or the posterior centrosome at various phases of the cell cycle. During anaphase B, ablation of the anterior centrosome caused posterior movement of the posterior centrosome and ablation of the posterior centrosome caused anterior movement of the anterior one; these results are similar to those obtained by Grill *et al*. However, during prophase (before pronuclear envelope breakdown), ablation of the anterior centrosome resulted in posterior displacement of the posterior centrosome, while ablating the posterior centrosome at this time did not cause anterior movement of the anterior centrosome. The anterior centrosome did not move until later, posibly at anaphase onset. This result suggests that a pulling force acts on the posterior aster before anaphase. One posible mechanism for this could be that components of the posterior cortex might modulate the dynamics of microtubules in this region of the embryo, which could asymmetrically modulate

pulling forces. PAR-1, a protein with similarity to MARK kinases that localizes to the posterior cortex of the embryo (3), is a good candidate to have a local effect on posterior microtubules. MARK kinases have been implicated in the phosphorylation of microtubule-associated proteins (MAPs) and in the destabilization of microtubules *in vivo* and *in vitro* (4). We monitored dynamics of individual microtubules by imaging embryos expressing an alpha-tubulin:gfp fusion (kindly provided by Karen Oogema) with a spinning-disk confocal microscope. Thus far, preliminary quantitative data analysis suggests a similar stability between anterior and posterior astral micrutubules during the time that the spindle becomes asymmetric in wild-type embryos. We will further address this issue by monitoring microtubule dynamics in *par* mutants. Furthermore, we are developing an *in vitro* assay to test whether PAR-1 can directly affect microtubules to modulate their stability. To this end, we have isolated *C. elegans* MAPs and will determine if they can play a role in this process. Progress toward these goals will be presented.

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465. Identification and characterization of *lin-14* genetic interactors

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Heterochronic genes regulate the timing of cell fate determination. Mutations in heterochronic genes cause alterations of cell fates in which stage-specific events are either omitted or reiterated, resulting in precocious or retarded phenotyes respectively. LIN-14 is a key heterochronic regulatory factor that promotes early larval fates in *C. elegans.* LIN-14 protein decreases over time and specifies the timing of the L1 to L2 cell lineage transition. Mutations that perturb the level of LIN-14 protein perturb the temporal sequence of cell lineages during development. LIN-14 is a novel nuclear protein and the method by which LIN-14 acts to specify stage-specific cell fates is unknown. To elucidate the role of *lin-14* in heterochronic control of development, we are identifying factors that mediate *lin-14* action as genetic suppressors or enhancers of *lin-14* mutant phenotypes.

To identify genes negatively regulated by *lin-14*, we performed a screen for suppressors of the heterochronic phenotypes conferred by a *lin-14(lf)* mutation. Preliminary screening of 4.4 x 10^4 haploid genomes produced 36 mutants in at least three complementation groups: Class 1: 2 alleles of *let-7 X;* Class 2: 8 alleles of a gene that we have tentatively called *sol-1* (**s**uppressor **o**f ***l****in-14*)*;* Class 3: 26 unmapped alleles (Non-*let-7*). This work focuses primarily on the Class 2 *sol-1* alleles. *sol-1* mutations cause a weak retarded heterochronic phenotype when not in the presence of decreased *lin-14* gene activity. Based on genetic mapping and complementation tests we are currently determining, whether *sol-1* corresponds to a known gene or defines a new one. That the *sol-1* phenotype is unlike any of the published heterochronic mutants suggests that we have identified a new heterochronic gene.

egl-35 mutations synthetically enhance the precocious phenotype of *lin-14* mutations. This synthetic enhancement with the two alleles suggests that *lin-14* and *egl-35* genes interact in the same pathway and that this interaction is a positive one. We are characterizing the *egl-35* phenotype in more detail and intend to fine map and clone *egl-35*. The *C. elegans timeless* homologue, *tim-1*, maps to the *egl-35* genetic region. *tim-1* is a good candidate for *egl-35* because another heterochronic gene, *lin-42*, which also enhances a *lin-14* phenotype encodes the *C. elegans period* homologue (*period* and *timeless* interact to control circadian timing in flies and mammals). We are attempting to rescue the *egl-35* phenotype with genomic DNA spanning the *timeless* gene, and we are also sequencing the *timeless* gene from *egl-35* alleles to identify potential lesions. We are testing the hypothesis that *tim-1* is a heterochronic gene by performing RNAi with *tim-1* and asking if this leads to a heterochronic phenotype. We are also testing if RNAi of *tim-1* enhances a *lin-14* phenotype, as would be expected if *tim-1* is *egl-35*.

We are investigating whether other *C. elegans* homologs of circadian genes have a function in the heterochronic pathway. We are doing RNAi with *C. elegans* ORFs that have sequence similarity to circadian genes and evaluating the effect of RNAi on the phenotypes of key heterochronic pathway regulators, such as *C. elegans lin-14*, *lin-28*, *let-7*, etc. In preliminary experiments, we have found that RNAi with the *C. elegans* homolog of the circadian gene *doubletime* suppresses the mutant (lf) phenotype of the heterochronic gene *let-7*.

466. A *C. elegans* Chromokinesin Required for Chromosome Segregation

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A search of the *C. elegans* genome, using the *Kinesin heavy chain* gene (*unc-116*), identified 18 kinesin family proteins. We have tentatively identified two of these, CeChromoKinesin-A (T01G1.1, klp-12) and CeChromoKinesin-B (Y43F4B.6, klp-8), as homologues of the vertebrate chromokinesins. In vertebrates these proteins bind chromosomes and are thought to function as mitotic motors. Depletion of the worm homologues by RNA interference (RNAi) suggests that CeChromoK-B is essential for development of early embryos.

Chromosome segregation in each division is abnormal in CeChromoK-B(RNAi) animals. In embryos, meiotic and subsequently mitotic chromosomes do not become organized into a tight metaphase plate, and then form bridges and fragment during anaphase. These defects, which have been monitored in living embryos using GFP::histone, often lead to the formation of multiple micronuclei. The same defects are evident in the mitotic nuclei at the distal tip of the gonad. Based on anti-tubulin staining and GFP::tubulin movies, spindle organization appears normal.

Antibodies to CeChromoK-B show a cell-cycle dependent association with chromosomes. During the telocentric meiotic divisions, CeChromoK-B is localized in a narrow band between homologs during meiosis I and between sister chromatids during meiosis II. During the holocentric mitotic divisions, CeChromoK appears to surround the chromosomes, starting at late prophase and continuing through anaphase. CeChromoK also localizes to the midzone and persists on the midbody. CeChromoK is not detectable in interphase nuclei.

Our results suggest that CeChromoK-B functions during late stages of prometaphase in organizing chromosomes on the metaphase plate. We speculate that it modulates microtubule/chromosome interactions to resolve bipolar chromatid attachment or chromosome catenation. In the absence of this function, chromosome segregation fails, either because individual chromosomes become attached to both poles or because chromosomes become tangled.

467. Understanding the Role of a Cyclin B Homologue during Postembryonic Development in *Caenorhabditis elegans*

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Cell cycle regulators can be both maternal and/or zygotic. The knock out consortium recently provided the community with a deletion of a *cyclin B* homologue. This allele is located at genetic position 4.7 LG IV (ZC168.4), and we have found that it is required for postembryonic development. Previous studies from the van den Heuvel Lab showed that the maternal *cyb* homologue is required for early embryonic development. RNAi experiments showed that removing maternal product caused multiple nuclei, and the affected embryos arrested development at an early embryonic stage. Homozygous *cyb* mutants (*gk35*) from heterozygous parents can survive embryogenesis, but show an early larval lethal phenotype. By injecting heterozygotes with a construct that contains a wild type copy of this *cyb* homologue, we found that most homozygous animals which contain both maternal product and the transgene grow up into sterile adults. Embryos from few fertile homozygous adults are either viable sterile animals or embryonic lethal which is consistent with the RNAi experimental results. All the viable animals expressed the co-transformation marker indicating the presence of the *cyb*-containing transgene. The possible explanation of these results is that maternal product of this *cyb* homologue allows the animal to go through embryogenesis, and the zygotic product is absolutely required for postembryonic development and sterility. Since transgenes can be expressed in the germline, albeit very inefficiently, few homozygous worms become fertile and give rise to the next generation. Transcriptional fusions of GFP showed that expression of the *cyb* homologue, which is very strong during embryogenesis, becomes weak after hatching and remains low throughout postembryonic development. At present, it is not clear whether the larval arrest phenotype is cell-cycle related, so we are presently engaged in determining the molecular and developmental basis of the early L1 arrest

associated with *cyb (gk35)*.

468. The beta-tubulin gene, *tbb-2* is an activator of the *C. elegans* female meiosis specific genes, mei-1 and mei-2

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mei-1 and *mei-2* are specifically required for the *C. elegans* female meiotic spindle formation. MEI-1 and MEI-2 show homology to the two subunits of the microtubule-severing protein, katanin, and disassemble interphase microtubules when co-expressed in Hela cell.[1] In the wild-type embryo, MEI-1 and MEI-2 localize to meiotic spindle but disappear before the onset of the first mitotic cleavage. However, a *mei-1* gain-of-function (*gf*) mutant results in ectopic localization of the proteins into the mitotic spindles and abnormal mitotic spindle formation. This mimics defects observed when embryos are treated with nocodazole, suggesting an ectopic spindle microtubule-destabilizing activity in mitosis.[2]

We isolated three extragenic suppressors of *mei-1*(*gf*). All genetically behave as activators of *mei-1* and *mei-2* function but are phenotypically wild-type by themselves. One of the suppressors, *sb26*, has a missense mutation in the β-tubulin gene, *tbb-2*. The amino acid change is in the carboxyl terminus, which has been shown to be required for the microtubule severing activity of sea urchin katanin.[3] Genetic interactions suggest that *tbb-2*(*sb26*) behaves as a *gf* suppressor that impairs the microtubule-severing activity of MEI-1 and MEI-2. TBB-2 specific antisera stain microtubule structures through out the worm life cycle. By using RNA interference and indirect immunoflurescence, we showed that *tbb-2* and another β-tubulin gene, *tbb-1*, function redundantly during early development.

The two other extragenic suppressors of *mei-1*(*gf*) are possibly allelic and map to the vicinity of an α-tubulin gene. Our work demonstrates genetic evidence that katanin requires specific interaction with spindle microtubules.

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469. Separase is required for the segregation of homologous chromosomes in meiosis I

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A combination of chiasmata and sister chromatid cohesion hold homologous chromosomes together in meiosis. At the metaphase-to-anaphase transition of meiosis I, sister chromatid arm cohesion distal to chiasmata must be dissolved to allow separation of homologues. The observation, that in higher eukaryotes, arm cohesion but not centromeric cohesion can be dissolved in a separase-independent manner led to the question as to whether separase has a function in promoting the segregation of homologous chromosomes in the first meiotic division of higher eukaryotes.

dsRNA-mediated interference was used to functionally inactivate the two C. elegans separase homologues and the consequences on chromosome segregation in the first meiotic division were analysed. Fluorescence time-lapse microscopy using a strain of worms expressing GFP-tagged histone H2B as well as fluorescence in situ hybridisation (FISH) reveal that separase is required for chromosome segregation in meiosis I. The meiotic spindle was also analysed in separase deficient embryos using GFP-tagged tubulin. Whereas in wild-type embryos, two meiotic spindles are sequentially formed and disassembled, in the separase deficient embryos only one meiotic spindle is formed. This spindle looks morphologically normal, but persists for much longer than in wild-type. Although the spindle persists, chromatin dynamics suggest that pulling forces are exerted on chromosomes. We, therefore, believe that the defects observed in segregating homologous chomosomes are not due to a non-functioning of the spindle.

The terminal phenotype of separase deficient embryos is characterised by a large mass of re-replicated DNA in the centre of an osmosensitive embryo which has not undergone

cytokinesis. Cytokinesis can be restored if embryos are incubated in appropriate osmotic conditions, thus we conclude that the cytokinesis defect is a secondary defect caused by the osmosensitive nature of the embryos.

470. A transgenic line produces post-prophase I chromosome segregation abnormalities in the female germline

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We have isolated a transgenic that produces a high proportion of self-progeny males due to a defect in segregation of the X chromosome. The gene responsible for this phenotype is encoded by C05D2.5, which is located to the right of dpy-17 on chromosome III (Left). RNAi assays confirm the role for this gene in meiotic chromosome segregation as worms treated with C05D2.5 dsRNA also exhibit a high proportion of self-progeny males. Chromosome defects are not seen in prophase I of meiosis and therefore, this gene must be acting at some point past this phase. Furthermore, recombination, which occurs during prophase I, proceeds normally. C05D2.5 expression is enhanced in the germline. Furthermore, chromosome segregation abnormalities occur only in the oocyte line of the hermaphrodite and involve the autosomes as well as the X chromosome. Our numbers indicate that there is a high incidence of chromosome loss, however, it appears that chromosomes do segregate in a normal fashion most of the time. We are currently testing whether the phenotype produced by this transgenic is a result of co-suppression. We are also attempting to look at the one-cell embryo to observe any chromosome or spindle defects in meiosis I or II that occur after fertilization in these transgenic animals.

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471. Control of M-Phase Entry during Oocyte Meiotic Maturation

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Oocyte meiotic cell cycle progression must be precisely coordinated with ovulation and fertilization in order to form a diploid zygote. In *C. elegans*, fully grown oocytes arrest at diakinesis of meiotic prophase I. Oocyte meiotic maturation (nuclear envelope breakdown, cortical rearrangement, and meiotic spindle assembly) is triggered by an extracellular signal provided by the major sperm cytoskeletal protein (MSP) (1). Following meiotic maturation, oocytes undergo ovulation and fertilization. How does MSP signaling promote oocyte meiotic maturation? MSP signaling has been shown to activate the conserved MAP kinase pathway (1), which plays a critical role in meiotic maturation in other systems. Nuclear envelope breakdown during meiotic maturation involves the function of the *cdc2* homolog *ncc-1* (2) and the polo-like kinase homolog *plk-1* (3); however, the intermediate steps are not clear. We screened a large-collection of temperature sensitive (ts) sterile mutations for strains that exhibit defects in oocyte meiotic maturation and ovulation. One of the mutant strains isolated from this screen, *std-1* (*tn691*ts), for stuck in diakinesis, is defective in the transition from prophase to M-phase, when hermaphrodite L4 larvae are shifted to the restrictive temperature (25^{O}C). Mating to wild-type males fails to rescue the Std mutant phenotype, suggesting that the diakinesis block results from a defect in the oocyte or somatic gonad. When embryos are shifted to 25^{O}C, however, the animals complete spermatogenesis and grow to sterile adults. In both shifts, no apparent somatic defects are observed in the sterile adult animals. Since the control of M-phase entry is regulated by MSP signals, and MSP activates MAP kinase in oocytes, *std-1* could be involved in a highly conserved signal transduction pathway. We will present the preliminary results from our genetic and phenotypic analyses on *std-1*.

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472. *daz-1* , a *C. elegans* homolog of DAZ (Deleted in Azoospermia), localizes to the cytoplasm of mitotic germ cells, and required at the entry phase of meiosis during oogenesis

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The *DAZ* family genes encode an RNA-binding protein and are involved in germ cell development in a variety of organisms. Human *DAZ (Deleted in Azoospermia)* is located on the Y chromosome and essential for spermatogenesis. *Drosophila boule* is required for the entry to meiosis during spermatogenesis, and is expressed only in males. Mice deficient in *Dazla*, a mouse homolog of *DAZ*, are sterile and exhibit defects in germ cell development and survival in both sexes. We isolated *C. elegans DAZ* homolog, *daz-1* , and showed previously that the hermaphrodite *daz-1* null mutants were sterile due to the arrest of oogenesis at the pachytene stage of meiosis I. In contrast, spermatogenesis was not affected in both *daz-1* males and hermaphrodites.

Close observation of the DAPI-stained gonads of the *daz-1* mutant revealed that a large fraction of the nuclei near the mitotic region appeared abnormally condensed, although some "crescent"-shaped nuclei were observed, as normally seen in the transition zone. The further proximal area of the *daz-1* gonad was filled with "pachytene"-like nuclei, which were smaller than the wild type. These observation suggest that *daz-1* may be required at the entry of meiosis to facilitate the progression of the pachytene stage during oogenesis.

We produced affinity-purified polyclonal antibodies against the DAZ-1 protein. In immunoblot analysis, these antibodies recognized triple bands close to the expected molecular mass in the wild type extract. Using these antibodies, we analyzed expression and localization of DAZ-1 by immunostaining. DAZ-1 was detected only in the cytoplasm of the germline, starting from L2 through adulthood. In the hermaphrodite germline, DAZ-1 was stained intensely in the mitotic region and in the transition zone. Progression of meiosis was accompanied by a decrease of DAZ-1 expression, and DAZ-1 staining faded out around the middle of the pachytene stage. DAZ-1 staining was observed also in the male germline, but the level of expression was much lower than that in the hermaphrodite.

To examine the regulation of DAZ-1 expression, we stained gonads of various germline mutants. In the *gld-2 gld-1* mutant, whose germ cells fail to enter meiosis and continue mitosis, DAZ-1 staining was observed throughout the germline. On the other hand, DAZ-1 expression was much lower in the *glp-1(ts)* mutant than in the wild type. We concluded that DAZ-1 is expressed intensely in mitotic germ cells but weakly in germ cells that has already entered meiosis. In the *gld-2 gld-1; glp-1* mutant, which showed the same phenotype as the *gld-2 gld-1* double mutant, DAZ-1 expression was also observed throughout the germline, indicating that DAZ-1 expression is not directly regulated by *glp-1* .

These results suggest that, in the mitotic and early meiotic germ cells, DAZ-1 may regulate post-transcriptionally the expression of genes that are needed for the progression of early meiosis.

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473. Hyper-resistance of meiotic cells to radiation due to a strong expression of a single *recA*-like gene in *Caenorhabditis elegans*

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Double-strand breaks (DSBs) of DNA, caused by ionizing radiation and some chemical agents, are the most dangerous forms of damage to genetic material. All cells possess repair systems for such breaks, however, in certain cells DSBs occur normally in processes such as meiosis and the generation of diversity in the immune system in vertebrates. Little is known about the sensitivity of such cells to ionizing radiation and DNA damaging agents. Therefore, we examine the sensitivity of both meiotic cells and somatic cells of the nematode *C. elegans* to X-ray irradiation and its correlation to the expression of the *Ce-rdh-1 (Ce-rad-51)* gene (*C.elegans rad51 dmc1/lim15* homolog *1*)using an RNA interference (RNAi) method. As the results, the meiotic pachytene nuclei in the *C. elegans* gonad were hyper-resistant to X-ray irradiation, but not to UV irradiation, whereas the early embryonic cells after fertilization and the full grown oocytes were not. The hatching rate of the eggs laid during 0-8 hours after irradiation with 40 or 100 Gy of X-ray (early embryonic cells and full grown oocytes) was 61 and 12%, respectively. The hatching rate during 8-22 hours (pachynene nuclei) did not decrease by more than 5% at 100Gy. The *Ce-rdh-1* gene, which is essential for the meiotic process, is the only bacterial *recA*-like gene in the nematode genome, and was strongly expressed in the meiotic cells. Following silencing of the *Ce-rdh-1* gene with an RNA interference method, the meiotic pachytene nuclei became more sensitive to X-ray irradiation than the early embryonic cells. The hatching rate of eggs laid by *Ce-rdh-1* RNAi adult hermaphrodites during 0-8 hours and 8-24 hours after 20 Gy of X-ray irradiation was 70% and 10%, respectively. These results indicate that meiotic cells are hyper-resistant to extragenic DNA double strand breaks due to the high level of expression of the enzyme(s) involved in meiotic homologous recombination. In this presentation, we would like to also discuss the function of *Ce-rdh-1* gene for meiotic process.

474. him-8 and him-5

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The autosomal genes him-5 and him-8 affect X-chromosome disjunction and recombination. him-5 mutants also have detectable, but less severe, effects on autosomal disjunction. Each of the genes has been localized to specific chromosomal regions by mapping and transformation rescue experiments. Further molecular analysis will be described. For him-8, two different but overlapping cDNAs have been identified, each of which has given a Him phenotype by RNAi. One transcript from the putative gene has a very long (200 nt) 5' UTR which appears to be highly conserved in C. briggsae. The 3' end of the gene has not yet been identified. For the him-5 rescuing region, three different candidate genes were predicted by Genefinder or by the Intronerator. Despite extensive effort, we have been unable to verify that two of these predicted genes are transcribed, and him-5 mutants do not have lesions in these candidate genes. The third gene is not only transcribed but also alternatively spliced. This is our best candidate for him-5, and several different cDNAs have been isolated from this gene. RNAi is being attempted with these cDNAs. We hope to be able to report those results.

475. Special handling for the X chromosome in meiosis

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During prophase of meiosis I, homologous chromosomes must locate their partners, associate tightly along their lengths, and undergo recombination to ensure proper segregation at the first meiotic division. We are interested in the mechanisms that underly these processes, and have used a functional genomics approach to screen for components of the meiotic machinery in C. elegans. Microarray analysis has identified a set of genes whose expression is enriched in the C. elegans germline relative to somatic tissues (1). We used RNAi to inhibit the function of a subset of these genes that had expression profiles similar to those of known meiosis genes. For a high percentage of the genes analyzed, RNAi elicited germline phenotypes including meiotic defects, gonadogenesis defects, and abnormal chromatin in the mitotic zone of the germline.

Among the genes for which RNAi caused a defect in meiotic chromosome segregation, one gene, T09E8.2, had a property that set it apart. Specifically, the relative frequencies of inviable embryos and males produced by affected animals suggested that depletion of T09E8.2 activity preferentially affected the X chromosome. It is not altogether surprising that the X chromosome could be more susceptible to perturbations that affect chromosome behavior, since the X has several properties that distinguish it from the autosomes. For example, each of the autosomes has a central gene-rich region in which recombination is suppressed, whereas both physical density of genes and recombination are more uniformly distributed on the X (2). Further, germline-enriched genes are notably absent from the X chromosome (1). X chromosomes also exhibit distinct chromatin properties during meiotic prophase (3,4). T09E8.2 therefore provides an entry point for understanding how general meiotic mechanisms intersect with special properties of sex chromosomes.

The T09E8.2 RNAi phenotype was reminiscent of the meiotic defects we had previously observed in him-17 mutants. The meiotic segregation of both the autosomes and the X chromosome are affected in him-17 mutants, but the X chromosomes are much more strongly affected. There is a high frequency of achiasmate chromosomes in oocyte nuclei, presumably reflecting a defect in a mechanism that ensures crossing over. Interestingly, there is an alteration in both the frequency and distribution of crossovers along the X chromosome in him-17 mutants: recombination levels are elevated in a small region at the left end but are reduced along the remainder of the chromosome, suggesting a defect in associations between X chromosomes.

Our genetic mapping data had localized him-17 to a 2 Mb interval containing T09E8.2. We therefore sequenced T09E8.2 in three independently isolated him-17 mutants and identified mutations in all three alleles. This demonstrates how the data from our RNAi screen can greatly aid in the cloning of genes identified by forward genetic screens by reducing the need for extensive mapping of mutants. We are currently investigating the role of HIM-17 during meiotic prophase. Initial analysis using fluorescence in situ hybridization indicates that in him-17 mutants, X chromosomes can initially pair but this paired state may not be stabilized. We are examining different chromosome regions for premature loss of pairing, and are exploring whether loss of pairing might reflect premature dissociation of synaptonemal complex proteins.

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476. Genetic interplay between *rad-51* and other genes involved in meiosis

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The Rad51 protein is the eukaryotic homolog of Escherichia coli RecA; it mediates DNA strand-transfer and is a key molecule in homologous recombination and in double strand breaks repair. *RAD51* homologous genes have been found in several eukaryotes (fungi, plants, anfibes, birds, and mammals). However, the exact function and regulation of these genes in higher eukaryotes is still to be elucidated. In order to define the steps of gametogenesis in which the only *C. elegans RAD51* homolog (*Cerad-51*) is required, we decide to study epistasis relationships between this gene and other well characterized *C. elegans* genes in meiosis.

Genes supposedly acting in closely spaced steps of meiotic recombination are expected to exhibit similar phenotypes. The appropriate choice of phenotypes to be followed and of mutants to be analysed is crucial in epistasis studies.

Based on our observations we can outline a simple model of action of RAD-51 during gametogenesis:

a) RAD-51 is required at the end of the replicative stage of oocyte maturation, in order to eliminate accidental DNA damages occurred before the onset of meiotic prophase

b) RAD-51 performs its strand transfer activity after DSBs induction and it is required both for inter-homolog exchange and for sister chromosome exchange, failure of both these events leads to gross abnormalities in chromosome shape and triggers a meiotic check-point.

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477. Identifying new genes that function in meiotic DNA repair and double-strand break initiation

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Meiotic recombination is accomplished by a modified version of the general pathway for repair of double-strand DNA breaks (DSBs). During meiosis, recombination events are initiated by a deliberate induction of DSBs by a conserved topoisomerase-like enzyme, called SPO-11. Repair of these breaks is achieved using core components, such as MRE-11 and RAD-50, that are also crucial for repair of DNA damage induced by exposure to genotoxic insult. These general repair activities work in conjunction with meiosis-specific proteins, some of which function specifically to promote the crossover outcome of initiated recombination events, such as *msh-5* and *him-14*. While a core of widely conserved proteins that play key roles in this process have been identified, for a variety of reasons, it is likely that there are additional important players yet to be discovered. Thus, we are implementing two strategies to identify additional components of the meiotic machinery with particular emphasis on genes that function in DSB formation and/or meiotic DNA repair.

The first strategy employs a collection of mutants identified in the lab that are known to be defective in meiotic chromosome segregation and formation of chiasmata (the cytological manifestations of crossovers), but are otherwise largely uncharacterized. Studies from our lab of the conserved recombination genes *mre-11*, *spo-11*, and *msh-5* have provided us with a framework for designing two key assays to identify genes that function either in meiotic DNA repair and/or in DSB formation. The first assay exploits differences in the responses of mutant germ cells to ionizing radiation (IR) as a means of classifying mutants defective at different steps in the recombination process. Mutants that are defective in meiotic DNA repair, such as *mre-11*, exhibit both cytologically visible chromosomal abnormalities (reflecting failure of normal repair) and a complete loss of progeny survivorship in response to IR during meiotic prophase. In contrast, for mutants defective only in meiotic double-strand break initiation, such as *spo-11*, IR will bypass the defect in recombination initiation resulting in increased chiasma formation and a corresponding increase in progeny survivorship (reflecting improved segregation). These two types of responses are distinct from the response of mutants competent for repair but specifically defective in crossing-over, such as *msh-5*, which exhibit neither the improvement in chiasma formation seen in *spo-11* nor the meiotic radiation sensitivity seen in *mre-11* in response to IR treatment during prophase.

A second assay for mutants with possible DNA repair defects is based on our observation of a progressive loss of reproductive capacity in *mre-11* mutants. Although *mre-11* homozygotes derived from heterozygous parents are fully viable and produce a normal number of embryos (most of which die because of meiotic segregation defects), there is a marked drop both in the number and in the survivorship of embryos produced by succeeding generations. As a result, the strain cannot be propagated as a homozygous stock. This progressive loss of fecundity and viability sets *mre-11* mutants apart from many other meiotic recombination-defective mutants and implies that *mre-11* plays a role in an essential cellular process. An example of such a role that has been proposed for *mre-11* is a role in repairing DSBs that arise as a by-product of replication. Other mutants that cannot be propagated as a homozygous stock may also play a role in an essential DNA repair process. We are currently screening our collection of Him mutants for a DNA-repair-defective or initiation-defective phenotype using these two assays.

The second strategy for identifying new meiotic repair proteins involves a collaboration with Simon Boulton and Marc Vidal, who have provided us with a list of candidates shown to interact with MRE-11 in the yeast 2-hybrid system. We are using RNA interference to test whether any of these genes function in meiotic recombination.

478. Regulation of gene expression, cellular localization, and in vivo function of *Caenorhabditis elegans* DNA topoisomerase I

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The transcriptional regulation of *C. elegans* DNA topoisomerase I gene was investigated by mRNA localization and reporter gene expression in *C. elegans*. The mRNA was expressed in the gonad and in the early embryos, followed by a rapid decrease in its level during the late embryonic stage. A reporter gene expression induced by the 5'-upstream DNA sequence appeared at the comma stage of embryos, continued through the L1 larval stage, and began to decrease gradually afterwards. The DNA topoisomerase I protein was immuno-localized in nuclei of meiotic gonad cells and interphase embryonic cells, and unexpectedly in centrosomes of mitotic embryonic cells. Double-stranded RNA interference of the DNA topoisomerase I gene expression resulted in pleiotropic phenotypes showing abnormal gonadogenesis, oocyte development, and embryogenesis. These phenotypes, along with expressional regulations manifest that the DNA topoisomerase I plays important roles in rapidly growing germ cells and embryonic cells.

479. The yolk proteins and the vitellogenin receptor of *Rhabditis (Oscheius) pseudodolichura*, strain CEW1

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----*R. (O.) pseudodolichura* yolk polypeptides are very similar in size to the *C. elegans* homologs (Winter, 1992; Comp. Biochem. Physiol. **103B**:189-196, 1992). Nevertheless, *CEW1-vit-6* and *Ce-vit-6* differ in their codon preferences and intron positioning (Winter et al., Mol. Biol. Evol. **13**:674-684, 1996). In *C. elegans, vit-6* codes for the precursor of YP88 and YP115 (Spieth and Blumenthal, Mol. Cell. Biol. **5**:2495-2501, 1985). Thus, the homologs of YP88 and YP115 in CEW1, VT2 and VT3, must be coded by *CEW1-vit-6*.

----We expressed three small polypeptides corresponding to terminal and central portions of CEW1-VIT-6. P40 corresponds to amino acid residues 20 to 665; P49 to amino acid residues 366 to 778 and P26 to amino acid residues 1413 to 1648. Antisera against each of the recombinant polypeptides were raised in Balb/c mice. Anti-P26 recognizes VT2, the homologous of YP115. Anti-P40 recognizes VT3, the homologous to YP88. This positioned VT2 at the C-terminal portion and VT3 at the N-terminal portion of CEW1-VIT-6. This arrangement was corroborated by N-terminal sequencing of VT2 and VT3 by automatic Edman degradation. The specificity of those antibodies was confirmed by immunofluorescence microscopy over CEW1 adult hermaphrodite worms. Neither of the antibodies recognized the *C. elegans* homolog yolk polypeptides. P49 is insoluble even in 8M urea, showing a highly hydrophobic nature. When an alignment of CEW1-VIT-6 is made

against the lamprey vitellogenin we observe that P49 amino acid sequence aligns to a lipid interacting region of the cyclostome vitellogenin (Anderson et al., Structure **6**:895-909, 1998), suggesting that nematode and chordate vitellogenins show the same 3D arrangement.

----Automatic Edman degradation of VT1, the homolog of YP170 from *C. elegans*, unexpectedly showed a single amino acid sequence. This would mean that, different from *C. elegans*, the CEW1 YP170 homolog (VT1) is composed of a single polypeptide chain. Recently, M.-A. Félix found a cDNA clone which 5´-end theoretical translation matches the VT1 N-terminal sequence.

----Using Concanavalin A as a probe against the yolk polypeptides transferred to a nylon membrane after SDS-PAGE we have been able to detect the presence of mannose and/or galactose covalently linked to VT1, VT2 and VT3. Although VT1 is the major yolk polypeptide, the Con A reaction was more intense with VT3 than either VT1 or VT2.

----The vitellogenin receptor of CEW1 was characterized through a ligand-blotting assay using purified fluorescein labeled yolk proteins and anti-fluorescein peroxidase labeled secondary antibodies. Using this approach on SDS-PAGE fractionated proteins we have been able to show that CEW1 contains a 107 kDa membrane polypeptide that interacts specifically with the fluorescein labeled yolk proteins. This 107 kDa polypeptide have almost the same size of the *C. elegans* vitellogenin receptor, coded by *rme-2* (Grant & Hirsh, Mol. Biol. Cell **10**:4311-4326, 1999).

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480. The ClC channel CLH-3 is activated during meiotic maturation in *C. elegans oocytes*

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C. elegans oocytes express an inwardly rectifying Cl^- channel that is activated by cell swelling and is encoded by the ClC gene *clh-3*. The biophysical characteristics of CLH-3 are virtually indistinguishable from heterologously expressed mammalian ClC-2. CLH-3 and ClC-2 share 40% amino acid identity. We have suggested that CLH-3 and ClC-2 are orthologs.

The amount of swelling required to activate CLH-3 varies by >50-60 fold between different oocytes. CLH-3 in small, early stage oocytes requires substantially more volume increase to activate compared to larger, later stage oocytes. Upon completion of growth, oocytes undergo meiotic maturation and are ovulated and fertilized. In full-grown, maturing oocytes, CLH-3 is constitutively activated. Mean whole-cell Cl^- currents at -70 mV were -3.2 ± 0.3 pA/pF and -14.6 ± 2.9 pA/pF in non-maturing and full-grown, maturing oocytes, respectively. These findings suggest that either completion of growth or induction of maturation activate the channel.

To determine the role of cell growth versus meiotic maturation in CLH-3 activation, we carried out patch clamp studies on oocytes isolated from *fog-2* mutant worms. Mutations in certain *C. elegans* sex determination genes such as *fog-2* block sperm production in hermaphrodites (Schedl and Kimble, *Genetics* 119:43-61, 1988). The presence of sperm is required for normal progression of meiotic maturation. Late-stage *fog-2* oocytes reach full-grown size, but arrest in diakinesis for many hours to days (McCarter et al., *Dev Biol* 205:111-128, 1999). Since ovulation is triggered by maturation, full-grown oocytes accumulate in the gonad of *fog-2* worms. We reasoned that if completion of growth activates CLH-3, then the channel should be active in

late-stage *fog-2* oocytes. However, *fog-2* oocytes exhibited no channel activity.

Meiotic maturation was induced in *fog-2* oocytes by injecting recombinant Major Sperm Protein (MSP)38 into the uterus. CLH-3 was constitutively activated in late stage, maturing oocytes from MSP38-injected *fog-2* worms (Cl^- current = -39.7 ± 1.5 pA/pF). These results indicate that CLH-3 is activated by induction of meiotic maturation.

To further examine the role of maturation in channel activation, we carried out a series of experiments on wild type oocytes undergoing maturation *in vitro*. Shortly before maturation begins, the nucleus increases in size and migrates to the cell periphery. Meiotic maturation is marked by Nuclear Envelope Breakdown (NEBD). Oocytes with centrally located nuclei were never observed to undergo maturation after isolation from the gonad. However, when oocytes with off-center nuclei were isolated and placed in a bath chamber, all of them underwent maturation within 2-10 min (mean time to beginning of NEBD = 6.2 ± 1 min; n=9). Cell volume did not change significantly prior to and during the maturation process.

In separate experiments, we patch clamped oocytes with off-center nuclei and oocytes that had undergone NEBD *in vitro*. CLH-3 activity was not detected in oocytes with intact nuclear envelopes (Cl^- current = -2.1 ± 0.3 pA/pF). However, in oocytes undergoing maturation *in vitro*, CLH-3 activity was detected immediately upon obtaining whole-cell access. After membrane rupture, CLH-3 continued to activate until the current reached a stable plateau level (Cl^- current = -44.4 ± 6.2 pA/pF).

Taken together, our results demonstrate clearly that CLH-3 is activated during meiotic maturation rather than after completion of oocyte growth. We are currently characterizing the signaling pathways responsible for channel activation.

481. Regulation of the ClC channel CLH-3 by serine/threonine dephosphorylation

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CLH-3 is a ClC-2 Cl^- channel ortholog expressed in *C. elegans* oocytes. The channel is activated by oocyte swelling and during meiotic maturation. Activation of CLH-3 in maturing oocytes modulates ovulatory contractions of surrounding gap junction-coupled sheath cells.

The CLH-3 protein contains consensus motifs for phosphorylation by cyclin-dependent and MAP kinases, both of which play critical roles in regulating meiotic maturation and other cell cycle processes. Given these observations, we postulated that CLH-3 activation is regulated by protein phosphorylation events. To begin testing this hypothesis, we carried out a series of pharmacological studies. In control, non-maturing oocytes, CLH-3 is inactive (mean whole-cell current at -70 mV = -3.1 ± 0.3 pA/pF). When oocytes were pretreated with metabolic inhibitors for 15-20 minutes, CLH-3 activity was detected immediately upon obtaining whole-cell access (mean current = -51 ± 12 pA/pF). Dialysis of oocytes with an ATP-free, metabolic inhibitor-containing pipette solution activated CLH-3 in the absence of swelling. Incubation of oocytes with 100 nM calyculin A, a PP1/PP2A phosphatase inhibitor, inhibited swelling-induced current activation by 88 ± 4%. However, extracellular incubation and intracellular dialysis of cells for 10 min with 1 μM okadaic acid, a more potent PP2A inhibitor, had no effect on current activation induced by cell swelling.

To examine the role of phosphorylation in channel activation during meiotic maturation, we carried out a series of experiments on oocytes maturing *in vitro*. Shortly before maturation begins, the oocyte nucleus increases in size and migrates to the cell periphery. Oocytes with off-center nuclei undergo maturation within 6.2 ± 1 min after isolation from the gonad. CLH-3 is constitutively active in maturing oocytes (mean current = -44.4 ± 6.2 pA/pF). Exposure of maturing oocytes to 100

nM calyculin A completely inhibited maturation-induced channel activation whereas 1 μM okadaic acid had no effect.

Taken together, these experimental results demonstrate that serine/threonine dephosphorylation events activate CLH-3. The differential effect of calyculin A versus okadaic acid suggests strongly that protein dephosphorylation is mediated by a PP1-type phosphatase.

Almost nothing is known about the signaling pathways that regulate ClC-type Cl^- channels. We have begun to use RNA interference in an effort to identify the phosphatases involved in CLH-3 regulation. Analysis of the *C. elegans* genome revealed the presence of 15 genes that encode PP1-like phosphatases. Recent microarray studies (Hill et al., *Science* 290:809-812, 2000) have demonstrated that at least four PP1-like phosphatases are expressed in oocytes. In addition, oocytes express two novel phosphatases encoded by C24H11.1 and R13A5.11. RNA interference of CePP1β and CePP1γ produced an embryonic lethal phenotype, but had no effect on swelling-induced channel activation. Channel activity was normal and no obvious phenotypes were detected with knockdown of C24H11.1 and R13A5.11 expression.

In addition to RNA interference, we are also using yeast 2 hybrid analysis, heterologous expression and mutagenesis methods, and biochemical approaches to identify phosphatases and kinases that modulate channel activity. Our long term goal is to identify the signaling pathways and interacting proteins responsible for regulation of CLH-3 and its mammalian ortholog ClC-2.

482. Control of Oocyte Meiotic Maturation and Gonadal Sheath Cell Contraction by MSP Signaling

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Oocyte meiotic maturation and ovulation are essential biological processes required for reproduction. During meiotic maturation, *C. elegan*s oocytes undergo nuclear envelope breakdown, cortical rearrangement, and meiotic spindle assembly in an assembly-line-like fashion. Recently we found that the major sperm cytoskeletal protein (MSP) is the sperm derived signal that induces both oocyte meiotic maturation and gonadal sheath cell contraction (1). Many questions remain about this newly discovered signaling pathway. For example, how does a cytoskeletal protein function as a signaling molecule? How does MSP signal two distinct responses?

To examine MSP signaling *in vivo*, we analyzed extracellular MSP localization. Previous studies reported the intracellular localization of MSP during spermatogenesis (2). We stained mated *fog-2* females with anti-peptide antibodies generated to the N- and C- terminal regions of MSP, as well as previously described antibodies (2). In addition to intracellular staining within spermatozoa, we observed an extracellular MSP gradient, with the highest levels of MSP within the spermatheca and lower levels in the proximal gonad arm. The average extent of the observable MSP gradient is approximately 30 μm. Detection of this extracellular MSP gradient is dependent on the number of sperm present and their position in the spermatheca. By mating females with Mr. Vigorous, we have been able to detect the MSP gradient extending as far as 90 μm. These data suggest that a fraction of MSP is released from sperm *in vivo*, and the most proximal oocyte receives the highest concentration of released MSP.

MSP is a bipartite signal: MSP (106-126) is sufficient to promote gonadal sheath cell contraction, and MSP (1-106) is sufficient to promote oocyte maturation (1). In solution MSP exists as a dimer (3). To determine if dimerization is necessary for signaling, we

injected dimerization mutants (4) into females and measured oocyte maturation and sheath cell contraction rates. The dimerization mutants tested signal both oocyte maturation and gonadal sheath cell contraction at rates similar to wild-type MSP. To further identify regions responsible for promoting oocyte maturation and gonadal sheath cell contraction, we tested whether *Ascaris suum* MSP could signal in *C. elegans*. *Ascaris* MSP shares about 80% identity with *C. elegans* MSP at the amino acid level. Our results indicate that *Ascaris* MSP can signal maturation and sheath cell contraction in *C. elegans* at rates comparable to *C. elegans* MSP. We are currently targeting regions for deletion experiments that are conserved between both *C. elegans* and *Ascaris*.

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We would like to thank T. Roberts and P. Griffin for *Ascaris* clones and S. Ward and H. Smith for dimerization mutants and antibodies.

483. Reverse genetic analysis of a ubiquitin C-terminal hydrolase gene and an hnRNP homologue gene, which are important for gametogenesis in *C. elegans*.

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Gametogenesis is a highly concerted process which requires the coordination of cell cycle progression and germ cell differentiation. We have been screening the whole *C. elegans* genome for genes involved in gametogenesis, by a comprehensive RNAi method (Maeda et al., 2001). In this screen, we identified a *C. elegans* homologue of the *Drosophila squid* gene, which encodes an hnRNP, as a candidate for such genes. RNAi-mediated disruption of this gene did not inhibit growth markedly but resulted in the formation of an abnormal germline with neither sperm nor oocytes but only vacuolous germ cells in the proximal region of the gonad arm. The distal region of the RNAi animals appeared relatively normal, suggesting an indispensable role for the *squid* homologue in gamete differentiation. *Drosophila* Squid is known to have an important role in the evolutionarily conserved pathway for the export and/or cytoplasmic localization of transcripts. This cytoplasmic localization mechanism is proposed to involve the recognition of transcripts in the context of nuclear partner proteins.

Meanwhile, we isolated deletion mutants for three ubiquitin C-terminal hydrolase (UBP) genes in *C. elegans*, F07A11.4, K09A9.4 and F30A10.10. UBP functions to remove ubiquitin from its substrate by reverting the C-terminal covalent conjugation, and is one of the key modifiers of protein degradation. UBPs form a large family in *C. elegans* and other species, and are thought to have diverse biological roles in vivo. The F07A11.4 and K09A9.4 mutants showed no obvious phenotype, but the

F30A10.10 mutant showed vacuolous germ cells similar to those of *squid* homologue-RNAi animals.

To see the possible functional relation of F30A10.10 and the *squid* homologue, we performed RNAi of the *squid* homologue in the F30A10.10 mutant. Worms defective in both F30A10.10 and the *squid* homologue showed synthetic phenotypes; namely, small body size (nearly half of the N2 adult), protruded vulvae and unelongated gonads. The F30A10.10 gene product and the Squid homologue may function in the same pathway that regulates RNA export and/or localization. To further characterize the function of these genes, a screen for a mutant of the *squid* homologue is in progress.

484. The *spe-5* Gene Encodes a Vacuolar (H+)-ATPase Beta Subunit that is Required for Spermatogenesis

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Spermatogenesis in all animals requires the orderly segregation of cytoplasm during a series of asymmetric cellular divisions. During *C. elegans* spermatogenesis, retention of essential components for spermiogenesis occurs, in part, because many reside within the fibrous body-membranous organelle (FB-MO) complexes. The FB-MO complexes segregate to spermatids during their formation and much of the work in our laboratory is focused on the morphogenesis and function of these Golgi-derived organelles. Prior work indicated that *spe-5* mutants contain defective FB-MO complexes (K. Machaca and S. W. L'Hernault *Genetics* 146: 567-581), and we have cloned this gene in order to understand its molecular nature. *spe-5* was previously mapped to a 0.5 cM region of chromosome I between *unc-38* and *dpy-5*, and single nucleotide polymorphism (SNP) mapping was used to narrow this interval. Four classes of homozygous recombinant worms were created from mates between *unc-38 spe-5 dpy-5* Bristol hermaphrodites and wild type Hawaiian (strain CB4856) males. The recombinant strains were mapped against five SNPs found between N2 and CB4856 to place *spe-5* within a ~65 kb region covered by one cosmid and part of a YAC. Differential display data (M. Jiang, 2001, et. al. *PNAS USA* 98: 218-223) suggested that only one sperm-specific ORF was located in this interval, and all six *spe-5* alleles contained mutations in this gene. Five of the six *spe-5* alleles were EMS induced, and the sixth is a Tc1 insertion that arose spontaneously in a *mut-3* mutator background. The sequence of the *spe-5* gene reveals that it encodes a vacuolar (H+)-ATPase beta subunit. Vacuolar ATPases are >750 kDa proton pumping "machines" composed of at least 13 different subunits. They are found in a wide range of organisms, and utilize the energy produced by ATP

hydrolysis to acidify the lumen of membranous organelles (review by, T. H. Stevens and M. Forgac *Annu. Rev. Cell Dev. Biol.* 13: 779-808). A pH-sensitive fluorescent probe has shown that wild-type MOs become acidic during spermatid formation (K.L. Hill and S. W. L'Hernault, unpublished observations), and we presently are determining if *spe-5* spermatids have acidified MOs. While there appears to be another *C. elegans* beta subunit that is expressed somatically, there are no other sperm-specific V-ATPase subunits. Prior data suggests that a pH change accompanies spermatid maturation (Ward et al., 1983, *Dev. Biol*. 98: 70-79) and we are exploring if the spermatid vacuolar ATPase participates in this process.

485. Progress towards the cloning of *fer-14*, a gene required for fertilization in *C. elegans* sperm

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In the last act of spermatogenesis, competent sperm recognize, bind, and fuse to the oocyte. *fer-14* is a mutant that is defective in this final stage of spermatogenesis. Like the EGF-repeat containing *spe-9* gene, *fer-14* mutant sperm exhibit wild type motility, form a wild type pseudopod, localize to the spermatheca upon insemination into the female reproductive tract, and participate in sperm competition (Singson et al, *Genetics* 152;201-208, 1999). *fer-14* has been mapped to a .5 map unit interval on the right arm of chromosome I, between *lev-11* and *sur-2*. Various triply marked lines have been contructed for use in polymorphism mapping and this approach has narrowed the *fer-14* containing physical interval down to 40Kb. Utilizing the male/female microarray data from Jiang et al., a candidate gene that is expressed in a strongly sperm-enriched manner has been identified. Efforts are currently underway to generate transgenic lines containing this candidate gene and rescue *fer-14*. *fer-14* shows a unique deficiency complementation pattern to LGI deficiencies. *eDf4* and *eDf15* appear to have the same left breakpoint. However, *eDf4* complements *fer-14* whereas *eDf4* fails to complement. Physical mapping of these breakpoints using PCR should further confirm and narrow the physical region containing *fer-14*. *fer-14* is currently defined by one slightly temperature sensitive allele, which when put over a non-complementing deficiency, also has a *spe* phenotype. We are engaged in a screen for new alleles to unambigously determine the null phenotype for *fer-14*.

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486. *spe-39* and Orthologs: A New Player in Intracellular Membrane Morphogenesis in Metazoans

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spe-39 was first identified as a mutant that usually arrests spermatogenesis at the spermatocyte stage. Although the cell division that forms spermatids (budding) is often attempted, it either fails or the buds are abnormally small. Mutations in *spe-39* are unlikely to interfere with meiosis because four condensed nuclei are often seen in the arrested spermatocytes. Electron microscopy suggests that the defects in cytokinesis is related to abnormalities in the morphogenesis of ER-Golgi-derived fibrous body-membranous organelle (FB-MO) complexes, specialized organelles that play an essential role in budding and packaging of cytoplasmic constituents required for sperm function. In wild type, each FB develops in close association with a MO. In contrast, *spe-39* mutants do not form MO's, so FB's exist as isolated structures in the cytoplasm. Additionally, *spe-39* spematocytes are full of tiny vesicles, whose formation is presumably related to the defects in MO morphogenesis. In order to understand the molecular basis of this interesting phenotype, we cloned *spe-39* by transgenic complementation. This gene encodes a 522-amino acid protein and, although it is involved in membrane morphogenesis, it lacks transmembrane region(s) according to hydrophobicity analysis. Northern analysis indicates that *spe-39* is not limited in its expression to the testis. Our RNAi results indicate that the gene is essential for somatic development and suggest that the null phenotype of *spe-39* is lethal. *spe-39* has orthologs in fly, mouse and human, but not in yeast, and the worm and human proteins are 24% identical. cDNA clones of the human gene are found in more than 20 libraries including brain, germ cell, muscle, embryo, skin and kidney. This suggests that *spe-39* and its orthologs play an essential role, perhaps in all metazoans. Presently, the subcellular localization of both the worm and human proteins is being established.

487. A *C elegans* Inositol 5-Phosphatase Homologue Involved In Inositol 1,4,5-triphosphate Signaling and Ovulation.

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Ovulation in *C. elegans* is dependent on inositol (1,4,5) triphosphate (IP_3) signaling. Here, we report the genetic characterization of a Type I inositol polyphosphate 5-phosphatase (5-Ptase) and its function in ovulation. The *Caenorhabditis elegans* Type I 5-Ptase gene, *ipp-5* , is expressed in the spermetheca, pharynx, and vulva. The *ipp- 5* deletion mutant, *sy605* , shows a novel ovulation phenotype whereby the spermetheca extends further to ovulate two oocytes during a single ovulation cycle; thus indicating that IPP-5 helps to regulate spermethecal contractile behavior during ovulation and is necessary to prevent hyper-extension of the spermetheca. This defect is opposite to the result of decreased EGFR function in which the spermetheca fails to dilate. Genetic analysis indicates *ipp-5* acts downstream of *let-23. ipp-5* suppresses the sterile defect of *lin-3(rf)* and *let-23(rf). ipp- 5 interacts with others genes in the let-23 -mediated IP3 signaling pathway: it synergizes with let-23(gf), lfe-1/itr-1/dec-4 , and lfe-2 . Our data support the hypothesis that increased IP3 signaling causes increased extension of the spermetheca during ovulation and that either too much signaling or too little signaling prevents ovulation. We propose that IP3 signaling levels modulate spermetheca contractions and dilations during ovulation possibly through regulating calcium release. The expression of IPP-1 is consistent with its function in ovulation in the adult spermetheca.*

488. The F-box protein FOG-2 binds the *C. elegans* multi-ubiquitin chain binding protein-1 (Mcb1) homolog to promote spermatogenesis in the hermaphrodite

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In the C. elegans hermaphrodite, first sperm then oocytes are produced within the same somatic gonad. Based on genetic and molecular analysis, the genes *gld-1* and *fog-2* are required to promote male sex determination in the hermaphrodite germ line by the down regulation of *tra-2*, thus allowing for a transient period of spermatogenesis (Schedl and Kimble, 1988; Francis *et al.*, 1995; Jan *et al.*, 1999; Clifford *et al.*, 2000). GLD-1 directly binds the *tra-2* 3UTR causing its translational repression. The FOG-2 protein binds GLD-1 and is associated with the *tra-2* 3UTR in a ternary complex, but unlike GLD-1, FOG-2 does not make direct contact with the *tra-2* mRNA.

The FOG-2 protein contains two domains; an N-terminal F-box motif and a novel C-terminal 200 amino acid putative protein-protein interaction domain. Traditionally, F-box motif containing proteins have been associated with the ubiquitin mediated degradation pathway. Canonical F-box proteins interact with Skp1, a core component of SCF (Skp1/Cdc53/F-box, E3 ubiquitin ligase) complex, via their N-terminal F-box and recruit specific substrates for ubiquitin modification via their C-terminal protein-protein interaction domains.

The functional definition of an F-box requires that it contain the minimal sequence elements required to interact with the SCF core component Skp1. Therefore, we are conducting a directed screen of all Skp1 homologs in the *C. elegans* genome using FOG-2 as bait. Interestingly, FOG-2 is able to bind F46A9.5 and F46A9.4, which share the highest overall identity to the defining member yeast Skp1 (44% and 39% respectively). This suggests that the F-box motif in FOG-2 is functional and therefore could play a role in the ubiquitin

mediated degradation of specific substrates involving the SCF and 26S proteasome. However, RNAi phenotypes of the aforementioned Skp1 homologs were embryonic lethal so that FOG-2 related functions in sex determination remain unclear.

To define the broad scope of FOG-2 interactions, a *C. elegans* two-hybrid cDNA library (R. Barstead) was screened. The *C. elegans* Mcb1 homolog was identified as a FOG-2 interacting protein. RNAi analysis of *C. elegans* Mcb1 resulted in feminization of the hermaphrodite germ line and partial suppression of the *fem-3(q20gf)* phenotype, which is analogous to what is observed for *gld-1* and *fog-2* lf mutations. This provides *in vivo* support for an interaction between *C. elegans* Mcb1 and FOG-2. In *S. cerevisiae*, Mcb1 is a nonessential protein that co-purifies with the 19S regulatory cap of the 26S proteasome, binds multiubiquitin chains in vitro, and plays an ancillary role in substrate-specific protein turnover (van Nocker *et al.*, 1996).

While the yeast Mcb1 protein has been implicated in protein turnover, the *C. elegans* Mcb1 homolog is unlikely to be involved in GLD-1 degradation as, similar to FOG-2, the Mcb1 homolog appears to promote hermaphrodite spermatogenesis. When RNAi of the Mcb1 homolog is performed in a strain carrying GLD-1::GFP the feminizing phenotype is recapitulated but changes the GLD-1 accumulation or degradation pattern are not observed. Thus, the *C. elegans* Mcb1 homolog promotes hermaphrodite spermatogenesis and may represent another component of the FOG-2/GLD-1/*tra-2* 3'UTR translational control complex.

We propose two working models for FOG-2 as a scaffold in recruiting proteins such as Mcb1 into the framework of GLD-1 binding the *tra-2* 3'UTR and mediating its translational regulation: 1) FOG-2, perhaps in concert with Mcb1, degrades a positive regulator of *tra-2* translation. 2) FOG-2 and/or Mcb1 are involved in the ubiquitin modification, but not degradation, of another component(s) of the *tra-2* 3'UTR ternary complex. A role for F-box proteins in the direct translational regulation of a specific mRNA represents a novel function for this family of proteins.

489. The role of SPE-9 in C. elegans sperm-egg interactions.

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Fertilization is a process of fundamental importance in biology and an attractive model for understanding the molecular mechanisms of specific cell-cell interactions. Despite intense study, sperm-egg interactions are still poorly understood at the molecular level. The spe-9 gene encodes a sperm specific transmembrane protein with cysteine-rich sequences related to the epidermal growth factor (EGF) motif. The EGF motif is characterized by a conserved arrangement of six cysteine residues that form three specific disulfide bonds.

In order to gain a better understanding of the role of SPE-9 during fertilization, we are engineering specific mutations to determine the important functional domains of the SPE-9 protein. Transgenic constructs that delete several of the EGF motifs in SPE-9 (EGF motifs 2-6, 6-10, 2-10) are nonfunctional and transgenic worms are sterile. Furthermore, we have made constructs with point mutations in every EGF motif. This has provided a more precise method of determining the relative importance of each motif for SPE-9 function. These structure-function experiments are revealing important functional information and mutants for future misexpression experiments and genetics screens. To determine exactly where SPE-9 functions, we are conducting immunofluorescence experiments to localize the SPE-9 protein. We hypothesize that SPE-9 will function on the surface of the sperm to interact with molecules on the egg cell surface. These experiments should also reveal the region of mature sperm that are important for gamete interactions. Furthermore, in order to identify other genes involved in sperm function, we are focusing on the phenotypic characterization of spe-13 and spe-38 genes. Both genes produce sperm with normal morphology and motility that cannot fertilize oocytes even after contact between gametes. Worms with mutations in the spe-13 gene phenocopy spe-9 mutants. Additionally spe-13 has been shown to genetically interact with spe-9. The spe-13 gene

mapped to a small region of chromosome I. We will clone the spe-13 gene using transgenic rescue. We will use SNP (single nucleotide polymorphism) to localize and clone the spe-38 gene. In summary, the reproductive biology of C. elegans offers a unique opportunity to define sperm components required for sperm-egg interactions. Mutation analysis experiments will determine important functional domains of the SPE-9 protein. A simple model for SPE-9 function in C. elegans suggests that SPE-9 protein on sperm interacts with a receptor on the oocyte and mediates gamete recognition, adhesion and/or signaling during fertilization. Finally, this work will provide new insight into our understanding of fertilization and complement studies in other organisms.

490. MAPPING spe-19; FURTHER PROGRESS, NEW APPROACHES

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A primary goal of our lab is to identify those genes required for fertilization. Several of the spe genes fall into this class. I have been focusing my attention on the characterization and cloning of spe-19.

Hermaphrodite worms with mutations in either of the two known alleles of spe-19 (hc41 & eb52) are self-sterile and lay unfertilized oocytes. However, fertilization is rescued (cross-progeny are produced) when these otherwise healthy worms are mated to wild-type males.

Prior genetic mapping located spe-19 to the right of chromosome V, approximately 29 map units to the right of dpy-11. Additional three-factor mapping using dpy-21 and rol-9 as markers has localized spe-19 to the interval between these two genes (corresponding to approximately 12 map units), closer to rol-9. Additional three-factor mapping using unc-51 and rol-9 has further localized spe-19 to the interval between these two genes as well, closer to unc-51.

Two-factor mapping using recombinants generated during the above analysis has corroborated our results, suggesting that spe-19 lies approximately 11.5 map units to the right of dpy-21.

The putative spe-19 chromosomal location contains three deficiencies, and we have recently begun deficiency mapping to further narrow this interval. Spe-19 has been found to lie within the interval covered by the deficiency ozDF1. Additionally, primers are being designed for high-resolution mapping via PCR polymorphisms in anticipation of YAC injection and subsequent cloning via transgenic rescue.

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491. Screening for sperm competition mutants

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C. elegans hermaphrodites produce a supply of self sperm, which are normally used with extremely high efficiency (>99%). However, if a hermaphrodite mates with a male, its self sperm must compete with any transferred male sperm to fertilize oocytes. In this situation, sperm provided by the male are used preferentially(1). Male sperm precedence requires sperm motility(2) and involves displacement of self sperm from the hermaphrodite spermatheca. However, successive mating experiments have shown that sperm from different males do not exhibit any precedence relationship, indicating that sperm usage is not dictated by a "last in" mechanism of placement within the spermatheca and that there is a bona fide difference between hermaphrodite sperm and male sperm(1,3). Male sperm precedence is nearly complete: after mating, very few if any self progeny are produced until a mated hermaphrodite runs out of transferred male sperm. The strength of this effect provides a robust assay for alterations in the normal pattern of sperm usage.

To identify factors that confer male sperm advantage, we are screening for mutants that do not show the normal precedence of male sperm. Specifically, we are assaying males from mutagenized *him-5* lines for lack of precedence in crosses to *spe-8; dpy-4* hermaphrodites. *spe-8* hermaphrodites are defective in sperm activation, but *spe-8* self sperm can be *trans*-activated upon mating to a male; *dpy-4* provides a readily-scored marker for self vs. cross progeny. We expect that sperm precedence-defective males should produce sperm that are capable of fertilizing oocytes but do not suppress the production of hermaphrodite self progeny. Matings to such males should result in a mixture of self and cross progeny, and the relative numbers of self and cross progeny produced should be dependent on the numbers of each sperm type present within the hermaphrodite spermatheca. Sperm precedence mutants should be distinct from the previously described *spe* and *fer* mutants, which show defects in various aspects of spermatogenesis and/or fertilization(4).

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3. LaMunyon, C.W. and S. Ward, *Experientia* **51**:817-823, 1995.

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492. The *vab-22* gene encoding a zinc-finger like protein is required for coordinated body rotation in embryonic morphogenesis

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C. elegans morphogenesis is a complex process requiring numerous short-range migrations, rearrangements, and concerted cell movements. Several vab (variable abnormal) mutations have been identified, which disrupt essential morphogenic events, resulting in severe body deformities. We have isolated a novel vab mutation *vab-22*. The body morphology in *vab-22* mutants is variably abnormal in the region between posterior end of pharynx and vulva. In addition, morphologies of ventral and dorsal nerve cord, alae and muscle are also abnormal in this region. We examined the embryonic morphogenesis of *vab-22* embryos by using *jam-1::GFP*. When wild-type embryos enclose the ventral hypodermis, their bodies rotate 90^{o} in the eggshells (termed as body rotation). In *vab-22(km6)* mutant embryos, the body rotation occurs at posterior regions but not at anterior regions, suggesting that *vab-22* is involved in the control of coordinated body rotation. We cloned the *vab-22* gene by SNP. The VAB-22 protein contains two zinc-finger like domains similar to the DNA-binding region of mammalian DRE-binding factor DREF. These results suggest that *vab-22* may regulate the transcription of components which participate in the embryonic body rotation.

493. Lineage control of cell behavior during gastrulation

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Cells within the animal embryo separate into distinct germ layers during gastrulation. Although the morphogenetic movements accompanying gastrulation have been described for many species, the molecular mechanisms used to drive these rearrangements are poorly understood. Gastrulation in *C. elegans* involves the movement, or ingression, of cells from the ventral surface of the embryo to its interior. Since these cells can be followed individually in live embryos, the *C. elegans* embryo provides a useful model for investigating gastrulation.

In some types of animal embryos, gastrulation involves coordinated shape changes and movements by a sheet of surface cells. By contrast, gastrulation in *C. elegans* includes several examples of non-contiguous cells that ingress at different times. Since all of these cells enter from the ventral surface, we are interested in whether gastrulation is controlled by cell position or lineage. We present evidence indicating that the normal sequence of gastrulation events is not essential; at least some cells that normally ingress at relatively late stages of embryogenesis can do so even when earlier cells fail to ingress.

We have found that some cells undergo a reproducible flattening of their exterior-facing surface as they ingress. Non-muscle myosin accumulates at the cortex underlying the flattened surfaces. This flattening does not occur in cells that fail to ingress in certain mutant backgrounds. We are interested in whether flattening is required for gastrulation, and whether the mechanism that results in flattening is controlled by factors that specify cell lineage.

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494. A possible role for *gcy-31* in embryogenesis

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The nitric oxide (NO) cGMP signaling pathway is involved in many important physiological processes including neuronal signaling, axon guidance and maintenance of vascular tone. Nitric oxide synthase (NOS) synthesizes NO which diffuses to target cells, activating soluble guanylyl cyclase (sGC), generating cGMP. Soluble GC is classically a heterodimeric protein and requires an α and β subunit for catalytic activity. However, the discovery of a novel homodimeric, NO-insensitive sGC (MsGCβ_3) from the tobacco hawk moth, *Manduca sexta*, implies that other control mechanisms may exist for these proteins.

C. elegans appears to have a modified NO-cGMP pathway because no NOS gene is evident in its genome. Also, worm sGCs contain structural features likely to render them insensitive to NO. Of the seven sGC genes in *C. elegans*, we are primarily interested in *gcy-31* because it shows the highest homology to MsGCβ_3. We are using genetic and biochemical approaches to establish roles for *gcy-31* in *C. elegans* development and physiology.

We previously localized *gcy-31* expression to a pair of bilaterally symmetric neurons in the worm head using extrachromosomal array line qaEx2201[*gcy-31(3' deletion)*::GFP *rol-6(su1006)*]. Detailed analysis of this line revealed low level embryonic lethality, with some survivors showing deformations in the head similar to **v**ariable **ab**normal (vab) mutants. 4D-microscopy of developing qaEx2201 embryos identified defects in hypodermal enclosure, similar to those seen in *vab-1*, *2* and *3* mutants. Control experiments revealed these defects were caused by over expressing just the first 4 exons of *gcy-31*, as extrachromosomal array lines expressing full length *gcy-31* had almost no phenotype. These defects may be caused by titering out a transcription factor such as the Pax-6 homologue *vab-3*. However, array lines containing just the *gcy-31* promotor region were wild type. Furthermore, when qaEx2201 was crossed into *vab-3(e643)* worms their phenotypes were additive, implying that *vab-3* is not directly involved in *gcy-31* transcription.

We hypothesize that the N-terminal fragment of *gcy-31* in qaEx2201 is dimerizing with wildtype *gcy-31* to generate a catalytically inactive species which is somehow responsible for the phenotype observed. We are currently screening for *gcy-31* deletion alleles to further characterize its role during *C. elegans* embryogenesis.

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495. An analysis of the epithelial movement defect in the mutant *ct254*

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We have identified a mutant, *ct254*, that shows defects in the process of ventral enclosure, an epithelial morphogenetic event that occurs during *C. elegans* embryogenesis. Ventral enclosure is the process by which hypodermal (epidermal) cells originating on the dorsal surface of the embryo migrate along the exterior, and eventually form adhesive junctions along the ventral midline. Mutations in this process display abnormalities both in the migration of epidermal cells and in the formation of junctions along the ventral midline.

4D time lapse recordings show that *ct254* displays variable embryonic lethal phenotypes, including complete hypodermal retraction (completely unenclosed embryos in which the hypodermis retracts to the dorsal surface), head enclosure defects (incomplete migration of the anterior hypodermis), and ventral midline rupture (imperfect junction formation). The variability of phenotypes is reminiscent of the range of phenotypes observed in other ventral enclosure mutants identified in our laboratory. The organization of seam cells in *ct254* embryos appears disorganized based on analysis of expression of JAM-1-GFP. Dynamic multiphoton analyses using JAM-1-GFP suggest that rupture phenotypes may be due to a delay in migration of the ventral hypodermal cells. This delay prohibits the ventral hypodermal cells from reaching the ventral midline and forming junctions by the initiation of elongation, and thus the hypodermis is retracted. The stress of this retraction may cause the seam cells to misalign. A similar analysis using HMP-1-GFP (alpha-catenin-GFP) has shown no abnormalities in the filopodia of migrating ventral hypodermal cells, thus the reason for the delayed migration can not readily be explained.

To identify the molecular identity of this mutation, *ct254* has been mapped to +0.41 on the right arm of LGIII. Transformation rescue using cosmid DNA is currently being attempted. In addition, candidate embryonic lethal genes in the region are being tested by RNAi.

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496. Genes Mediating Elongation of the *C. elegans* Embryo

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Genes mediating elongation of the *C. elegans* embryo

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Morphogenesis of the *C. elegans* embryo results from an actin-mediated contraction of the epidermal cells that transforms a ball of cells into a long, thin worm. We have previously identified two genes, *let-502* and *mel-11*, which are essential to this process[1]. These genes also function during other contractile events including cytokenesis (see abstract by Piekny and Mains) and in the somatic gonad[2]. Based on our genetic results and analogies to smooth muscle contraction, we proposed that contraction (elongation) occurs when myosin light chain kinase phosphorylates myosin light chain. Myosin phosphatase (MEL-11) removes the activating phosphate, blocking contraction, and so loss of *mel-11* results in hypercontraction. Elongation is triggered when Rho induces Rho-binding kinase (LET-502) to inhibit myosin phosphatase (MEL-11), thereby removing the MEL-11 brake to contraction. Consistent with this, *let-502* mutants hatch (and arrest) with minimal elongation. However, the double null phenotype of *let-502* and *mel-11* appears to be near-normal elongation, implying that a redundant pathway can mediate morphogenesis[3].

To identify more genes involved in elongation, candidates similar to genes involved in contractile events in other systems were tested for genetic interactions with *let-502* and *mel-11*[2,3]. We also constructed triple mutants to determine how the genes functioned during elongation: those acting downstream or in a pathway redundant with *let-502* or *mel-11* would block the near normal elongation of *let-502; mel-11* while mutations of upstream genes would have no effect. We found that *daf-2* and *age-1* (insulin pathway) and *mig-2* (Rho/Rac-like) act upstream. *unc-73* (Rho/Rac GEF) and *fem-2* (PP2C phosphatase) appear to act in parallel to *let-502 - mel-11*, while *mlc-4* (myosin light chain) acts downstream[3]. More recently, we have found that the Rac-mediated pathway required for engulfment of cell corpses (*ced-2, ced-5* and *ced-10)* may function upstream of *let-502 - mel-11*. We are also characterizing several new genes isolated in a screen for *mel-11* suppressors.

We have generated polyclonal antibodies to LET-502 and MEL-11 and examined their expression throughout development. LET-502 and MEL-11 co-localize at cleavage furrows during early cell divisions. During morphogenesis LET-502 and MEL-11 are expressed in the epidermis, pharynx and Z2/Z3 cells. In adults, they are expressed throughout the developing germ line and spermatheca. Therefore, the expression of LET-502 and MEL-11 are consistent with their mutant phenotypes during different stages of the life cycle.

[1] Wissmann et al. (1997) Genes Develop. 11:409; [2] Wissmann et al. (1999) Dev. Biol. 209:111; [3] Piekny et al. (2000) Genetics 156:1671

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497. *vab-19* functions in epidermal elongation and encodes an ankyrin repeat containing protein.

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Epidermal elongation requires the coordination of the morphogenesis between epidermis and underlying body wall muscles. This process is mediated in part by hemidesmosome-like structures, called fibrous organelles, found in regions of the epidermis adjacent to muscle. We report here that the *vab-19* gene functions in epidermal elongation and encodes a novel conserved, ankyrin repeat containing protein that appear to be associated with fibrous organelles.

vab-19 is defined by a single cold-sensitive allele, *e1036* (isolated by Jim Lewis). At 15° C almost all of *e1036* animals arrest during embryogenesis. At 22.5 °C 90% of the *e1036* animals survive to adulthood and show variable notched-head and notched-tail morphological phenotypes (Vab). Four-dimensional Nomarski microscopy analysis shows that *vab-19* mutants are defective in elongation and have no obvious defects in earlier embryogenesis. Preliminary results of *vab-19* temperature shift experiments also suggest that *vab-19* is required in elongation.

vab-19 maps to chromosome II. We rescued the mutant phenotypes with an 11 kb DNA fragment containing a gene that encodes a novel protein with four ankyrin (ANK) repeats in the C-terminus. The *e1036* mutation results in a stop codon, creating a truncated protein lacking the ANK repeats. VAB-19 homologs, of unknown function, exist in flies, mice, and humans. A rescuing VAB-19::GFP transgene is expressed in epidermal cells, within which it is localized to or close to fibrous organelles. The C-terminal ANK repeats are not essential for this sub-cellular localization. The function of these repeats in VAB-19 is thus not yet clear.

To understand how *vab-19* acts in embryonic morphogenesis, we have begun to examine genetic interactions with *vab-19* by constructing double mutants with other known elongation defective mutations. We found that loss of function in the β_H-spectrin *sma-1* specifically suppresses *vab-19* Vab and cold sensitive phenotypes. The suppression of *vab-19* by *sma-1* suggests that VAB-19 and SMA-1 may play antagonistic roles in the epidermal cytoskeleton.

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498. A Role for VAV-1 in Pharyngeal Contraction

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VAV, VAV-2 and VAV-3 comprise a family of vertebrate guanine nucleotide exchange factor proteins that function in the activation of Rho signaling pathways. This activation occurs in the form of a VAV-mediated exchange of GDP for GTP on Rho in response to cellular stimulation. Once activated, Rho pathways promote an organized remodeling of the actin cytoskeleton, which results in processes such as cellular motility, proliferation and growth. Misregulation of these signaling pathways through specific mutations of the *vav* gene results in the constituative activation of Rho family members and oncogenesis. To better understand the role of VAV in the regulation of Rho signaling, we have undertaken a genetic and molecular analysis of VAV in *C. elegans*.

We have previously reported the cloning and expression pattern of the *C. elegans vav* homolog (*vav-1*). Briefly, *vav-1* encodes a 1000 amino acid protein that shares 34% identity with vertebrate family members and contains the domains predicted to be important for nucleotide exchange factor activity. VAV-1 is localized to pharyngeal muscle, g1 gland cells, body wall muscle, and is sometimes found in vulval tissues and somatic gonad. We have also reported that the deletion of *vav-1* results in larval lethality, presumably caused by the disruption of synchronous pharyngeal muscle contraction.

Electrophysiological analysis of *vav-1* null animals suggests that the pharyngeal defect may result from inadequate conduction between individual muscle cells. This hypothesis is based on results obtained from Electropharyngeograms (EPG) performed on the *vav-1* mutant: EPG analyses of *vav-1* animals produce traces in which the depolarization wave is extended over an abnormally long period of time relative to wild type EPGs. This might indicate that each pharyngeal muscle cell is depolarizing at its own pace, uncoupled from other cells. That *vav-1* mutant pharyngeal muscle tends to fibrillate, rather than pump, supports the uncoupled hypothesis.

We are now performing experiments designed to further study the *vav-1* null phenotype. Recently, we identified that a limited expression of *vav-1* in the pharynx was enough to rescue this lethality. Further tissue-specific expression studies are now attempting to localize the *vav-1* requirement to pharyngeal muscle cells or g1 gland cells. We are also performing a directed mutational analysis to identify the potential for VAV-1 to function in nucleotide exchange.

499. Pharynx tubulogenesis during C. elegans development

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During C. elegans embryogenesis, the pharyngeal primordium develops from a ball of cells into a linear tube connected anteriorly to the buccal cavity and posteriorly to the midgut. Using GFP reporters localized to discrete subcellular regions, we have shown that pharyngeal tubulogenesis occurs in three stages: i) lengthening of the nascent pharyngeal lumen by reorientation of apicobasal polarity of anterior pharyngeal cells (Reorientation), ii) formation of an epithelium by the buccal cavity cells, which mechanically couples the buccal cavity to the pharynx and anterior epidermis (Epithelialization), and iii) a concomitant movement of the pharynx anteriorly and the epidermis of the mouth posteriorly to bring the pharynx, buccal cavity and mouth into close apposition (Contraction) (1). We call this three-step process pharyngeal extension. We have undertaken two approaches to identify loci required for pharyngeal extension. First, we have used RNA interference to determine the role, if any, of candidate genes previously shown to be expressed in the pharynx. Second, we are undertaking a mutagenesis screen to identify mutants that generate pun (pharynx unattached) phenotypes but are otherwise largely normal. From 2000 haploid genomes, we have recovered seven Pun mutants. Our current goals are to characterize the phenotypes of the mutants and to continue screening. 1. MF Portereiko and SE Mango, Morphogenesis of the C. elegans Pharynx, Dev. Biol, in press.

500. Molecular analysis of *pel-2* and *pel-3*, two genes required for embryonic pharynx differentiation and morphogenesis

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The pharynx is comprised of five different cell types, all of which derive from two blastomeres in the early embryo, ABa and MS. How are cells that arise from disparate lineages specified and patterned to form this single, coherently functioning organ? While some of the genes that impart pharynx-specific identity to the five pharynx cell types have been characterized, the mechanisms that regulate pharynx morphogenesis and differentiation are largely unknown. To address these problems, we are investigating two genes required for embryonic pharyngeal development, *pel-2 IV*and *pel-3 II* (pharynx and elongation defective). Both genes were identified from mutations isolated in a genome-wide screen for zygotic embryonic lethal mutants (JH Rothman, 8th International C. elegans Meeting).

The *pel-2* mutant shows a block in embryonic elongation, with arrest occurring at the comma to 1.5-fold stage. The pharynx neither elongates nor displays any signs of differentiation or morphogenesis (e.g., a lumen, grinder, buccal capsule, or muscle striations). The *pel-3* mutant arrests at the two- to three-fold stage with a partially elongated pharynx that never attaches to the buccal opening and with variable defects in differentiation and morphogenesis (e.g., the grinder, lumen, and buccal capsule are variably absent).

We are analyzing the expression of pharynx markers and the behavior of pharyngeal cells in an effort to assess whether the defects we observe arise from defective differentiation or morphogenesis of the pharynx. To test whether the *pel* genes act within the known pathway of regulators for pharynx development (e.g., *pha-1, pha-4*, and *ceh-22*), we are examining expression of these genes in *pel* mutants and are investigating genetic interactions between them.

We have narrowed the chromosomal positions of both *pel* genes using SNIP-SNP mapping in an effort to clone them. By dissecting the function of *pel-2* and *pel-3* genetically and molecularly, we hope to gain further insights into how organ development and assembly are molecularly orchestrated.

501. Comparative and functional analyses of the novel pharyngeal factor PEB-1

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The *C.elegans* pharynx is a complex neuromuscular organ consisting of five distinct cell types: muscles, neurons, glands, epithelial, and marginal cells. Pharyngeal cell-type specific gene expression is regulated in part by organ-specific signals. In the pharyngeal muscle myosin gene, *myo-2*, these signals target an enhancer sequence called the *C* subelement. The *C* subelement contains overlapping binding sites for PHA-4, a winged helix transcription factor, and PEB-1, a novel factor that may define a new class of DNA binding proteins. PEB-1 and PHA-4 are co-expressed in many pharyngeal cell types and we hypothesize they participate in organ-specific regulation of gene expression.

PEB-1 is a novel 427 amino acid protein with a unique DNA binding domain. To help recognize functionally important regions in PEB-1, we have identified a *peb-1* homologue from the related nematode *C. briggsae* (1). *peb-1* intron/exon structure, splice acceptor and splice donor sites are highly conserved between the two species. Overall, the predicted *C. briggsae* PEB-1 protein is 77% identical to *C. elegans* PEB-1. The PEB-1 DNA binding domain exhibits 84% identity over 158 amino acids. While this level is higher than the overall sequence identity, it is lower than that found in other DNA binding domains that have been characterized in both species. A match to a consensus sequence found in the *Drosophila Mod(mdg4)* proteins is found in both *C. elegans* and *C. briggsae* PEB-1. Among the most highly conserved regions are a putative PEST sequence located near the N-terminus and a Cys-rich region at the C-terminus. We hypothesize the DNA binding domain and these conserved regions are important for PEB-1 function.

To better understand PEB-1s role in regulating pharyngeal gene expression, we are characterizing how PEB-1 interacts with PHA-4 and with DNA. We have observed no evidence for cooperative binding between PEB-1 and any

of the three PHA-4 isoforms *in vitro* (2). Rather PEB-1 competes effectively for PHA-4 binding to the *C* subelement, suggesting these proteins may not bind the *C* subelement together. Indeed when PEB-1 and PHA-4 are coexpressed *in vivo* using a heat shock promoter, PEB-1 interferes with PHA-4s ability to activate the *C* subelement enhancer (3). We are currently identifying a consensus PEB-1 binding site by PCR-assisted binding site selection to facilitate identification of other genes that may be targeted by PEB-1.

1. Genome Sequencing Center, Washington University, personal communication.
2. We thank J. McGhee and J. Kalb for PHA-4 protein.
3. See abstract of A. Fernandez and P. Okkema.

502. Involvement of septins in development of the pharynx

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The septins are a family of proteins often essential for cytokinesis in organisms ranging from yeast to mammals. They are also expressed in some post-mitotic cells, indicating that they function in other cellular processes . Two septins, encoded by the *unc-59* and *unc-61* genes, were recently identified in *Caenorhabditis elegans*, and have been described as having no essential function in embryogenesis . Many of the septin mutant phenotypes are common, but not restricted, to mutants affecting cell division. These include defects in the male tail, protruding vulvae, uncoordination, and gaps in the alae. A new phenotype that we found at low penetrance for the septin mutants, defects in the hermaphrodite tail, has not been previously described for cell cycle mutants, but is seen in a hypodermal fusion mutant, as are male tail and vulval defects (W. Mohler, personal communication).

We now find that loss of septin function results in approximately 20% embryonic lethality. Of those larvae that hatch, up to 50% do not survive the first larval stage. Closer examination revealed that the L1 lethality was due to defects in the formation of the pharynx. The defects range from pharynges that do not appear to have properly elongated and have not attached to the buccal cavity, to pharynges that appear morphologically normal, but are detached, to those that are blocked by deposition of cuticle in the buccal cavity. L1 lethality in all cases results from the worms inability to feed. This phenotype has not been described before for mutations affecting the cell cycle or cell fusions, and may indicate a direct role for the septins in organogenesis of the pharynx. We are currently using *pha-4::GFP::CAAX* strains (gift of Susan Mango) to determine if these defects result from failures in cytokinesis, and performing indirect immunofluorescence to localize the septins in the developing pharynx. Preliminary results suggest that, in the case of the most extreme mutant phenotype, there are no obvious early failures in cell division. By analogy to studies on septin function in other organisms, potential roles for the septins in morphogenesis include

polarized secretion, activation or assembly of machinery necessary for the synthesis or assembly of cuticle structures, a scaffold for assembly of other proteins (such as signaling molecules), interaction with the actin cytoskeleton, and the specification of membrane subdomains.

1. Longtine, M. S., DeMarini, D. J., Valencik, M. L., Al-Awar, O. S., Fares, H., De Virgilio, C. & Pringle, J. R. (1996) *Curr. Opin. Cell Biol.* **8,** 106-119.
2. Nguyen, T. Q. & White, J. G. (2000) *J. Cell Sci.* **113,** 3825-3837.

503. The cloning and characterization of *gob-1*, a gene affecting intestine development.

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Our lab is interested in intestine development and discovering genes, perhaps transcription factors, that direct differentiation of the intestine. A genetic screen to isolate mutated genes that give rise to gut specific defects has been developed. With a quarter of the screen completed, three candidate genes have been found and one candidate, *gob-1 (g*ut *ob*structed), is being characterized in detail. The *gob-1* homozygous mutation shows predominately an L1 lethal phenotype with a blocked or malformed lumen that results in a stuffed appearance. The mutant intestine appears distinct from the phenotype produced by the *elt-2* null mutant. For example, the *gob-1* mutant intestine has intense gut granules and antibody staining with MH33 (a gut specific intermediate filament) appears weak and patchy, suggesting a disorganization of the intestinal cell terminal web.

gob-1 is in the process of being cloned. Using a combination of genetic markers and SNIP-SNPs, *gob-1* was located at the far right hand side of the X chromosome. Using deletion complementation, the gene was found to lie between the end point of mnDf43 (21.7609) and *lin-15* (22.33). This area covers 230 Kb and contains 12 predicted open reading frames. Sequencing and transgenic complementation are being used to confirm the mutation that is causing the Gob-1 phenotype.

504. Analysis of *glo-1* which lacks autofluorescent and birefringent intestinal granules

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GFP reporters are very useful in determining the expression pattern, localization, and function of genes in *C. elegans*. However, the analysis of GFP expression can be hindered by the strong fluorescence emitted from the birefringent and autofluorescent granules (called gut granules) present within the cytoplasm of intestinal cells. Here we report the identification and characterization of *glo-1*, a mutant that lacks gut granules. The loss of gut granules in the *glo-1* strain make it ideal for the analysis of GFP expression patterns.

Gut granules first appear in the intestine of bean stage embryos at a time when the polarizing intestinal cells associate into an epithelium. During epithelial polarization, gut granules become asymmetrically positioned near the future basal membrane. In screens for intestinal polarity mutants, we identified a mutant called *glo-1* (for Gut granule LOss) that causes gut granules to be targeted apically and to be secreted into the intestinal lumen during embryogenesis. *glo-1* mutants also lack autofluorescent and birefringent gut granules in larval and adult stages. Homozygous *glo-1* animals are reasonably healthy and fertile indicating that gut granules are not essential for viability. We are currently cloning the *glo-1* gene.

Gut granules may represent a specialized endocytic compartment involved in storage or degradation. Therefore, defects in polarized vesicular transport or targeting might result in the secretion of gut granules seen in *glo-1* mutants. In the future, we will analyze the role of *glo-1* in these processes.

505. *elt-4*, the worm's (and possibly the world's) smallest GATA factor.

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We are studying transcriptional regulation during formation of the *C. elegans* intestine. The *elt-2* gene encodes a single zinc-finger GATA factor that is involved in gut development beginning at the 2E cell stage; *elt-2* mutants die as L1 larvae with obstructed and malformed intestines. Genefinder predicts that *elt-2* really has two zinc fingers (the rule in vertebrates), connecting a small ORF 5 kb upstream to the main body of *elt-2* that we previously described. However, we could find no evidence (RT-PCR and Northerns) that such a two-finger GATA factor exists, even as a minor alternative product of *elt-2*. What RT-PCR did show, however, is that message for this small upstream ORF (named *elt-4*) could be detected in worm RNA. The transcript begins ~ 160 bps upstream of the predicted ATG, has only one intron (in the same position as in other GATA factor zinc fingers) and is poly-adenylated. The latest ACeDB version shows that a Yuji Kohara cDNA has also been identified.

elt-4 is predicted to encode a **very** small GATA factor, containing only 72 amino acids (~8kD). The predicted size is perilously close to the lower size limit necessary to bind DNA (determined by Omichinski et al. with the second finger of chicken GATA-1). Essentially, ELT-4 is a zinc finger + adjacent basic domain with very little else. However, parts of the sequence are highly conserved with ELT-2; the fingers are identical in 24/25 residues and a pentapeptide at the N-terminus is completely conserved. A plausible model is that, at some point in evolution, *elt-2* was duplicated to produce *elt-4*, which subsequently suffered gradual pruning to produce its current minimalist state. Perhaps, this is an intermediate step in the process of evolving a two-finger GATA factor. So, what does *elt-4* do? *elt-4*::GFP constructs (~5 kb 5'-flanking region) are expressed in the embryonic gut, as well as ~ 6 cells in the posterior pharynx. RNAi produces

a low-penetrance "gob" phenotype ("gut obstructed", the same phenotype produced by *elt-2*). However, it is difficult to rule out cross reactivity with *elt-2*. We have recently isolated a deletion mutant that completely removes the *elt-4* coding region, by PCR screening of Bernie Lemire's library. First indications are that *elt-4* deletions are viable; they are now being outcrossed before closer examination of any possible gut phenotype. Ectopic expression of *elt-4* cDNA within the embryo (by means of the heat shock promoter) does **not** produce ectopic expression of our standard gut differentiation markers, such as *ges-1* and MH33; heat shocked embryos reach 3-fold stage and often fail to hatch but this phenotype is much less severe than with heat shock *elt-2* or heatshock *end-1*. When *elt-2* and *elt-4* are co-expressed ectopically, the phenotype is no more severe than with *elt-2* alone; perhaps the expression level of *elt-2* and MH33 are reduced somewhat (perhaps suggesting that *elt-4* may act as a negative regulator) but the effect is not pronounced. Our current view is that *elt-4* plays a minor role in gut development, perhaps more in the nature of fine tuning of expression levels. Future work will centre on the *elt-4* phenotype, on determining whether *C. briggsae* also has an *elt-4* homolog and on investigating the binding properties of the protein in vitro.

506. Does ELT-7 act redundantly with ELT-2 to regulate gut differentiation?

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The E founder cell is the sole progenitor of the endoderm in the *C. elegans* embryo, giving rise exclusively to the 20 cells of the intestine. While several maternal pathways, and their zygotic gene targets, have been shown to specify the identity of the E cell, and many other genes are known to be expressed later in the developing gut, the precise genetic events that cause descendants of the E cell to differentiate into a functional organ are not yet understood. In an effort to understand the regulatory network of factors involved in gut formation, we are investigating the GATA-type transcription factor ELT-7.

Previous work identified a number of GATA factors involved in *C. elegans* endoderm development. A pair of redundant GATA factors, MED-1 and -2, acts zygotically to specify the identity of EMS, the mother of E. The redundant END-1 and END-3 GATA factors in turn specify the identity of the E cell. Eliminating the function of both *end* genes, but not either alone, results in a penetrant E to C transformation and loss of the gut. *end-1::gfp* and *end-3::gfp* are expressed for only a brief period, from shortly after the time that E is born until the 8E cell stage. At the 2E cell stage, immediately after the *end* genes are activated, another GATA factor, *elt-2*, becomes expressed; however, unlike the *end*s, *elt-2* expression persists throughout the life of the animal. Elimination of ELT-2 results in a gut that degrades upon hatching, causing L1 lethality (Fukushige, et al., 1998). ELT-2 was identified by virtue of its interaction with GATA binding sites that are essential for wild-type expression of the gene encoding a gut-specific esterase, *ges-1* (Hawkins and McGhee, 1995). However, *elt-2* is not essential for *ges-1* expression, suggesting that it, and perhaps many other

aspects of gut differentiation, is activated by a genetically redundant mechanism.

We have identified a candidate redundant partner of ELT-2, the ELT-7 GATA factor. An *elt-7* reporter construct reveals an expression pattern that is indistinguishable from that of *elt-2*. Moreover, like the *end* genes and *elt-2*, ectopic *elt-7* is sufficient to promote widespread gut differentiation. While *elt-2(0)* animal show a gut defect, RNAi of *elt-7* results in morphologically normal animals and *elt-7(RNAi)* does not appear to enhance the degenerated gut phenotype of an *elt-2* mutant. However, partial elimination of *elt-2* and *elt-7* function by RNAi results in greatly diminished expression of a *ges-1::gfp* reporter. This observation, the similar expression patterns of *elt-2* and *-7*, and the genetic redundancy seen with the *med*, *end*, and other GATA factor-encoding genes (see abstract by Koh et al.), lead one to speculate that a similar redundant relationship might exist between *elt-2* and *elt-7*. We are in the process of examining the relationship between *elt-7*, *elt-2*, and the *end* genes by ectopic expression experiments, RNAi, and "nuclear spot" assays to examine how the network of GATA factors functions to regulate differentiation of the gut.

Fukushige, T., Hawkins M. G., McGhee, J, D. (1998) The GATA Factor *elt-2* is Essential for Formation of the *Caenorhabditis elegans* Intestine. Dev. Biol. **198**, 286-302.

Hawkins, M. G., McGhee, J. D. (1995) *elt-2*, a Second GATA Factor from the Nematode *Ceanorhabditis elegans*. J. Biol. Chem. **270**, 14666-14671.

507. Characterization of Intestinal Morphogenesis Defects in *zu450*

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The C. elegans intestine is a bilaterally symmetric tube of 20 polarized epithelial cells. Cell polarity in the intestine involves a reorganization of the microtubule cytoskeleton, followed by migration of the intestinal nuclei apically, and other organelles basally. Small apical membrane separations coalesce into a continuous compartment to form the lumen of the intestine. Cell ablation experiments suggest that morphogenesis of the intestine requires interactions between intestinal cells and interactions between intestinal and non-intestinal cells surrounding the intestine[1,2].

We have been conducting screens for embryonically-expressed genes required for intestinal morphogenesis, and are currently characterizing one mutant, *zu450*, in more detail. The intestines of *zu450* homozygotes appear slightly disorganized, and have abnormally wide intestinal lumens. Analysis of *zu450* intestines using two indicators of epithelial polarity, the adherens junction marker MH27 and the lumenal marker MH33, reveal defects in the attachment of the intestine to the pharynx and rectum as well as discontinuities in the intestinal lumens. Preliminary analysis of the mutation *zu450* suggests that it may play a role in multiple epithelia; *zu450* homozygotes have elongation and enclosure defects in addition to intestinal morphogenesis defects.

[1] Leung, B., G. J. Hermann, and J. R. Priess (1999). Developmental Biology **216**: 114-134.

[2] Hermann, G. J., B. Leung, and J. R. Priess (2000). Development **127**: 3429-3440.

508. Role of PDZ domain proteins in establishing epithelial cell polarity

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Epithelial cells are polarized cells that set up and maintain distinct basolateral and apical membrane domains. The PDZ protein motif, originally recognized in the postsynaptic density protein PSD-95, the *Drosophila discs-large* (Dlg), and the tight junction protein ZO-1, mediates protein-protein interactions and is found in many proteins having a central role in localizing proteins to the basolateral membrane domain of epithelial cells or to neuronal synapses. For example, our work has shown that three proteins with PDZ domains (LIN-2, LIN-7 and LIN-10) are involved in basolateral localization of the LET-23 EGF receptor in the vulval precursor cells. We are currently testing the hypothesis that other PDZ proteins may also be involved in epithelial cell polarity. Analysis of the C. elegans genome revealed 58 open reading frames predicted to encode PDZ domains. To examine the role of each of the PDZ proteins, the loss of function phenotype of each gene was determined by RNAi. Preliminary data from this screen suggests that each of the four membrane associated guanylate kinases (MAGUKs) in *C. elegans* has a role in cell polarity. MAGUKs contain one to three PDZ domains, an SH3 domain and a guanylate kinase domain. The *C. elegans* MAGUKs include *lin-2, dlg-1,* and two uncharacterized genes. We are examining the RNAi phenotypes of the two uncharacterized MAGUKs in greater detail to assess their role in cell polarity. Additionally, we are examining possible redundancy of function between the MAGUKs.

509. The three *C. elegans* beta-catenin homologs make distinct protein interactions but retain functional redundancy *in vivo*

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bar-1 encodes a *C. elegans* homolog of the β-catenin/Armadillo family of proteins which are known to function in cell adhesion and Wnt signaling. *C. elegans* has three β-catenin family members (BAR-1, WRM-1 and HMP-2), while *Drosophila* has only a single homolog which performs both functions. Interestingly, the homologs in *C. elegans* show only about 17-28% amino acid identity to vertebrate β-catenins and to each other. We are interested in determining if the three highly diverged β-catenin family members in *C. elegans* have segregated β-catenins function amongst themselves or if all three can perform both functions as in *Drosophila* and vertebrates.

Using a directed yeast two hybrid assay, we determined if the β-catenin homologs in

C. elegans have similar or different interacting factors. Results show that BAR-1 interacts with

POP-1 (TCF homolog) and APR-1 (APC homolog) and not with HMP-2 (α-catenin), suggesting a Wnt signaling function and not a cell adhesion function for BAR-1. WRM-1 however interacts with LIT-1 and POP-1, consistent with both POP-1 and LIT-1 functioning in Wnt signaling in embryogenesis. Finally, HMP-2 interacts only with vertebrate and *C. elegans* α-catenin (HMP-1) implying a cell adhesion function for HMP-2. Similar results have been reported by Korswagen *et al*.(1). These results support the idea that the three β-catenin homologs may have evolutionarily diverged from an ancestral β-catenin to perform different functions. However, *wrm-1, hmp-2*and *armadillo* driven by the *bar-1* promoter can rescue the *bar-1* vulval phenotype and can thus substitute for BAR-1 function *in vivo*. This suggests that the homologs may still share conserved functions. However, HMP-2 can rescue only when injected at high concentrations. This suggests that high

levels of the protein may result in forced interactions between HMP-2 and BAR-1 interacting factors allowing HMP-2 to substitute for BAR-1 function.

We also found that BAR-1, WRM-1 and HMP-2 can all activate transcription in yeast, a function attributed to a role in Wnt signaling indicating they all share this function of β-catenin. Further characterization of BAR-1 shows that the N- and C-terminal domains possess transcriptional activation function and are required for BAR-1 activity *in vivo*. We have isolated BAR-1 interacting proteins (BIPs) from a yeast two hybrid screen and are testing them for their function in the vulva by RNAi. We will test these BIPs for interaction in yeast with HMP-2 and WRM-1 to determine which are common interacting factors and which are specific to BAR-1.

(1) Korswagen et al. Nature 406, 527-32 (3rd Aug 2000)

510. Characterization of Several *C. elegans* Cadherin Genes

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Cell-cell interaction is one of the fundamental processes required for development. Cadherins represent a major superfamily of transmembrane glycoproteins that mediate Calcium-dependent homophilic cell-cell adhesion in both vertebrates and invertebrates. Specifically, cadherins are responsible for cell segregation during morphogenesis, formation and maintenance of tissues, and cell signaling pathways during development.

Protein sequence predictions have identified 14 cadherin genes in *C. elegans* (Hutter et al, 2000; Hill et al, 2001). Two cadherins, *cdh-3* and *hmr-1a*, have so far been studied. CDH-3, which was studied by Pettitt et. al., is expressed in developing epithelial cells and a number of neuroectodermal cells that extend processes along some of these epithelial cells. In addition, loss-of-function mutant in *cdh-3* was found to affect the hyp10 cell, resulting in defective morphogenesis of the tail. HMR-1a, studied by the Priess lab, is essential for adhesion of leading cells during ventral enclosure.

We have done expression pattern studies of several cadherin genes by promoter::GFP fusion. R05H10.6 (*cdh-7*) is expressed in seam cells during larvae to adult stages. F18F11.3/Y66H1B.1 (*cdh-8*) expression is seen in larvae and adult pharynx. C45G7.6/C45G7.5 (*cdh-10*) is expressed in hypodermal seam cells throughout the body and its strongest expression is seen in larvae. B0034.3 (*cdh-11*) expression is faint in late embryos; in adults, it is expressed in the tail neurons PVT, PHA, PHB, PVD, PDE. We also see *cdh-11* expression in the ovarian sheath and SDQR neuron, and neurons in the head lateral ganglia. We are currently attempting to further characterize the functions of these genes.

511. Identification of discs large binding partners during epithelial development in C.elegans

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MAGUKs ("membrane-associated guanylate kinase homologues") are proposed to act as scaffolds, which seem to have a dual role in membrane-associated protein assembly and signal transduction (1). Proteins of the dlg-subfamily contain three PDZ (PSD-95, Dlg and ZO-1) domains, one SH3 domain ("src homology-3") and one GUK domain ("guanylate kinase"). These motifs are known to be involved in protein-protein interactions. We have identified C. elegans dlg-1, the homologue of Drosophila discs large. Immunohistochemical analysis demonstrate localization of DLG-1 to the zonula adherens (ZA) and dlg-1(RNAi) experiments reveal an important function of DLG-1 in the maintenance of ZA integrity in all epithelial tissues of the C.elegans embryo (2).To gain further insight into the role of DLG-1 in ZA organization and to identify proteins involved in epithelial cell polarity, we have started to screen for direct binding partners of DLG-1. We are using two different domains of the DLG-1 protein as baits in a yeast two-hybrid screen. The first bait contains the three PDZ domains and the second bait the SH3- and GUK-domains. Preliminary results about DLG-1 interacting partners and their role in epithelial differentiation will be presented.

(1) Dimitratos et al., 1999, Bio Essays 21, p912;
(2) Bossinger et al., 2001, Dev Biol 230, p29

512. The role of erm-1 during zonula adherens formation in the gut epithelium of C. elegans

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We study epithelial cell polarity in the embryonic gut of C.elegans. Interactions of polarity cues with the executive cytoskeleton are mediated by adaptor molecules. Members of the B4.1/ERM (Ezrin-Radixin-Moesin) protein family are generally described as crosslinkers between plasma membrane and actin cytoskeleton. We have started in-depth analysis of erm-1 that has an essential function during development of the junctional complex in the intestine of C.elegans. ERM-1 is expressed on the apical membrane domain and loss of erm-1 causes larval lethality. Worms die with an expanded gut lumen and uptake of food is usually blocked. Staining of erm-1(RNAi) embryos with MH27 visualize defects in zonula adherens organization, while FITC-phalloidin reveals strongly reduced actin filaments in the apical cytocortex of the gut epithelium. We hypothesize that ERM-1 interferes with the refinement of the zonula adherens pattern. Instead of forming a continuous belt around the apex of epithelial cells, zonula adherens components remain clustered apically, thus causing blockade of the gut lumen.

513. DLG-1 is a PDZ-Containing Protein Responsible for Proper Adherens Junction Formation

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Cellular junctions are critical for intercellular communication and for the assembly of cells into tissues. Cell junctions often consist of tight junctions, which form a permeability barrier and prevent the diffusion of lipids and proteins between cell compartments, and adherens junctions, which control the adhesion of cells and link cortical actin filaments to attachment sites on the plasma membrane. Proper tight junction formation and cell polarity require the function of MAGUKs, a PDZ-containing protein family. In contrast, less is known about how adherens junctions are assembled. Here we describe how the PDZ-containing protein DLG-1 is required for the proper formation and function of adherens junctions. DLG-1 is localized to adherens junctions, and embryos that lack DLG-1 fail to recruit the proteins JAM-1 and the guanine aminohydrolase CPI-1 to adherens junctions. DLG-1 is required for the proper organization of the actin cytoskeleton and for the morphological elongation of embryos. We have examined embryos that lack DLG-1 by electron microscopy and found that they have disorganized adherens junctions or often lack them altogether. In contrast to other proteins that have been observed to affect adherens junction assembly and function, DLG-1 is not required to maintain cell polarity. We believe that DLG-1 plays a relatively specific role in assembling adherens junction proteins, and suggest a new function for MAGUK proteins distinct from their role in cell polarity.

514. Is UNC-52 produced by the hypodermis?

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The *unc-52* gene encodes components of the body-wall muscle extracellular matrix that are essential and required for proper myofilament lattice assembly during embryogenesis (Rogalski et al., 1993, Genes and Development 7:1471). Studies using antibodies that recognize different regions of UNC-52 have indicated that the UNC-52 proteins found in the basement membrane between muscle and hypodermis in early embryos are produced by muscle (most recently Moerman et al., 1996, Dev. Biol. 173:228 and Mullen et al., 1999, Mol. Biol. Cell 10:3205). These studies demonstrated that faint intracellular staining of UNC-52 could be seen in the body-wall muscle cells (but not hypodermal cells) of early morphogenesis stage embryos. We have been focusing on *mec-8*, a gene that encodes a probable RNA binding protein that may interact directly with *unc-52* pre-mRNA. Our studies of MEC-8 have led us to hypothesize that a significant amount of UNC-52 protein is produced by the hypodermis. Loss-of-function mutations in *mec-8* enhance the phenotypes of hypomorphic mutations in *unc-52* found in alternatively spliced exons. It was shown that *mec-8* function is required to generate *unc-52* mRNA isoforms that skip these exons (Lundquist et al., 1996, Development 122:1601). We recently found that expression of MEC-8 by the main body hypodermis, but not by body-wall muscles or their precursors, is sufficient to rescue the genetic interaction between *mec-8* and *unc-52*, and that high levels of MEC-8 in the hypodermis can delay or completely suppress the onset of paralysis conferred by the hypomorphic *unc-52(e669)* allele. These experiments were performed by expressing a *mec-8* cDNA under the control of either *lin-26* (main body hypodermis) or *hlh-1*(body-wall muscle) regulatory elements (generous gifts from M. Labouesse and A. Fire, respectively); antibody staining of embryos indicated that MEC-8 was expressed appropriately in each case. We previously reported making a minigene containing the

alternatively spliced region of *unc-52(e444)* translationally fused to *gfp* and driven by a muscle-specific promoter, *myo-3* (Davies et al., 1997, IWM abstract 116). *unc-52(e444)* is a nonsense mutation in an alternatively spliced exon that must be removed by MEC-8-promoted alternative splicing for expression of GFP. The production of GFP in morphogenesis stage embryos containing this minigene was diminished by *mec-8* mutation. We have found that expression of MEC-8 by the body-wall muscles and their precursors, but not by the main body hypodermis, results in extremely strong activation of this minigene in morphogenesis stage embryos. This result strongly suggests that overexpression of MEC-8 protein results in enhanced *unc-52* exon-skipping, but only when these two gene products are present in the same cell. Since MEC-8 is able to rescue *unc-52(e669)* when it is made by the hypodermis but not when it is made by muscle we infer that a significant amount of UNC-52 must be produced by the hypodermis. We are currently performing mosaic analysis on *unc-52* to test this hypothesis.

515. Identification of interacting molecules with GEI-4, a possible regulatory protein of intermediate filaments in *C. elegans*

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Tissue formation accompanies rearrangement of cytoskeltons. We have been interested in function and regulation of intermediate filaments, one of the major cytoskeletal components. Recently we found one possible regulatory protein of intermediate filaments, GEI-4, as an interacting molecule with GEX-2 in *C. elegans*. We revealed that RNAi of *gei-4* causes an embryonic lethality with disorganization of tissue formation and that GEI-4 interacts with *C. elegans* intermediate filaments in two hybrid system (Qadota *et al*., this meeting). To clarify the molecular mechanism of tissue formation involved in GEI-4, we performed two hybrid screening of interacting molecules with GEI-4 and identified 4 interacting molecules (*gfi* genes, GEI-4 (four) interacting molecule). We found that one of interacting molecules, GFI-2, contains ankyrin repeats in the amino acid sequence. To examine functional relationship between GFI-2 and GEI-4, we performed RNAi experiments of *gfi-2*. RNAi of *gfi-2* caused an embryonic lethality with disorganization of tissue formation like *gei-4*(RNAi). Thus, we propose that this ankyrin repeat protein, GFI-2, associates with GEI-4 and is involved in rearrangement of intermediate filaments during embryogenesis.

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516. GATA factor function and hypodermal development.

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elt-1 is a GATA transcription factor family that is essential the formation of most hypodermal cells (Page et al 1997, Genes and Development, 11,1651-1661). Based on the expression of a number of hypodermal markers (MH33, elt-3::gfp, dpy-7::gfp), we have found that up to 16 hypodermal-like cells can still be formed in elt-1(zu180) mutant embryos. elt-3 is another GATA transcription factor that is expressed in most hypodermal cells (except the lateral seam cells) immediately after the cell division that gives rise to their formation (Gilleard et al 1999, Dev Biol, 208,265-280.). However, elt-3 is not an essential gene (Gilleard and McGhee, Mol.Cell.Biol, 21,(7) 2533-44). In order to further elucidate the role of these two genes in hypodermal development, we have investigated whether they are independently sufficient to activate a program of hypodermal cell differentiation (or at least part of it). Forced expression these genes in early embryos, under control of the heat shock promoter, causes embryos to arrest development as a "ball of cells" with no visible signs of morphogenesis. These embryos show widespread expression of a number of independent hypodermal markers (dpy-7::GFP, elt-3::GFP, LIN-26 and MH27) and repression of non-hypodermal markers such as muscle and neuronal specific reporter genes. Laser ablation experiments show that forced expression of either elt-1 or elt-3 is sufficient to activate hypodermal gene expression in cells derived from blastomeres that do not normally give rise to hypodermis in wild type embryos (eg. EMS blastomere). Thus both elt-1 and elt-3 are independently capable of activating hypodermal gene expression.

We are also studying elt-1 function later in development after its early role in cell fate specification. A chromosomally integrated elt-1::GFP reporter gene is expressed in hypodermal cell precursors (consistent with the antibody staining of Page et al 1997). From the comma stage onwards, GFP expression is confined to the lateral seam cells. Post-embryonically, there is continued expression in seam cells and additional expression in the ventral nerve chord and many cell bodies of the retro-pharyngeal ganglion. There is also expression in the sex muscles surrounding the vulva. We are performing RNAi experiments to address postembryonic elt-1 function. Finally, we are studying functional conservation between vertebrate and nematode GATA factors. We have placed cDNAs corresponding to the mouse GATA factors mGATA-1 to mGATA-6 under the control of the heat shock promoter. We are currently examining the ability of these genes to activate programs of differentiation in early C.elegans embryos.

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517. Screening for Dorsal Intercalation Defective Mutants

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During *C. elegans* embryogenesis, the hypodermis undergoes three major morphogenetic processes that lead to formation of the worm's tubular shape: dorsal intercalation, ventral enclosure and elongation. Dorsal intercalation occurs when cells in two adjoining anterior-posterior columns of ten hypodermal cells each change shape to point towards each other, interdigitate via migration of basal protrusions, and elongate to form a single column. This process involves cell shape and adhesion dynamics, but the specific molecules involved are largely unknown. For example, certain adhesion proteins required for other morphogenetic steps, such as the worm cadherin and catenin homologs, are not required for successful intercalation. The physical cell movements involved in dorsal intercalation have previously been characterized through techniques such as laser ablation, serial section TEM analysis, and pharmacological studies. Additionally, two mutants have been found that exhibit intercalation defects. Mutants in *apr-1*, the worm homolog of the Wnt signaling pathway member APC, display a range of embryonic phenotypes, including intercalation failure. *apr-1(zh10)* mutants have general hypodermal organization defects and *apr-1* may represent one of the genes required for initiation of intercalation. Additionally, a mutation in the putative transcription factor gene *die-1* blocks completion of intercalation. *die-1* mutant embryos eventually enclose, but fail to elongate properly and arrest prior to hatching.

Because little is known about the molecular players involved in this process, we are performing a genetic screen seeking mutants defective in dorsal intercalation. Our screen takes advantage of an integrated *JAM-1::GFP* reporter gene, which provides an excellent marker for hypodermal cell boundaries; an *elf-1* allele (courtesy W. Mohler) that is defective for the fusions of dorsal cells (which would normally mask intercalation defects), but otherwise develops normally; and the *lin-2(e1309)* allele, which results in Vul animals, so that arrested embryos will be conveniently contained in the mother's carcass. We have treated these animals with EMS and score F3 embryos in F2 carcasses in order to be able to detect both maternal effect and zygotic mutants. We have screened 5800 haploid genomes and are in the process of outcrossing candidate mutants, with phenotypic analysis and cloning to follow.

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518. A family of beta-catenin interacting proteins involved in hypodermal morphogenesis

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In both vertebrates and *Drosophila* a single protein, beta-catenin/Armadillo, is a key component of both Wnt signalling and cadherin-mediated cell adhesion. In *C. elegans* the signalling and adhesion functions of beta-catenin appear to have been distributed between separate proteins. HMP-2 appears to function exclusively in cadherin adhesion complexes, while BAR-1 and WRM-1 act in Wnt signalling pathways. We have identified three genes whose products interact with both BAR-1 and HMP-2 in yeast two hybrid screens. These genes have been designated *bin-1*, *-2* and *-3* (*b*eta-catenin *in*teracting). The three genes encode related gene products that do not show any significant similarities to other non-worm proteins in the publicly available sequence databases. In order to determine the role of these genes we have examined the effect of depleting their function using RNA interference. *bin-1(RNAi)* resulted in variable defects in hypodermal cell positioning, particularly in the seam and ventral hypodermis, often resulting in ventral enclosure, or elongation defects. Neither *bin-2(RNAi)* nor *bin-3(RNAi)* resulted in an obvious mutant phenotype, and neither did the double RNAi of both *bin-2* and *bin-3*. However, double RNAi of both *bin-1* and *bin-2* resulted in an enhancement in the penetrance and severity of the defects observed with *bin-1(RNAi)* alone. RNAi of *bin-1* and *bin-3* resulted in a synthetic phenotype not seen in *bin-1(RNAi)* animals. These embryos all arrested with very pronounced defects in the pattern of hypodermal cell junctions, and never initiated ventral enclosure. These results demonstrate that *bin-1* shares functions in regulating hypodermal patterning and morphogenesis with *bin-2* and *bin-3*. We do not know how these gene products act to regulate hypodermal development. They do not appear to regulate the activities of HMP-2 or BAR-1, since the mutant phenotypes of these genes do not cause the same phenotypes. We are currently attempting to determine whether the products of the *bin* genes can interact with other Armadillo repeat containing proteins, such as WRM-1, APR-1 and the *C. elegans* homologue of p120 catenin, as well as examining where they are expressed.

519. A Genetic Screen for New Molting Mutants in *C.elegans*

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Periodic molts are a hallmark of the post-embryonic development of Ecdysozoans, the evolutionary clade including nematodes and insects. In insects, hormones govern execution of the molting cycle by modulating the transcriptional activity of nuclear hormone receptors. Molting in *C. elegans* also involves nuclear hormone receptors (Kostrouchova et al., 1998, Gissendanner and Sluder, 2000), but the neuroendocrine pathways that function upstream of these receptors remain unclear.

When cholesterol is excluded from the culture medium of *C. elegans*, worms arrest development in the second generation (Chitwood, 1992). Arrested larvae fail to complete the molting cycle and often become encased in old cuticle (Yochem et al., 1999).

We used cholesterol deprivation to create a genetic screen sensitized for molting mutants. Conditional mutants were isolated that arrest development at the L1 or L2 stage when cultured on medium lacking cholesterol, but recover to form reproductive adults when transferred to medium containing cholesterol. Five mutants were isolated that displayed an increased sensitivity to cholesterol deprivation in the first generation. Three of these mutants displayed molting defects when cultured in the presence or absence of cholesterol. These mutants could be defective in the regulation and synthesis of or response to steroid hormones that control molting in *C. elegans*. Alternatively, these mutants could be primarily defective in cholesterol uptake or homeostasis.

520. A role for *par-1* in vulval development

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par-1 is a maternally-acting gene that plays a well-established role in asymmetric cell division during early embryonic development. Although PAR-1 is expressed at other times in development, apparent null mutations do not show consistent phenotypes outside of embryogenesis. Furthermore, a temperature-sensitive allele (zu310ts) shows no obvious phenotypes if grown at restrictive temperature after the two-cell stage (G. Mirani and K. Kemphues, unpublished observations). We discovered that depleting PAR-1 by growing newly hatched worms to adulthood on bacteria expressing par-1 dsRNA causes a protruding vulva phenotype. We had noticed a poorly penetrant Egl defect in some of our par-1 mutant strains, but we were never able to determine whether it was due to par-1. Immunolocalization of PAR-1 in developing larvae has revealed a cortical enrichment in vulval cells at the L4 stage that persists into young adulthood and is absent after RNAi depletion. Thus far we have not observed staining in larvae younger than L4, which suggests that PAR-1 is required for morphogenesis and not specification of vulval cell fates. We will present results of our ongoing experiments to characterize the defects underlying the protruding vulva phenotype caused by depleting PAR-1.

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521. The *ty10* mutation results in an unfused anchor cell that degenerates or detaches in L4 stage hermahprodites

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During organogenesis, the differentiation of any one cell must be coordinated with that of other cells of the developing organ. The anchor cell (AC) of the *C. elegans* hermaphrodite has a pivotal role in formation of fhe uterine-vulval connection, and its own terminal differentiation is also critical. The AC induces the vulva (using LIN-3/EGF) and the uterine π cells whose daughters connect to the vulva (using LIN-12/Notch), thereby organizing the uterine-vulval connection. A subset of the π cell daughters fuse to form the utse (uterine-seam cell) whose thin laminar process separates the uterine and vulval lumens. Formation of a functional uterine-vulval connection requires that the utse be specified and that the AC fuse with the utse. In animals lacking π cells, the uterine and vulval lumens are separated by thick tissue with an unfused AC in its midst. The AC also fails to fuse with the utse in *egl-13/cog-2* and in *lin-11* mutants; this is likely due to defects in maintenance of the π cell fate (*egl-13/cog-2*) or differentiation of the utse (*lin-11*).

ty10 mutants have a phenotype that is distinct from those previoulsy described. In these mutants, the thin laminar process of the utse is observed but the AC remains unfused. During the time of utse differentiation, the AC is initially observed as an unfused cell blocking the uterine-vulval connection. Subsequently, one of the following occurs: 1) The AC remains in this position but begins to degenerate; ultimately, only cellular debris remains; or 2) the AC detaches from the uterine surface and is observed floating free in the uterine lumen. It is possible that the floating AC also dies. The CED-3 caspase is not required for the *ty10* mutant phenotype. Thus, the AC appears to die but not through programmed cell death. Animals containing *ty10* in trans to a deficiency die as embryos or young larvae. We infer that the corresponding gene functions broadly in development. We will test whether it is required generally for cell fusions. We have mapped the gene to the interval between *gld-1* and *unc-55*, which is spanned by eight cosmids. We are currently attempting to clone it through injection of these cosmids.

At present, we are considering two general models. First, the direct result of the *ty10* mutation might be the inability of the AC to fuse with the utse. The subsequent degeneration or detachment of the AC might be a consequence of its isolation (If, for example, the AC needs to contact other cells in order to survive). Alternatively, the direct consequence of the *ty10* mutation might be degeneration of the AC. In either case, our results suggest that the biology of the multi-functional AC may be even more complex than previously realized.

522. The *C.elegans* Mi-2 chromatin remodelling proteins LET-418 and CHD-3 have essential developmental functions

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The Mi-2 protein is the central component of the recently isolated NuRD (nucleosome remodeling and histone deacetylase) complex .Although the NuRD complex has been the subject of extensive biochemical analyses,little is yet known about its biological function. Therefore we have initiated an analysis of the two *C.elegans* Mi-2 homologues LET-418 and CHD-3. We found that they play essential roles during development and that the two proteins possess both shared and unique functions during vulval cell determination. LET-418 is involved in antagonizing theRTK/Ras /MAP kinase pathway via the synMuv pathway, whereas LET-418 and CHD-3 together appear to be important for the proper execution of the LIN-12 Notch dependent secondary cell fate of P5.p and P7.pvulval precursor cells,. Currently, we are investigating a possible connection between let-418, chd-3 and the lin-12 Notch signalling pathway during vulva development. Furthermore,we have started an yeast two hybrid screen to identify proteins interacting with both *C.elegans* Mi-2 homologs . The results will be discussed.

523. A *C.elegans* Sterol reductase homologue involved in vulva development

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Sterol metabolism in *Caenorhabditis elegans*(*C.elegans*) is different from the other species such as mammals and yeast. It is unable to synthesize sterol through de novo pathway but is able to use sterols such as cholesterol, sitosterol and stigmasterol from the environment. Until now, a few data has been reported about sterol metabolism in *C. elegans*. However, research on the cloning of cDNA and functional studies of any enzyme involved in sterol metabolism has not been reported. Through the search of Sanger database, the B0250 cosmid clone, which contained a homologous gene to human 7-dehydrocholesterol reductase, human lamin B receptor and yeast ERG24 was identified. From the sequence data, *C. elegans* sterol reductase homologous cDNA (CeSR) was cloned by screening of embryo cDNA library followed by 5’-RACE. The deduced peptide was 457 amino acids and exhibited identities of 29, 28 and 27%, and similarity of 47, 43 and 45 % with those of human 7-dehydrocholesterol reductase, human Lamin B receptor, and yeast ERG24, respectively. The function of CeSR is not known at present. To examine the localization of CeSR expression, 5’ flanking region of CeSR was fused with green fluorescent protein (GFP) and injected into gonad. CeSR expression was appeared in the head neurons, hypodermis and body wall muscles during the larva stages (L1 to L3). CeSR was also expressed in the vulva muscle beginning in late larva stage (especially L4) and maintained through the adult stage. But the expression in the body wall muscle was disappeared after L4 stage. Our results indicate that CeSR is a nerve cell- and vulva-specific protein. We are in process of RNA mediated interference by transgene.

524. Involvment of PlexinA in vulval morphogenesis

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Involvment of PlexinA in vulval morphogenesis

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The plexin family transmembrane proteins are putative receptors for semaphorins, which are implicated in the morphogenesis of animal embryos including axonal guidance. We have generated and characterized putative null mutants of the *plx-1 (*pka. *cep-2*) gene encoding *C. elegans* plexinA. *plx-1* mutants exhibited morphological defects in several organs of epidermal origin, such as Ray1 in the male tail (see Fujii *et al*. in this Meeting) and alae. Here we report on the defects in vulvae of *plx-1* mutants.

*plx-1*adult hermaphrodites sometimes had a deformed vulva or extra vulva-like structures. *plx-1::gfp* transgene was expressed in the vulval primordial cells. Since the epidermal cells composing the vulval primordium are known to undergo dynamic changes in shape and position during the vulval morphogenesis, we analyzed the vulval primordium with *MH27::gfp*. The epidermal cells of the vulval primordium were aberrantly arranged in *plx-1* mutants: the rotation-symmetric configuration of the primordum was often disrupted, and some cells were not in contact with each other. Some cells failed to participate in the formation of the main vulva and formed an extra vulva-like depression. The results indicate that *plx-1* regulates the arrangement and/or migration of epidermal cells in this system.

525. Regulation of Hox gene *lin-39* activity by Ras and Wnt signaling pathways in vulval development

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We are studying how extracellular signaling regulates cell fate specification during vulval development. The vulva arises from a group of six Vulval Precursor Cells (VPCs) located in the ventral midline of the body. The Hox gene *lin-39* is expressed in the VPCs and is required for their cell fate specification. At the L3 stage, activation of a conserved RTK/Ras signaling pathway induces vulval formation and the Kenyon lab has shown that LIN-39 levels are upregulated by activation of the Ras pathway. A Wnt signaling pathway also acts during VPC fate determination. *bar-1* encodes a β-*catenin* homologue, and in *bar-1* mutants the level of LIN-39 is very low in some VPCs and these VPCs adopt abnormal cell fates. Therefore, *lin-39* is regulated by Wnt and Ras pathways. Our goal is to understand how these signaling pathways converge in the regulation of the Hox gene *lin-39* to specify VPC fates. Specifically, we want to know if *lin-39* regulation by these pathways is at the transcriptional or post transcriptional level.

MAP kinase acts downstream in the Ras pathway by phosphorylating transcription factors such as LIN-1 and LIN-31. The LIN-39 protein sequence has a single phosphorylation site and a single docking site for MAP kinase, suggesting that the Ras pathway may regulate *lin-39* activity at the post-transcriptional level by phosphorylation. Preliminary results show that

6HIS-LIN-39 purified from *E. coli* was weakly phosphorylated by vertebrate MAP kinase in an *in vitro* phosphorylation assay. The addition of CEH-20, a protein known to interact with LIN-39, enhanced LIN-39 phosphorylation under specific binding conditions, either alone or in the presence of a DNA fragment containing putative LIN-39 binding sites. Experiments are underway to determine if CEH-20, DNA or both enhance LIN-39 phosphorylation. We are also mutating the

LIN-39 phosphorylation and docking sites to test if phosphorylation requires these sequences. Our goal is to understand the significance of LIN-39 phosphorylation. We wish to determine if phosphorylation of LIN-39 affects 1) the interaction of LIN-39 with DNA, 2) the interaction of

LIN-39 with CEH-20, and 3) the stability of LIN-39 *in vivo*.

The *lin-39* genomic sequence contains recognition sites for TCF transcription factors, which are known to mediate Wnt signaling, suggesting a transcriptional mode of regulation of

lin-39. We are cloning *lin-39* genomic regions containing TCF recognition sites into a *pes10::GFP* reporter to determine if these TFC sites are required for *lin-39* expression. Preliminary results using a fragment containing six TCF sites located 5.5 kb upstream of the LIN-39 start codon showed GFP expression in different tissues but only a weak expression in the VPCs of one animal. Finally, we are making two *lin-39*::GFP reporter constructs that will help us to differentiate between a transcriptional and a post-transcriptional regulation of *lin-39* activity.

526. The Rhomboid homolog ROM-1 facilitates the long-range action of LIN-3 EGF during vulval development

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During the development of the hermaphrodite vulva, the fates of the vulval precursor cells (VPCs) are specified by the combined action of the receptor tyrosine kinase/Ras/MAP kinase and the LIN-12 Notch signalling pathways. The gonadal anchor cell (AC) induces the underlying VPCs to undergo vulval differentiation. The AC signal LIN-3 EGF activates the receptor tyrosine kinase LET-23 EGFR to specify the primary vulval fate in the closest VPC P6.p.

In Drosophila, Rhomboid is required for the the EGF ligand Spitz to activate the EGF receptor on neighbouring cells. It has been proposed that Rhomboid may act in concert with Star to promote the cleavage of a membrane bound form of Spitz and thus allow long-range signalling by secreted Spitz to occur. Four genes encoding putative homologs of Rhomboid have been identified by the *C.elegans* sequencing project. They are termed *rom-1* (F26F4.3), *rom-2* (C48B4.2), *rom-3* (Y116A8C.14) and *rom-4* (CEY116A8C). We are investigating the role of *rom-1* during vulval development. Around the time of vulval induction (in late L2 or early L3 larvae), a *rom-1::gfp* transcriptional fusion is expressed in all six VPCs with relatively higher expression in P6.p than in the other VPCs, suggesting that the inductive signal may up-regulate ROM-1 expression in P6.p. On the other hand, no ROM-1::GFP expression can be detected in the AC at this stage. Thus, ROM-1 is expressed in the signal receiving rather than the signal sending cell. A *rom-1* deletion that is predicted to inactivate gene function exhibits no phenotype as a single mutant. However, *rom-1(lf)* or RNAi against *rom-1* partially suppresses the multivulva (Muv) phenotype caused by overexpression of LIN-3 EGF or by a *let-60 (n1046^{gf})* mutation, but *rom-1(lf)* fails to suppress the *lin-15(rf)* Muv phenotype. Thus, *rom-1(+)* is not required for the reception of the AC signal by P6.p or for the AC-independent induction of vulval cell fates.

Rather, the induction of the VPCs that are further away from the AC (P3.p, P4.p and P8.p) appears to depend on *rom-1(+)* function. We propose that ROM-1 in P6.p may facilitate the conversion of a membrane-bound, locally acting form of LIN-3 EGF into a secreted form that can act at a distance.

527. A second function of the APC-related gene *apr-1* during vulval development

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During the first larval stage, a Wingless signal specifies the identity of the six equivalent vulval precursor cells (VPCs) P3.p through P8.p by inducing the expression of the Hox gene *lin-39*. The LIN-39 protein is a key regulator of VPC identity. Towards the end of the second larval stage, a signal from the gonadal anchor cell (AC) activates the conserved RTK/RAS/MAP kinase pathway to induce the primary and secondary vulval cell fates in P5.p, P6.p and P7.p.

apr-1 encodes the *C. elegans* homolog of the human Adenomatous Polyposis Colon (APC) tumor suppressor gene. APC mutations are found in most cases of human colorectal cancer. We have previously reported that *apr-1* is required for the transduction of the Wingless signal in the VPCs to induce the expression of *lin-39* (Fröhli Hoier et al., *Genes & Development*, 14: 874-886). While performing a structure-function analysis, we have discovered a new function of *apr-1*. Expression of an *apr-1* deletion that removes 198 amino acids from the C-terminus of the protein (*apr-1CT*) in *apr-1(lf)* mutants allows the generation of the VPCs to occur normally, as determined by staining of the adherens junctions with the MH27 antibody. Thus, APR-1CT can promote Wingless signalling with similar efficiency as full-length APR-1. However, in *apr-1CT* animals P5.p, P6.p and P7.p frequently adopt the 3° uninduced instead of the 1° or 2° induced cell fates. A mutation in *gap-1* which encodes an inhibitor of LET-60 RAS suppresses the *apr-1CT* vulvaless phenotype. Taken together, these observations indicate that APR-1 performs a second, probably Wingless-independent function that is required for the reception of the inductive AC signal or for signal transduction by the RTK/RAS/MAP kinase signalling pathway.

528. Suppression of mutation-induced vulval defects by activated EGL-30(Gq).

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During the course of other studies, we observed that hermaphrodites containing a gain-of-function allele of *egl-30* occasionally (<0.5%) develop ectopic vulval tissue. To determine directly whether activated Gq affects vulva development, we constructed double mutant combinations of *egl-30(gf)* with hypomorphic mutations in the EGF pathway. We find that *egl-30(gf)* partially suppresses loss-of-function mutations in *lin-3(n378)*, *let-23(sy1)*, *let-341(ku231)*, and *let-60(n2021)*; however, activated Gq neither suppresses all hypomorphs in *lin-3* nor synergize with *gap-1(n1691)*, gain-of-function *let-23(sa62)* or with *lin-15(n765)* to generate ectopic vulval tissue. Gq-mediated suppression requires wild-type EGL-19 voltage-gated calcium channels, but not synaptic transmission components such as UNC-64 (syntaxin) or UNC-13, suggesting that excitable cells are involved in this phenomenon, but that the mechanism of suppression does not strictly require synaptic transmission. We hypothesize that in the mutant strains we have constructed, activated Gq may amplify weak EGF signaling in P5.p, P6.p, and P7.p through a parallel pathway to induce vulva development.

529. SITE-DIRECTED MUTAGENESIS OF LIN-31, A WINGED-HELIX TRANSCRIPTION FACTOR INVOLVED IN *CAENORHABDITIS ELEGANS* VULVAL DEVELOPMENT

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In order to understand cell fate specification during vulval development, we are conducting a structure/function analysis of LIN-31, a member of the winged-helix family of transcription factors, which is required for the proper specification of vulval cell fates in *C. elegans* (Miller *et al.*, Genes and Dev., 7:933, 1993). The LIN-31 protein contains a DNA-binding domain, an acidic region, four MAP kinase consensus phosphorylation sites, and a small region of homology conserved among other winged-helix proteins.

We are using site-directed mutagenesis to create plasmids carrying specific mutations in the MAP kinase consensus phosphorylation sites, acidic domain, or the homology region of the LIN-31 protein. These plasmids are then injected into an animal with no functional LIN-31 protein to test for their ability to provide LIN-31 function.

LIN-31 is phosphorylated by the MAP kinase MPK-1 in response to an inductive signaling event (Tan *et al.*, Cell 93: 569, 1998). Tan *et al*. have already shown that removing all four MAP kinase consensus phosphorylation sites inactivates one of LIN-31's functions, but the individual contribution of each site is not known. Four individual clones are being created, with each one containing a different disrupted MAP kinase consensus phosphorylation site. In addition, it is appealing to imagine that the small acidic domain (six consecutive aspartic acid residues) adjacent to the DNA-binding domain is functioning as a transcriptional activator, as is the case with acidic regions in some other transcription factors. There is no proof, however, that this rather small acidic domain in LIN-31 is even required for function. Five mutant clones, in

which different portions of the acidic domain have been removed or replaced will address this question. Finally, the carboxy-terminus of LIN-31 contains a small region of homology that shows similarity to a subset of winged-helix proteins. The function of this homology region is also unknown and is being explored in this project.

This structure/function study is especially appealing since the current model (Miller *et al.*, 1993; Tan *et al.*, 1998; and Miller *et al.*, Genetics 156: 1595, 2000) proposes that LIN-31 has two functions: 1) to activate vulval cell fates in P5.p, P6.p, and P7.p and 2) to repress vulval cell fates in P3.p, P4.p, and P8.p. Microinjection of these clones into *lin-31(null)* animals will allow us to test if each specific mutation disrupts one, both, or none of LIN-31's functions. Thus, this approach will allow us to identify the roles of specific domains or sites in the LIN-31 protein.

530. Genetic approaches to identify vulva-specific targets of LIN-39

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During vulval development, multiple signaling pathways function cooperatively to establish the vulval fate in the P3.p-P8.p cells and their progeny. The LIN-39 Hox protein appears to be a central player in this process since these pathways, including those mediated by Ras and Wnt, interact with LIN-39[1,2,3]. LIN-39 is required in the L1/2 stage for preventing the P3.p-P8.p cells from fusing to hyp7, thereby maintaining the potential of these cells to be induced, and in the L3 stage for the vulval induction itself[4,5,1,2]. To identify targets downstream of LIN-39, we are undertaking various screens for genetic modifiers of *lin-39* vulval phenotypes.

Since it is possible that LIN-39 negatively regulates gene activities required for cell fusion, mutations in genes required for cell fusion may partially suppress the effect of a *lin-39(lf)* allele. To date, we have screened 14,000 haploid genomes and isolated three suppressors that partially suppress the Egl phenotype of the hypomorphic *lin-39(ku69)* allele. To further increase the sensitivity to detect the changes in the potential LIN-39 target genes, we have also constructed a *lin-39(n709ts)*; *lin-1(ar147)* double mutant that displays a weak Muv phenotype. Mutations that alter the effect of the weak *lin-39(n709ts)* allele are expected to enhance or suppress this Muv phenotype. So far, we have screened 3,000 haploid genomes and isolated one enhancer and one suppressor mutations. We are attempting to map these modifier mutations and characterize them further.

Since LIN-39 acts during two different stages, it may have two sets of target genes. If this is the case, a mutation in one of the target genes is unlikely to suppress or enhance the effects of *lin-39* mutations dramatically. To circumvent this problem, we are trying to use a *lin-39* mutant, in which the early fusion defect is rescued by an integrated array containing *lin-39(+)* driven by a *col-10* promoter. This

approach should allow us to screen specifically for mutations in genes that function in the later vulval induction.

1: Clandinin et al. (1997) Dev. Biol. 182: 150-161.

2: Maloof and Kenyon (1998) Development 125: 181-190.

3: Eisenmann et al. (1998) Development 125: 3667-3680.

4: Wang et al. (1993) Cell 74: 29-42.

5: Clark et al. (1993) Cell 74: 43-55.

531. *vex-2* mutations perturb more than just vulval fate execution

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For many years, the RAS/MAPK signaling cascade and its role in vulval induction has been intensely studied. On the other hand, less is known about what controls the later stages of vulval development, including the execution of vulval lineages. We have been studying a mutation, *vex-2(ku233),* that causes defects in the first round of vulval specific cell divisions. In *ku233* hermaphrodites, the Pn.px nuclei appear normal, but P(5-7).pxx cells often have abnormal nuclear morphology and spacing. These cells give rise to abnormal progeny cells that form a disorganized invagination at the L4 larval stage.

We performed a non-complementation screen and identified two new alleles of *vex-2, cs53* and *cs54*, that appear to be stronger than *ku233*. Homozygotes for these new alleles are completely sterile and have vulval defects similar to those of *ku233* mutants. Furthermore, males have grossly abnormal tail morphologies with crumpled spicules and missing rays. This spectrum of defects suggests that VEX-2 could be a general regulator of the cell cycle. Further experiments are being done to determine the role *vex-2* plays during the development of the worm.

The *ku233* mutation maps very close to *unc-45* on chromosome III. We rescued *ku233* with a single gene, F54C4.3, which encodes a C2H2 zinc finger protein. RNAi against this candidate gene causes protruding vulva and sterile phenotypes in hermaphrodites and abnormal male tails, consistent with this gene being *vex-2*. However, the RNAi animals appear sicker than all three *vex-2* mutants and have several new phenotypes including seam cell defects, gonadal defects and a partial vulvaless phenotype. Upon sequencing the F54C4.3 coding region in *vex-2* mutants, we did not find any lesions. Thus F54C4.3 appears to be an overexpression suppressor of *vex-2* rather than the actual *vex-2* gene.

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532. Genetic study of connectivity of ray sensory neurons in the *C. elegans* male

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The *C. elegans* male exhibits stereotyped mating behaviors, comprised of a series of discrete steps. The generation and modification of such complicated mating behaviors depends on the precise connectivity of neural circuits. It has been shown that many male specific sensory structures and neurons are required for mating behavior. The sensory rays in the tail mediate the response to contact with hermaphrodite and turning (Liu and Sternberg, 1995). However, little is known about the connectivity of ray sensory neurons and their postsynaptic partners**.**

The two neurons (RnA and RnB, n=1-9) of each ray have distinct positions and possible neurotransmitters. We have shown by means of GFP reporter genes that all the axons of the B neurons and at least some of the A neurons project to the same region in the ventral preanal ganglion, consistent with previous studies (Sulston *et al*, 1980). Each ray axon has a distinct but stereotyped pathway into the preanal ganglion. The axon of the most anterior (ray1) first migrates anteriorly out of the cell body in the subdorsal lumbar ganglion, then traverses commissurally to the ventral side before turning posteriorly and running into the preanal ganglion from the anterior, whereas axons of the middle rays 2-5 traverse ventrally and run directly into the preanal ganglion. Axons of the posterior rays 7-9 navigate anteriorly and ventrally to the same region. We could not determine whether different ray neurons have different target cells by reporter gene expression because GFP in ray axon terminals makes a large, featureless fluorescent area in the preanal ganglion. We plan to identify the postsynaptic cells of ray neurons by studying the expression patterns of putative receptors for ray neurotransmitters such as dopamine and serotonin.

In order to determine the mechanism by which ray neuron processes migrate and select appropriate target cells, we have focused on the identification of molecules that control ray axon guidance and target selection. We have first performed a genetic screen utilizing a *pkd-2*::GFP expressing strain, which labels all RnB neurons except R6B. This screen led to the isolation of 14 mutants. These mutants exhibit a variety of RnB axonal defects ranging from wandering, abnormal pathway, and missing target to ectopic outgrowth from the preanal ganglion. Among these was an allele of *unc-6*. We also find that Unc-6 receptors Unc-40/DCC and Unc-5 affect ray axon circumferential migration. We are currently characterizing mutant defects, using a series of other reporters to define whether they are ray specific, and mapping them to facilitate cloning genes.

In a second approach to identify molecules involved in ray axonal targeting, we are taking a candidate gene approach. We have focused on the *C. elegans* immunoglobulin (Ig) containing proteins. It has been shown that the Ig-domain superfamily plays a conserved role in axon guidance and also in the selection of neuronal connections. For instance, in *Drosophila*, Dscam has been shown to display multiple isoforms and may specify target selection. We are searching the *C. elegans* genome, as well as a male-specific set of genes (generated by the Kim lab) and our own set of ray specific genes (see abstract by Portman and Emmons). The expression patterns and functions in directing axon targeting of these candidate genes will be further characterized by reporter gene and RNAi experiments.

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533. MAB-18 BI-DIRECTIONALLY REGULATES MALE TAIL COLLAGEN GENE AND SPERM SPECIFIC PROTEIN GENE IN *CAENORHABDITIS ELEGANS*

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Gene-expression controls in tissue, stage and sex specificities are one of the most interesting subjects. Bi-directional promoter is defined as 1) two genes are located bi-directionally 2) both promoters are overlapped each other. Bi-directional promoter is found in lambda phage, bacteria, fruit fly, mouse, and human. None of more than 1,000 candidates in the *C. elegans* genome was analyzed. We found *spe-17*-like gene (designated *spe-TI*) and *sqt-3 (col-1)* -like gene (designated *col-TI*) in the cosmid clone C46C8 maps in the center of chromosome V of *C. elegans*. SPE-TI is a Ser/Thr rich hydrophilic protein and COL-TI is a cuticular collagen similar to SQT-3 at the rate of 84%. Both genes are separated by 1.3 kb and are transcribed bi-directionally. It is of interest to know how and which factors control the gene expression of both. Using the promoter/*lacZ* fusion plasmids, we analyzed the promoter activity of each of genes. *spe-TI* was expressed in about 80 cells of spermatheca after 4th larval stage of hermaphrodite under the control of 0.7kb upstream fragment. *col-TI* was expressed only in the male tail tip of adult under the control of the 0.6kb upstream fragment. These results suggest that both promoters may overlap to each other and both genes are regulated by sex-specific bi-directional promoter. Transcription factors regulating *spe-TI* and *col-TI* were identified with yeast one-hybrid screen system by using the promoter specific regions. After screening approximately 1.4×10^6 yeast transformants for *spe-TI* and 8.5×10^5 yeast transformants for *col-TI* , 4 plasmids and 5 plasmids were identified respectively. Surprisingly, all 9 plasmids came from cDNA of *mab-18* isoform II promoting male tail ray6 formation and are most closely related to the vertebrate Pax-6. In vertebrate, Pax-6 is used for eye crystal formation with HMG class transcription factors but did not use for spermatogenesis nor collagen gene expression. We also found the Pax-6 homeodomain binding sequence on 1.0kb upstream of *col-TI* and 1.1kb upstream of *spe-TI* respectively. *col-TI::lacZ* was not expressed in *mab-18* mutant animals but heat shock promoter derived ectopic MAB-18 cannot activate *col-TI::gfp* expression ectopically. These results suggest that MAB-18 stimulates *col-TI* expression directly in tissue or stage-specifically, but another factor(s) are necessary for normal *col-TI* expression. We believe that bi-directional promoter presented here is a useful example of understanding the sex-specific morphological changes in terminal differentiation.

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534. The roles of a Semaphorin and an ephrin in patterning cell-cell contacts in the C. elegans male tail

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Formation of certain cell-cell adhesive contacts and avoidance of others are essential to morphogenesis. Differences in adhesive specificity often correlate with differences in cell fate, suggesting these two processes are linked. We are using the cells that give rise to the *C. elegans* male sensory rays as a model to study this linkage. The sensory rays derive from cells that display adhesive specificity: adherens junctions form between cells of the same ray, and not between cells of different rays. The Hox gene *egl-5* and the Pax6 homolog *mab-18* are required for adhesive specificity in the cells in which they are expressed, but how they perform this function is unknown. Both genes encode transcription factors, suggesting that they transcriptionally activate mechanisms that promote adhesive specificity.

Signaling pathways involving the Semaphorin MAB-20 and the ephrin MAB-26 are candidates for such mechanisms. Absence of either MAB-20 or MAB-26 causes a high frequency of ray fusion, indicating that both proteins are necessary for adhesive specificity. Ephrins and Semaphorins mediate repulsion to axons and migrating cells in other systems. Hence, as a predicted secreted protein expressed in cells of all rays, MAB-20 was hypothesized to prevent the initial formation of inappropriate cell-cell contacts, via inhibiting extension of processes from ray cells (Roy et al, *Development* 127, 755-67). We found that a rescuing *mab-26* reporter is expressed in ray cells. As a membrane-bound factor, we hypothesize that MAB-26 acts as a contact repellent between cells of different rays. Interestingly, the *mab-26*reporter is expressed at levels that differ between rays, with an alternating OFF-ON pattern in cells of rays 1, 2, 3 and 4, strong expression in ray 6 cells, and intermediate expression in cells of rays 5 and 7.

We tested the hypothesis that *egl-5* and *mab-18* promote adhesive specificity through activating expression of *mab-20* or *mab-26*. In *egl-5* mutants, rays 2, 3, 4 and 5 form a single fusion; in*mab-18* mutants, rays 4 and 6 fuse. Expression of a *mab-20* reporter is wild-type in *egl-5* and *mab-18* null mutants, indicating that neither gene operates through activating*mab-20* expression.

In contrast, *mab-26* reporter expression is altered in both *mab-18* and *egl-5*. *mab-26* reporter expression is reduced in ray 6 cells in *mab-18*, suggesting that *mab-18* ray fusion might result from insufficient *mab-26*-mediated repulsive signaling from ray 6 cells to ray 4 cells. However, a transgene that drives *mab-26* expression in ray 6 cells and rescues ray 6 fusion in *mab-26* does not rescue ray 6 fusion in *mab-18*. We conclude that *mab-18* promotes adhesive specificity of ray 6 cells through a mechanism in addition to activating *mab-26* expression.

In contrast to *mab-18*, *egl-5*appears to repress *mab-26*expression. In *egl-5*, the *mab-26* reporter is ectopically expressed in ray 3 cells. Because ephrin-Eph interactions can mediate adhesion, we considered the possibility that fusion of ray 3 with either ray 2 or ray 4 in *egl-5* results from an adhesive interaction between ectopic MAB-26 in ray 3 cells and a receptor in cells of rays 2 or 4. Consistent with such an interaction between cells of rays 3 and 4, driving ectopic expression of *mab-26*in ray 3 cells in a wild-type background causes a low but significant frequency of ray 3-4 fusion. However, ray 3 cells still make ectopic contacts with ray 4 cells in the *egl-5*; *mab-26*double null mutant, indicating that ectopic *mab-26* expression alone cannot account for ray fusion in *egl-5*. We conclude that *egl-5* promotes adhesive specificity between cells of rays 3 and 4 through a mechanism in addition to repressing *mab-26* expression.

Our results indicate that *mab-18* and *egl-5* control expression of *mab-26* in distinct manners that promote adhesive specificity. However, this control is not sufficient for adhesive specificity, indicating that *mab-18* and *egl-5* perform additional functions. These functions could regulate activity of *mab-20* or *mab-26*-dependent pathways, or other pathways yet to be identified.

535. Regulation of the ray position by two Plexin/Semaphorin systems

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Plexin is a family of transmembrane protein conserved across a wide range of animal species. Recent studies on Drosophilae and mice showed that plexins act as receptors for semaphorins, a group of proteins originally identified as a chemorepulsive factor for growing axons. *C. elegans* has two genes for plexins, *plx-1* and *plx-2*, (pka. *cep-2* and *cep-1*, respectively); two genes for transmembrane semaphorins, *Ce-sema-1a*, *Ce-sema-1b*; and one gene for a secreted semaphorin, *mab-20/Ce-sema-2a*. We have generated deletion mutations for *plx-1* and *plx-2* in order to examine their function in vivo. Here we report the ray morphogenesis in these mutants.Putative null mutants of the *plx-1* gene exhibited anterior displacement of Ray1 in the adult male tail. The similar ray phenotype was observed by suppression of *Ce-sema-1a* and/or *Ce-sema-1b* by RNAi. In *plx-1* mutants, precursors for Ray1 were often mispositioned, suggesting that plx-1 regulates the arrangement of the ray precursors. Showing no defects in other rays, the phenotype of plx-1 mutants is distinct from that of mab-20 mutants reported previously. *plx-2 (nc7)* is presumed to cause a deletion within the extracellular region of PLX-2. While *plx-2 (nc7)* animals exhibited no apparent defects in the male tail by itself, *nc7* enhanced the ray fusion traits of *mab-20(bx-24)*, an allele of medium strength, suggesting that these genes interact.

By using cultured cells, we also showed that Ce-Sema-1a binds to PLX-1, but not to PLX-2, while Ce-Sema-2a binds to PLX-2 but not to PLX-1. The results indicate that PLX-1 interacts with the transmembrane semaphorins and PLX-2 interacts with the secreted semaphorin. Although both plexins are required for the proper positioning of rays, they appear to function rather independently.

536. Dissection of RAM-5 protein domains required for subcellular localization and protein interaction during sensory ray morphogenesis.

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C. elegans male animals develop specialized sensory rays in their tails. Each ray has a simple and stereotyped cellular architecture. The clear understanding of the cellular components and organization of a ray makes it an ideal model to study morphogenesis. Our group previously reported the cloning of *ram-5* gene, which encodes a novel transmembrane protein on the structural cell surface. Male animals with loss of *ram-5* activity exhibit a lumpy ray phenotype in their tails. The ray tip localization of RAM-5 is crucial for its function to establish the wild type ray morphology. Analysis on subcellular defect of lumpy ray revealed that abnormal cell morphology was found in both hypodermis and structural cell. Hence, RAM-5 molecule has been proposed to be involved in cell-cell communication, probably through protein-protein interaction.

We present here the analysis of the protein domains required for rescuing activity and ray tip localization respectively. We demonstrate that RAM-5 molecule has a large extracellular and a short dispensable cytoplasmic domain. Both extracellular domain and its own transmembrane segment are essential for its biological function and localization. Deletion studies suggest that either a synthetic signal sequence or RAM-5's own SP, CUT-1 and D1 together with its specific TM segment can confer full biological activity during ray morphogenesis, and revert null mutant phenotype into wild type features. SP was only used for ensuring proper orientation of RAM-5. CUT-1 and D1 domains provide interaction interface and can confer dominant negative phenotype when they are expressed alone. D1 domain together with RAM-5 TM segment was used to localize the protein properly on the cell surface of the ray tip. The PEST sequence controlling its stability and the D2 domain redundantly required for ray tip localization, on the other hand, appear to be nonessential for the

wild type RAM-5 biological activity during ray morphogenesis.

537. Additional phenotypes associated with *ram-3* mutants

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In *Caenorhabditis elegans*, five *ram* (Ray Morphology Abnormal) genes have been identified to play key roles guiding ray morphogenesis in the male tail. There are additional collagen and *dpy* genes involved in this process. In this study, we focus on two of these *ram* genes, *ram-1* and *ram-3,* to characterize their mutant phenotypes, which may provide clues to their functions. Genetic rescue by cosmids is currently performed for these genes.

Using ray cell specific reporters, we have examined the cellular defects in the *ram* mutant ray cells. Rays of *ram-1* mutant worms have both the structural cells and hypodermis are both swollen. Similar defects are noted in *ram-3* mutant males, which has the most severe Ram phenotype among all *ram* mutants. Individual rays are usually indistinguishable. In addition, *ram-3* maled had abnormal cell bodies trapped within their fans, another features that has been observed in strong *dpy-11* and *ram-4* mutants. These extra cells are not the result of incorrect cell lineage or failure of cell death. Instead, they are confirmed to be neurons and structural cells, which should normally migrate anteriorly into the body. Such results prompt us to investigate if *ram-3* has additional phenotype associated with cell migration.

We constructed double mutants of *ram-3* and *unc-5,* which turns out to have reduction in progeny viability. A reduced hatch rate was observed although a normal number of eggs were laid by the double mutants. These data suggest that there may be possible interactions between these two genes. Since *unc-5* encodes a membrane bound protein (netrin receptor) required for autonomous function in neurons going through migration, genes involved in such axonal migration process are tested for potential interaction with *ram-3*. Additional genes, such as the *unc-6* nectrin, *unc-40 DCC, unc-44*

ankyrin and *unc-34* are being tested. Our preliminary data suggest that they do produce more severe phenotype including lethality. The cellular defects and their potential biochemical interaction will be further investigated.

538. Cloning and characterization of the *mab-7* gene that regulates sensory ray morphogenesis.

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Morphogenesis is a complex process requiring active cellular communication and cell shape changes. Any of these processes disrupted by mutation of specific genes will result in abnormalities in tissue organ function, morphology, or both. *C. elegans* male tail sensory ray is a model organ tissue for studying such a developmental process. A group of genes, the *ram*s (ray morphology abnormal), have been shown to be essential for determining normal ray morphology. Mutations of these genes affect all these male specific sensory organs, which display a swollen morphology. Among these *ram* genes, *mab-7* appears unique. Although mutant males have a similar Ram phenotype, the swelling is more localized at the base of the rays. The phenotype suggests a more localized effect in the ray cell processes.

In this study, we try to characterize the phenotypes of *mab-7* mutant animals using tissue specific markers. Our results show that different cell types in a sensory ray are affected in *mab-7* mutant animals. Both the structural cell and the neuronal processes in a ray appear abnormal and slightly swollen. Hypodermal sheath of the rays is also dramatically enlarged. The results suggest that wherever *mab-7* is expressed, it has the impact on all three ray cell types, possibly through interference of the cellular communication.

We have already rescued *mab-7* mutants with cosmid transformation. From the pattern of ray rescue, *mab-7* is predicted to be made by the hyp syncytium. Further deletion and RNAi experiments will allow us to narrow down the *mab-7* locus and to determine its molecular features. Subsequently, its tissue expression pattern will be verified. Molecular analysis of the product should uncover potential functional property of this gene in ray morphogenesis. Its interactions with other *ram* genes controlling this developmental process will also be examined.

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539. Membrane association of a thioreodoxin-like protein, DPY-11, is important for ray morphogenesis in *C. elegans*.

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Protein disulfide bond formation or reduction are important modification steps for controlling protein function and assembly. Proteins expressed in the extracellular matrices are usually good target substrate for such modifications. One of the enzymes that facilitates this process is thioredoxin, which may be an important player in matrix organization and thus morphogenesis of *C. elegans*.

dpy-11 is one of many *ram* genes that affect sensory ray morphogenesis. Mutations of it give swollen sensory rays at the male tail. *dpy-11* has been cloned and is defined as the predicted thioredoxin-like-protein encoding gene, F46E10.9. Using a *gfp* reporter, we determine its expression pattern to be in the hypodermal cells in all larval and adult stages. To characterize the cellular defect of *dpy-11* mutants, different cell-type specific GFP markers were employed to show that all ray cells within a ray process have abnormal morphology. It implies that the *dpy-11* mutants have defects in protein modification and possibly cell differentiation facilitated by cell-cell communication.

The protein structure of DPY-11 was predicted to have a putative signal peptide, thioredoxin-like domain, a spacer, transmembrane domain and a 46 amino acid carboxyl terminus. To understand the function of *dpy-11*, phenotypic characterization and mutation mapping of 16 existing alleles have been carried out. Mutations on the signal peptide and transmembrane domain affect the function of DPY-11 on body shape, but not the rays. The null mutants have most severe dumpiness and severe swollen rays while the animals with point mutations on thioredoxin-like domain have mild dumpiness and mild swollen rays. Heterozygous mutants of severe and mild alleles have been examined, and the results show that DPY-11 acts in a dosage dependent manner. For further characterization of each protein domain, deletion constructs are made for mutant rescue assay. The enzymatic activity of thioredoxin-like domain is expressed in bacteria and purified for reduction assay. Different versions of DPY-11 GFP fusion protein will also be employed to reveal the membrane-bound property and subcellular localization of this protein. These studies will provide us a clear picture when, where and how dpy-11 takes part in the morphogenesis of male sensory rays.

540. Characterization of nuclear pore membrane protein, gp210

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The inner and outer nuclear membranes merge to form nuclear pores, which are the sites of nuclear pore complex (NPC) assembly and nucleo-cytoplasmic transport. In mammals, the pore membrane domain contains two known integral membrane proteins: POM121, which is not apparently conserved in *C. elegans*, and gp210, which is conserved among metazoans and plants. The *C. elegans* gp210 protein (Ce-gp210) is 19%, 21% and 25% identical (36%, 40% and 44% similar) to the *Arabidopsis*, *Drosophila* and rat gp210 proteins, respectively. Gp210 is proposed to play a fundamental role during membrane fusion, to generate nuclear pores. However, this proposed role is unproven, and is disputed based on the slow rate of gp210 re-accumulation during nuclear assembly in mammalian cells. We are testing the function of gp210 in *C. elegans*. Antibodies raised against different regions of Ce-gp210 reveal a 209-kDa protein on immunoblots of total worm lysate proteins. By immunofluorescence, the antigens colocalize with NPCs during interphase, consistent with specificity for Ce-gp210. During mitosis in *Drosophila* and mammalian cells, gp210 disassembles during early prophase, similar to other nucleoporins. In contrast, Ce-gp210 completely disassembles only during late anaphase, similar to the inner nuclear membrane proteins and lamins, and different from soluble nucleoporins, which disassemble during metaphase. Preliminary results in gp210-deficient (*RNAi*) embryos show that gp210 is essential for viability during embryonic and larval development. Further characterization of the gp210-*RNAi* phenotype is underway, to determine the essential role of gp210 during development.

541. Protein-Protein Interaction Screening for Effectors of NUD-1: A Functional Link Between Nuclear Positioning, Cell Division, and Neuronal Migration

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The brains of children born with lissencephaly lack the stereotypical surface folds associated with brain morphology. Mutations in the gene responsible for this disease, *LIS-1*, result in defective neuronal migration in the developing cerebral cortex. Lissencephaly is more appropriately termed a syndrome because malformations of the heart, kidneys, and other organs, as well as polydactyly and unusual craniofacial appearance, have been associated with it. In this regard, studies from our lab have previously shown that RNAi depletion of the*C. elegans* ortholog of *LIS-1* results in reproducible and distinct post-embryonic phenotypes including a malformed cuticle, everted vulva, sterility, and uncoordination, as well as embryonic lethality due to defective pronuclear migration the predominant RNAi-induced phenotype. An associated gene product that we named NUD-1 exhibits these same phenotypes and has been shown to physically interact with LIS-1 in mammalian systems. *C. elegans* LIS-1 and NUD-1 share significant amino acid identity to proteins that are necessary for nuclear migration in the fungus *Aspergillus nidulans*, In fact, *C. elegans* NUD-1 can functionally complement the nuclear positioning defect of an *A. nidulans nudC* mutation. The human NUD-1 homologue (HNUDC) has been implicated in both normal and malignant hematopoiesis, further suggesting that nuclear migration genes provide an essential function related to cell division processes that have cytoskeletal repercussions in a variety of cell types and tissues. We are investigating the evolutionarily conserved relationship between these genes in the context that they represent a functional link between fundamental processes governing nuclear positioning, cell division, and neuronal migration.

In an effort to elucidate possible cellular pathways involving LIS-1 or NUD-1, we have undertaken a yeast two-hybrid screen to identify putative interacting gene products. We previously isolated a NUD-1 full-length cDNA and have used a fusion of this gene product to the DNA-binding domain of transcription factor LEXA as bait in this screen. After rigorous testing to verify that NUD-1 does not self-induce reporter gene expression, over 1 x 10^6 yeast transformants of *S. cerevisiae* two-hybrid strain L40 were tested for their ability to induce both transcriptional activation of chromosomally integrated *HIS-3* and *lacZ* reporter genes. Clones passing these criteria were scrutinized for false interactions by isolation of all library vector DNAs, retransformation of yeast, retesting for interactions, and by verifying a lack of interaction with non-specific proteins. The results of this analysis yielded evidence that NUD-1 interacts strongly with itself, perhaps as a dimer or multimer, since it was identified as an interacting clone in multiple isolates. This result is interesting, as LIS-1 has also been shown to function as a dimer. Furthermore, the region of NUD-1 in the interacting clones corresponds to a N-terminal extension found only in metazoan homologs of this protein. Another strong positive interactor was a predicted open-reading frame encoding a protein that exhibits homology to PGL-1-like proteins. Given the role of NUD-1 in embryonic cell division, it is interesting to speculate that this protein may interact with a possible component of P granules and considering their role in the establishment of embryonic asymmetry. Weaker, but reproducible protein interaction was also identified between NUD-1 and a gene encoding *C. elegans* beta-tubulin, another known component of the nuclear migration machinery. Simultaneous co-immunopreciptiation experiments and phenotypic analyses by RNAi and GFP expression are underway to clarify these interactions and examine putative functional relationships between NUD-1 and these proteins.

542. Phenotypic Crosstalk Between the LIS-1 and Cdk5 Pathways in *C. elegans*

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Cdk5 and its activators p35 and p39 are well-studied genes found to be expressed predominantly in neuronal cell types and to play roles in both neuronal migration events and myogenesis. However, unlike other activator/cyclin-dependent kinase complexes, Cdk5, complexed with either p35 or p39, has not been implicated in cell cycle regulation. Previous work has indicated that the *C. elegans* homologues of Cdk5 and p35 are both neuronally expressed by translational GFP reporter constructs and may play a role in neuronal development (Harbaugh and Garriga, 2000 West Coast Worm Meeting). Interestingly, our lab has found embryonic phenotypes associated with these genes through both p35 and *cdk-5* RNAi experiments and an analysis of *mig-18(k140)* embryos (a putative allele of *cdk-5*). The embryonic phenotypes associated with deficiencies in *cdk-5* include pronuclear migration and rotation defects in one-celled embryos. In p35 (RNAi) embryos, centrosome mispositioning also occurs. Furthermore, offspring of worms fed either p35 or *cdk-5* dsRNA, individually or in combination, exhibit uncoordinated phenotypes.

Recently, several groups (Neuron 28:665-711) have shown a connection between the Cdk5 and LIS-1 pathways in higher organisms, previously believed to be separate mechanisms in brain development. Our work supports these findings, as we have examined the consequence of p35 and *cdk-5* RNAi on *lis-1*::GFP expression patterns, wherein we find altered neuronal expression. Moreover, we observe enhanced uncoordinated phenotypes when combining *lis-1* dsRNA with either p35 or *cdk-5* dsRNA. In addition, we have found phenotypic changes in embryos from parental worms fed combinations of *lis-1*, p35, and *cdk-5* dsRNA, including centrosome duplication and positioning errors. Taken together, these results seem to indicate a connection between the neuronal and embryonic development pathways mediated by *cdk-5* and

lis-1 in

C. elegans.

543. *C. elegans* NUD-1 and LIS-1 are Neuronally Expressed and are Required for Embryonic Development and Nuclear Positioning

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Accurate nuclear positioning is necessary for correct segregation of genetic material in dividing cells and is critical to the vertebrate and invertebrate embryonic development. We discovered the presence of genes related to this mechanism within the genome of the nematode *Caenorhabditis elegans.* Our work has focused on the *C. elegans* orthologs of two genes, *lis-1* and *nud-1*, originally identified as being required for nuclear migration in the multinucleated hyphae of the filamentous fungus, *Aspergillus nidulans*. We have shown cross-species conservation of function for one of these genes and both are highly expressed in early embryos and neurons, in addition to other tissues. *C. elegans* LIS-1 protein is 68% identical to human LIS-1, a gene product linked to a severe defect in neuronal migration termed lissencephaly. We used RNA-mediated interference (RNAi) to generate mutant phenocopies of the *lis-1* and *nud-1* genes. These data indicate that both these genes are essential for a variety of developmental processes including proper embryonic cell division, gonad morphogenesis, and neuronal function. Using time-lapse video microscopy, we have determined that depletion of *C. elegans* LIS-1 and NUD-1 results in embryonic lethality and phenotypically mimics results obtained by blocking dynein and dynactin activity, respectively. Specifically, these genes exhibit defective pronuclear migration and rotation abnormalities. We are in the process of cloning candidate mutations corresponding to these genes and further defining their function cytologically. These results serve as a foundation for future investigation into the cytoskeletal mechanisms governing nuclear positioning, cell division, and neuronal migration.

544. The expression, lamin-dependent localization, and loss-of-function phenotype for emerin in *C. elegans*

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Emerin is an integral protein of the inner nuclear membrane. In humans, loss of emerin causes the X-linked form of Emery-Dreifuss muscular dystrophy. The emerin gene (*emr-1*) is conserved in *C. elegans*. We show that Ce-emerin protein is expressed ubiquitously in *C. elegans*, and that Ce-emerin localization at the nuclear envelope requires the *C. elegans* lamin protein, Ce-lamin. We determined the loss-of-function phenotype for emerin in *C. elegans*, by double-stranded RNA inhibition (RNAi) of the *emr-1* gene. In embryos lacking Ce-emerin, we detected normal nuclear envelope localization for Ce-lamin, UNC-84, nucleoporins and Ce-MAN1, which is related to emerin through the conserved LEM-domain. The RNAi-induced loss of Ce-emerin had no apparent effects on development, since Ce-emerin-deficient embryos developed normally into fertile adult nematodes. Furthermore, the RNAi-induced loss of emerin persisted into adulthood, with no detectable phenotype. Based on its lamin-dependent localization at the nuclear envelope, biochemical properties and non-essential phenotype, Ce-emerin closely resembles human emerin. These findings establish *C. elegans* as a suitable genetic organism in which to dissect the molecular mechanisms of emerin function *in vivo*.

545. A novel nuclear envelope protein with germline-specific expression

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The Lamina is an essential architectural element of the nucleus, which is involved directly or indirectly in nuclear structure, cell cycle regulation, transcriptional repression, cells differentiation and apoptosis. The nuclear lamina is composed of nuclear lamins and lamina-associated proteins. We have recently identified a 55-kDa novel *C. elegans* protein named matefin by its homology, in the SUN domain, to UNC-84. Antibodies against matefin revealed that it is an integral membrane protein of the nuclear envelope that colocalizes with the nuclear lamina. In early embryos, matefin is present in all cells. In late embryos and throughout the rest of development, matefin's expression is confined to the germ cells. This pattern of expression is similar to that of the *C. elegans* MES proteins, which are predicted transcriptional regulators that are essential for germline survival. Matefin expression in the gonad is essential for their survival since matefin depletion induced by feeding animals with *mtf-1* dsRNA (*mtf-1 (RNAi))* , caused sterility in the progeny hermaphrodites.

We hypothesize that that matefin may function, along with the MES proteins, in regulating germline transcription.

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546. Essential roles for *C. elegans* lamin gene in nuclear organization, cell cycle progression and spatial organization of nuclear pore complexes

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We are interested in using *C. elegans* as a model system to study the function of nuclear lamin and lamin-associated proteins during development. *C. elegans* has a single lamin gene, designated *lmn-1* (previously termed CeLam-1). Antibodies raised against the *lmn-1* product (Ce-lamin) detected a 64 kD nuclear envelope protein. Ce-lamin was detected in the nuclear periphery of all cells except sperm and was found in the nuclear interior in embryonic cells and in a fraction of adult cells. Reductions in the amount of Ce-lamin protein produce embryonic lethality. Although the majority of affected embryos survive to produce several hundred nuclei, defects can be detected as early as the first nuclear divisions. Abnormalities include rapid changes in nuclear morphology during interphase, loss of chromosomes, unequal separation of chromosomes into daughter nuclei, abnormal condensation of chromatin, an increase in DNA content, and abnormal distribution of nuclear pore complexes (NPCs). Under conditions of incomplete RNA interference a fraction of embryos escaped embryonic arrest and continue to develop through larval life. These animals exhibit additional phenotypes including sterility and defective segregation of chromosomes in germ cells. Our observations show that *lmn-1* is an essential gene in *C. elegans*, and that the nuclear lamins are involved in chromatin organization, cell cycle progression, chromosome segregation, and correct spacing of NPCs.

547. ANC-1 is an over 800 kDa coiled-coil protein required for anchorage of nuclei

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The *C. elegans* adult has many syncytial cells. In the hypodermis alone, over 100 nuclei are in syncytia. Normally nuclei are anchored in a stereotypical pattern evenly spaced throughout the syncytium. Mutations in *anc-1* disrupt this anchorage, and the nuclei move through the syncytia (pushed around by underlying body muscles or forces from crawling) and often clump together (Hedgecock and Thomson, 1982 *Cell* 30:321-330). Certain mutations in *unc-84,* which encodes a nuclear envelope protein that functions in nuclear migration events, also disrupt nuclear anchorage (Malone et al., 1999 *Development* 126:3171-3181).

We mapped the *anc-1* gene between *unc-73* and *let-502*, but were unable to rescue the phenotype by traditional cosmid injections. We therefore fine-mapped *anc-1* using single nucleotide polymorphisims (SNPs) to a 100 kb region between two SNPs. We used RNAi to knock out candidates in this small region. RNAi against any one of three regions of a predicted 25 kb open reading frame in this region gave a strong ANC phenotype. This suggests that the *anc-1* alleles are null. The *anc-1* gene is predicted to encode a 7530 amino acid protein. The *anc-1* genomic region contains six 3 kb tandem repeats which are nearly 100% conserved, even in a 123 bp intron, suggesting that there are evolutionary forces to keep the ANC-1 protein as large as possible.

We have raised antibodies against the repeat region in rats and western analysis confirms that we have cloned *anc-1*. The anti-ANC-1 antibodies localize to most somatic post-embryonic cells. ANC-1 was detected in unorganized, fibrous structures throughout the cytoplasm and was often enriched at the nuclear periphery. We propose two alternative models for ANC-1 function. ANC-1 could form a filamentous network and act as a net to anchor nuclei or ANC-1 could tether nuclei by directly attaching the nucleus to the plasma membrane.

548. THE *C. elegans* LISSENCEPHALY (LIS1)-LIKE GENE *lis-1* IS REQUIRED FOR EMBRYONIC DEVELOPMENT

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In the human disease lissencephaly, abnormal neuronal migration during brain development leads to reduced cerebral convolutions. Patients with lissencephaly suffer from epilepsy and mental retardation. The most common cause of lissencephaly is haploinsufficiency of the gene LIS1. The predicted *C. elegans* LIS-1 protein is 58% identical to human LIS1. We have isolated two deletion alleles of *lis-1*. One deletion removes 1,465 nucleotides, including predicted exons 4-5, and a second deletion removes 2,019 nucleotides, including predicted exons 4-6. These two mutations fail to complement each other for the defects described below.

Deletion homozygotes are Unc, Egl, and Mel. Progeny of deletion homozygotes arrest at the 50-100 cell stage and contain enlarged, heterogeneous, asymmetrically distributed nuclei. We have analyzed the cell biological basis of the defects in embryonic development and in egg-laying. A *lis-1::gfp* transcriptional fusion gene driven by the *lis-1* promoter is expressed in multiple tissue types in transgenic worms; expression in the nervous system is restricted to a subset of neurons. We are now attempting to confirm this expression pattern using antibody staining. To test whether the human and worm genes might be orthologous, we are attempting to rescue the phenotype of the worm deletion mutants with the human LIS1 cDNA driven by either the heat-shock promoters or the endogenous *lis-1* promoter.

We are seeking *lis-1* interactors both by screening for suppressors of the *lis-1* Mel phenotype and by testing candidate genes. A preliminary F1 screen of ~10,000 haploid genomes failed to yield suppressors of the *lis-1* Mel phenotype. We now plan to extend this screen to the F2 generation.

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549. The cDNA sequence and expression of the AAA peroxin genes *pex-1* and *pex-6* from the nematode *Caenorhabditis elegans*

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We cloned cDNA encoding two peroxins, PEX-1 and PEX-6, of the nematode *Caenorhabditis elegans*. Peroxins are proteins that play essential roles in peroxisome biogenesis and are encoded by increasing numbers of *pex* genes. Among peroxins, Pex1p (yeast protein)/PEX1 (mammalian protein) and Pex6p/PEX6 constitute the subfamily 2 (SF2) of AAA proteins. SF2 presents two AAA cassettes of which the one located closest to the C-terminus is highly conserved, while the other one diverges from the consensus sequence considerably. Disorders of peroxisome biogenesis cause abnormal neuronal cell migration in brain development. This could be studied with the model organism *Caenorhabditis elegans* whose cell lineage has been completely established. However, none of the peroxins of this organism has been identified. In the present study we cloned cDNAs encoding two putative peroxins that belong to SF2; the genes are termed *pex-1* and *pex-6*.

The length of each cDNA agreed well with the apparent size of the respective mRNA. From this and other lines of evidence we concluded both of the cDNAs to be full-length. The *pex-1* cDNA was composed of 24 exons, which were coded by a genomic region containing three ORFs, c11h1.4, c11h1.5, and c11h1.6, predicted by the *C. elegans* Sequencing Consortium. The ORF c11h1.5 that has a different orientation from the others was encompassed in the 15th intron of the *pex-1* cDNA. Although many exon-intron borders in *pex-1* were inconsistent with those predicted for c11h1.4 and c11h1.6, those in *pex-6* coincided with those for the ORF f39g3.7. The *pex-1* and *pex-6* genes encoded proteins with 996 and 720 amino acid residues long, respectively. Both *pex-1 mRNA and pex-6 mRNA were detectable throughout the life cycle of C. elegans, however the amount relative to that of an the egg-lying adult stage than at the embryo, L1 larva, or young adult stages. Whole-mount in situ hybridization using antisense RNA probes suggested that both pex-1 mRNA and pex-6 mRNA accumulated mainly in intestinal cells.*

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550. Modeling peroxisome biogenesis disorders and identification of novel peroxisomal proteins in *C. elegans*

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The peroxisome is a single-membrane bound orgenelle that functions in both anabolic and catabolic pathways in eukaryotic cells. The peroxisome is the primary site for the beta-oxidation of very long chain fatty acids and is the site where hydrogen peroxide metabolism is carried out by oxidases and catalase. The importance of the peroxisome in human health is illustrated by the occurrence of several severe genetic disorders associated with defects in the peroxisome. Neuronal migration defects and degeneration in the CNS are among the clinical manifestations found in peroxisomal biogenesis disorders in humans. We are modeling these disorders in the nematode in order to obtain a better understanding of the toxic effects that peroxisome dysfunction causes for both the cell and the organism. This will lead to a clearer understanding of the pathogenesis of these lethal disorders.

There are at least 23 proteins (peroxins) that are required for correct peroxisome assembly in both humans and yeast. We have identified the *C. elegans* homologs to many of these genes. Knockdown of five of these genes in the worm using RNAi results in developmental arrest at the first larval stage supporting an essential function for the *C. elegans* peroxisome. When we performed the knockdown experiments in the background of a peroxisome-localized GFP, the GFP becomes mislocalized to the cytoplasm, suggesting a block of peroxisomal import and/or biogenesis in the worm. This is the same cellular phenotype found in fibroblasts from patients that have the peroxisome biogenesis disorders such as Zellweger Syndrome.

C. elegans appears to use only one targeting signal to mark matrix proteins for import to the peroxisome. This peroxisomal targeting signal (PTS1) is made up of a tripeptide sequence (S/A/C - K/R/H - L/M) located at the extreme C-terminal end of a protein. A search of all *C. elegans* ORFs resulted in 92 predicted proteins that contain this signal at their C-terminal end. Our analysis using both the PSORT and BLAST algorithms indicates that among these are 27 that have direct peroxisomal homologs in other systems. In addition, there are 15 novel proteins that are predicted by PSORT to be peroxisomal proteins. These proteins are either proteins of unknown function or proteins that have not been previously known to be peroxisomal in any other organism. We are analyzing these proteins using GFP fusions to confirm their peroxisomal locations.

551. A *Caenorhabditis elegans* catalase with an unusual feature

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Earlier attempts to detect peroxisomes in the free-living nematode *Caenorhabditis elegans* by the alkaline-diaminobenzidine method that exploits the peroxidase activity of only peroxisomal catalase at pH 9 were not successful. This motivated us to purify catalase, a peroxisomal marker form *C. elegans* whose genome has genes *ctl-1* and *ctl-2* encoding catalases closely similar to each other. A near to homogeneity preparation was obtained with a purification fold of 2900 and a yield of 15%. The purified enzyme is a protein of molecular weight of 220kDa and is composed of four subunits. Antiserum raised against a synthetic pentadecapeptide corresponding to the C-terminal sequence of the *ctl-2* product specifically recognized the purified enzyme, and was used in immunoelectron microscopy to confirm the peroxisomal localization of catalase obtained in cell fractionation. The enzyme is similar in squence to other catalases from various sources, except that it showed an unexpectedly low pH optimum (pH 4.0) for its peroxidase activity. This feature is different from catalases thus far known and explains why the *C. elegans* peroxisome was undetectable when the widely used alkaline-diaminobenzidine staining method was applied.

552. Biochemical and functional analysis of SKP1 family in *Caenorhabditis elegans*

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The ubiuquitin-proteasome pathway is a key mechanism for substrate-specific degradation to control the abundance of a number of proteins. SCF complex, one of ubiquitin-protein ligases (E3s), regulates cell cycle progression, signal transduction, and many other biological systems. The SCF complex consists of invariable components, such as Skp1, Cul-1 and Rbx1, and variable components called F-box proteins that bind to Skp1 through the F-box motif. F-box proteins are substrate-specific adaptor subunits that recruit substrates to the SCF complex. Surprisingly, we found that the genome of *Caenorhabditis elegans* (*C. elegans*) contains at least 20 Skp1-like sequenses, whereas one or a few Skp1 is present in humans. Therefore, we studied *C. elegans* Skp1-like proteins (CeSkp1) that are likely to be variable components of SCF complex in addition to F-box proteins. At least, seven CeSkp1s were associated with *C. elegans* Cul-1 (CeCul-1) in yeast two-hybrid system as well as co-immunoprecipitation assay in mammalian cells, and these expression patterns were different in *C. elegans*. By RNA interference (RNAi), two of these CeSkp1s showed embyonic lethality and four showed the phenotype of slow growth. There were differences among CeSkp1s in ability to interact with F-box proteins. These results suggest that CeSkp1s, like F-box proteins, act as variable components of SCF complex in *C. elegans*.

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553. Emergence of an Ancient Intracellular Antiproteinase Defense System

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Background: High molecular weight serine proteinase inhibitors (serpins) such as antithrombin III, α2-antiplasmin and α1-antitrypsin are critical regulators of intravascular (clotting and thrombolytic) and extravascular proteolytic cascades. We have characterized a family (n>13) of human ovalbuminlike Serpins. Unlike the fluid phase serpins, the ov-serpins lack N-terminal signal peptides and reside within the cytosol of epithelial cells. However, the function of these molecules is unknown.

Objective: Determine whether the function of the ov-serpins can be elucidated using a comparative genomics approach and a simpler organism, *C. elegans*.

Design/Methods: Database analysis and molecular cloning were used to identify serpins.

Results: Screening of ACeDB revealed the presence of 9-10 functional serpin genes (*srp-1-10*). They are tandemly arrayed along Chromosome V. By a combination of cDNA cloning and RT-PCR, cDNAs corresponding to 6 of the genes were isolated. The genes contain 5-6 exons with conserved splice sites at the 5' and 3' ends but some variation within. Like the ov-serpins, none of the worm serpins encode for typical N-terminal signal peptides. Thus, *C.elegans* serpins also are likely to reside intracellularly. Each serpin contains different residues in the reactive site loop (the reactive site loop is that portion of the serpin that binds to the active site of the proteinase). This variation suggests that each serpin will inhibit different types of proteinases. Indeed, kinetic analysis in vitro, using recombinant proteins expressed in *E.coli*, showed that *srp-2* and *srp-3* are inhibitors (k_{ass} ~$1x10^4 M^{-1} sec^{-1}$) of granzyme B and chymotrypsin-like serine proteinases, respectively. Preliminary transgenic studies using a vector containing the *srp-2* promotor fused to a GFP reporter gene showed that, like the ov-serpins, *srp-2* is highly expressed in the hypoderm.

Conclusions: Members of the ov-serpin family are functionally and topologically conserved in *C.elegans*. Reverse genetic studies by the generation of null mutations should reveal the functions of this intracellular anti-proteinase defense system that is highly conserved through out metazoan evolution.

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554. Activation of protein degradation in muscle: A non-transcriptional output of the Ras-MAP kinase signaling pathway

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Many disease states cause muscle wasting associated with increased mortality, but the intracellular signals that trigger muscle wasting remain largely uncharacterized. To discover and study such signals in *C. elegans* we have employed a transgene that fuses a portion of the unc-54 myosin heavy-chain gene to *E. coli* lacZ, producing a soluble fusion protein with beta-galactosidase activity expressed only in body-wall and vulval muscles. This reporter protein is completely stable in well-fed wild-type animals, but its degradation is triggered by starvation or denervation, which also promote muscle wasting in mammals. In well-fed worms carrying a temperature-sensitive activating mutation (ga89) in the Ras oncogene homologue let-60, reporter protein degradation is initiated upon shift to non-permissive temperature (25C). Normal growth rates at 25C imply that these mutants are not physiologically "starved", and the reporter is degraded in all muscle cells rather than showing the anterior-posterior distinctions characteristic of starved animals. This degradation is not prevented by the addition of cycloheximide at the time of temperature shift, and thus uses pre-existing proteolytic systems and signaling components. Furthermore, degradation is triggered in animals shifted to 25C as adults, confirming that Ras "acutely" promotes protein degradation rather than acting by altering some aspect of muscle development.
Reduction-of-function mutations in the downstream protein kinases Raf (lin-45), MEK (mek-2) or MAP kinase (mpk-1) prevent Ras-induced protein degradation, whereas a transgenic strain with heat-inducible activated-MEK and overexpressed wild-type MAP kinase degrades reporter protein in muscle after heat induction. This indicates that the Raf-MEK-MAPK cascade is the principal route by which Ras signaling triggers muscle protein degradation. There is so far no evidence that relates Ras-MAPK signaling to starvation-induced protein degradation in muscle, insofar as reduction-of-function mutations in Ras (let-60(n2021)), Raf, MEK or MAPK affect neither the rate nor the anterior-posterior cellular specificity of starvation-induced lacZ degradation. The nicotinic agonist levamisole does not prevent Ras-induced protein degradation, in contrast to its protective action in animals with genetically disrupted cholinergic signaling; thus, Ras activation does not simply phenocopy the effect of denervation. There is some indication that activation of Ras-Raf-MEK-MAPK signaling also provokes protein degradation in mouse muscle cells, suggesting that *C. elegans* will continue to be useful for studying signaling systems that regulate intracellular proteolysis in muscle.

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555. CHARACTERIZATION OF RNF-5, A RING FINGER PROTEIN WITH E3 LIGASE ACTIVITY WHICH CONTRIBUTES TO CELL PROLIFERATION AND DEVELOPMENT IN C. ELEGANS.

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In searching for RING finger proteins in C. elegans that resemble mammalian RINGs known to exhibit E3 ligase activities, we characterized rnf-5 (C16C10.7), the nematode homolog of RNF5, an unknown protein that shares features within the RING domain with BRCA1, Cbl and Mdm2. Three different spliced transcripts of rnf-5 were expressed during development, with the highest expression in young adults. Interestingly, L2d pre-dauer larvae preferentially expressed an rnf-5 mRNA that lacks the RING domain. We used RNAi to determine the function of rnf-5 during C. elegans development. We found that rnf-5(RNAi)-treated worm populations showed an increased fraction of dauer and dauer-like larvae. Adult progeny of animals treated with rnf-5 dsRNA had an overcrowed germ line containing a higher density of germ cell nuclei. We also noticed a weak egg-laying defect in these animals. Over-expression of rnf-5 resulted in slower growth. In vitro ubiquitination assays revealed that RNF-5 exhibits E3 ligase activity, as reflected by efficient self-ubiquitination upon the addition of E1, E2 and ubiquitin. The E3 ligase activity of RNF-5 was confirmed in 293T cells, which exhibit a high basal level of ubiquitination under normal growth conditions. A marked decrease in RNF-5 ubiquitination was noted following UV-irradiation, suggesting that stress inhibits the E3 ligase activity of the protein. PI3K p110 or AKT efficiently inhibit RNF-5 E3 ligase activity, suggesting that RNF-5 might be regulated by the insulin pathway. Taken together, our results identify a new RING finger protein that exhibits E3 ligase activity and is likely important to the regulation of cell proliferation and development in C. elegans.

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556. Identification and characterization of a Ubiquitin C-terminal Hydrolase in *C. elegans*

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Ubiquitin, a highly conserved protein composed of 76 amino acids, is important for many cellular processes including the regulation of intracellular protein breakdown, cell cycle regulation, and stress response.

Ubiquitin C-terminal hydrolase (UCH) is a thiol protease that recognizes and hydrolyzes the peptide bond at the C- terminal glycine of ubiquitin. This enzyme is involved in the processing of poly-ubiquitin precursors as well as ubiquitinated proteins. UCHs have two homologous regions; the first region contains a conserved cysteine domain, the second contains two conserved residues. These two homologous regions are likely implicated in the catalytic mechanism.

We searched the worm genome database and located a putative homologue of UCH in the cosmid ZK328 (LGIII). *C. elegans* UCH (ZK328.1) consists of 14 exons encoding 1178 amino acids and contains two functional domains that are well conserved in other organisms.

Expression pattern was investigated using GFP and is shown to express in excretory cells, coelomocytes, hypodermal cells, pharynx, and neuronal cells. Northern blot experiments revealed an mRNA transcript of 3.7 kb. To better understand the function of UCH in *C. elegans*, RNA-mediated interference (RNAi) technology was conducted and resulted in high embryonic lethality suggesting that *Ce-uch-1* has an essential role in the early embryo development of *C. elegans*. The RNAi phenotype was rescued by mating with wild type males. Furthermore, whole mount immunostaining showed that UCH stains the microtubule organizing center (MTOC) in fertilized embryo and in the sperm cytosol. Our data suggests that UCH is indispensable for the formation of a sperm-contributed functional MTOC and early cleavage during embryogenesis in *C. elegans*.

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557. Peptide metabolism in *C. elegans*: expression and functional characterisation of a cytoplasmic aminopeptidase P

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Aminopeptidase P (AP-P; X-Pro aminopeptidase; EC 3.4.11.9) is a metallopeptidase that specifically removes N-terminal amino acids from peptides normally protected from attack by general aminopeptidases through the presence of proline in the penultimate N-terminal position. Since many biologically active peptides contain N-terminal Pro residues, AP-P can have a key role in initiating the metabolism and subsequent inactivation of such peptides. There are two forms of mammalian AP-P, an integral membrane form and a less abundant cytosolic enzyme. The membrane AP-P is a kininase, degrading the potent vasodilator bradykinin in the rat pulmonary vascular bed, the rat heart and in the human skin. It is also involved in collagen turnover by hydrolysing collagen derived peptides and may have a role in regulating peptides of the immune system. In contrast, the physiological roles of the mammalian cytosolic AP-P are unknown.

The sequencing of the entire genome of *Caenorhabditis elegans* has identified several aminopeptidase genes, one of which (*W03G9.4*) shows high homology to mammalian cytoplasmic AP-P. We have generated His-tagged AP-P through high-level expression in *Escherichia coli*. The purified enzyme hydrolyses the Arg-Pro, Lys-Pro and Ala-Pro bonds of bradykinin, the *C. elegans flp-9* gene product KPSFVRFamide and locustatachykinin (GPSGFYGVRamide) respectively. Using bradykinin as the substrate, we have shown that the *C. elegans* AP-P has many of the properties of mammalian AP-P. For example, it is inhibited by chelators of divalent metal ions, has a neutral pH optimum and is inhibited by the selective AP-P inhibitor, apstatin (IC_{50} = 1 μ M).

Spatio/temporal expression pattern analysis using a *W03G9.4* promoter::*GFP* transgene showed expression to be predominantly in the most anterior cells of the intestine (Int 1) from the L2 stage through to the adult. This restricted expression in the intestine indicates a specific role for the enzyme in gut function. Interestingly, the highest levels of expression of mammalian cytoplasmic AP-P occur in the pancreas.

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558. Expression of *C. elegans* ADAM proteins in a mammalian system.

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ADAMs are integral membrane proteins which contain **a** **d**isintegrin **a**nd **m**etalloprotease domain, and have the potential to participate in proteolysis, adhesion, fusion and signaling events. So far thirty ADAM genes have been described from across the animal kingdom, but their precise functions and mechanisms remain largely unknown. A small number of mammalian ADAMs however, have been shown to be important in development, signal transduction, sperm and egg binding and muscle cell fusion. In addition, some *C. elegans* ADAMs have been investigated recently and these are thought to be involved in sperm and epithelial cell fusion events (*adm-1*), in early embryonic development (*adm-2*), and in vulval development (*sup-17*). *C. elegans* also possesses homologues of ADAM-like genes known as ADAM-TS. Proteins encoded by this gene family are not membrane bound and have an additional thrombospondin-like motif, for example GON-1 and MIG-17. These proteins play essential roles in the morphogenesis of the *C. elegans* gonad.

We are studying three other ADAMs genes in *C. elegans*: *adm-4* (a tumour necrosis factor alpha converting enzyme or TACE homologue); and two soluble ADAM-TSs, (*C02B4.1* and *T19D2.1*). GFP reporter data indicates that *adm-4* is highly expressed in most tissues in all post-embryonic stages. The expression pattern for *C02B4.1*::*GFP* however is more confined and is observed in body wall and pharyngeal muscle. We aim to complement the reporter data with RNAi/knockout mutagenesis work, but our main goal is to biochemically analyse the recombinant proteins in a mammalian expression system. Using this approach we hope to identify which proteins are cleaved by these ADAMs proteins, as well as providing information about their targeting and processing. Ultimately we hope to elucidate their physiological function and relate this information to mammalian ADAM proteins.

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559. Systematic RNAi analysis of maternal genes reveals the function of proteasome in oocyte maturation and fertilization.

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Our cDNA/expression pattern project has provided unique sets of limited numbers of genes that possibly participate in specific stages, lineages, tissues and so on. Such sets of genes are suitable to systematic analysis to elucidate the molecular mechanisms governing the biological processes. Since we are particularly interested in early embryogenesis, we have focused on a set of genes whose mRNA are maternally supplied and disappear or localize to specific cells during early stages (2-cell~early gastrulation). We found out 477 (about 10%) such genes when the expression analysis of about 5,000 genes was finished, and have done RNAi (RNA mediated interference) analysis on the 477 genes.

We microinjected double-stranded RNA into the gonads of N2 worms. Phenotypic analyses were performed on the injected worms themselves, F1 embryos, larvae and adults that survived (called "escapers") and F2 embryos, with respect to embryogenesis, larval growth, sterility, morphogenesis, and so on. Out of the 477 genes, 5% showed the reduction of the number of F1, 33% showed F1 embryonic lethality, 27% showed one or various phenotypes with only escapers and 35% did not show any phenotype. Interestingly, genes in X chromosome showed much higher ratio of no-phenotype than those in autosomes (X chromosome: 42/71, autosomes: 125/404).

We further analyzed early cell division using the 4D microscope for the genes that showed more than 50% embryonic lethality (61 genes) in the RNAi screening. Out of them, 12 genes showed the arrest at fertilized egg and no division, 29 genes showed aberrant process until 8-cell stage and 20 genes showed no phenotype until 8-cell stage.

Interestingly, 5 out of the 12 genes of the severest phenotype (arrest at fertilized egg) related to proteasome; 2 genes are proteasome components and 3 genes are proteasome regulatory subunits. Proteasome is known to participate in the regulation of meiotic maturation and fertilization in fish and frog oocytes. Thus, we examined all possible proteasome genes. In *C.elegans*, 14 genes for proteasome components are predicted. cDNA are available in our collection for 13 genes including the 2 genes in the above analysis. Their mRNA were detected in gonads (12/13), strongly detected in extreme early stage embryo and disappear by early gastrulation (6/13) or localize to specific cells (6/13). We did RNAi experiments for these genes, and they showed high F1 embryonic lethality (12/13) and strong reduction of the number of F1 (12/13). The affected embryos did not show any cell divisions and seemed to be arrested during meiosis like the previous ones. Immunostaining for one of the genes showed strong staining at nuclei of oocytes. These results indicate that proteasome plays important roles during meiosis in *C.elegans*. The experiments on the genes of proteasome regulatory subunits are in progress. Some of them show similar results to that of proteasome components.

560. Functional analysis of potential E2 ubiquitin conjugating enzymes using RNAi.

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We are investigating how genes predicted to be involved protein degradation effect embryogenesis in *Caenorhabditis elegans*. Within the cell, protein degradation is primarily accomplished through the ubiquitin-proteasome pathway. Studies in other systems show that E2 and E3 enzymes work in tandem to attach ubiquitin to a specific protein substrate, thereby condemning the substrate to degradation by the proteasome. We have identified 26 potential E2 genes within the completed genome of *C. elegans*. We are assessing the function of these genes through the use of RNAi-mediated interference (RNAi). E3 ligases are less conserved and more numerous than E2s. One class of E3 enzymes contains proteins with RING finger domains. We have previously identified 112 genes containing a RING finger in the *C. elegans* database. Four of the RING finger proteins were found to be required for embryogenesis (Moore, ECWM 2000, 154). By comparing E2 RNAi phenotypes with the RING finger mutant phenotypes, we hope to determine which E2 ubiquitin-conjugating enzymes partner with specific RING finger proteins. One of the four essential RING finger containing genes is *par-2*, a gene involved in establishing anterior-posterior polarity in the embryo. PAR-2 protein is localized asymmetrically to the posterior cortex in embryos. In order to understand if protein degradation is involved in PAR-2 localization, we are using a transgenic strain expressing PAR-2:GFP to observe PAR-2 localization in E2 RNAi embryos.

561. A screen for natural targets of NMD using *C. elegans* cDNA microarrays

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mRNA transcripts that contain premature termination codons are degraded in a regulated manner by a specific decay pathway termed *mRNA surveillance* or *nonsense mediated mRNA decay* (NMD). mRNA surveillance has been described in all eukaryotes tested including plants, flies, yeast, nematodes, and mammals. Loss-of-function mutations affecting any of seven *smg* genes eliminates mRNA surveillance in *C. elegans*. While much progress has been made in defining the *cis*-acting elements and *trans*-acting factors necessary for mRNA surveillance, less is know about the mRNAs produced during the normal course of gene expression that serve as substrates of NMD.

In order to systematically investigate these natural targets of NMD, I have probed high density microarrays of *C. elegans* cDNA clones. These arrays, made in Stuart Kim's lab at Stanford University, consist of PCR fragments from 17,871 genes currently annotated in the *C. elegans* genome (Jiang et al., 2001). We performed nine different hybridizations, each comparing expression profiles of poly(A)$^+$ mRNA from *smg(-)* and *smg(+)* samples. The microarray screen identified 402 mRNAs whose abundance is 2-fold or greater in *smg(-)* mutants. Several observations suggest that microarrays are a reliable method for identifying natural targets of mRNA surveillance:

1. Control mRNAs known previously to be natural targets of mRNA surveillance (*rpl-12* and *srp-20*) were consistently elevated on the microarray screen.

2. Both northern blots and quantitative PCR analyses confirm the microarray results.

These results indicate that the microarray screen will be useful for the systematic investigation of the *C. elegans* natural targets of NMD. We are presently analyzing *smg(-)*affected mRNAs to establish whether they are direct or indirect

targets of NMD, and understand why their abundance is increased in *smg(-)* mutants. We anticipate that analysis of the ~400 candidate genes will identify broad categories of mRNAs produced in wild-type animals yet targeted for degradation by NMD and, possibly, new principles of post-transcriptional regulation of gene expression.

Jiang M, Ryu J, Kiraly M, Duke K, Reinke V, Kim SK. Genome-wide analysis of developmental and sex-regulated gene expression profiles in *Caenorhabditis elegans*. *Proc. Nat. Acad. Sci. USA.* (2001) **98**(1):218-23.

562. Molecular, genomic and genetic analysis of alternative splicing in *C. elegans*

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Our lab is interested in regulation of alternative splicing and we have begun several projects to study alternative splice site choice using *C. elegans* as a model system. To this end, we have developed the Intronerator as a computational system for using EST information in *C. elegans* to identify alternatively spliced genes. We have identified over 800 alternatively spliced genes using this system (1). By comparing genomic sequence of *C. briggsae* and *C. elegans* we have identified regions of conservation near some of these spliced genes that are candidates for putative *cis*-regulatory elements for alternative splicing (2). We are developing splicing reporter assays to test the activity of these conserved elements in regulation of alternative splicing.

One alternatively spliced gene we are studying in depth is the *let-2* gene. Alternative splicing involves the use of two mutually exclusive exons, exons 9 and 10. In embryos, exon 9 is primarily used and in adults exon 10 is primarily used, with a gradual switch in the usage occurring during the larval stages (3). We have created a splicing reporter construct in which the alternatively spliced region of *let-2* is fused to green fluorescent protein. The almost exclusive usage of exon 9 in embryos is maintained in this construct. A series of *cis*-mutations that we have devised in this reporter indicate that the unusual beginning of the intron between exon 10 and exon 11, the intron starts with GC instead of the canonical GT, is important for promoting the splicing of exon 9. *Cis* mutagenesis of the alternatively spliced regions of this gene to identify splicing regulatory elements is continuing. In addition, we have made a splicing reporter for this gene in which only the adult form of the message is in frame with GFP. Animals containing this reporter do not express detectable GFP until the L2 stage. We are using this reporter in a visual screen to identify genes involved in the regulation of *let-2* alternative splicing. Our progress in this screen will be presented.

1. Kent, W.J. and Zahler, A.M. 2000. Nucleic Acids Research 28: 91-93.

2. Kent, W.J. and Zahler, A.M. 2000. Genome Research 10: 1115-1125.

3. Sibley M.H. et al. 1993. Journal of Cell Biology 123: 255-264

563. Functional knockout of the C. elegans homologues of two eucaryotic pseudouridine synthases.

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Pseudouridine synthases catalyze the conversion of uridine to pseudouridine in tRNA, rRNA, and snRNAs. The sites of modification on these RNAs are highly conserved in eucaryotes. Many pseudouridine synthases have been cloned and characterized, but only two tRNA pseudouridine synthases have been studied from metazoans, mouse pseudouridine synthases 1 and 3 (mPus1p and mPus3p; Chen and Patton (1999) RNA, 5, 409; ibid (2000), Biochemistry, 39, 12723). These enzymes have a high degree of homology with their yeast homologues and have stretches exhibiting near perfect identity. Pus1p and Pus3p are in the same family of pseudouridine synthases (truA) and actually share significant homology, but each modifies very specific regions of tRNA, and those regions do not overlap. When the gene for Pus1p is deleted in yeast, there is no apparent phenotype but when the gene for Pus3p is deleted, the yeast have a 3X slower growth rate (Lecointe et al. (1998) JBC, 273, 1316; Simos et al. (1996) EMBO J., 15, 2270). Gene knockouts of these two enzymes in metazoans have not been published.

C. elegans genes that code for homologues of Pus1p (W06H3.2) and Pus3p (E02H1.3) were identified by BLAST search and clones coding for the corresponding cDNAs were obtained from Y. Kohara. The inserts were cloned into the pPD129.36 vector that contains T7 RNA polymerase promoters on either side of a multiple cloning site. In addition, two recombinant plasmids were constructed that contain coding sequence from uaf-2 and cyp-13 (gift of T. Blumenthal), which are the C. elegans homologues for U2AF35 and cyclophilin. In previous RNAi injection experiments, uaf-2 was shown to be essential for development, whereas cyp-13 was not (Zorio and Blumenthal (1999)

RNA, 5, 487). L4 stage hermaphrodite worms were used in RNAi feeding experiments as described (Kamath et al. (2000) Genome Biol., 2, 1) and neither of the C. elegans homologues of the pseudouridine synthases were essential for viability in this initial test. As expected, none of the eggs hatched when the worms were fed on bacteria expressing the RNA for uaf-2, whereas all of the eggs hatched when the cyp-13 plasmid was used. Worms grown on the plates were picked and subjected to two more rounds of the RNAi feeding protocol and the worms grown on the Pus1 homologue and the cyp-13 plates showed no observable phenotype. However, the worms grown on the plates expressing the RNA for the Pus3 homologue appear to mature more slowly, lay a smaller number of eggs, and have a significant number of unhatched eggs. Experiments that involve feeding the worms bacteria containing one plasmid that expresses the RNAs for both of the pseudouridine homologues are underway. In addition, in vitro experiments to characterize the enzymatic activity of expressed C. elegans Pus1p and Pus3p homologues are also in the process of being completed.

564. Cloning and Characterization of *smg-6*, a Gene Involved in mRNA Surveillance in *C.elegans*

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Eukaryotic transcripts that contain premature stop codons are less stable than their wild-type counterparts, a phenomenon termed "nonsense-mediated mRNA decay" (NMD) or "mRNA surveillance". NMD in *C. elegans* requires functions of seven *smg* genes. *Smg-6* is possibly unusual among described *smg* genes, in that lethal alleles of *smg-6* have been isolated. Thus, *smg-6* may have essential functions in addition to its role in NMD, which is known to be a nonessential process.

We mapped *smg-6* between *dpy-1* and *laf-1* on chromosome III. Using cosmids from the region as hybridization probes, we detected a 1.8 kb deletion in *smg-6(r1217)*, a viable *smg-6* mutation. DNA fragments of N2 that cover the region deleted in *smg-6(r1217)* rescue *smg-6* viable mutants, demonstrating that *smg-6* derives from the deleted region. The *smg-6*-rescuing region contains two open reading frames that appear to constitute an operon. Three distinct mRNAs emanate from this operon: (i) a transcript of the upstream ORF; (ii) a transcript of the downstream ORF; and (iii) a chimeric transcript generated by *cis*-splicing that fuses both upstream and downstream ORFs into a single long ORF. *smg-6(r1217)* affects mRNAs (ii) and (iii). All open reading frames of mRNAs (i), (ii), and (iii) encode novel proteins. Experiments are in progress to identify which transcript(s) are affected by *smg-6* viable and lethal "point" mutations and to clarify which mRNAs of the *smg-6* region are necessary and sufficient for *smg-6(+)* function.

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565. Increased sensitivity to RNAi in lines that express transgenes in the germline.

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JH1327 is a transgenic line that carries an integrated "complex" array containing a PIE-1:GFP transgene, the roller marker pRF4, and digested genomic DNA. Unlike other lines carrying germline-expressed transgenes that can become silenced over time, this line has maintained robust GFP expression in the germline for over 100 generations. In the course of working with JH1327, we noticed that it consistently shows higher penetrance of phenotypes induced by RNAi (feeding method) compared to N2. For example, under conditions where *nos-2(RNAi)* causes only 3% sterility in N2, we observe on average 64% sterility in JH1327.

Outcrossing of JH1327 to N2 showed that the RNAi hypersensitivity segregates with the complex array. To determine whether the RNAi hypersensitivity was due to the roller DNA, the PIE-1:GFP transgene or some other property specific to the array, we compared the RNAi sensitivity of JH1327 to that of 3 other transgenic strains. JR1186 is a line carrying a high copy, simple array containing pRF4 and MED-1:GFP transgenes (thanks to Morris Maduro and Joel Rothman); KK866 is a line carrying an integrated, complex array containing pRF4 and a GFP:PAR-2: fusion driven by the *pie-1* promoter (thanks to Ken Kemphues); JH1461 is a line carrying an extrachromosomal, complex array containing pRF4 and a GFP:PAR-6 fusion driven by the *pie-1* promoter. Like JH1327, KK866 and JH1461 show robust GFP expression in the germline that has not become silenced over time. We found that, whereas JR1186 shows the same sensitivity to RNAi as N2, KK866 and JH1461 show increased sensitivity to RNAi, in some cases as high as JH1327. These observations suggest that strains that express transgenes in the germline tend to show higher sensitivity to RNAi (at least as assayed by feeding) compared to wild-type and other transgenic strains. We are currently testing this hypothesis further by comparing the RNAi sensitivity of other complex array strains and of low copy transgenic strains generated by bombardment.

In addition to providing potential insights into RNA-mediated interference, lines with increased RNAi sensitivity such as JH1327 may prove useful in RNAi screens (e.g. abstract by Pellettieri et al.).

566. A genetic screen to identify genes required for RNAi in the somatic tissue of *C. elegans*

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RNA interference (RNAi) is a type of post-transcriptional, gene silencing that is induced by double-stranded RNA. RNAi can be used to inhibit gene function in a number of organisms, including worms, flies, planaria, and trypanosomes, and possibly vertebrates. This suggests that the mechanism of RNAi is conserved across eukaryotic phyla. Two classes of RNAi deficient (*rde*) mutants have been isolated in previous genetic screens. The first class consists of 6 mutants that lack RNAi in all tissues, *rde-1* (5 alleles) and *rde-4* (1 allele). The second class consists of 14 mutants that lack RNAi primarily in the germline. To identify additional alleles of *rde-4* as well as other genes that are required for RNAi of somatic genes, we mutagenized a transgenic strain that expresses both sense and anti-sense strands of the muscle specific gene *unc-22* and screened for mutants that fail to exhibit twitching and paralysis due to *unc-22*(RNAi). So as not to recover additional alleles of *rde-1*, we engineered the transgene to contain extra copies of *rde-1*(+). Using this screen, we have isolated two new alleles of *rde-4* as well as several new mutants that define at least one new locus, *rde-9*. The *rde-9* mutants are strongly resistant to RNAi targeting all of the somatic genes that we have tested but, interestingly, are fully sensitive to RNAi targeting germline genes. These results provide further evidence that tissue-specific, or redundant, components of the RNAi machinery are expressed in the germ-line and soma of *C. elegans*. We have mapped *rde-9* to LG I, between *unc-13* and *lin-11*. The characterization and cloning of this and other new genes involved in RNAi will be presented.

567. A Screen for Mutations that Enhance and Suppress Systemic RNAi

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One of the hallmarks of RNAi in *C. elegans* is the systemic effect: injecting gene specific dsRNA into one tissue interferes with the expression of that gene in other tissues (Fire, A. *et. al*, 1998). In order to elucidate the mechanisms of systemic RNAi, we have developed an assay that has allowed us to identify mutants that are specifically suppressed in their ability to execute systemic RNAi, but are still able to maintain cell autonomous RNAi. This assay has also been used to identify mutants that are apparently enhanced for RNAi. We have screened approximately 600,000 genomes in search of suppressor mutants and approximately 100,000 genomes for enhancer mutants. Towards our goal of identifying the genes necessary for systemic RNAi, we are placing the mutations into complementation groups, mapping representative mutants to linkage groups, and characterizing the gene and tissue specificity of the suppressor mutants.

Fire, A., Xu, S., Montgomery, M.K., Kostas, S.A., Driver, S.E., Mello, C.C. (1998) Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. Nature 19;391(6669):806-11

568. Pulling the Trigger of Transposon Silencing

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Transposons are mobile genetic elements that are widespread among prokaryotes and eukaryotes. *C. elegans* contains different types of transposons among which are Tc1, Tc3 and Tc5. These elements belong to the Tc1/mariner family. Members of this family encode a transposase that is flanked by terminal inverted repeats. The transposase binds near the inverted repeats mediating the ability to jump from one position in the genome to another through a 'cut-and-paste' mechanism. In *C. elegans*, the activity of these intragenomic parasites is highly regulated by the mechanism of RNA interference (RNAi), e.g. in the laboratory strain Bristol N2, transposition activity is only detectable in somatic tissue and not in the germline.

The mechanism of RNAi involves the silencing of gene expression in response to double stranded RNA (dsRNA). There are at least two possible ways by which transposons can produce dsRNA. First, readthrough transcription from flanking promoters in the genome may yield both sense and antisense transcripts that can basepair and form dsRNA. Alternatively, either a sense or an antisense transcript of one transposon could snap back by basepairing between the two terminal inverted repeats, thus forming dsRNA.

Here, we use Tc5 of which only four copies are present in N2. Preliminary results show that all four copies are transcribed in both the soma and germline. In addition, dsRNA of the terminal inverted repeats of Tc5 was detected in soma and germline. We are currently generating strains that contain only one element each in order to determine the origin of the dsRNA that triggers transposon silencing.

569. The three faces of RNAi: A comparison of dsRNA delivery systems

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The three most convenient methods for obtaining a RNAi phenotype in *C. elegans* involve feeding, soaking or micro-injection. These methods differ in their effectiveness; differences have been observed in the penetrance and expressivity of the mutant phenocopy produced by RNAi, which are dependent on the gene and the delivery system chosen. To compare these systems, four genes were selected because they each act at different stages of development and in different tissues: *him-14*, *unc-22*, *tra-2*, and *fem-1*.

him-14 mutants display meiotic defects and a high percentage of embryonic lethality; surviving progeny have a Him phenotype (high incidence of males). One of the reasons for selecting *him-14* for this study was that we wanted to know if *him-14 (RNAi)* could provide a potential source of XO males for genetic studies. *unc-22* mutants undergo constant muscular twitching and have a thin appearance. Mutations in *tra-2* masculinise hermaphrodites; tissues affected include the tail, gonad, vulva and the germ line. Conversely, mutations in *fem-1* feminise both XX and XO animals; hermaphrodites and males are transformed into females that fail to undergo spermatogenesis.

It has been reported that inhibiting the activity of two genes simultaneously by RNAi feeding reduces the penetrance of each mutant phenocopy (Kamanth et al, 2000. Genome Biol.2:1) Modifications that may improve the penetrance of RNAi by feeding two genes at the same time are currently under investigation.

570. Identification of New *rde* (RNAi deficient) mutants in *C. elegans*

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In a number of organisms, the introduction of double-stranded RNA into cells causes the post-transcriptional silencing of the corresponding gene. This experimental phenomenon is called RNA interference (RNAi). We are particularly interested in molecular mechanism of this phenomenon. In order to elucidate the mechanism of RNAi we screened for *rde* (RNAi deficient) mutants and identified over 100 mutants. Previously, 30 of these mutants were analyzed and five complementation groups (*rde-1,-2,-3,-4* and *mut-7*) were identified. In this study, we have characterized 30 more mutants that define three new rde loci (*rde-5, -6,* and *-7*). All three of these new mutants are primarily deficient in RNAi in the germline. The *rde-5* and *rde-6* mutants are similar to previous mutator class RNAi mutants such as mut-7 in that they exhibit transposon mobilization and increased frequency of males. The *rde-7* locus differs from other germline specific RNAi mutants in that it does not exhibit any phenotypes other than RNAi resistance (For more on tissue specific RNAi mutants see the abstract by Conte et al.). *rde-7* is on LGV and is defined by 5 alleles. *rde-5* maps on LGV near unc-76, and *rde-6* maps on LG I near lin-11. While trying to use the Hawaiian strain CB4856 for single nucleotide polymorphism (SNP) mapping of the new *rde*-loci we discovered that this natural isolate of *C. elegans* is resistant to germline RNAi and contains at least two RNAi deficient loci, one that maps on LGI and one on LGIV. Further mapping studies are underway to determine if the Hawaii mutants represent new *rde*-loci. SNP mapping and rescue experiments are now underway to further define and clone the *rde* mutants.

571. *rde-4* encodes a double-stranded RNA-binding protein required for RNAi in *C. elegans*

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In many eucaryotic species, the introduction of double-stranded RNA (dsRNA) induces potent and sequence-specific gene silencing, a phenomenon called RNA interference (RNAi). Natural functions of RNAi-related mechanisms may include antiviral immunity, as well as developmental regulation mediated by natural dsRNA encoding genes (See abstract by Grishok and Pasquanali et al.). To elucidate the mechanism of RNAi, we have isolated RNAi deficient mutants, (*rde-1* through *rde-9*). The *rde-1* and *rde-4* mutants are the strongest RNAi deficient strains, lacking RNAi in all tissues examined. Genetic analysis suggests that *rde-1* and *rde-4* function in the initiation of interference after exposure to dsRNA. The *rde-1* gene is a member of a functionally novel but highly conserved eucaryotic gene family with numerous homologs in *C. elegans* as well as fungi, plants and animals.

Here we report that the *rde-4* gene encodes a 385 amino acid protein with two double-stranded RNA-binding motifs (dsRBDs). A PCR product predicted to contain this single gene rescues *rde-4* and both alleles of *rde-4* cause premature stop codons within this gene. The ne301 allele truncates the protein prior to the dsRBDs and behaves like a null allele when placed in trans to a chromosomal deficiency. *In vitro* gel shift and north-western blot analyses suggest that RDE-4 strongly binds to dsRNA. In order to ask whether RDE-4 interacts with dsRNA during RNAi *in vivo*, we raised RDE-4 antibodies and used them to immuno-precipitate the RDE-4 protein from worms exposed to *pos-1* dsRNA. The RDE-4 precipitate contained abundant *pos-1* dsRNA whose average length

was about 100bp. We are currently analyzing this precipitate to determine whether it contains the trigger dsRNA, the endogenous target mRNA or both. We are also examining whether the *in vivo* interaction between RDE-4 and dsRNA depends on other *rde(+)* activities. Localization studies suggest that both RDE-1 and RDE-4 are broadly expressed cytoplasmic proteins and that they co-localize *in vivo*. Preliminary analysis suggests that the *in vivo* interaction between RDE-4 and *pos-1* dsRNA depends on *rde-1* activity. The genetic and biochemical data are consistent with a model in which RDE-1 and RDE-4 act directly with the trigger dsRNA in forming an interfering complex that mediates mRNA destruction.

572. Hypergravity responses in *C elegans*

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All organisms on earth have evolved at unit gravity (1xG), and thus are probably adapted to function optimally at 1xG. However, with the advent of space exploration, it has been shown that organisms are capable of surviving at much less than 1xG, as well as at greater than 1xG. Organisms subjected to increased G levels exhibit alterations in physiological processes, that compensate for novel environmental stresses such as increased weight and density-driven sedimentation. These physiological adaptations illustrate the plasticity of organisms when presented with environmental conditions in which they could not possibly have evolved. Investigating the mechanism(s) behind these adaptations may uncover biological pathways that have not been identified previously, or highlight novel uses for known pathways. An easily-cultured and well-studied organism, such as C. elegans, would be a desirable model system for these studies. However, the effects of increased G are dependent on size, and the lower size limit for responding to increased G has not been established. *C elegans* are routinely subjected to transient accelerations of several thousand times G, during common laboratory procedures requiring centrifugation, and show no obvious ill effects. Even a transient exposure to 100,000xG did not result in permanent damage (L. Avery, personal communication). To establish whether *C elegans* exhibit altered physiological processes as a result of several days exposure to low levels of increased G (2-50xG), we have developed a centrifugation protocol for using axenic liquid cultures of *C elegans* in the centrifuge facilities at NASA-Ames Research Center. During exposure to 10, 20, or 50x G for 4 days, *C elegans* cultures exhibited retarded growth relative to stationary control cultures. Immediately after being removed from the centrifuge, *C elegans* were immobile and did not display a

'tap-response' to brief mechanical stimulation. Mobility and the tap-response were regained by two hours after the return to unit gravity, and the behavioral responses and growth of cultures were subsequently indistinguishable from controls. Growth at 10xG for 4 days produced alterations in steady-state mRNA levels, as evaluated by whole-genome microarray analysis on mRNA from centrifuged *C elegans* compared to parallel cultures of stationary controls. Fewer than 100 genes out of the entire genome were upregulated or downregulated to a statistically significant degree. Only a few stress-response genes were upregulated, indicating that the centrifuged cultures were under only mild stress. Most of the genes are of unknown function, but a number of them show similarity to proteins possibly involved in signal transduction, including G-protein receptor subunits and transcription factors. These data demonstrate that *C elegans* do respond to increases in G level at both a behavioral and molecular level. A series of time-course and G-level exposures are being performed, to establish how the genes that we have identified may be involved in the responses of *C elegans* to increased gravity.

573. Downstream targets of HOX genes in *C. elegans*

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We are studying gene expression profiles caused by overexpression of homeobox genes using full genome DNA microarrays in *Caenorhabditis elegans*. In all metazoa, HOX genes specify anterio-posterior identity during early development. Despite their importance, downstream targets of these HOX genes are poorly understood. We wish to define a comprehensive list of genes that are regulated by HOX genes using DNA microarrays. Currently we are focusing on four major HOX genes: *ceh-13, lin-39, mab-5* and *egl-5*.

We used DNA microarrays that contain nearly every gene of *C. elegans* to identify gene expression changes following induction of HOX gene expression by heat shock. We showed that heat shock expression of *ceh-13, lin-39* and *mab-5* in embryos causes strong lethality and malformation of the hatched larvae, showing that embryonic cells are responsive to excessive or ectopic HOX gene expression. Preliminary data have identified a large number of genes that show significantly changes in gene expression following *lin-39* and *mab-5* expression. These genes define a molecular profile of the genetic cascade that executes the patterning instructions from the HOX genes.

The HOX proteins have very similar DNA binding domains and yet specify different regions of the body. How much overlap is there among the target genes for each of the different HOX genes? We are currently examining the targets of each of the four major HOX genes to determine which are common to all HOX genes and which are specific to individual HOX genes.

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574. Tissue expression, interaction and RNA interference of troponin I genes in *Caenorhabditis elegans*

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In *Caenorhabditis elegans* troponin I has four isoforms which are encoded by four different genes *tni-1, tni-2, tni-3* and *tni-4*. Two isoforms, *tni-1* and *tni-2* encode 250 and 242 amino acid residues respectively and are expressed exclusively in body wall muscles. After elucidating the genome structure by using 5' RACE we determined the tissue expressions of *tni-3* and *tni-4*, interactions between troponin I and troponin C, as well as the function and necessity of all four isoforms. Microinjection with *lacZ* fusion plasmids, a protein overlay assay and RNA interference experiments respectively were employed. *tni-4* (LG IV) included four exons with the initial ATG located within the first exon. Both of *tni-3* and *tni-4* were trans-spliced by SL1 and encoded 193 and 260 amino acid residues respectively. The *tni-3::lacZ* and *tni-4::lacZ* fusion genes were expressed in body wall and pharynx respectively. Interactions between each of troponin I and troponin C isoforms were determined by using GST-fusion proteins and were consistent with the tissue specific expressions. RNAi animals of *tni-1* and *tni-2* showed loopy, sluggish and poor backward movements and were larger in size at the posterior part than that of wild type. RNAi worms of *tni-3* were shorter in size, characteristic of a dumpy phenotype. Collectively *tni-1, tni-2* and *tni-3* animals showed similar phenotypes, but with higher penetrance than that of individual injected worms. RNAi worms of *tni-4* produced 80% of dead embryos and had developmental arrest around the 400-cell stage at 300 minutes. These results indicate that troponin I plays an essential role in interaction with troponin C and is also necessary for muscle filament assembly and motility during animals development.

575. Microarray Analysis of Ethanol-treated Worms

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We used cDNA microarray analysis to identify genes that are either up- or down-regulated on varied time-lengths of exposure to 7 vol% ethanol. Several gene families, including heat shock protein families and gene families of known or unknown function, were up- or down-regulated by ethanol. These ethanol-effected genes were further examined by Northern analysis. Northern results of a total of 100 genes - 50 each of genes either up- or down-regulated by 6 hour exposure to ethanol - showed 95% consistency with microarray results. By analyzing microarray data according to varied lengths of exposure to ethanol, we were able to classify the effected genes largely into 5 groups. Class I genes show rapid increase in gene expression. Class II genes show a steady increase, and Class III genes show an increase in expression and then fall back to normal. Class IV genes show reduced expression and then go back to normal, and Class V genes show a steady decrease in expression. We are currently studying genes that belong to each group by Northern analysis and expression studies. Also, we are functionally analyzing some candidate genes using previously isolated mutants. Through these results, we are planning to identify genes that are regulated by ethanol, and study their inter-relationships.

576. Expression of *C. elegans* LGR: A homolog of vertebrate glycoprotein hormone receptors.

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Vertebrate reproduction and metabolism are tightly controlled by glycoprotein hormones that interact with leucine-rich repeat G-protein coupled receptors (LGRs). A *C. elegans* homolog of the vertebrate hormone receptors (*Ce*LGR) was identified by the *C. elegans* Genome Sequencing Consortium (1). When expressed in mammalian cells *Ce*LGR constitutively activates production of the second messenger cAMP, but does not respond to vertebrate hormones (2).

We have taken advantage of available tools to study the possible function of *Ce*LGR in growth and development of *C. elegans*. *Ce*LGR:: *gfp* reporter constructs were made using 4.5kb of the 5' promoter sequence and 12 of the 13 exons (including the predicted transmembrane regions). Following co-injection with the *rol-6* transformation marker, GFP expression patterns were determined from the roller progeny. Strong expression was observed in many tissues associated with sensory projections to the external environment including nose sensillia, inner labial sheath and socket cells, CEP neurons, amphid sheath and socket cells, ADE and PDE neurons, vulva and male hook, spicule sensillum and ray sensory neurons. In addition, bright staining was also observed in lumenal cells of intestine and CAN neurons in both hermaphrodites and males.

RNAi using a full-length coding sequence in N2 animals did not result in a detectable effect on development, general behavior, or reproduction. RNAi in the roller animals of *Ce*LGR::GFP strain resulted in marked suppression of intestinal GFP signal, but little or no change in

expression at other sites.

Studies are in progress to observe the possible phenotype using heat shock promoter fused to *Ce*LGR cDNA. Also, there are a number of mutants available in the vicinity of the *Ce*LGR locus that affect one or more cell types that expressed the *Ce*LGR::GFP reporter. Results from rescue studies of some of these mutants will be presented.

1. Wilson R., et al. Nature 368:32-38. (1994).

2. Kudo M., et al. Molecular Endocrinology 14:272-284. (2000).

577. A *C. elegans* Whole Genome DNA Microarray for Expression Profiling

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The sequencing of the entire *C. elegans* genome has facilitated the ability to investigate gene function and gene interactions on a global scale. Advances in whole genome screening technology, in particular DNA microarrays, enable investigators to analyse the expression profiles of thousands of gene sequences simultaneously. This, combined with the genetic data available for *C. elegans*, makes the technique extremely powerful.

DNA microarrays have been produced following protocols originally developed in the laboratory of P. Brown (Stanford, CA). Our arrays are composed of approx. 1kb genomic DNA fragments corresponding to each of the 19,000 predicted *C. elegans* ORFs. The microarrays were generated by robotically gridding each of these fragments onto poly-lysine coated glass slides. The complete complement of fragments was arrayed on two slides, and includes various controls. The microarrays are being used to compare steady-state RNA populations expressed by animals with different genetic backgrounds or physiologies by differential hybridisation of cDNAs. The cDNAs used in the experiments are labelled with either a Cy3 or Cy5 fluorescent tag, incorporated during reverse transcription. Fluorescence hybridisation signals are detected by confocal laser scanning; the resulting data are expressed as two-colour ratios. We are presently optimising a number of parameters affecting array gridding, RNA labelling, hybridisation and data analysis.

We have developed a core database for analysing array data using the GeneSpring(TM) (Silicon Genetics) commercial software package. GeneSpring(TM) allows the user to perform hierarchical clustering, correlate data across multiple experiments and view data graphically. The Sanger Centre is developing additional software suites that will facilitate array data handling and analysis, including a

web based browser.

Microarrays produced at the Sanger Centre will be made available as a scientific resource for members of the UK research community, and the data obtained will be freely accessible to all. We are grateful to Stuart Kim and members of his laboratory for providing reagents and advice.

578. Microarray analysis of MAP kinase signaling in the germline

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Our lab uses microarray analysis to study germline development in C. elegans. Through microarrays, we can examine the global response of all genes to a specific condition or mutation. Previous microarray experiments have compared global gene expression patterns of wild type animals to mutant animals lacking a germline. Analysis of these data has identified a set of genes that are involved in germline development.

The MAP kinase pathway is involved in many developmental processes, including meiotic progression. We are examining the effects of MAP kinase signaling using a temperature sensitive MAP kinase mutant allele, mpk-1(ga111), that produces a phenotype only in the germline. When these mutant animals are raised at the restrictive temperature, germ cells arrest at the pachytene stage of meiosis. By comparing gene expression of these MAP kinase mutants to wild type through microarray analysis, we can examine the global genome response to loss of MAP kinase signaling in the germline. To do this, we will compare mRNA extracts from the wild type and mpk-1 mutant at both L4 and adult stages of development. Through this experiment we hope to obtain candidates for genes acting downstream of the MAP kinase pathway in the germline. We will distinguish between germline and somatic genes by comparing the MAP kinase responsive genes to the known set of germline genes. Upon identification of MAP kinase-responsive germline genes, we plan to examine their individual roles in MAP kinase signaling in the germline.

579. Nuclear Receptors Required for Molting in *C. elegans*

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C. elegans, like other nematodes, undergoes a molting process that marks the transition between developmental stages. During the molt, cuticle of the next developmental stage is synthesized while cuticle from the previous stage is shed (ecdysed). The regulation of molting in nematodes has been an important focus in nematode parasitology research for many years, but has not been extensively investigated in *C. elegans*.

Using a functional genomics approach, we have identified two putative transcriptional regulators of the molting process. NHR-25 and NHR-41 are conserved members of the nuclear receptor (NR) superfamily of transcription factors. The insect orthologs of NHR-25 and NHR-41, FTZ-F1 and HR78 respectively, function in the ecdysone cascade during insect metamorphosis and molting. Strikingly, the *C. elegans* genes also appear to be required for the molting process, suggesting the existence of a conserved transcriptional pathway that regulates the molting process in invertebrates.

nhr-25 and *nhr-41* are expressed in different cell types. *nhr-25* is expressed in the epidermis while *nhr-41* is expressed in specific anterior and posterior neurons as well as the gut. Loss of gene function for *nhr-25* and *nhr-41* generate similar molting defects; however, their distinctive expression patterns suggest the regulation of different molting processes. Although *nhr-25* also has other epidermal development functions, we have demonstrated that *nhr-25* and *nhr-41* have a molting-specific function and that their expression levels cycle with the molt. A discussion of the possible roles of these genes during the molting process will be presented.

580. A systematical gene expression screen of the *Caenorhabditis elegans* cytochrome P450 genes.

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Cytochromes P450 (CYP) are a super family of heme containing NADPH dependent monooxygenases which catalyze the oxidative metabolism of many exogenous and endogenous compounds. These proteins have been particularly implicated in the biotransformation of many drugs and other xenobiotics. Moreover, P450 gene expression can be induced by the presence of these compounds in the medium. The soil nematode *Caenorhabditis elegans* is probably the simplest animal having the status of a laboratory model. Its genome contains the remarkable number of 80 cytochrome P450 genes.

In order to study CYP gene expression worms were exposed to 18 different cytochrome P450 inducers. Using subfamily-specific primers we could amplify a pooled set of exon-rich CYP fragments to create a P450 subfamily specific gene filter. In that way we were able to check systematically the influence of the different inducers to CYP expression at the same time. Interestingly, the well known CYP1A inducers β-naphtoflavone, PCB52 and Lansoprazol were the most active ones, especially in case of family CYP35. Taking advantage of the *C. elegans* whole genome DNA microarray created in Stuart Kim's lab (Stanford University, USA) we could confirm and extend our results significantly. Differentially expressed CYP genes were further analyzed by a semi-quantitative RT-PCR. The strongest expression were observed for the genes CYP35A1-4 and 35C1. In addition, a transgenic *C. elegans* line expressing GFP under control of the CYP35A2 promoter showed a strong induction of the fusion by β-naphthoflavone in the intestine. Altogether, xenobiotic inducible gene expression of *C. elegans* could be a useful tool to develop a biomonitoring screening

system.

581. NOVEL HELIX-LOOP-HELIX PROTEINS IN CAENORHABDITIS ELEGANS.

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Helix-loop-helix (HLH) proteins are transcription factors that are involved in cellular specification, differentiation, and differential gene expression in development. Normal pancreatic functioning, brain and eye morphogenesis, and skeletal muscle development are just some aspects of human development that are regulated by HLH proteins. The nematode, Caenorhabditis elegans, is an ideal model to study myogenic related HLH proteins since it has a simple genetic system and is known to produce numerous transcriptional regulators. After identifying six DNA sequences that are likely to encode myogenic HLH genes in C. elegans, we used RT-PCR, RNA interference, and promoter fusions to determine which of the genes are expressed, and the timing and localization of their expression. (Research supported by NSF grant # MCB9986640 and by MBRS/RISE grant # R25GM58094)

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582. Using Genomics to Study Homeostatic Plasticity

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U.C. San Francisco and HHMI

In an effort to understand how *C. elegans* sensory neurons regulate their excitability, we used DNA microarrays to compare the transcriptional profiles of wild-type animals to those of animals lacking components of the TAX-2/TAX-4 cyclic-nucleotide-gated channel.

We find that *tax-2* and *tax-4* mutants have similar transcriptional profiles. Of 23 genes which showed fourfold enhanced signal in *tax-2* mutants, 22/23 were overexpressed in *tax-4* mutants as well. Of 21 genes which showed fourfold reduced signal in *tax-2* mutants, 19/21 were also reduced in *tax-4* mutants.

The reproducibility of gene-expression-ratio measurements is strongly related to gene expression level: strongly-expressed genes yield reproducible expression ratio measurements, and weakly expressed genes do not. We are synthesizing expression-ratio measurements and overall spot intensity into a single measure of statistical significance for differential expression.

tax-4 and *tax-2* mutants exhibit growth defects, and despite the fact that we harvest RNA from all strains at an equivalent developmental stage (young adult), we still find that our lists of differentially regulated genes are enriched for developmentally regulated genes. We are using developmental transcriptional profiles from Jiang et al. (PNAS 98: 218-23, 2001) to filter these gene lists, by identifying genes which show rapidly changing expression levels during the transition from L4 to adult. This filtering significantly enriches our gene lists for genes of potential relevance to the nervous system.

To see whether this technique allows us to identify neuronal genes, we constructed promoter:gfp fusions for three of the genes identified in these experiments. All three show expression specific to the nervous system. One of these genes, which encodes a protein with homology to G-protein-coupled receptors of the srd family, shows strong and specific expression in the ASJ chemosensory neurons. Expression of gfp from this construct is extinguished in *tax-2* mutants.

We are doing more DNA microarray experiments to understand how other perturbations of neuronal excitability and its reporters affect neuronal gene expression, and how these transcriptional pathways might regulate neuronal excitability in an adaptive way.

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583. Gene Expression in Single *C. elegans* Neurons

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Genome sequences lack information about when and where genes are expressed. To fill this gap, we are working on methods to determine the expression profiles of individual cells, especially neurons. We harvest single, GFP-labeled neurons (using the same techniques used to record from neurons in vivo) and use the method of C. Dulac for single-cell RT-PCR. Successful amplification is verified by secondary PCR with primers directed against adjacent 3' exons of GFP and of known cell-specific genes. Transcripts encoding GFP (expressed from a multi-copy array) are detected in most GFP+ neurons (41/62) and few unlabelled neurons (2/18). Endogenous transcripts encoding known cell-specific genes are detected in ~1/5 neurons examined. We tested for the following genes: *gcy-8* in AFD cells (9/47); *gcy-5* in ASER cells (2/10); and *odr-10* in AWA cells (1/3). Amplified material is used for further analysis only when both GFP and at least one endogenous, cell-specific transcript are detected by secondary PCR.

Verified cellular cDNAs can be used either to screen for new genes by secondary PCR or to construct cell-specific cDNA libraries. For example, we screened AFD cDNAs for expression of two gene families implicated in sensory transduction: transient receptor potential channels (*trp*) and transmembrane guanylyl cyclases (*gcy*). We found three new AFD genes: the *C. elegans trp* ortholog (R06B10.4) and two *gcy* genes (*gcy-23* and *gcy-26*). We are currently expanding our search for new genes by constructing and screening AFD, ASER, AWA and AWC-specific cDNA libraries. We will conduct differential screens with these libraries to identify genes expressed specifically by each sensory neuron. These experiments are a first step toward determining the expression profiles of individual neurons and mapping the genome onto the nervous system.

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584. Morphological Evolution of Rhabditidae

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As a foundation for understanding how macroevolutionary changes have occurred, we are building a matrix of morphological and molecular characters to reconstruct the phylogeny and evolution of Rhabditidae, the family that includes *C. elegans*.

Of the 145 morphological characters we have defined for over 70 taxa, 60 involve aspects of the male tail. These character definitions have been based on considerations of homology (aided by developmental analyses) and presumed independence of variation. Molecular characters include well over 3 kilobases from small subunit ribosomal RNA and RNA polymerase II genes.

We find that the molecular characters resolve some relationships not resolved by morphological characters and vice versa, suggesting that data combination may provide better resolution than using individual data sets. The phylogeny of Rhabditidae is notably different from those previously proposed on the basis of morphology alone, suggesting some homoplastic morphological changes not previously recognized or mistaken assumptions about character homologies. In particular, Rhabditidae is paraphyletic; i.e., it forms the backbone of a phylogeny from which several diverse groups have derived.

The resulting phylogeny can be used to trace the evolution of any character. For example, we find that hermaphroditism has evolved independently from gonochorism in several lineages, but there is no unequivocal case where gonochorism has evolved from hermaphroditism. Phasmid position relative to the rays in males has changed a few times in a saltational manner, consistent with an evolutionary shift in blast cell polarity, a hypothesis we are currently testing. Test your own evolutionary hypotheses directly on the poster!

585. Ray pattern and HOM-C gene variation in *Caenorhabditis briggsae*.

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Ray pattern is one of the most variable morphological characters in rhabditid nematodes. Within *Caenorhabditis*, a modified ray pattern in which ray 3 is posterior of the anus and frequently fused with ray 4 is diagnostic for *C. briggsae*. This diagnostic character is based on observations of a limited number of *C. briggsae* strains. Recently, a *C. briggsae* strain, PB800, was obtained that exhibited an *elegans* ray pattern at a high frequency. Prompted by this observation, ray pattern variation has been assessed in four other *C. briggsae* strains, AF16, HK104, HK105, and VT847. AF16 and VT847 exhibited predominately the canonical *briggsae* pattern whereas HK104, HK105, and PB800 exhibited *briggsae* and *elegans* patterns at approximately equal frequencies. PB800 also exhibited a sixless phenotype, in which ray six is morphological transformed and fused with ray 4, at an approximately 1% frequency. In *C. elegans* this phenotype results at high frequency from mutations *mab-18* and *mab 21* and at low frequency from haploinsufficiency of *egl-5*. Moreover, altered levels *egl-5* and *mab-5* expression can mimic the canonical *C. briggsae* ray pattern. Thus, ray pattern variation in *C. briggsae* may result from allelic variation in *C. briggsae* HOM-C genes. To test this hypothesis, PCR product length polymorphisms have been identified in the *C. briggsae* homolog of *egl-5* (*Cb_egl-5*). These *Cb_egl-5* polymorphisms will be tested for linkage to variation in ray pattern. If linkage is detected, additional experiments will be conducted to map the *C. briggsae* ray pattern variants to a single locus.

586. Abundance, Distribution and Mutation Rate of Homopolymeric Nucleotide Runs in the Genome of *Caenorhabditis elegans*

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Homopolymeric nucleotide runs are a ubiquitous feature of eukaryotic genomes. Although their dominance in nuclear genomes is clear, homopolymer origins and mechanisms of maintenance and mutation are less obvious. To address these questions, we comprehensively examined the abundance and distribution of homopolymer loci e8 nucleotides in length in the genome of *C. elegans*. There are 148,625 homopolymer loci in the *C. elegans* genome. The four specific homopolymers are evenly distributed with respect to (+/-) strands of each chromosome. Homopolymer loci are under-represented in exons, show significant clustering in the arms of autosomes, and have significantly different densities between chromosomes. Homopolymers are over-represented in *C. elegans* genome compared to expectations based on individual nucleotide frequencies. A/T homopolymers vastly outnumber C/G homopolymers, and the size distributions of AT and CG homopolymers differ significantly, particularly for homopolymer loci less than 20 nucleotides in length. A direct investigation of mutation rates at numerous homopolymer loci in a set of *C. elegans* mutation accumulation lines (Denver, et al. 2000) revealed a significantly higher rate of mutation at C/G loci than at A/T loci. The abundance and distribution of homopolymer loci taken together with direct measures of their mutation rate suggest that differential stability may play a significant role in the maintenance of homopolymer loci.

Literature cited:

Denver, D.R., Morris, K., Lynch, M., Vassilieva, L., Thomas, W.K. "High direct estimate of the mutation rate in the mitochondrial genome of *Caenorhabditis elegans*" Science 289:2342-2344.

587. Microevolutionary analysis of the nematode genus *Pristionchus*

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To identify the mechanisms by which molecular variations are introduced into developmental systems, microevolutionary approaches to evolutionary developmental biology have to be taken. We describe the molecular and developmental characterization of laboratory strains of the nematode genus *Pristionchus*. 13 laboratory strains of the *Pristionchus* genus were obtained from natural isolates from around the world. Mating experiments and ITS sequence analysis indicated that these 13 strains represent four different species; the gonochoristic species *P. lheritieri* and three hermaphroditic species, *P. pacificus*, *P. maupasi* and an as yet undescribed species *Pristionchus sp.*, respectively. *P. pacificus* is represented by five different strains isolated from California, Washington, Hawaii, Ontario and Poland. Developmental differences during vulva formation are observed between strains from different species but also between strains of *P. pacificus* , like the strains from California and Poland. In particular, redundant developmental mechanisms present during vulva formation in *P. pacificus* var. California, are absent in other strains. AFLP analyses of the *P. pacificus* strains revealed that the American strains are highly polymorphic, whereas the developmentally distinct strain from Poland is identical to the Californian strain. Thus developmental differences rely on a small number of changes in developmental control genes rather than the accumulation of changes at multiple loci.

588. Intraspecific sperm size variation supports sperm competition model

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Intraspecific variation in male sperm size was examined in *C. elegans*, *C. briggsae*, and *C. remanei*. *C. elegans* and *C. briggsae* are hermaphroditic species with males present at extremely low frequencies, whereas *C. remanei* is a gonochoristic species with males and females at approximately equal frequencies. It has been shown that males from gonochoristic species of nematodes tend to have larger sperm than do males from hermaphroditic species, perhaps due to increased levels of sperm competition in a gonochoristic mating system. The reasoning is as follows: Males from the hermaphroditic species need only compete with hermaphrodite sperm most of the time, whereas males from a gonochoristic species must compete with the sperm of rivals on a regular basis; hence, if sperm size plays an important role in sperm competitive ability, then the sperm of gonochoristic males should tend to evolve towards larger sizes in a coevolutionary "arms race" with rivals. Another prediction of this model is that gonochoristic species will exhibit greater intraspecific variation in male sperm size, since different strategies for dealing with high levels of male-male competition can evolve, each balancing sperm size, sperm manufacture rate, and other reproductive variables with the amount and types of male competition present. In a hermaphroditic species, on the other hand, males need only produce sperm that can outcompete hermaphrodite sperm. To test this model further, we measured cross-sectional areas of 300-700 sperm from multiple males across 5-7 strains from each species. Consistent with the sperm competition model, *C. remanei* exhibited significantly more variation in male sperm size than did *C. elegans* or *C. briggsae*.

589. Sperm competition causes evolution of large sperm

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Larger sperm are found in rhabditid nematode species that experience more sperm competition, and sperm size is the single most divergent character among the group. In *C. elegans*, larger sperm outcompete smaller sperm but are more costly to produce. This suggests that pressure from sperm competition drives the evolution of sperm size, but such data do not demonstrate cause and effect. We tested the effect of sperm competition on male sperm size evolution in three outcrossing lines of *C. elegans*. These lines were forced to outcross using a *spe-8* mutation (which renders hermaphrodites self-sterile, but has little effect on male fertility), and genetic variation was introduced from wild isolate strains CB4855, DR1345, DR1350, and AB1. Sperm volume increased nearly 20% in the outcrossing lines over the course of 60 generations. No such sperm size evolution occurred in selfing control lines. These data demonstrate that increased sperm competition causes larger sperm to evolve. Sperm morphology is extremely variable both within and among animal taxa, while ova show much less variability. Perhaps the pressures of sperm competition explain some of this morphological variation not only in nematodes but in other animals as well.

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590. There are at least four eEF1A genes in *Rhabditis (Oscheius) pseudodolichura*, strain CEW1 (Nematoda; Rhabditida)

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----*Rhabditis (Oscheius) pseudodolichura*, strain CEW1, is a free-living rhabditid nematode used as a model organism in molecular (Winter, Comp. Biochem. Physiol. **103B**:189, 1992; Winter et al., Mol. Biol. Evol. **13**:674, 1996; Evans et al., PNAS **94**:9751, 1997) and developmental studies (Félix, Nematology **2**:89, 2000; Dichtel et al. Genetics **157**:183, 2001). The 50kDa eEF1A (a.k.a. EF-1alpha) is a multifunctional, highly-expressed, ubiquitous G-protein, involved in protein synthesis (Moldave, Ann. Rev. Biochem. **54**:1109, 1985), cytoskeleton organization (Furukawa et al, Int. Rev. Cytol. **175**:29, 1997), oncogenic transformation (Tatsuka et al, Nature **359**:333, 1992) and apoptosis (Duttaroy et al, Exp.Cell Res. **238**:168, 1998). An eEF1A gene from CEW1 was isolated from a genomic library through screening with a total cDNA probe. This gene is temporarily called *CEW1-eft-1* and its complete sequence has been determined, revealing four small introns, a putative TATA-box and one VPE-1 motif (MacMorris et al, Mol. Cel. Biol. *12*:1652, 1992). Primer extension experiments have been done with total mRNA extracted from an asynchronic CEW1 culture, revealing a transcription start site 57 nucleotides upstream from the start codon.

----PCR reactions made over genomic DNA with two *CEW1-eft-1* primer pairs amplified two fragments, one of them slightly bigger than expected. This larger fragment has been purified and, after sequencing, shown to correspond to another eEF1A gene, called *CEW1-eft-2*. Its coding sequence is almost identical to *CEW1-eft-1*, differing only in the position and size of the introns. Screening of a CEW1 cDNA library (a kind gift from Marie-Anne Félix, Inst. Jacques Monod, Univ. de Paris IV) with a *CEW1-eft-1* probe allowed us to isolate five putative eEF1A clones. Three of these cDNA clones corresponds to *CEW1-eft-2*, suggesting that it is the most expressed eEF1A gene in CEW1. No *CEW1-eft-1* cDNA clone has been isolated to date.

----The 5' and 3' end sequences of the two other cDNA clones do not match the eEF1A genes already identified. The results obtained suggest the existence of two more eEF1A genes in CEW1 genome. PCR reactions done with primers made from the 5' and 3' UTR of the cDNA clones, amplified the complete sequence of *CEW1-eft-2* and of the two other genes, named *CEW1-eft-3* and *CEW1-eft-4*. Sequencing of the amplified fragments confirmed the existence of four eEF1A genes in CEW1. These genes present a high conservation in their nucleotide sequence and in the position of the introns. In some cases even the sequence of the introns is conserved. The deduced amino acid sequences are almost identical.

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591. Evolution of Hox genes through the phylum Nematoda indicates that C. elegans has lost at least three of its Hox genes.

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The genomic structure of the C. elegans Hox cluster and molecular phylogeny data suggest that the Hox cluster is undergoing rapid evolutionary change in nematodes. This provides an opportunity to study the molecular basis of this change. How different are nematode Hox clusters? Are there differences in the membership of nematode Hox clusters? How conserved are the Hox genes between nematodes with respect to sequence and function? These questions have been addressed by performing a series of comparisons with other nematode species. We have employed a degenerate PCR approach to identify Hox cluster cDNAs from Pristionchus pacificus, Brugia malayi, Trichinella spiralis and Strongyloides ratti representing a spectrum of distant to very close relationships to C. elegans. Two of the B. malayi and five of the T.spiralis Hox like genes have no orthologues in the C. elegans genome. Thus it appears that C. elegans has a derived cluster membership (even for a nematode) indicative of hox gene loss during evolution. The implications of this in refining our view of the relationship of nematodes to the other invertebrate phyla will be discussed.

592. USING *C. ELEGANS* IN THE UNDERGRADUATE LABORATORY CLASSROOM: PROJECT LAB AND GENETICS

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I have used *C. elegans* in two different undergraduate lab courses at Santa Clara University. One is an upper division research-focused "Project Lab" course described below and the other is an upper division Genetics course that uses *C. elegans* for its laboratory component.

The "Project Lab" course was developed with an NSF grant to integrate research and education by designing and implementing an intensive new laboratory course in which undergraduate students participate in supervised research projects. These research projects are directly related to ongoing research in my laboratory. Project Lab has a class size of 7-12 students and, each year, it focuses on research activities appropriate for that particular stage of the research project. The addition of this course to our biology curriculum enhances the science education of undergraduate students at Santa Clara University by providing hands-on laboratory experience and exposure to real research problems. It also enhances the research program of the principal investigator by training and motivating students for research.

Specifically, we are using sequencing and site-directed mutagenesis to conduct a structure/function analysis of LIN-31, a member of the winged-helix family of transcription factors, which is required for the proper specification of vulval cell fates in *C. elegans*. The first two Project Lab classes made considerable progress, resulting in a paper on which three Project Lab students were authors (Miller *et al.*, Genetics 156: 1595, 2000). Results from the two most recent Project Lab class will be presented as a poster at this meeting (see Mora-Blanco *et al.*).

I have also successfully used *C. elegans* in my upper division genetics laboratory course. This is a 10-week course in which students use *C. elegans* to study basic genetic principles such as

dominance, independent assortment, recombination, and complementation. In order to do this, they first find and then analyze their own genetic mutant. As the quarter progresses, they use their mutants to study the basic genetic principles listed above. The first lab introduces them to *C. elegans* and teaches them how to grow, manipulate, and sex the animals. In the second lab, they "find" their mutant by doing a mock mutant screen. In subsequent labs, they carry out genetic manipulations (crosses) to better understand their mutants. By the end of the quarter, they can answer the following questions about their mutant: Is the mutant allele dominant or recessive to the wild-type allele? What chromosome does it map to? Between what other genes does it map? Is it an allele of a known gene?

In summary, *C. elegans* is ideally suited for an undergraduate laboratory course. With appropriate training, undergraduate students can quickly learn to manipulate nematodes for use in genetic and/or molecular experiments. Furthermore, the flexibility of the 3-, 4-, or 7-day life cycle makes *C. elegans* particularly appealing for institutions on the quarter system.

Access to teaching materials for both courses will be available at the teaching poster session and on my web page (www-acc.scu.edu/~lmiller/homepage.html).

593. A simple lab using single worm PCR and *dpy-5*

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A simple lab has been designed for use in a sophomore genetics course. There are four primary objectives for this laboratory exercise:

1) students predict genotypes based on phenotype

2) students design primers for PCR

3) students use the PCR technique

4) students analyze results using gel electrophoresis

The learning elements of the lab can be broken down into two main areas. First, it serves as a demonstration of the relationship between genotype and phenotype. Students observe the phenotype of worms and then make predictions about the genotype which they subsequently test. A second valuable learning element has been having the students design primers for PCR. Students must have a good understanding of both DNA structure and DNA polymerase activity to design primers successfully.

This laboratory exercise makes use of a *dpy-5* mutation that contains a deletion of 1009 bp (gracious thanks to Colin Thacker and Ann Rose for supplying the strain).. The mutation (e907) is semidominant. Therefore, students can identify all three genotypes in a population of worms containing the wild type and e907 alleles (+/+; +/e907; e907/e907). The investigation occurs over three lab periods. In the first lab, students become familiar with observing the Dpy phenotype and with picking worms. They also design primer pairs that will amplify both the wild type and e907 alleles. In the second lab, students use these primer pairs in single worm PCR reactions. They are asked to pick worms that represent each of the three genotypes and use these for PCR. In the third period, an agarose gel is run of the PCR reactions. Students determine the genotypes of the worms they chose and find out if their genotype

predictions were correct. I have found that sophomore students are able to do this lab with a high percentage of success. If you would like more information or would like the lab handout, please contact Lynn Boyd at boydl@uah.edu.

594. An Upper-level Undergraduate Project-Based "Laboratory in Genetics" Course using *C. elegans*

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I designed a new upper-level 14-week undergraduate "Laboratory in Genetics" course using *C. elegans*, and the course was offered for the first time this spring semester (2001). The course is geared for juniors and seniors with prerequisites of Introductory Biology I and II, Molecular and Cell Biology I and II, and Genetics.

The course covers many genetic principles by means of a project-based laboratory in two parts: forward and reverse genetics projects. After familiarizing themselves with a variety of mutant phenotypes, teams of 2-4 students consider a biological process, select mutants that perturb a process, and characterize the mutant phenotype. Next, they begin a classical genetic analysis. First they describe their mutant phenotype in terms of penetrance and expressivity and assess dominance and X-linkage. Then they carry out further linkage tests and three-factor mapping experiments. Finally, they perform complementation tests, deficiency mapping and dosage analysis to further characterize their mutant. Two demonstrations (worms under Nomarski optics in the first class and injections later in the semester) round out the first part of the course. The design of this part of the course was drawn from a similar course offered by Leilani Miller at Santa Clara University.

The second part of the course consists of a reverse genetics experiment, made possible by the availability of the full sequence of the *C. elegans* genome and RNAi. First, students select a gene of interest from a provided list of possibilities (e.g., human disease-related genes). Then, using gene databases and information available on the web, they identify a closely related gene in the *C. elegans* genome. Finally, they examine the consequences of an RNAi feeding experiment with appropriate controls.

The class meets once/week for 4 and one quarter hours and is run with the help of a graduate teaching assistant. Approximately 45 minutes of the class time consists of a lab-lecture. In addition, during the class time teams of students prepare, pour and seed plates. The experiments for the first part of the course are accomplished more or less within the allotted time with occasional follow-up scoring the day or two after the regularly scheduled lab. The second set of experiments requires a more flexible schedule, allowing the students to come in each day for a shorter period of time.

Grading for the course is based on (1) four required written lab reports (three of which have a re-write option), (2) a final exam and (3) laboratory conduct. The reports are written individually although the lab work is done in teams. The final examination consists of problems to test students knowledge of the concepts applied during the semester.

I am deeply grateful to Leilani Miller for sharing material, suggestions and encouragement during the preparation of the course.

595. Using *C elegans* to teach genetics: SNP mapping in three weeks!

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C. elegans has been used to the teach fundamentals of genetic mapping for the last three years in the Neurobiology summer course at the Marine Biological Laboratories (MBL). The nine week course is primarily aimed at graduate students and postdoctoral fellows. The molecular section of the course I help teach lasts three weeks including lectures in the morning and lab work in the afternoons and evenings. Two students work with each of the six faculty members in the molecular section of the course. The two students assigned to me spend about 120 hours each working in the lab spread out over a three week period. Consistently, the students have had no experience in *C. elegans* and, often, little or no prior experience in molecular biology.

Given the short duration the molecular section, and the desire to accomplish something of substance, the project has to be chosen with care. The short life cycle of *C. elegans* makes it an ideal organism to teach the fundamentals of genetic mapping in a limited amount of time. The project chosen last year was recombinant mapping of an interesting mutation, *rt70*, using single nucleotide polymorphisms (SNPs) from the Hawaiian strain, CB4856. *rt70* causes ASH neuron degeneration and is described in an abstract/poster by Emily Bates, *et al.*

Prior to the start of the course last year, a *lon-2(e678) rt70 egl-15(n484)* chromosome was generated. All mutations are recessive. Heterozygous *lon-2 rt70 egl-15* / CB4856 animals were generated and picked one per plate. These heterozygous animals were transported to MBL along with hundreds of seeded plates. The students practiced picking *C. elegans*, then singled animals carrying recombinant chromosomes/*lon-2 rt70 egl-15* for the first few days (putative recombinant lines). We attempted to generate homozygous recombinant lines by picking single 9 animals in

the next generation and were successful about 80%of the time; the remaining 20% were repicked for analysis at a later date. The homozygous recombinant lines were 1) frozen in a 96 well format using Michael Koelle's protocol and 2) allowed to starve and lysed for PCR analysis using previously defined SNPs identified by Stephen Wicks. To facilitate analysis, only previously characterized SNPs creating a restriction site polymorphism were utilized. Screening more than 100 lines by PCR was not a problem and we were able to refine the *rt70* map position. The biggest difficulty we had was scoring *egl-15(e484)*. In retrospect, I should have chosen marker mutations with very obvious phenotypes so they could be scored unambiguously and easily.

PCR reactions were optimized while we were waiting for the recombinant strains to grow. The MBL students also had time to learn how to generate transgenic *C. elegans* by microinjection. PCR machines for the course were generously loaned by MJ Research, Taq was kindly provided by Fisher and NEB generously donated restriction enzymes. Thanks to all who donated their time, reagents and help- especially MBL students Michael Long and Ryohei Yasuda for their unflagging enthusiasm!

596. Collaborative Research with Undergraduate Students: An Assembly Line Model

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Working with undergraduates in a research program can be rewarding but also presents some unique challenges. Although senior students are capable of carrying out fairly sophisticated research projects, beginning students lack technical expertise, a deep understanding of the discipline, and have limited time to devote to research. Faculty at predominantly undergraduate institutions learn how to divide up their research programs into small digestible problems that undergraduate researchers can more easily tackle. A complementary approach is to introduce students to just one or two different techniques initially; then, after performing small projects that allow them to acquire some expertise, let students work collaboratively to carry out larger projects from beginning to end, in an assembly line-like fashion. For example, my research program is focused on a comparative study of genes regulating early development using RNAi and a variety of other techniques. I will train one student in some molecular techniques, another to perform immunocytochemistry and/or in situ hybridization, and a third student to do microinjections. A typical research project in my lab will involve (1) one student cloning a gene and making dsRNA that (2) another student injects into worms with (3) a third student antibody staining the progeny of the injected worms to reveal the nature of the resulting RNAi phenotype. Each student is respected for his/her contribution and everyone is invested in analyzing the results. Eventually, the students learn many of the other techniques as well, mostly from looking over the shoulders of each other during the inevitable laboratory "down time." Thus students learn from me intially, become experts by repeating the same protocol several times, and then are able to teach each other. Students learn about shared responsibility and the true nature of collaboration. Eventually students develop a deeper understanding of the nature of the research and can begin to both generate and pursue their own experimental questions.

597. Surfing the Genome: Using the *C. elegans* Genomic Sequence to Teach Molecular Genetics

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There is considerable interest in inquiry-based learning in current undergraduate science pedagogy. The idea is that students learn science better by participating in the process, rather than taking endless notes in a large lecture hall. This approach conveys the notion that science is not static, but rather an ongoing process. Moreover, it is widely-believed that students retain information more effectively if they have experienced science first-hand.

The completion of the *C. elegans* genomic sequence provides a unique opportunity to engage students in the process of science. In my Advanced Molecular Genetics class five students (all sophomores and juniors) learned molecular genetics and protein structure through an independent project approach. Each student was provided with a "roadmap" for designing their own project. Their assignment was to identify a poorly-characterized gene from the *C. elegans* database that was a member of an orthologous gene family, research their gene and the gene family, perform RT-PCR to isolate clones of their gene, characterize their clones, and finally investigate gene function by RNAi. Current technology has advanced to the point where such a project is not as unrealistic as it might sound at first glance.

In the process of performing their projects, students learn about genomics, protein structural motifs and functional domains, computer-based sequence analysis and retrieval, and experimental design. It is my hope that this approach will create better-trained future scientists and physicians.

This project was funded in part by an ILI grant from the NSF.

598. THERE'S A HUMP IN YOUR BACK!! RNA INTERFERENCE IN THE CLASSROOM

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Undergraduate students often have difficulty understanding the relationship between nucleotides, genes, and the regulation of developmental processes. In an undergraduate sophomore level laboratory exercise, I used RNA interference assays to reinforce concepts in gene expression. Over four laboratory periods, students were able to (1) build a three-dimensional model of a eukaryotic gene, (2) use the BLAST and OMIM databases to identify genes in C. elegans with significant homology to selected human genes, (3) perform RNA interference assays by soaking, and (4) statistically evaluate the data obtained from RNAi.

599. Using PCR in an undergraduate lab course to detect deletions in the unc-93 gene

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PCR is a powerful and common molecular biology technique with many applications. We have developed an exercise for an undergraduate molecular biology lab course using PCR to detect deletions in the unc-93 gene of C. elegans. unc-93 encodes a putative transmembrane protein muscle protein of unknown function.

Wildtype and three different unc-93 deletion mutant strains (carrying alleles lr12, lr28, and lr81) were grown, harvested, and frozen at -20 C by the instructor. Students isolate genomic DNA from each strain using a genomic DNA isolation kit from Gentra Systems, Inc. (Minneapolis, MN). We have used this kit for several years and it seems to be almost foolproof in the hands of undergraduates. Following proteinase K and RNase A treatment, proteins in the worm extract are precipitated by high salt and the genomic DNA is recovered by isopropanol precipitation.

PCR reactions using two sets of primers (A and B) from two different regions of the unc-93 gene are carried out on the genomic DNA from wildtype and mutant strains and the results analyzed by agarose gel electrophoresis. Primer pair A yields a 789 bp fragment, while primer pair B yields a 728 bp fragment. The A primers detect a 173 bp deletion in lr28 and a 78 bp deletion in lr81 while the B primers detect a 517 bp deletion in lr12. The use of wildtype DNA and primers for amplifying two different regions of the unc-93 gene provide internal controls.

Five student groups carried out this exercise in a molecular biology lab course during the Spring 2001 semester and their results will be presented.

We gratefully acknowledge the assistance of Dr. Beth DeStasio, Biology Dept., Lawrence University, Appleton, WI, who provided the unc-93 strains and primers used in this exercise.

600. *Caenorhabditis elegans* in the Undergraduate Physiology Curriculum

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Given the broad range of mutant strains with deficits in behaviors relevant to an undergraduate course in physiology, we sought to develop two discovery-based experiments with *C. elegans* that would both amplify concepts being learned in class and permit students to participate in experimental design.

The first experiment challenges students to characterize quantitatively the deficits in muscular function arising from selected missense mutations of *unc-54* (myosin heavy chain B). Cyclic interaction of myosin with actin is the basis of tension development in muscle cells, as well as eukaryotic cells generally, and the mechanical events are coupled to the hydrolysis of MgATP by myosin. The experiment exploits the availability of *unc-54* mutations (*e.g., s74* and *s95* alleles) that have salient functional deficits without disruption of muscle structure. Following discussion of viscosity and Reynolds number, students develop swimming assays in which tail-beat frequency is examined as a function of bulk solution viscosity. The concepts that the students consider when designing their experiments include the following: osmolarity and its measurement; bulk solution viscosity and its measurement; muscular fatigue and endurance; and force-velocity relations and their interpretation in the context of muscular efficiency. Because of the linkage between mutations of human myosin isoforms and a range of muscle and non-muscle diseases, including lethal heart disease and hereditary deafness, students have found the experiment engaging. Moreover, the accessibility of the crystalline structure of the myosin head (containing the catalytic domain and the actin-binding regions) permits students to correlate their results with the emerging structure-function relationship of myosin. The experiment can also be extended to the analysis of other mutations affecting muscular function (*e.g., unc-27*/Troponin-I-2; please see abstract

presented at this meeting by Priest *et al.*, "Structural and Functional Disruption of Muscle Caused by Troponin I Mutation"), as well as to evaluation of muscle structure in wild-type and mutant muscles with rhodamine-phalloidin staining of filamentous actin.

The second experiment, which spans portions of 3-4 laboratory sessions, aims: (1) to increase familiarity of students with computational tools used by physiologists to glean information from genomic databases, and (2) to illustrate how genomic information can be used to design physiological experiments probing human diseases with a model organism. Students "adopt" a disease linked to a mutant transporter, channel, or contractile protein and then use computational tools to identify a candidate homologue in *C. elegans*, to analyze protein domain architecture, and to formulate an hypothesis concerning the physiological role of the protein. Subsequently, students test their hypothesis by eliminating expression of the candidate homologue through RNA interference. Use of forward and reverse PCR primers incorporating the T7 RNA polymerase promoter sequence allows conservation of time and money when cDNA is transcribed, since double-stranded RNA can be prepared in a single reaction. Following an overnight soak in phosphate-buffered saline containing 100-1000 ng/microliter dsRNA, worms and progeny are examined for deficits in assays of chemotaxis, osmoregulation, egg-laying, and swimming, as well as in structural analyses of actin organization with rhodamine-phalloidin staining. Examples of adopted diseases include Bartter’s Syndrome (linked to mutation of Na-K-2Cl cotransporter), affecting kidney function in humans, and Familial Hypertrophic Cardiomyopathy (linked to mutation of myosin, tropomyosin, troponin I, and troponin T), affecting human heart muscle. Two qualities of the experiment are its practical relevance, because of consideration of human diseases, and its integration of molecular, cellular, and organismal biology.

601. A discovery oriented laboratory: from gene to mutant in the classroom

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The Rutgers University Department of Molecular Biology and Biochemistry has developed an undergraduate lab course that fully integrates *C. elegans* into a discovery-based research project that spans a full semester. "Introduction to Molecular Biology and Biochemistry Research" is taught by faculty members of the Department of MBB. The goal of this course is to allow students to investigate actual "unknown genes" while learning the basics of scientific research in the field of molecular biology.

The lab is designed so that students, working in groups of two, randomly pick their own clones from a *C. elegans* embryonic cDNA library (gift of F. Piano) and analyze these clones over the course of the semester. In individual lab experiments the students are required to isolate the plasmid DNA, perform restriction enzyme digests, perform gel electrophoresis and prepare the DNA for sequencing. Students are then able to perform computer-based bioinformatic analysis of the obtained sequences. Finally, students are able to directly asses the developmental function of their gene by performing RNAi feeding experiments with *C. elegans*.

The specifics of how the laboratory experiments are carried out will be presented. Briefly, the cDNA library was constructed in the TriplEx vector. This vector contains two T& promoter sites flanking the insert to allow expression of dsRNA when transformed into an appropriate bacterial strain. Students are therefore able to interpret the role of their gene in embryonic development by examining worms grown on bacteria that produce dsRNA of their specific genes. This is accomplished through use of the *lin-2* strain that normally produces a bag of worms phenotype. If the dsRNA disrupts

embryonic development, then a bag of eggs phenotype will be observed.

This version of the Introduction to Molecular Biology and Biochemistry Research laboratory course has been completed twice. We have found on average that about one third of the clones tested by the students exhibit an embryonic lethal phenotype.

602. Vesicle Transport and Fusion: An Organismal and Cell Biological Study for an Undergraduate Laboratory Exercise

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We developed a laboratory exercise to illustrate how defects in the nervous system affect movement. The experiment engages students in a study which combines organismal and cell biology. It allows students to gain hands on laboratory experience exploring animal movement, neuron function, and vesicle transport. Students gain experience using a fluorescent microscope to visualize GFP in cells. The exercise has been used successfully with both first year and second year undergraduate students in classes ranging from 12 to 24 people. Students characterize the movement of *C. elegans* and then determine the nature of nervous system defects. Using a dissecting microscope, students distinguish between healthy worms and partially paralyzed worms. The partially paralyzed strains are either *unc-104* mutants, which have a defect in vesicle transport, or *unc-13* mutants, which have a defect in vesicle fusion. The synaptic vesicles in these strains are labeled with GFP. Students next observe the strains under a fluorescent microscope to distinguish between worms with vesicle transport or vesicle fusion defects. The nervous system of the healthy worms and the *unc-13* worms appear the same, because the vesicles are transported to the synapses. In *unc-104* mutants, most vesicles are trapped in the neuron cell bodies and few reach the synapses. Based on their observations of movement and vesicle localization, students determine which worms have defects in vesicle transport and which worms have defects in vesicle fusion.

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603. *Caenorhabditis elegans* as a Model Host for Human Bacterial Pathogens

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During the past few years, our laboratory and others have focused on the development of pathogenicity models using *C. elegans* as the host because of the extensive genetic and genomic resources available and because of the relative ease of identifying *C. elegans* mutants that exhibit altered susceptibility to pathogen attack. For example, several *C. elegans* mutants have been identified that exhibit enhanced susceptibility or enhanced resistance to killing by the human opportunistic pathogen *Pseudomonas aeruginosa* [M.-W. Tan, G. Alloing, R. Feinbaum and F. M. Ausubel, unpublished data].

In addition, we demonstrated that the highly specialized vertebrate pathogen *Salmonella enterica* serovar *typhimurium* is capable of killing *C. elegans*. When *C. elegans* is placed on a lawn of *S. typhimurium*, the bacteria accumulate in the lumen of the worm intestine and the nematodes die over the course of several days. The worms die with similar kinetics when placed on a lawn of *S. typhimurium* for a relatively short time (3-5 hours) before transfer to a lawn of *E. coli*. In contrast to *P. aeruginosa*, a small inoculum of *S. typhimurium* can proliferate in the *C. elegans* intestine and establish a persistent infection. Genetic analyses of *S. typhimurium* indicate that many virulence factors are important for both mammalian nematode pathogenesis.

Despite the differences between the *P. aeruginosa*-mediated killing and the *S. typhimurium*-mediated killing, some of the *C. elegans* mutants more susceptible to *P. aeruginosa* turned out to be more susceptible to *S. typhimurium*, suggesting that some of the mechanisms underling host defense responses in *C. elegans* might be required to fight against bacterial infections in general.

Also, we found that *S. typhimurium* colonization of the *C. elegans* intestine leads to an increased level of cell death in the worm gonad. Using a variety of *C. elegans* mutants in which cell death is blocked, we observed that *S. typhimurium*-induced germline cell death is dependent on the *ced-3*/*ced-4* cell death pathway. Moreover, *ced-3* and *ced-4* mutants are hypersensitive to S. *typhimurium*-mediated killing. These results suggest that PCD may be involved in the *C. elegans* defense response to pathogen attack.

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604. *Caenorhabditis* species as an infection model for the investigation of genes conserved between pathogens and their hosts

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During the infection process, bacterial pathogens make use of host cellular processes and may acquire virulence factor sequences by horizontal gene transfer. Our Pathogenomics Project (www.pathogenomics.bc.ca) has developed software which aids identification of horizontally acquired sequences. Our approach has enabled us to not only identify new potential virulence factors, but also gain insight into the frequency of horizontal gene transfer within the bacteria, and between the three domains of life of Bacteria, Eukarya, and Archaea. From our analysis, we have determined that horizontal gene transfer between bacteria and multicellular eukaryotes, including *Caenorhabditis elegans*, is rare; however, we detected some interesting cases of genes that share high sequence similarity between bacteria and such eukaryotes, and may have been selectively maintained in certain bacterial pathogens.

Candidate virulence genes identified are being targeted for further functional study as part of this interdisciplinary project, using a *Caenorhabditis* model for infection. Recent published literature has demonstrated that *C. elegans* can be successfully infected with *Pseudomonas aeruginosa*, *Bacillus megaterium*, and *Salmonella typhimurium*. To examine the pathogenicity process, we are using a thermotolerant *Caenorhabditis* species as an infection model with the hypothesis that such organisms may more accurately reflect the infection process by permitting pathogenic bacteria to express their virulence factors at a temperature closer to the mammalian infection environment.

This project is funded by the Peter Wall Institute for Advanced Studies at University of British Columbia, Vancouver, Canada.

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605. A bummed pathogen meets with resistance: Towards the characterization and cloning of *bus-3* and *bus-5*.

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C. elegans is a host for the bacterial pathogen *Microbacterium nematophilum* [1]. Unlike other bacterial pathogens of *C. elegans*, *M. nematophilum* does not kill the worm; instead, the bacteria adhere to the tail of the worm causing constipation and a distinct deformed anal region (Dar) phenotype. Therefore, this interaction is reminiscent of a chronic disease state rather than a general toxicity mechanism as observed with other bacterial pathogens such as *P. aeruginosa* and *S. marcescens* [2]. To begin to understand the molecular basis of the interaction between *C. elegans* and *M. nematophilum*, a number of mutants were isolated that conferred resistance to *M. nematophilum*. From a pilot screen for resistant mutants, 30 EMS-induced mutations were identified and placed into 13 complementation groups. Four mutations are alleles of the *srf-2*, *srf-3* and *srf-5* genes, which are required for the proper display of surface proteins on the cuticle of the worm [3]. The fact that mutations in these loci confer resistance to the bacterial infection suggests that their gene products may be exploited by the bacteria during the infection process. The remaining mutations have been roughly mapped, defined as new genes, and termed *bus* for *b*acterially *u*nswollen. A screen for transposon-induced mutations defined three new *bus* loci. In addition, multiple new alleles of *srf-2*, *srf-3*, *srf-5* and most of the previously identified *bus* loci were identified in this screen. Transposition induced mutations of *bus-3* and *bus-5* were not obtained, suggesting that null alleles of these loci may be lethal. We are in the process of characterizing and cloning *bus-3* and *bus-5*. These genes are represented by multiple EMS alleles, which will aid in determining the genomic sequence. In addition, these mutations cause other phenotypes that should assist in mapping and rescue of the mutations. *bus-3* has been mapped to LGI and is represented by two EMS alleles, *e2695* and *e2696*, both of which exhibit an Unc (slow loopy backward) phenotype. *bus-5* has been mapped to LGX and is represented by four alleles, *e2685*, *e2686*, *e2688* and *e2699*. All these alleles cause the worms to exhibit an Unc (weak jerky, slow loopy backward), and weak Con phenotype. In addition, these worms appear squirmy, as if they have no traction, when not on the bacterial lawn. We are mapping these genes using single nucleotide polymorphisms. Finally, the screens for bacterially resistant loci have not been saturated, so we have begun a screen for more *bus* loci using Mos1 mutagenesis (J-L. Bessereau, D. Williams, and E. Jorgensen personal communication). Our progress on these projects will be presented.

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606. Molecular cloning of *C. elegans bus* genes using Transposon Insertion Display.

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Microbacterium nematophilum is a novel bacterial pathogen of *C elegans* (Hodgkin *et al*, 2000). This pathogen adheres to the rectum and post-anal region of the worm causing swelling of the underlying tissues, severe constipation and slow growth rates. Affected worms are described as having a Dar (Deformed Anal Region) phenotype. To define the molecular events involved in this host: pathogen interaction, we have used the mutator strain, *mut-7*, to activate *C. elegans* transposons and screen for mutant worms that are resistant to infection and exhibit a Bus (Bacterially UnSwollen) phenotype. Mutations in at least 8 *bus* genes have been obtained from this screen.

To identify, clone and analyse the *bus* loci we have chosen to use the transposon insertion display (TID) technique, which has been previously used to identify *C. elegans* genes (Wicks *et al*., 2000). Southern blots of genomic DNA from *bus* and wt strains were probed with transposon specific probes. *bus* recombinants exhibiting unique Tc1 bands that co-segregated with the mutant phenotype were picked for TID analysis. Our initial probing of Southern blots with transposon probes illustrated the importance of multifactor crosses in obtaining strains with clean backgrounds before proceeding to TID.

We will describe our use of TID and our progress in the molecular cloning of several of the *bus* genes.

Hodgkin, J. Kuwabara, P. E. Corneliussen, B. *Current Biology* 2000 10 ;1615-1618 A novel bacterial pathogen, *Microbacterium nematophilum*, induces morphological change in the nematode *C. elegans.*

Wicks SR, de Vries CJ, van Luenen HG, Plasterk RH *Dev Biol* 2000 May 15;221(2):295-307. CHE-3, a cytosolic dynein heavy chain, is required for sensory cilia structure and function in *Caenorhabditis elegans*.

607. Developing genetic methods for a novel *C. elegans* pathogen, the bacterium *Microbacterium nematophilum*

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Recently a specific bacterial pathogen of *C. elegans* has been discovered and named *Microbacterium nematophilum* (Hodgkin *et al.*, 2000). The mechanism of pathogenicity seems to be completely different from the more general pathogenic effects described for *Pseudomonas aeruginosa* and *Salmonella typhimurium*. Cells of *M. nematophilum* adhere to the surface of worms in the rectum and post-anal region and cause massive swelling of the underlying tissue, but they do not usually kill the infected worms. The bacterium, which belongs to the coryneform group of gram-positive eubacteria, is genetically uncharacterized and we are developing the first genetic approaches for it. Transformation of *M. nematophilum* has been demonstrated using the broad host-range plasmid pE194, which confers resistance to erythromycin. Both electroporation and a freeze-thaw protocol yield transformants with low efficiency (10^1 and 10^2 clones per μg DNA, respectively). The plasmid pE194 appears to undergo structural rearrangements in the host cell, during or after transformation. A few of the resulting resistant bacteria produce sectored colonies with two types of cell that differ in color and correspond to virulent and avirulent variants. Such loss of virulence can also rarely occur in *M. nematophilum* cells during routine plating and is usually associated with decrease in colony pigmentation. We are investigating the mechanism of this loss of pathogenicity, as well as screening directly for bacterial mutants with altered virulence. We are also investigating the genome size and genomic organization of *M. nematophilum*.

608. *C. elegans* - *M. nematophilum* interaction: Isolation and characterization of disease resistance mutants

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The coryneform bacterium*Microbacterium nematophilum* exhibits a specific interaction with its host*C. elegans*. In the susceptible condition, worms undergo morphological changes seen both at the anal and post-anal region and causing a Dar (Deformed Anal Region) phenotype. In contrast to this, resistant worms fail to exhibit such morphological changes and exhibit Bus (Bacterially UnSwollen) tails. We expect that this genetically tractable host-pathogen model will provide a good opportunity for understanding interactions between *C. elegans* and pathogenic microrganisms.

To this end, we have undertaken a forward genetic approach to find resistant mutants and have performed screens using transposon-induced mutagenesis. Using this strategy, a total of 70 alleles have been identified. We have mapped these mutations by conventional means or by using a subset of validated snip-SNPs. Genetic mapping and complementation tests defined 10 loci. Among them, are mutants of *C. elegans* with altered surface antigenicity (*srf-2* and *srf-3*). However, the remaining mutants appear specific to this *C. elegans*-pathogen association and define *bus* genes. The distribution of transposon-induced alleles has been compared with results from an EMS screen. We find that some *bus* genes (*bus-1* and *bus-10*) are hot spots for *mut-7* mutagenesis. Mutations unique to the transposon mutagenesis have also been identified. One such mutation is *bus-12* (*e2740*). *e2740* males, but not hermaphrodites, exhibit bacterial clumps at the dorsal side of the tail, in addition to male mating impairment. *bus-12* has been mapped to a small chromosomal interval that lacks obvious candidate genes. Molecular identification of *bus-12* is underway and should provide clues to the molecular nature of the

worm surface and mechanisms that control adherence by bacteria.

609. Multiple defects in a *C. elegans* mutant resistant to infection by *M. nematophilum*

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Microbacterium nematophilum is a recently-identified bacterial pathogen of *Caenorhabditis elegans* (Hodgkin *et al*., 2000). These bacteria adhere to the rectal and post-anal cuticle of the worm and induce swelling of the underlying hypodermal tissue. Screens for mutant worms resistant to infection (bacterially un-swollen; Bus) have been carried out using a mutator strain (*mut-7*) in which germline transposition of Tc1 and other transposons is activated. One strain obtained from this screen, was initially named *bus-11*, and has a complex phenotype. In addition to exhibiting the Bus phenotype, the hermaphrodites are egg-laying defective, uncoordinated and constipated. The males have tail developmental abnormalities.

Probing of a Southern blot of genomic DNA isolated from *bus-11* worms with a Tc1-specific probe demonstrated the presence of a novel Tc1 insertion that co-segregated with the *bus-11* phenotype. Transposon insertion display was used to sequence the genomic region flanking this insertion. The sequence obtained lay in the region to which *bus-11* had been mapped (LGIII between *unc-32* and *lon-1*) and indicated that the insertion was within the open reading frame of two genes encoding a prenyltransferase (B0280.1) and a KH-domain-containing protein (B0280.11). Preliminary RNA interference experiments to knock out both these genes failed to emulate the *bus-11* phenotype suggesting that this Tc1 insertion may be closely linked to *bus-11* but not causative of the phenotype.

Inspection of the genetic map in this region identified the *egl-5* gene as an alternative candidate on account of the striking phenotypic similarities between *egl-5* and *bus-11* mutant strains. *egl-5* is a *Hox* gene involved in the patterning of posterior structures. *egl-5* mutants were duly tested for response to the pathogen. Of four mutant strains tested, three (*n1066*, *n945 e1239*, *n988 e499*) demonstrated a Bus

phenotype suggesting that the resistance mutation in our strain is indeed in the *egl-5* gene. The fourth strain tested (*n1439*) contains a weak *egl-5* mutant allele. While egg-laying defective, these worms are not uncoordinated nor do males show the tail abnormalities of other *egl-5* mutant strains (Chisholm, 1991). Interestingly, these animals showed marked swelling of the anal region on exposure to the pathogen. This allele may thus provide a tool for the dissection of *egl-5* function and elucidation of its role in susceptibility to *M. nematophilum* infection. We are now investigating the response to the pathogen of other *egl-5* mutants and mutants of additional genes that have been implicated either in the regulation of *egl-5* function or as *egl-5* targets.

Hodgkin, J., Kuwabara, P.E. and Corneliussen, B. (2000) A novel bacterial pathogen, *Microbacterium nematophilum*, induces morphological change in the nematode *C. elegans*. *Current Biology* 10:1615-1618

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610. Understanding Bt-toxicity: Molecular and Cellular Analysis of Bt-resistance genes.

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Bacillus thuringiensis (*Bt)* toxins are organic insecticides that have been genetically engineered into a variety of agricultural crops that have become widely commercialized. The increasing rate at which farmers are utilizing *Bt*-crops leads to the fundamental concern that targeted insects will develop *Bt*-resistance. Strategies for resistance management have been devised and are currently undertaken, however, given our limited understanding of how *Bt* toxins operate these strategies may not provide long-term solutions.

Insect studies have shown that after ingestion by a susceptible host, toxin becomes solubilized and proteolytically activated in the gut. Active toxin binds to gut receptors and inserts itself into the membrane leading to cell lysis and death. While we understand the general mode of *Bt*action, there is still very little information regarding the genes required for *Bt*-toxicity. Our lab is interested in defining the molecular mechanisms underlying *Bt*-toxicity and resistance in the nematode *Caenorhabditis elegans*.

We have taken a genetic approach in *C. elegans* to identify molecular components required for *Bt*-toxicity. *C. elegans* is susceptible to a class of *Bt* toxins and we have identified five genes that confer resistance to one *Bt* toxin, Cry5B. Two of these genes, *bre-3* and *bre-5*, have been cloned, and I am currently investigating where these proteins are expressed in the wild-type animal. *bre-3* encodes a novel protein containing multiple potential transmembrane domains and *bre-5* encodes a likely glycosyltransferase. RNAi experiments conducted for both genes demonstrate the null phenotype of *bre-3* and *bre-5* is *Bt*-resistance with no other detectable phenotypes. Antibodies directed against *bre-5* were created and used to show that *bre-5* localizes to a perinuclear compartment that might be ER/golgi in most

and/or all cells of early and late embryos. This localization pattern is consistent with where a carbohydrate modification enzyme would be expected to function. Further experiments with antibodies are underway to localize the *bre-3* protein.

Cloning of the *bre-2* gene is currently in progress and close to being identified. Three-factor mapping and deficiency data position *bre-2* to the right of *unc-64* on the right arm of chromosome III. Transgenic rescue experiments are ongoing to determine the molecular identity of this gene. Concomitantly, we are performing RNAi experiments of the predicted genes in this region to clone *bre-2*.

611. Effects of *Stenotrophomonas maltophilia* on *Caenorhabditis elegans* and *Heterodera schachitii*.

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The influence of the chitinase-producing bacterium *Stenotrophomonas maltophilia* on the behavior, reproduction, egg hatch, and survival of the bacterial-feeding nematode *Caenorhabditis elegans* and the sugar beet cyst nematode *Heterodera schachitii* was assessed. The strain C3 of *S. maltophilia* suppressed and eliminated almost all reproduction in *C. elegans*, and was lethal to juveniles and adults, suggesting activity of proteases and lipases produced by the bacterium. When C3 was evaluated for its biocontrol potential against plant-parasitic nematodes, egg hatch in *H. schachitii* was significantly reduced by the presence of C3 as compared to the control, *E. coli* strain OP50. In addition, the viability of newly-hatched juveniles and eggs of *H. schachitii* was affected by C3. *Caenorhabditis elegans* served as a bioassay model to assess potential biocontrol activities of this chitinase-producing bacterium.

612. Insulin/IGF-like peptides of C. elegans

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Insulin/IGF-like peptides of C. elegans

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An insulin/IGF-I receptor homolog of C. elegans, DAF-2, is involved in the diapause, aging, and fat metabolism of the organism. We have searched candidates of the DAF-2 ligands and identified three insulin/IGF-like peptides designated Ceinsulin-1, -2, and -3. These peptides possess six cystein residues conserved among insulin/IGF peptide family. Ceinsulin-1 and -2 do not have a definite D domain characteristic of IGFs and show some degrees of sequence identify. On the other hand, Ceinsulin-3 has a definite D domain and shows little sequence similarity to Ceinsulin-1 and -2. Predicted tertiary structures of Ceinsulin-1 and -2 by computer modeling are quite similar to each other, suggesting that these peptide should recognize a same receptor. The developmental expression patterns of these peptides were examined by RT-PCR and Western blotting. The expression pattern of Ceinsulin-3 is somewhat different from those of Ceinsulin-1 and -2. The RNAi experiments revealed that the Ceinsulin-1 and -3 are involved in aging and fat metabolism, respectively. The physiological function of Ceinsulin-2 remains unknown. We hypothesize that each of the peptides has its own physiological function in the diapause, aging, or fat metabolism via the DAF-2.

613. *daf-2* signalling into the cell: looking for the second pathway

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The *daf-2/age-1/daf-16* pathway regulates numerous aspects of life history including early development, the dauer/non-dauer decision, fertility and life span (1). Mutations in *daf-2*, a homologue of vertebrate insulin and IGF-I receptors, or in *age-1*, a subunit of phosphoinositide 3-kinase (PI3K), cause dauer larva arrest, and can double adult life (2-4). Other components of the pathway include *daf-18* (PTEN phosphatase), *pdk-1* (phosphoinositide-dependent kinase), and *akt-1* and *akt-2* (protein kinase Bs). The latter inactivate DAF-16, a forkhead transcription factor, probably by phosphorylation.

Although this signal transduction pathway between *daf-2* and *daf-16* has been well defined, several findings suggest the existence of a second *daf-2-daf-16* pathway. For example, phenotypic analysis of 15 *daf-2* mutants, which fall into two distinct classes, suggested that the gene has two functional components, *daf-2A* and *daf-2B* (4). These potentially correspond to different signalling pathways emanating from *daf-2* into the cell; *daf-2B* is likely to correspond to the PI3K pathway (4). In addition, a gain-of-function allele of *akt-1*(5), and the hypomorphic allele *daf-18(e1375)*, fully suppress the dauer constitutive (Daf-c) phenotype of several severe *age-1* alleles yet only weakly suppress the relatively weak allele, *daf-2(e1370).*

To investigate and characterise the putative second *daf-2* pathway, we are carrying out interaction studies between several class 1 and class 2 *daf-2* alleles and various components of the PI3K and other signalling pathways. Preliminary results suggest a possible identity for the *daf-2A* pathway. We will present the results of these interactions studies.

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614. Identifying pathways through which neuronal *daf-2*/insulin-like signaling controls lifespan and dauer arrest.

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Lifespan and dauer arrest in *C. elegans* are controlled by the *daf-2*/insulin-like signaling pathway. In adults, mutations in *daf-2*, an insulin/IGF-I receptor homolog, or *age-1*, a homolog of the p110 catalytic subunit of PI(3)K, significantly extend lifespan. In addition, null mutations in these genes cause constitutive arrest as dauer larvae.

Previously, we showed that the long lifespan of *daf-2* and *age-1* mutants is rescued by restoring *daf-2* or *age-1* activity to neurons. In addition, dauer arrest in these mutants is rescued by *daf-2* or *age-1* expression in either neuronal or non-neuronal cell types, although rescue from neurons was most potent. To identify specific neurons that control dauer arrest and lifespan, we have expressed an *age-1* cDNA from several neuronal subtype-specific promoters in *age-1* null mutants. Expressing *age-1* from the promoters for *mec-7* (mechanosensory neurons), *unc-4* (motor neurons) or *osm-3* (chemosensory neurons) can rescue *age-1(0)* dauer arrest, but not lifespan. This suggests that *daf-2* pathway signaling in these cell types is not sufficient for wild-type lifespan. We are currently investigating whether wild-type lifespan requires *age-1* activity in other neurons, or in combinations of neurons.

The *daf-2* pathway controls lifespan and dauer arrest non-cell autonomously from neurons, suggesting that the *daf-2* pathway may regulate downstream genes for dauer remodeling and aging of target cells. To identify these genes, we screened for mutations that revert the ability of the neuronally-expressed *unc-14* promoter*::age-1* transgene to rescue dauer arrest in *age-1* null mutants. This screen is predicted to identify mutations in genes that are required for signaling from the neuronal *daf-2* pathway to the target cells that are remodeled during

dauer morphogenesis, such as the pharynx, hypodermis, gonad and intestine. In addition, these genes may act in the *daf-2* lifespan pathway. We are currently mapping and characterizing the mutants we identified in this screen.

615. *daf-12* appears not to be involved in male ageing

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C. elegans males look to be intrinsically longer-lived than hermaphrodites. A range of *unc* mutations extend male but not hermaphrodite lifespan, apparently by inhibiting some lifespan-reducing elements of wild-type male behaviour (1). The greater longevity of males requires *daf-16*(+), but appears independent of *daf-2*: previous studies showed that *daf-2* mutant males remain longer-lived than *daf-2* hermaphrodites (1). Conditional alleles of *daf-2* fall into two classes. In class 1 mutant hermaphrodites [e.g. *daf-2(m41)*] the larval arrest phenotype is fully suppressed by *daf-12(m20),* while in class 2 mutant hermaphrodites [e.g. *daf-2(e1370)*] it is not. Furthermore, in class 2 mutants, the *daf-2* extended lifespan is increased by *daf-12,* while in class 1 mutants it is either unaffected or partially suppressed (e.g. *m41*)(2,3).

We examined the effect of *daf-2*-*daf-12* interactions on male lifespan, in monoxenic liquid culture (on plates a high proportion of males climb up the walls and die from desiccation). In *daf-2* mutants, lifespan increases in both sexes were mostly similar to those seen previously on plates. One exception was as follows: while *daf-2(e1370)* males were much longer-lived than hermaphrodites, as previously seen (1), *daf-2(m41)* males and hermaphrodites lifespans were identical. *daf-2*;*daf-12* hermaphrodite lifespans were comparable to previous work (2,3).
Surprisingly, the presence of *daf-12(m20)* had no effect on male lifespan, in either *daf-2(e1370)* or *daf-2(m41)* males (22.5oC). We also examined the effect of *daf-12* on male lifespan, either alone or in combination with *unc-32(e189),* which blocks male mating behaviour and increases lifespan by ~60% (1). While a slight increase in median (but not maximum) male lifespan resulted from *daf-12* (+20%, $p<0.01$), compared with a reduction in hermaphrodite lifespan, (-10%, $p<0.01$), no effect on male lifespan was seen in the presence of *unc-32*.

Thus, while *daf-12* plays an important role in hermaphrodite ageing (as shown by mutant interactions with *daf-2* and its requirement for lifespan extension resulting from germline ablation (4)), it appears to play no role in male ageing. *daf-12* encodes a nuclear hormone receptor (5), which is a candidate gene for the ultimate receipt and transduction of cell subset-specific signals from the insulin-like signalling pathway to control dauer formation and ageing (5). Our findings reveal sex-specific differences in genetic pathways regulating lifespan, and suggest the possibility that *daf-12* may not be expressed in adult males.

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616. Molecular Identification of Transcriptional Targets of the DAF-16 Winged Helix Transcription Factor

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Dauer arrest and longevity in *C. elegans* are controlled by an insulin-like signaling pathway transduced by the winged helix transcription factor DAF-16. Mutations in several genes within this pathway (*daf-2, age-1*, and *pdk-1*) result in constitutive dauer formation (Daf-c) and increased lifespan (Age) phenotypes. Both the Daf-c and Age phenotypes of these mutants are suppressed by loss of function mutations in *daf-16*. This suggests DAF-16 acts as the principal transcriptional output controlling both diapause and lifespan governed by this pathway. We are attempting to identify both direct and indirect transcriptional targets of DAF-16 utilizing two complementary molecular approaches. Recently, the DAF-16 consensus binding element (DBE) was identified by Furuyama et al (2000). Using the DBE sequence, we have searched the *C. elegans* genomic sequence and identified many genes that contain the DBE within putative regulatory regions. To refine this list of possible DAF-16 target genes, we have collaborated with the Kim lab at Stanford to examine *in vivo* differences in gene expression between *daf-2* and *daf-2; daf-16* strains using cDNA microarrays. These two approaches have yielded a small subset of genes that contain at least one DBE and show a reproducible expression difference. We are currently examining the role of these genes in dauer formation and lifespan determination using RNAi and transgenic overexpression.

617. An Adaptive Response Extends Life Span Through DAF-16 Transcription Factor in *Caenorhabditis elegans*.

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Short-term exposure to high-concentration oxygen (90%) produces an adaptive response to oxidative stress in *Caenorhabditis elegans*. Thermotolerance, UV resistance and resistance against reactive oxygen species (ROS) are all consequences of this adaptive response. In this study, we found that life span of *C. elegans* was elongated by the adaptive response derived from short-term exposure to high-concentration oxygen as well as to heat shock stress. This extension was dependent on the stages and periods exposed with high-concentration oxygen. Moreover, this response was different in several mutants (*age-1*, *mev-1*, *daf-16*) previously shown to have altered life spans. Accordingly, we speculated that the degree of the adaptive response plays an important role in determining life span. We measured mRNA levels of six genes (*sod-1* through *sod-4*, *ctl-1*, and *ctl-2*) known to be important in combating oxidative stress. Wild-type, *age-1*, *mev-1* and *daf-16* animals were grown under atmospheric (21%) and 90% oxygen. There were considerable differences in the expression patterns of these six genes, most notably *sod-1*, *2* and *3* levels were significantly reduced in *daf-16*. This suggests that the adaptive response may mediate the relative intensity of resistance to oxidative stress, which was signaled to the *daf-16*-dependent pathway. These data indicate that *daf-16* must be functional in order to appropriately regulate the expression of these and other genes important in conferring resistance to oxidative stress. They further illustrate the importance of oxidative stress in the aging process.

618. Osmoregulation by daf-2/ age-1 insulin pathway

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Water balance is an important variable for terrestrial and some marine animals. For example, there is exquisite regulation of thirst reflexes in mammals based on small percentage changes in hydration. We expected that water balance would be, just as food and temperature are, key environmental input to the decision to grow reproductively. Dauer larvae of *C. elegans* have distinct reprogrammed secretory-excretory systems, which play an important role in osmoregulation through all larval stages. So we tested whether dauer pathway, including daf-2 / insulin, daf-7/ TGFβ, and daf-11 / guanyl cyclase pathways, mutants affect sensitivity to osmotic pressure.

We found that worms with mutations in the daf-2 insulin pathway are specifically sensitive to high osmolarity compared with wild type animals (N2). Previous studies have suggested that signals through DAF-2 insulin receptor are mediated by AGE-1 (a PI-3 kinase), PDK-1 and AKT-1/2 (protein kinases); and DAF-16, a forkhead transcription factor, is the main target of the signals. Daf-2 (e1370) or age-1 (mg305) mutant worms on NGM plates with 200 mM NaCl showed 100% embryonic or L1 lethality while wild type (N2) animals were viable. Pdk-1 loss-of-function mutants were less sensitive to high osmolarity than daf-2 or age-1 mutants but were still more sensitive than N2. Interestingly, akt-1 loss-of-function mutants exhibited wild-type resistance to high osmolarity. The lethality of daf-2 or age-1 in response to high osmolarity can be suppressed by daf-16 (mgDf47) or rescued by neuronal-specific expression of an age-1 transgene, both of which are also able to suppress or rescue the daf-c and aging phenotype of the daf-2 or age-1 mutants. Thus relationship between dauer formation, longevity and osmoregulation might be indicated.

Finally, similar results were obtained using various concentrations of KCl, Na_2SO4, or sorbitol in NGM plates, indicating that the observed hypersensitivity is a general response to osmolarity rather than to toxic levels of a given salt constituent.

In order to identify other genes involved in osmoregulation, we have performed a genetic screen for suppressors of daf-2 (e1370) lethality caused by high osmolarity. We have mutagenized 50,000 haploid genomes and have identified 13 independent mutations. Eleven are allelic to daf-16 and we are in the process of characterizing the other two mutants.

619. Dauers: what makes them long-lived?

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The dauer larva is a stress resistant and seemingly non-ageing alternative third stage larva. We have been performing a number of experimental approaches in an attempt to reveal mechanisms that underlie sustained life in dauers, and to examine whether the diapause stage has any effect on post dauer metabolism and life span. We have measured several parameters that reflect metabolic condition, including oxygen consumption rates, heat output, ATP levels, light production potential, XTT reducing capacity and autofluorescence intensities. The potential to resist oxidative stress was assessed by measuring catalase and superoxide dismutase activities. The experimental data were normalised to protein content to account for size differences.

Dauers consume less oxygen and produce less heat than young adults, but unlike adults they can maintain fairly stable rates for several weeks. However, the light production potential declines almost as steeply with time in adults and dauers, suggesting increasing mitochondrial dysfunction in both developmental stages. This could be reflected by rapidly decreasing ATP levels in adults, but much milder decreases are observed in dauers. Reducing capacity is constantly high in dauers reflecting a potential for anabolic reactions. A strongly negative reduction potential would plausibly also protect against oxidative damage. Dauers also have greatly increased activity levels of superoxide dismutase and catalase to scavenge reactive oxygen species. Blue fluorescence due to lipofuscin and reduced nicotinamides increases rapidly with age in adults and more gradually in dauers.

We conclude that the superior antioxidant capacity of the dauer metabolism plays a major role in the upregulation of longevity during diapause.

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620. Role of DAF-9 CYP450 in the regulation of fat storage and life span in *C. elegans*.

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In *C. elegans*, dauer arrest and fat storage are controlled by TGF-beta and Insulin-like signaling pathways. The nuclear hormone receptor *daf-12* acts downstream of both of these pathways, mediating the choice between dauer arrest and reproductive growth. *daf-12* also plays a role in aging since mutants enhance the longevity of *daf-2(e1370)* (the insulin-like receptor) and abolish the life span extension of germline laser ablated animals. *daf-9* acts downstream of TGF-beta and insulin-like inputs but upstream of *daf-12* for dauer formation. It encodes a cytochrome P450 suggesting a function in the production of a ligand for *daf-12*. We investigated the role of *daf-9* in fat storage and in the gonad signaling pathway affecting life span.

Under dauer inducing conditions, wild type animals shift metabolism towards fat storage. L2 predauers (L2d) and dauers of TGF-beta (e.g. *daf-7*) and insulin-like (e.g. *daf-2*) pathway mutants are dark and stain with Sudan black. This phenotype is blocked by Daf-d mutants *daf-3* and *daf-16*, respectively. We find that *daf-9* L2d larvae store fat independently of *daf-3* and *daf-16* and dependent on *daf-12*. These results place *daf-9* downstream or parallel to *daf-16* and *daf-3* but upstream of *daf-12*. *daf-9* L3 dauers on the other hand, are somewhat lighter indicating that fat is mobilized during partial recovery from diapause.

We performed laser ablations of somatic gonad and germline precursors in *daf-9(rh50)* mutant animals. *rh50* is a hypomorphic allele of *daf-9*. Germline ablation did not lengthen life span and somatic gonad ablation did not further shorten life span. We conclude that *daf-9(+)* is necessary for life span extension. Since *daf-9* is expressed in the spermatheca it might define the life extending signal from the somatic gonad.

Finally we want to identify more genes in the putative hormone signaling pathway. We are currently analyzing one candidate that displays gonadal and Daf-c phenotypes similar to *daf-9*.

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621. Dissecting the Signaling Pathways Regulated by the PTEN Tumor Suppressor Homolog DAF-18 in *C. elegans*

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PTEN tumor suppressor protein acts as a phosphatase for phosphatidylinositol 3,4,5-triphosphate (PIP3) and phosphatidylinositol 3,4-bisphosphate, PI(3,4)P2. We have previously shown that in *C. elegans*, the PTEN homolog DAF-18 functions as a negative regulator for the signaling pathway controlled by DAF-2, an insulin receptor-like molecule and by AGE-1, a PI 3-kinase homolog. A deletion mutation in the catalytic domain of CePTEN/DAF-18, *daf-18(nr2037)*, completely suppresses the dauer-constitutive phenotype caused by mutations in *daf-2* or *in age-1* (1). The fact that inactivation of *daf-18* suppresses the null mutation in *age-1* PI 3-kinase suggest that there may be other enzymes involved in the production of PIP3 and PI(3,4)P2 in the absence of *age-1* activity. In the *C. elegans* genome database, we found that there is a type II PI 3-kinase homolog encoded by the F39B1.1 locus. AGE-1 is a type I PI 3-kinase homolog. While type I PI 3-kinases are known to be activated by association with the ligand-engaged growth factor receptor tyrosine kinases, very little is known about the function and regulation of the type II PI 3-kinases. To understand the function of the type II PI 3-kinase we undertook the approach of the reverse genetics and obtained a deletion mutation in the F39B1.1 gene,*vs 56*. Our characterization of *vs56* mutant shows that F39B1.1 is involved in maintaining PIP3 levels in cooperation with DAF-2 and AGE-1.

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622. Nuclear Lamina Characterization in *C. elegans* during Apoptosis

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During apoptosis, nuclei undergo specific morphological changes, including proteolytic cleavage of the nuclear lamina by caspases, clustering of nuclear pore complexes, detachment of chromatin from the nuclear envelope and DNA cleavage. In mammalian cells, expression of uncleavable mutant lamins results in delayed apoptosis, failure of chromatin condensation, and altered lamina structure. In *C. elegans*, the caspase CED-3 is necessary for programmed cell death. Activation of CED-3 follows the release of CED-4 from the mitochondria and translocation to the nuclear envelope. *C. elegans* provides an excellent system for the study of the role of the nuclear lamina in apoptosis, as this nematode possesses only one lamin protein (Ce-lamin) and three LEM-domain proteins (Ce-emerin, Ce-MAN1 and LEM3). In the current study, the roles of different lamina proteins (Ce-lamin, Ce-emerin, Ce-MAN1, UNC-84 and matefin) during *C. elegans* apoptosis was studied by determining whether these nuclear lamina proteins can be degraded *in vitro* by CED-3, by analyzing their kinetics of degradation *in vivo* and by studying their possible interactions with CED-4. We have also generated lamin constructs altering the aspartic acid residue at the putative CED-3 cleavage site and generated transgenic lines expressing these constructs. We will determine the effects of these mutant lamins on apoptosis by looking for any cell-death defects, either in execution, engulfment, or timing.

623. A *ced-4* SUPPRESSION SCREEN TO IDENTIFY NEW GENES INVOLVED IN PROGRAMMED CELL DEATH

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Many genes involved in programmed cell death in *C. elegans* have been identified by screening for mutations that allow survival of cells that are normally destined to die. The opposite approach, screening for mutations that result in an increase in the number of cell deaths, has been comparatively unexplored, in part because of the lack of efficient methods to identify such mutations. A *lin-11::gfp* reporter (generated by Scott Cameron) expresses in the Pn.aap cells of the ventral cord. In wild-type animals, the six Pn.aap cells of the P3-P8 lineages survive, differentiate into VC neurons, and express *lin-11::gfp*. In strong cell-death defective mutants, all 12 Pn.aap cells survive and express GFP. In the *lin-11::gfp* reporter strain the amount of cell death can be quantified by scoring the number of fluorescent nuclei in the ventral cord using a fluorescence-equipped dissecting microscope. Using the *lin-11::gfp* reporter, I have performed a screen for suppressors of a partial loss-of-function *ced-4* mutant by looking for mutants with a reduction in the number of GFP-positive Pn.aap cells.

To date approximately 40,500 mutagenized genomes have been screened; 5,000 were screened clonally and 35,500 non-clonally. Two strong suppressors were obtained that reduce the number of GFP-positive Pn.aap cells. The first suppressor, identified in the clonal screen, is recessive and is recessively sterile. The second suppressor, identified in the non-clonal screen, is dominant and is recessively sterile. Further characterization of these mutants will be described.

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624. Identification of transcriptional regulators of the cell-death activator gene *egl-1*

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The cell-death activator gene *egl-1* (*egl* *e*g*g-l*aying defective) encodes the most upstream component of the general cell-death machinery in *C. elegans*. An *egl-1* loss-of-function (lf) mutation prevents most if not all somatic cell death during development (1). In contrast, *egl-1* gain-of-function (gf) mutations result in ectopic cell death: they cause the hermaphrodite-specific neurons (HSNs) to inappropriately undergo programmed cell death in hermaphrodites (2). These *egl-1* (gf) mutations are located in a TRA-1A binding site downstream of the *egl-1* transcription unit (3). The characterization of these mutations revealed that the terminal global regulator of somatic sexual fate, TRA-1A (*tra*, *tra*nsformer) (4), represses *egl-1* transcription in the HSNs in hermaphrodites and thereby prevents these neurons from undergoing programmed cell death. It has been proposed that TRA-1A represses *egl-1* transcription by negatively regulating a cell-type specific factor, such as an HSN-specific activator of *egl-1* (3). The identity of this factor, however, is still elusive. Lf mutations in the Hox gene *egl-5* cause the phenotypes expected for lf mutations in an HSN-specific activator of *egl-1* transcription: About 40% of the HSNs survive in *egl-5(n945)* males and in *egl-1(n986gf)*; *egl-5(n945*) hermaphrodites (5). Using an *egl-1::gfp* reporter construct, we are investigating whether HSN survival in these animals is due to the repression of *egl-1* transcription. We will also try to determine whether *egl-1* is a direct target of *egl-5* using various biochemical and molecular methods.

In addition, we have identified potential cell-type specific transcriptional regulators of *egl-1* in a yeast one-hybrid screen using a 500 bp regulatory region (which is conserved between *C. elegans* and *C. briggsae*)of the *egl-1* locus as bait. We narrowed down the binding sites of two candidates to fragments of about 125 bp and one candidate binds to the TRA-1A binding site. We have cloned the corresponding cDNAs to confirm our results in Electric-Mobility-Shift assays. Currently, we are studying the possible function of these candidates *in vivo*, using RNAi or existing mutants.

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625. Identification and characterization of *cps-4* and *cps-5*

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Programmed cell death (apoptosis) is a complex and tightly controlled process that is vital for the proper development of an organism as well as for maintaining its homeostasis. *ced-3*, a key player in the execution of programmed cell death in *C. elegans*, encodes a member of the caspase family of cysteine proteases. One particularly important area that has not been studied is the *in vivo* targets of the death caspases. In order to reveal downstream targets of CED-3, our lab developed a novel and sensitized genetic screen to isolate mutations that partially suppress or delay cell death caused by constitutively activated CED-3 death protease. In this screen, at least eight new genes (*cps-1,-2,-3,,-8*; **C**ED-3 **p**rotease **s**uppressors) have been identified. Here we report the characterization of the *cps-4* and *cps-5* genes which appear to function in interesting yet different ways to affect apoptosis.

To study how *cps-4* and *cps-5* affect programmed cell death in nematodes, we performed time-course analyses of the appearance of embryonic cell corpses in *cps-4* and *cps-5* mutants. We found that both *cps-4* and *cps-5* mutants display a delay of cell death phenotype: the peak of cell corpses is shifted from the bean/comma embryonic stage (seen in wild type animals) to the 2-fold embryonic stage in *cps-4* and *cps-5* mutants. These phenotypes are similar but weaker than the delay-of-cell-death phenotype displayed by another cell death mutant, *ced-8*, in which the peak of cell corpses is found in late embryonic stage (late three-fold embryonic stage). We constructed double and triple mutants among *cps-4*, *cps-5*, *ced-8* and other *cps* genes and found that *cps-4*, *cps-5*, and *ced-8* can significantly enhance one another's phenotype in delaying cell death. In addition, *cps-5* but not *cps-4* can also enhance the delay-of-cell-death phenotype of *cps-6*, which is involved in apoptotic DNA degradation and encodes an endonuclease (please see the abstract by Parrish et. al.). These results suggest that *cps-4* and *cps-6* may function in the same pathway while *cps-5* and *ced-8* may function in different pathways. Consistent with this hypothesis, we found that *cps-4* mutants contained significantly higher number of TUNEL-positive cells than that of wild-type animals, while the *cps-5* mutant has a similar number of TUNEL-positive cells as the wild-type animals.

We mapped *cps-4* to linkage group I and *cps-5* to linkage group III and are in the process of fine mapping and cloning these two genes.

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626. TWO NEW CELL-DEATH GENES AND ENGULFMENT CONTRIBUTE TO CELL KILLING

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The gene *ced-3*, which is the most downstream known component in the cell-death execution pathway, encodes a cysteine protease or caspase. How the CED-3 protease kills cells is unknown, and the identities and mechanisms of genes affecting the downstream processes of cell death has been a long-standing problem in the cell death field. Mutations in genes controlling cell-killing activities downstream of or parallel to *ced-3* might not have been previously identified because of their redundant nature. Animals with strong loss-of-function mutations in *ced-3* lack most if not all programmed cell deaths. However, there are weaker *ced-3* mutants that lack only a small percentage of programmed cell deaths. We reasoned that mutations in genes controlling subtle or redundant cell-killing activities might enhance a cell-killing defect conferred by a partial loss-of-function mutation in *ced-3*.

We performed a screen for enhancers of a partial *ced-3* loss-of-function allele and isolated 37 mutations. At least three are mutations in *ced-9*, two in *ced-4*, and six in *ced-3*. Nine mutations confer defects in cell-corpse engulfment, indicating a novel role for engulfment in promoting the killing process of programmed cell death. Because cell corpses are generated in engulfment-defective mutants, the proposed function of engulfment has long been solely the removal of unwanted apoptotic cell bodies. We found that engulfment mutations lead to the low-penetrance survival of cells that normally die in the presence of an intact core cell-killing pathway. Cell lineage analysis showed that cell death is typically initiated but occasionally incompletely executed in the absence of engulfment.

On the basis of complementation tests and map positions, we also defined at least two new cell-killing genes. Like engulfment mutants animals mutant for only these genes have subtle but detectable defects in programmed cell death. Genetic analyses place these genes downstream of or parallel to the anti-apoptotic gene *ced-9* and partially redundant with the cell-killing activity controlled by engulfment. We will discuss our characterization of these genes.

627. Phagocytosis of necrotic corpses--a new gene involved in NCD corpse disposal

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We are interested in the mechanisms by which "injured" cells that have died by necrotic cell death (for example, as induced by channel hyperactivating mutations in the mec-4 touch channel) are recognized and removed by phagocytosis. There are seven known genes needed for phagocytic engulfment of both programmed cell death and necrotic cell death corpses. *ced-1, ced-6, and ced-7* act in one partially redundant pathway for corpse elimination and *ced-2, ced-5, ced-10*, and *ced-12* function in another. In a screen designed to identify genes that might specifically function in the phagocytosis of necrotic cells, we identified allele *bz84*, which significantly potentiates necrotic corpse persistence in a *ced-7* mutant background. *ced-7* encodes a member of the ABC transporter family that is required in both the engulfing and dying cell for removal of cells that have undergone programmed cell death. We are currently mapping and characterizing the genetic properties of *bz84*. In a complementary study we are probing members of the CD36 scavenger receptor family for roles in necrotic corpse elimination since CD36 family members influence elimination of dead cells in both flies and mammals. Six CD36 *C. elegans* counterparts fall into two subfamilies. One branch consists of the ORFs, Y49E10.20, Y76A2B.6, and C03F11.3, all of which are represented by ESTs. Members of the second branch, F07A5.3, F11C1.3, and R07B1.3 are not represented by EST clones. We are using RNAi studies to probe the potential roles of the CD36 family members on elimination of necrotic corpses. We made full length GFP constructs to identify the expression patterns of the different scavenger receptors and to test if the expression pattern changes in response to necrotic cell death.

628. CD36-like Proteins and alpha-integrin are Needed for Cell Death Engulfment

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Genetic analysis in *Caenorhabditis elegans* has identified several genes needed for the engulfment of apoptotic cells. Additional components have been identified through the study of phagocytosis in mammalian macrophages. For example, CD36 is one of several transmembrane receptors needed for the engulfment of apoptotic cells by macrophages. In addition, CD36 expressed on endothelial cells mediates endothelial cell apoptosis and inhibits cell migration in response to thrombospondin-1 in the extracellular matrix. We find that CD36-like genes in *C. elegans* are also needed for engulfment of programmed cell deaths and for the migration of the anterior arm of the gonad.

C. elegans has six CD36-like proteins: C03F11.3, Y49E10.20, Y76A2B.6, F11C1.3, F07A5.3, and R07B1.3. Bacteria-mediated RNAi, which we found to be superior to injection of dsRNA, revealed a germline cell corpse engulfment defect for Y49E10.20 and Y76A2B.6 but not C03F11.3, R07B1.3, F07A5.3, F11C1.3, *unc-22*, or the empty vector control. RNA interference of Y76A2B.6 also delayed embryonic corpse clearance in the three-fold embryo. In addition, RNAi of Y49E10.20 and Y76A2B.6 caused inappropriate migration of the anterior arm of the gonad.

GFP gene and promoter fusions with Y76A2B.6 are expressed in hypodermal cells, which are known to engulf apoptotic cells. A Y49E10.20 promoter fusion to GFP is expressed in many cells in the three-fold embryo, in the head and tail of L1 larvae, and in the hypodermis throughout development, consistent with functions in apoptosis and engulfment. C03F11.3 promoter and gene fusions are expressed in the intestine.

Since the thrombospondin type-1 repeat of UNC-5 is required to rescue *unc-5(e53)* mutants and *unc-5* animals have defective gonad arm migration, we examined whether RNAi of

CD36-like proteins could enhance this phenotype. We also looked for potential genetic interactions between CD36-like proteins and cell death engulfment mutants in both genetically determined engulfment pathways. Bacterial RNAi of the six candidates did not enhance either the *unc-5* gonad arm migration defect or the somatic engulfment phenotype of *ced-1* or *ced-5*. Because integrins are known to cooperate with CD36 in the binding and internalization of apoptotic cells by macrophages, we also examined *ina-1*, an a -integrin mutant, for an engulfment phenotype. *ina-1(gm199)* does produce a germline cell death engulfment phenotype which can be enhanced by bacterial-mediated RNA interference of either Y49E10.20 or F11C1.3.

629. Study of the function of phosphatidylserine receptor in *C.elegans*

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The recognition and clearance of apoptotic cells is an important step during apoptosis. In this process, it has been observed that apoptotic cells lose phospholipid asymmetry and expose phosphatidyserine (PS), an anionic phospholipid, on the outer surface of the plasma membrane. The exposed PS has been proposed to serve as a recognition marker for engulfing cells. How the exposed phosphatidylserine is recognized and mediates the corpse engulfment process is still largely unknown. Recently, a phosphatidylserine receptor (PSR) that appears to mediate specific recognition of PS on apoptotic cells by phagocytes has been identified in humans[1]. It is expressed on the surface of macrophages, fibroblasts and epithelial cells. When transfected into cultured cells, the PSR allows cultured B and T lymphocytes to recognize and engulf apoptotic cells in a phosphatidylserine-specific manner. This phosphatidylserine receptor is highly conserved throughout evolution. We have noted the presence of a homologous gene in *C.elegans* that shares high sequence homology with human PSR (46% identity).

In order to investigate the possible role of the putative worm PSR homolog in the engulfment of dying cells during *C. elegans* programmed cell death, we are currently performing RNAi experiments and looking for deletions in the PSR homolog locus to examine the loss-of-function phenotypes of worm PSR. We have made several PSR::GFP fusions and are in the process of determining its expression pattern and localization in nematodes. These experiments will help us better understand the function of PSR in nematodes and also will likely provide additional important information about the mechanisms of cell corpse recognition

and clearance in *C.elegans*.

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630. Characterization of a deficiency mutant that produces abnormally large cell corpses in late embryogenesis.

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Programmed cell death (PCD/ apoptosis) is a common cell fate in most if not all multicellular animals. To understand its steps genetically, we screened for regions of the genome required zygotically for normal PCD using *C. elegans* chromosomal deficiency mutants, which collectively delete ~80% of the genome. By this screen, we identified 27 individual strains that abnormally increase or decreased the number of PCD, or tha exhibited abnormal corpse morphology.

Among these, we characterized deficiency mutants *tDf6* and *nDf41*, which generated abnormally large corpses. We observed developing embryos of the *tDf6* mutant by 4D time-lapse recording. We found that the number of PCD corpses was significantly decreased in this mutant and that most corpses appeared at the late stage of embryogenesis and were larger than those seen in the wild type. Because the *ced-3*; *tDf6* and the *egl-1*; *tDf6* double mutants showed neither large nor normal corpses, the large corpses appeared to be produced by the known PCD genetic pathway. By deficiency mapping and series of RNAi of the candidate genes in the region, we found that some *cyk-4*(*RNAi*) embryos phenocopied *tDf6*. The *cyk-4* gene encodes a GTPase-activator protein (GAP) for Rho-like GTPase, and CYK-4 is thought to have a role to complete cytokinesis, as reported by Jantsch-Plunger V et al.. By temperature-shift experiments using the *cyk-4*(*t1689ts*) mutant, we confirmed that the large corpses in *tDf6* embryos were produced by the deprivation of zygotic CYK-4 in late embryogenesis. We also performed membrane and nuclei staining and found that terminally

arrested *tDf6* embryos had many large cells with 2-4 nuclei. Taken together, we conclude that the large corpses in *tDf6* embryos are produced by deaths of multinucleate large cells.

This phenotype of the *tDf6* mutant was similar to that of another deficiency mutant *nDf41*. By deficiency mapping and complementation tests, we found that *zen-4* is responsible for the large corpses observed in *nDf41* embryos. The *zen-4* gene is reported to encode the motor protein kinesine and the mutant fails to complete the late step of cytokinesis . In addition, according to the report of Jantsch-Plunger V et al., ZEN-4 and CYK-4 are thought to cooperate to complete the late step of cytokinesis, because their locations are similar and interdependent. Thus, through our deficiency screening, these two cytokinesis genes were identified as similar PCD-defective mutations.

Our screening covered ~80% of the genome, and no other Deficiency embryos exhibited large corpses similar to those of *tDf6* and *nDf41*. Therefore, we speculate that at most one or two more genes are required zygotically for the late step of cytokinesis during embryogenesis.

631. Analysis of the *C. elegans* homolog of the mammalian autophagy gene *beclin 1*

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Autophagy is the bulk degradation of cytoplasmic components through an autophagosomic-lysosomal pathway. Under stress or nutrient starvation, mammalian cells as well as yeast cells undergo major degradation of proteins and remodelling of cellular compartments. Autophagy is essential for differentiation, survival during nutrient deprivation, and normal growth control; however, nothing is known about autophagy in the development of multicellular organisms.

We are analyzing the role of evolutionarily conserved autophagy genes in *C. elegans* development, by studying the function of the worm*beclin1* homolog. The human *beclin 1* gene is the first identified mammalian gene to mediate autophagy and also has tumour suppresor and antiviral function (Liang et al., 1999). In yeast, the *beclin1* gene was isolated as an autophagy defective mutation, *Apg6*, or as a vacuolar protein sorting mutation,*Vps11*. Mammalian Beclin 1 promotes autophagy, but not vacuolar protein sorting in autophagy-defective yeast with a targeted disruption of the yeast ortholog, *APG6/VPS30* (Liang et al., 1999). Human Beclin1 shares 28% amino acid with the *C. elegans* Beclin1 (T19E7.3;*C. elegans* genome sequencing project). We have inactivated the *C. elegans beclin1* homologue by injecting T19E7.3 dsRNA or by soaking N2 worms with T19E7.3 dsRNA. *beclin1* RNAi-soaked N2 worms arrested at the L1 stage, whereas, the progeny of the injected *beclin1* RNAi N2worms reached adulthood with varying defects in the intestine, including the accumulation of large vacuolar structures in the intestine. Ce*beclin1* RNAi also decreases the endocytosis of a YP170(yolk protein) ::GFP fusion by oocytes, an assay that has been used to isolate mutants in receptor

mediated endocytosis (Grant and Hirsh, 1999).

As autophagy is required for survival under stress or nutrient starvation conditions, we hypothesized that autophagy might be required for dauer formation or survival of the dauer larva. To investigate this hypothesis, we injected *daf-2* mutants with Ce *beclin1* dsRNA. *daf-2* mutants are dauer constitutive when raised at the restricted temperature and have an increased lifespan at the permissive temperature (Riddle, 1997). Ce *beclin 1* (RNAi);*daf-2* double mutants arrested at different stages of dauer formation when grown at the restrictive temperature. Survival of the *daf-2* dauers was also affected after *beclin1* RNAi. We are now using electron microscopy to determine if autophagy is increased upon dauer formation, and if autophagy is affected in the Ce *beclin 1* RNAi mutants.

In summary, Ce *beclin 1* RNAi-soaked N2 worms arrest at the L1 stage and display intestinal defects, and the Ce *beclin 1* RNAi-injected *daf-2* worms arrest at different stages of dauer formation. While the molecular basis of these phenotypes is not yet known, these observations suggest a possible role for *beclin 1*-mediated autophagy in *C. elegans* development and in the survival or formation of the *C. elegans* dauer stage larvae.

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632. Oxygen Deprivation in *Caenorhabditis elegans*

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C. elegans respond to anoxia (0% oxygen) by entering into a state of recoverable suspended animation. Nematode exposure to anoxia causes all microscopically observable movement to cease, including cell division, developmental progression, feeding, and motility. Upon re-exposure to oxygen, animals resumed these processes. All nematode life cycle stages are capable of exhibiting this response to anoxia. However, the survival rate to prolonged exposure of anoxia depends on life cycle stage. To determine if blastomeres from embryos exposed to anoxia arrest during specific points in the cell cycle we used various cell cycle regulated antibodies for immunofluorescence analysis. We found that blastomeres can arrest during interphase, prophase, metaphase, and telophase but not anaphase. To investigate the mechanism by which so many diverse cellular processes are coordinately regulated during suspended animation we examined the phosphorylation state of several proteins. We found that phosphorylated forms of histone H3, RNA Polymerase II, and cell cycle regulated proteins recognized by the MPM-2 antibody were not detectable in anoxia-arrested embryos. In contrast, phosphorylated forms of SR proteins were detectable in anoxic blastomeres. This suggests that specific dephosphorylation of key proteins may play a critical role in the establishment and/or maintenance of a state of suspended animation.

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633. Programmed cell death in *Pristionchus pacificus*

Wu et al., Development. **126**, 2021-2031 (1999)

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To study the evolution of developmental processes, we analyse vulva development in *P. pacificus* and compare it to *C. elegans*. In *P. pacificus*, 7 of the 12 ventral epidermal cells undergo apoptosis, whereas the corresponding cells in *C. elegans* fuse with the surrounding hypodermis. Phylogenetic analysis suggests that cell death of these epidermal cells represents a derived character. To understand the evolutionary changes responsible for these evolutionary alterations in the apoptotic mechanism, we are characterising cell death mutants in *P. pacificus*. Mutations in the cell death pathway in *P. pacificus* lead to the survival of P(1-4,9-11).p, which normally die in wild-type. Complementation tests have shown, that these mutants fall into two complementation groups, one of which has been proven to correspond to *Ppa-ced-3* (Sommer et al., 1998). The second complementation group has been originally characterized as *ipa-2* (**i**nhibitor of **P**-ectoblast **a**poptosis) and is a good candidate for the *Ppa-ced-4* homolog. To test this hypothesis, we tried to clone *ced-4* using different approaches, *viz* low stringency hybridisation, PCR and yeast two hybrid approach with *Ppa-mac-1* as bait. In *C. elegans* it has been shown that MAC-1, a member of the AAA type ATPase family, interacts with *ced-4* at the protein level (Wu et al., 1999). However none of the used approaches were successful. We are presently trying to clone *Ppa-ced-4* using positional cloning. Preliminary data suggest a linkage of *ipa-2* and *Ppa-pal-1*. In *C. elegans, pal-1* is closely linked to *ced-4*. Further mapping is in progress to clone *P. pacificus ced-4* by chromosomal walking.

Ref.:

Sommer et al., Development **125**, 3865-3873 (1998)

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634. BAG1, AN HSP70 CO-CHAPERONE INVOLVED IN CELL GROWTH AND CELL STRESS

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Bag1 is a multifunctional protein that interacts with a variety of partner proteins. In mammalian cells, overexpression of Bag1 influences different signal transduction pathways, which in general leads to prevention of apoptosis or inhibition of cell cycle arrest. Interaction partners of Bag1 include Bcl-2, Raf-1 kinase, hormone receptors, and Hsp70. Bag1 enhances the anti-apoptotic function of Bcl-2, stimulates of the Raf-1 kinase activity, and inhibits Hsp70 chaperone activities.

We are using *C. elegans* as a model to investigate the function of Bag1 during development and survival of a complex multicellular organism. We have created and analyzed a deletion mutant of *Bag1* (F57B10.11). Null mutants appear similar to the wild type N2 strain with respect to viability, anatomy, timing of development, and life span. This indicates that *Bag1* is not an essential gene and, moreover that Bag1 is not essential for Hsp70 activity under physiological conditions. Interestingly, the *Bag1* null hermaphrodites show a 20% increase in the number of progeny compared to wild type N2 worms.

Given the effect of overexpression of Bag1 on signal transduction pathways in mammalian cells, often reflected in changes in transcriptional activities, we asked whether phenotypic changes in C.elegans caused by deletion of *Bag1* could be explained by changes in the mRNA expression profile. We are currently analyzing the mRNA expression profile of adult *Bag1* null worms, compared to adult wild type N2 worms, using full genome microarrays. We will report on our progress on the microarray analysis and on the characterization of the phenotype of the *Bag1* deletion strain.

635. Induction of a heat shock protein by hypoxia stress can be used as a new tool of conditional gene expression in *Caenorhabditis elegans*

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Many heat shock proteins(HSPs) are induced by various stresses. According to microarray analysis of ethanol-treated worms, the expression of the HSP16 family is upregulated. We interpret this to mean that the HSP16 family proteins are responsive to general stress. To confirm the microarry results, we made GFP constructs and tested GFP induction by ethanol and heat shock. From our experiments, we found that one HSP16 protein is induced by hypoxia and that others are not. Accordingly, we analyzed regulatory regions of this gene, and found that it has two heat shock responsive elements. We have arbitrarily named the two elements the distal promoter and proximal promoter. Heat shock induction needs only either one of the two promoter elements, but the induction by hypoxia needs both of the two promoter elements, or at least the region that includes the distal promoter element.

Using these characteristics of this promoter, we are now trying in vivo RNAi induced by hypoxia. Gene induction by hypoxia has many advantages compared to gene induction by heat shock. The major advantage are that it is easy , only involving putting worms into M9, and that it is less stressful on the worms, and therefore has almost no side-effects.

636. Heat Shock Factor in C. elegans and in parasitic nematodes

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Filarial parasites of the genus Brugia live in mammals (including humans) and are transmitted between hosts by the bite of a blood-feeding mosquito vector. The infective form for the mosquito is the L1 or microfilariae (Mf) a life cycle stage that is highly adapted for life in the bloodstream of the mammal. Mf are developmentally arrested and undergo no further development until ingested by a mosquito. The link between the progression of the developmental cycle and the transition between hosts implies that the Mf has a mechanism by which it can sense its changing environment. Results in other systems have shown that heat shock factor (HSF) can act as a cellular thermometer directly monitoring temperature and oxidative state. As temperature is one of the major differences between the mosquito and mammalian hosts we are interested in investigating the role of HSF in developmental progression. As the tools for functional genomics do not exist for Brugia, we are using C. elegans as a model system in which to define the role of HSF in a nematode. ACeDB identifies a single HSF-like gene with a highly conserved DNA binding domain. We have used an RNAi feeding vector to determine the likely function of HSF in the nematode. A number of different phenotypes were apparent at 20oC including: slow growth, scrawny appearance, thermo-sensitivity, decreased fertility and egg-laying defective. At 25oC these phenotypes were more pronounced. In an hsp-16/GFP reporter background, RNAi abolished GFP expression in the intestine but not in the pharynx or nerve ring. These results are consistent with a requirement for HSF function in the gut. Ongoing studies are focused on determining the spatial and temporal expression pattern of hsf throughout development. We aim to determine the pathways in which HSF is active under normal developmental conditions and to identify down-stream targets of HSF.

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637. HSP90 mRNA in the nematode Caenorhabditis elegans under heat stress

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To study changes in the amount of the C. elegans HSP90 mRNA induced by heat shock, nematodes cultured at 16 degrees were either given heat shock at various temperatures for 2 hours or continued to be cultured at 16 degrees. After the total RNA was extracted from the nematodes, the amount of the HSP90 mRNA was measured by the GeneAmp 5700 system.

Of heat shock treatment at 25, 32, and 37 degrees, the heat shock at 32 degrees induced the maximum expression of the HSP90 mRNA. The relative amount of the HSP90 mRNA to the total RNA after heat shock treatment at 32 degrees was about 3.6 times as much as that without the heat shock. The HSP90 mRNA was induced to increase rapidly within 30 minutes of the heat shock treatment and was not observed to increase thereafter. To normalize the total RNA with the heat shock treatment to that without it, the relative amounts of ACTIN and GDH mRNAs to the total RNA with and without the heat shock were also measured, and the amount with the treatment was found to be about 0.6 times as much as that without it. Assuming that the relative amounts of ACTIN and GDH mRNAs do not change under heat shock treatment, we estimate the increase in the amount of the HSP90 mRNA with heat shock to be about 6-fold as much as that without it. We also began to use the HSP90 gene for monitoring environmentally hazardous substances. To do so, we constructed a hybrid DNA by fusing an upstream region of the HSP90 gene to a GFP gene. The hybrid DNA was injected into the ovary of adult C. elegans to obtain transgenic C. elegans. Increased fluorescence was observed when heat shock, organic mercury compound, cadmium, or selenite was challenged against the transgenic C. elegans.

638. Genes Involved in Heat Shock Induced Transcription in *C. elegans*

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In a screen for suppressors of activated GOA-1 under the control of a heat shock promoter, we identified several genetic loci that affect heat-shock induction of GOA-1. *sag-4* (V) mutants are wild type in appearance, whereas *sag-3* (I) and *sag-5* (III) mutants are egg-laying defective. Western analysis indicated that mutations in all three genes suppress activated GOA-1 by decreasing heat-shock induced protein expression. Although endogenous GOA-1 expression is not affected, heat-shock induction of GOA-1 decreased in the suppressor strains.

We cloned *sag-4*, and found that it encodes a cyclin homologue most similar to cyclin L, a novel type of cyclin with unknown function, but also similar to cyclin T, K or C, which was identified as a subunit of TFIIH, part of the RNA polymerase II complex that functions in basal transcription. Only transgenes with the *hsp16-2* promoter can be affected by mutations in *sag-4*. These results suggest that SAG-4 is necessary for heat- shock mediated transcription in *C. elegans*. We propose that cyclin L is the type of cyclin acting in TFIIH during heat-shock induced mRNA transcription and that it functions similarly to cyclin T, K or C during basal transcription.

We also found that mutations in *sag-3* and *sag-5*, as well as *sag-4*, were able to suppress another transgene under the control of the *hsp16-2* promoter. The suppression by *sag-3* was especially striking. We have used single nucleotide polymorphisms to map *sag-3* more precisely and found that it maps to a small region (Y53C10A) that includes a putative heat shock transcription factor. We are in the process of sequencing this candidate gene in the *sag-3 mutant*. *sag-5* has been mapped to a small interval on the left end of III, between *unc-45* and *daf-7*. We will present our latest mapping and cloning results.

639. Calreticulin, a calcium-binding molecular chaperone, is required for stress response and sperm fertility in C. elegans

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Calreticulin, a Ca2+-binding protein known to have many cellular functions including regulation of Ca2+ homoeostasis and chaperone activity, is essential for heart and brain development during embryogenesis in mice. Here we report the functional characterization of C. elegans calreticulin (crt-1). A crt-1 null mutant does not result in embryonic lethality but shows temperature-dependent reproduction defects. In C. elegans CRT-1 is expressed in the intestine, pharynx, body-wall muscles, head neurons, coelomocytes, and in sperm. crt-1 males exhibit reduced mating efficiency and defects late in sperm development. Additionally, crt-1 and itr-1 (inositol triphosphate receptor) together are required for normal behavioral rhythms. crt-1 transcript level is elevated under stress conditions suggesting that CRT-1 may be important for stress-induced chaperoning function in C. elegans.

640. A study of the osmotic stress response in *Caenorhabditis elegans*

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Osmotic stress is one of the most common stresses suffered by living organisms. The removal of water from a cell causes such severe perturbation to molecular structures that organisms or organ cells may die within seconds of their exposure to the hypertonic environment. The only comprehensive study on osmotic stress response at the molecular and genome level has been conducted in *Saccharomyces cerevisiae*. Accordingly, very little is known on primary osmosensors, specific signal transduction pathways and the key genes involved in osmotic stress tolerance in complex organisms. As a consequence, we initiated a study on the osmotic stress response in *Caenorhabditis elegans* to understand how a complex multi-cellular organism responds and adapts to extreme water loss. In osmotic stress conditions (2 hours in 500 mM NaCl in NGM media), the wild-type hermaphrodite has a remarkable capacity to survive extreme water loss which reduces their body volume size by approximately 40%. The dramatic change in the nematode body size is observed during the first five minutes of exposure to osmotic shock and is accompanied by a complete cessation of motility (vigorous swimming movements). The shrunken dehydrated state of the nematodes in the hyperosmotic environment is reversible in isotonic conditions (50mM NaCl in NGM media). In those conditions, the dehydrated wild-type return after 10 minutes to their initial body size (2.3±0.25 μm^3) and to normal activity. In order to identify the components involved in the osmotic stress resistance (OSR) in *C. elegans*, we have conducted a forward genetic screen. In a screen of ~8000 mutagenized F2 we isolated five mutants that display a striking capacity to retain body water content and vigorous swimming movements in extreme osmotic stress conditions (500mM NaCl). We are currently characterizing other mutants and complementation tests among these mutations are ongoing.

641. Synergistic toxicity of multiple heavy metals is revealed by a biological assay using *C. elegans* and its transgenic derivatives.

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Caenorhabditis elegans with a short life cycle, low maintenance cost, high tolerance to pH, salinity, water hardness and temperature as well as its well-characterized genetics makes it an reliable model as bioindicator for monitoring effluent or water samples from freshwater or estuarine sources. It eliminates the problem of heterogeneous population occurred in other field collected species used in bioassay.

We fine-tuned the toxicity assay with the use of L1 larvae. Toxicity of 12 different metal ions have been tested by exposing *C. elegans* and its transgenic derivatives to individual metals. Based on the LC_{50} value in these tests, we showed that the toxicity of these metals could be ranked as Hg> Cu> Pb> Cr> Cd> Al> Co> Ni> Zn> Mn. We further demonstrated that there were combinatorial effects of these heavy metals on the survival of *C. elegans*. Both synergistic and rescue effect could be observed in the paired-metal toxicity bioassay. Mixed doses of two metals at concentrations with weak lethal effect could lead to massive killing of animals. Copper, coupled with other metals, elicits the strongest synergistic results, while zinc could rescue the toxic impact of other metals on *C. elegans*. Our observation indicates that the current practice of regular monitoring of heavy metal contents in environmental samples by physical or chemical means might not be effective in determining the actual toxicity, where bioavailablity and combinatorial effect are neglected.

In order to improve the sensitivity of our assay, we had generated and tested multiple transgenic strains carrying a heat shock promoter driving *gfp* reporter in substitution of wild type worms. The duration of the assay could be reduced about 10 folds and the assay completed in hours, while synergistic effect between toxicants could still be revealed. With further optimization, regular year round environmental monitoring

with these transgenic strains should be possible.

642. The unfolded protein response protein kinases IRE-1 and PEK-1 signal complementary pathways essential for C. elegans development

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The ability to maintain ER function and adapt to the demand for increased protein folding is a crucial part of the way in which cells respond to stress or damage. IRE1 and PERK are type I transmembrane protein kinases localized to the endoplasmic reticulum (ER) that are activated by the accumulation of unfolded proteins in the ER to signal transcriptional induction of genes encoding ER chaperones, such as BIP, which increases the total folding capacity. This pathway is called the unfolded protein response (UPR). IRE1 is essential to signal the UPR in S. cerevisiae, however, is dispensable for the mammalian UPR. Although absent in S. cerevisiae, in metazoan species PERK phosphorylates the a subunit of eukaryotic translation initiation factor 2 (eIF2a) to attenuate general protein synthesis, thereby reducing the amount of proteins that require folding. However, in mammalian cells PERK is also required to activate transcription of the UPR-responsive genes through preferential translation of selective transcription factors.

To develop a genetic method for dissecting the UPR in higher eukaryotes, we are studying C. elegans. C. elegans has two BIP genes that are induced 2- to 10- fold upon ER stress, suggesting that the UPR exist in C. elegans. We used RT-PCR and RACE to clone ire-1, the homolog of the IRE1 gene, and pek-1, the homolog of the PERK gene. RNA interference (RNAi) showed that ire-1 and pek-1 are redundant, and mediate a process that is essential for worm development. The ire-1(RNAi); pek-1(RNAi) double mutants arrest as L2 larvae, and their intestines rapidly degrade as the animals age. When we studied the expression of pek-1, with a promotor::GFP fusion construct, we found that it was strongly expressed in the intestine, as well as in several other tissues. Surprisingly, the UPR monitored by BIP induction remained intact in these double mutants, suggesting the existence of additional pathways that activate the UPR in C. elegans. However, at this point we cannot rule out that residual signaling through ire-1 and/or pek-1 occurs due to incomplete RNAi silencing. To address this question, we recently isolated a deletion mutant of ire-1, and are now using it to identify new genes that act in this response.

643. CLR-1 and Fibroblast Growth Factor Receptor (FGFR) Signaling in *C. elegans*

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Mutations in *clr-1* result in a dramatic Clr (**Cl**ea**r**) phenotype characterized by extremely short, immobile, and sterile animals that accumulate clear fluid within their pseudocoelomic cavities. *clr-1* encodes a receptor tyrosine phosphatase (RTP) that negatively regulates an EGL-15 FGFR signaling pathway in *C. elegans*; the Clr phenotype of *clr-1* mutants is the manifestation of hyperactivation of this pathway. Conversely, animals that completely lose *egl-15* function arrest early in larval development, and less severe reduction of *egl-15* function results in a Scr (**Sc**rawn**y**) body morphology. To understand the role of FGFR signaling in *C. elegans* better, we are investigating the cellular basis of these phenotypic defects.

The results of several different lines of experimentation suggest that CLR-1 acts in the excretory cell to regulate EGL-15 signaling. To identify the cells that express CLR-1, we generated a GFP reporter driven by the *clr-1* promoter. This *clr-1::gfp* construct is expressed in a wide variety of cells, including the excretory cell, ventral cord neurons, body wall muscle cells, some neurons in the head, adult vulval cells, and some rectal cells. This expression pattern is also observed using a *clr-1::lacZ* reporter construct and immunofluorescence studies of CLR-1 expressed from a rescuing transgene. To determine the cellular site of action of *clr-1*, a mosaic analysis was performed. This analysis suggests that *clr-1* activity is required in the AB.plp sublineage, which gives rise to a group of cells including the excretory cell. Laser ablation of the excretory cell has been shown to result in a similar Clr phenotype[1]. Based on these results, we hypothesize that the excretory cell is the site in which CLR-1 acts to regulate EGL-15 signaling.

To support this hypothesis further, we have tested a number of promoters that are capable of driving GFP expression in the excretory cell for their ability to drive CLR-1 expression to rescue *clr-1* mutants. Both the *vha-1* promoter (**V**acuolar-type **H**$^+$ **A**TPase) and *itr-1* promoter (**I**nositol 1,4,5-**T**riphosphate **R**eceptor) are able to drive GFP expression in the excretory cell[2,3]. We have shown that both promoters can rescue *clr-1(e1745ts)* when used to drive expression of *clr-1*. Furthermore, consistent with EGL-15 also acting in the excretory cell, we have also shown that the *vha-1* promoter can be used to drive *egl-15* expression to rescue the larval lethal phenotype conferred by *egl-15(null)*.

These data are consistent with EGL-15 regulating the function of the excretory system. It will be interesting to determine what this pathway responds to in order to regulate fluid flux in *C. elegans*.

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644. Identification of Tyrosine Phosphorylated Proteins in the EGL-15 Signaling Pathway

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Fibroblast growth factor receptors (FGFRs), like other receptor tyrosine kinases, respond to extracellular cues by forming active dimers that autophosphorylate tyrosine residues on the intracellular domain of the receptor. Active receptors tyrosine phosphorylate specific downstream substrates and recruit a signaling complex to the membrane, thus initiating a cascade of events which leads ultimately to cellular responses such as cell cycle progression, cell migration or differentiation.

In *C. elegans*, there appears to be one FGFR , EGL-15, which is involved in multiple developmental processes including an essential function (*egl-15* null alleles confer larval lethality) and a role in the guidance of the migrating sex myoblasts (SMs). A decrease in signaling through EGL-15 results in aberrant SM migration and confers the Egl phenotype. Additionally, EGL-15 plays a poorly understood role in fluid homeostasis and excretory function in which hyperactivity of the FGFR pathway results in a Clear (Clr) phenotype. Components that are responsible for mediating downstream signaling pathways have been identified based on their ability to confer either a Clr or a Suppressor of Clr (Soc) phenotype, the latter being indicative of a decrease in EGL-15 signaling.

While many components that mediate EGL-15 signaling have been identified in this manner, the mechanism by which EGL-15 is linked to these downstream signaling components is not known. To identify components which function downstream of the receptor and act as substrates for its kinase activity, I have taken both a candidate gene approach and am developing a method for examining proteins which are tyrosine phosphorylated in response to EGL-15 activation.

Mammalian FGFRs activate RAS signaling by phosphorylating FRS2, a multisubstrate adaptor protein. FRS2 is membrane bound via an N-terminal myristylation site, constitutively interacts with the receptor via its PTB domain, and is multiply phosphorylated on tyrosine residues along its long carboxy-terminal tail. The *C. elegans* genome project predicts the presence of an ORF with significant homology to the PTB domain of FRS2. Our lab has generated a partial cDNA by RT-PCR which includes this PTB domain as well as a C-terminal region. We are attempting to determine if RNAi of this predicted gene results in an *egl-15* -like phenotype as would be expected by loss of function of this FGFR signaling pathway.

An alternative approach to finding substrates of EGL-15 is to identify proteins which are tyrosine phosphorylated in response to EGL-15 activation. Since the endogenous level of activated EGL-15 might be too low, I have generated transgenic animals which express a constitutively activated form of EGL-15. As hyperactivation of the EGL-15 pathway in otherwise normal animals results in the Clr phenotype and sterile animals, activated transgenic arrays were generated in a *soc-2* background in order to suppress the potential Clr phenotype. Unexpectedly, transgenic animals expressing activated EGL-15 become Egl. Preliminary evidence suggests that the Egl phenotype may result from cell lineage abnormalities, SM migration defects, and vulval muscle differentiation or attachment defects. These transgenic animals will be studied both for tyrosine-phosphorylated proteins as well as to characterize the Egl phenotype that may reflect an additional role for the EGL-15 FGFR.

645. Characterization of the 13 *C. elegans* RGS Proteins: A reverse genetics approach

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Heterotrimeric G proteins mediate signaling through G protein-coupled receptors and are composed of three subunits (alpha, beta, gamma). Regulator of G protein Signaling (RGS) proteins interact with the G alpha subunit to stimulate its slow intrinsic GTPase activity, thus driving the heterotrimeric G protein to its inactive form.

There are exactly 13 RGS proteins in *C. elegans* and ~24 in humans. Many mammalian RGS proteins act on the same G proteins *in vitro* but this may not reflect their *in vivo* target specificity. An important question in the RGS field is why there are so many RGS proteins if their biochemical properties are so similar. We hypothesize that these proteins do have *in vivo* specificity for G alpha targets.

RGS proteins may play a role in the poorly understood mechanism of desensitization of G protein signaling in which cells stop responding to signals after prolonged stimulation. Most pharmaceuticals act through G proteins and desensitization thus limits their usefulness. Prolonged G protein signaling might affect the activity of RGS proteins to shut down signaling.

To address the issues of *in vivo* specificity and desensitization we are generating RGS knockout mutants by screening a ~1 million genome knockout library made in our lab. We have generated mutations in eight RGS genes and the remaining knockout mutations are in process. All of the mutants are viable and fertile and six show no gross abnormalities. Mutations in two RGS genes (*egl-10* and *eat-16*) were identified in classical genetic screens and have defects in egg-laying and locomotion. We also generated strains overexpressing each RGS gene. Overexpression of four of the RGS proteins resulted in egg-laying behavior defects, while the remaining eight showed no gross abnormalities. In addition, we determined the expression patterns of the 13 RGS genes using GFP fusion transgenes. Ten RGS proteins are expressed in many neurons, one is expressed

only embryonically, one is only in the chemosensory neurons, and one is expressed in two pharyngeal neurons, several ventral cord neurons and two cells in the tail.

To analyze the functions of the RGS proteins we are employing behavioral assays using stimuli known to act through G protein-coupled receptors. The knockout mutants and overexpressors are being tested for chemotaxis to various chemoattractants and for response to neurotransmitters such as dopamine and serotonin. Specifically, we are testing for the inability of mutant animals to respond normally to either high or low concentrations of these chemicals and also for their inability to undergo desensitization.

646. The differentially expressed C. elegans RapGEF, pxf-1, is required for molting.

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The Rap1-specific guanine nucleotide exchange factor (GEF), pxf-1, is the C. elegans orthologue of the mammalian PDZ-GEF. Differential promoter usage and alternative splicing result in the production of at least three different pxf-1 isoforms, which share a common catalytic domain. Green fluorescent protein (GFP) reporter construct demonstrate that pxf-1 is expressed in the hypodermis, gut, various neurons and somatic cells of the distal gonad. Particularly striking is the oscilating expression of the downstream promoter in the pharynx during the four molting stages L1 to L4. Worms homozygous for a mutation in pxf-1, that deletes part of the PDZ-domain and the complete catalytic domain, hatch normally but develop slower and move uncoordinated. They have severe problems in removing the old cuticle during molting. In addition, vesicles are frequently formed underneath the cuticle, indicating a defect in cell-matrix or cell-cell interaction. Most animals die before adulthood, but those that reach this stage are largely sterile. From these results we conclude that pxf-1 is not involved in processes directly related to cell proliferation or development, but plays an role in cell maintenance processes like cell adhesion and/or polarized secretion, which are essential for proper molting.

647. Genetic analysis of the Rap1-pathway in C. elegans

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The family of Ras-like GTPases functions in a diversity of signal transduction routes in which they transmit signals received by cell surface receptors to downstream effector molecules. Despite the high level of homology and conservation during evolution, the function of most Ras-like GTPases other than Ras remain poorly defined. This holds also for Rap1, for which functions have been claimed as diverse as antagonzing Ras, regulation of secretion and activation of integrins (1). As a first step in elucidating the function of Rap1in C. elegans, the expression patterns of the homologues of Rap1 (C27B7.8), the related Rap2 (C25D7.7) and of two of their guanine nucleotide exchange factors (GEFs) wer studied using GFP-reproter constructs. Rap1 appears to be expressed in various neurons in the head and tail, in the hypodermis and somatic cells of the gonad. Rap2 is also expressed in various neurons and in the pharynx. Of the tow Rap1-GEFs, pxf-1 (T14G10.2) is widely expressed, whereas the homologue of Epac (T20G5.5) is restrict to neurons in the head and ventral nerve cord. More recently we have begun to make trangenic animals overexpressing constitutively active and dominant negative versions of Rap1 and data from our first analysis of these transgenic animals will be presented.

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648. Does GOA-1, the *C. elegans* ortholog of the major human brain G alpha protein, signal through diacylglycerol kinase-1?

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The most abundant G protein of the human brain, $G\alpha_o$, signals through an unknown mechanism. The nematode *C. elegans* has a highly similar (~80% identical) ortholog of human $G\alpha_o$ called GOA-1. GOA-1 signaling modulates a variety of behaviors in *C. elegans*, including locomotion and egg-laying. *dgk-1* is the predominant gene identified in genetic screens for mutants with a *goa-1* like phenotype. Further, genetic screens for mutations that suppress the defects of constitutively active GOA-1 result in many alleles of *dgk-1* (1). Genetic analysis suggests that *dgk-1*, which encodes a diacylglycerol kinase, acts downstream of or in parallel to GOA-1(1). DGK-1 is 38% identical to DGKθ, an enzyme expressed in the human brain (2). We decided to test the possibility that GOA-1 signals through DGK-1 by directly activating it.

The GOA-1 and DGK-1 proteins were expressed recombinantly and purified as untagged (GOA-1) or affinity tagged fusion proteins (DGK-1). An *in vitro* assay for the activity of DGK-1 was developed. In this assay, the substrate, diacylglycerol (DAG) along with the lipid cofactor phosphatidylserine is presented as vesicles and as a monolayer at the air/aqueous interface. Diacylglycerol kinase that is present in the aqueous phase then uses γ^{32}P-ATP to phosphorylate DAG, producing radioactive phosphatidic acid (PA). This can be extracted into an organic phase and quantitated. The recombinant DGK-1 was active in this assay. The *in vitro* assay is linear over a significant range with respect to time, amount of enzyme and substrate (DAG). These characteristics are essential to detect possible effects of GOA-1 on the Km or Vmax of DGK-1. However, as the protein preparation of DGK-1 is prone to aggregation, we are optimizing purification strategies in order to obtain soluble and stable protein. We then plan to use the purified proteins in the *in vitro* assay

to test if GOA-1 activates DGK-1.

In *C. elegans* there are two G alpha proteins , $G\alpha_o$ (GOA-1) and $G\alpha_q$(EGL-30), that have opposing effects on egg-laying and locomotion. GOA-1 signals to reduce the rates of egg-laying and locomotion, while EGL-30 signals to increase the rates of egg-laying and locomotion. EGL-30 activates a PLCβ (EGL-8) which results in the production of the second messenger DAG, which may activate neurotransmission through UNC-13 (2). If GOA-1 directly activates DGK-1, then this provides a molecular mechanism to explain the antagonism between the two G alpha signaling pathways in *C. elegans.* The opposing effects of EGL-30 and GOA-1 on neurotransmission can be explained by EGL-30 activating EGL-8 to produce more DAG and GOA-1 activating DGK-1 to convert the DAG to inactive PA.

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649. EAT-16 as a link between GOA-1 and EGL-30 signaling pathways

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Mutations in the genes that encode the heterotrimeric G protein alpha subunits EGL-30 and GOA-1 produce opposite effects on a variety of the behaviors that they regulate: movement, egg-laying, and pharyngeal pumping. Previous genetic analysis has suggested that the GOA-1 signaling pathway acts to directly inhibit EGL-30 mediated signaling through the RGS (regulator of G-protein signaling) protein, EAT-16 (1). In support of this hypothesis, cotransfection of EAT-16 with EGL-30 inhibits EGL-30-dependent activation of endogeous PLCbeta in COS-7 cells and in Galphaq/Galpha11 knockout cell lines. Additionally, cotransfection of the constitutively active mutant, GOA-1(QL), enhances this inhibition.

Coimmunoprecipitation assays reveal that both GOA-1 and EGL-30 can interact with EAT-16. We found that GOA-1 and EGL-30 can interact with the C-terminal region of EAT-16 containing the GGL and RGS domains, but not with the N-terminal DEP domain. In addition, GOA-1(QL) demonstrates a higher affinity for intact EAT-16 than wild-type GOA-1. Conversely, wild-type EGL-30 demonstrates a higher affinity for EAT-16 than EGL-30(QL). These interactions are consistent with our genetic model that proposes EAT-16 as an effector for activated GOA-1, and as a negative regulator for EGL-30.

Mammalian Gbeta5 has been shown to interact with RGS proteins that contain GGL domains (2, 3). Moreover, the gene that encodes the *C. elegans* homologue of Gbeta5, GPB-2, has been shown to interact genetically with the two genes that encode RGS proteins with GGL domains, *eat-16* and *egl-10* (4). We have further shown that GPB-2 enhances the negative regulation of EGL-30 by EAT-16 in COS-7 cells. We also addressed conflicting initial reports as to the

mechanism of regulation of RGS proteins by Gbeta5. One report suggested that Gbeta5 affects the interaction of RGS proteins with Galpha subunits (3). Another report suggested that Gbeta5 enhances that GTPase-activating function of RGS proteins (2). Our cotransfection experiments indicate that GPB-2 does not affect the interaction of EAT-16 with either GOA-1 or EGL-30. We are currently doing additional experiments to explore how these interactions are regulated.

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650. Type I and Type II phosphatidylinositol phosphate kinases have diverse functions in *C. elegans*

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Phosphoinositides modulate several cellular behaviours including cell proliferation, cell survival, cytoskeletal changes and vesicular trafficking. Many of these processes are regulated by enzymes that use phosphatidylinositol 4,5 bisphosphate ($PI4,5P_2$) as a substrate or by proteins that bind $PI4,5P_2$ directly. $PI4,5P_2$ can be made by 5 phosphorylation of PI4P by Type I phosphatidylinositol phosphate kinase (Type I PIPK) or by 4 phosphorylation of PI5P by Type II PIPK. However, while Type I PIPK is required to produce functional $PI4,5P_2$ *in vivo*, no one has yet demonstrated a requirement for Type II PIPK, an enzyme found only in metazoans.

In mammals, there are at least three isoforms of Type I PIPK and three isoforms of Type II PIPK. *C. elegans* contains only one of each, named *ppk-1* (F55A12.3) and *ppk-2* (predicted as two genes Y48G9A.g and Y48G9A.h) respectively. *ppk-1* RNAi applied to L4 larvae results in a sterile phenotype (Fraser *et al.* 2000, Maeda *et al.* 2001), consistent with a requirement for $PI4,5P_2$ in the spermathecae (Clandinin *et al.* 1998). We have isolated a deletion allele (*pk1343*) of *ppk-2*, which lacks a large part of the catalytic domain. Unlike worms treated with *ppk-1* RNAi, worms homozygous for pk1343 are viable, develop and move normally, and respond like wild type to *ppk-1* RNAi. However, *ppk-2* (*pk1343*) larvae show an increased sensitivity to oxidative stress. Thus, we have confirmed that *ppk-1* and *ppk-2* have separate functions, and, for the first time in any organism, we have evidence for an *in vivo* function of a Type II PIP kinase. To investigate the effect of Type II PIPK on *in vivo* lipids, we will analyse *in vivo* phosphoinositide levels in

wild type and mutant worms.

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651. A Novel Ser/Thr Protein Kinase is Involved in the Genesis of Alae

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A 2.3 kb mRNA encodes a novel *C. elegans* Ser/Thr protein kinase (722 amino acids) named PKC4. Four domains govern intracellular location and activity of the kinase. Two Cys-rich regions near the N terminus of PKC4 create classical Zn-finger domains that bind diacylglycerol (DAG) and phorbol esters (TPA). Ligation of TPA elicits translocation of cytoplasmic PKC4 to plasma membrane, thereby promoting kinase activation via interaction with phosphatidylserine (PS). A centrally-located PH domain is predicted to bind $PI4,5P_{2.}$ This structural module is not essential for targeting PKC4 to plasma membrane. However, mutation of conserved residues within the PH module (K^{298} or W^{396} to A) abrogates a 7-fold activation of phosphotransferase activity by DAG/PS. Thus, the PKC4 PH domain may function as an "internal adapter" that either (a) transmits activating conformational changes from Cys-rich regulatory regions to the C terminal catalytic domain or (b) stabilizes the DAG/PS-induced active conformation of catalytic and substrate binding sites. Catalytic domains of PKC4 and a recently discovered mammalian kinase (PKD) are ~ 60% identical.

Cellular and temporal patterns of *pkc-4* gene transcription were determined to provide insight into physiological functions of the kinase. A 2.5 kbp DNA fragment (promoter/enhancer) that flanks the 5’ end of the PKC4 gene was inserted upstream from a nucleus-directed, GFP reporter cDNA in a *C. elegans* expression plasmid (designated *pkc4P:gfp*). Transgenic *C. elegans* that carry the chimeric *pkc4P:gfp* gene were created and assayed by immunofluorescence microscopy. High-level *pkc-4* promoter activity was evident almost exclusively in nuclei of hypodermal seam cells in late-stage embryos (pre L1/L1 transition) and young adults (L4/adult transition). The precisely-timed, cell-specific expression of PKC4 suggested an association of the novel kinase with the genesis and/or function of alae, seam-cell derived

cuticular structures ("treads") that are longitudinally oriented along lateral surfaces of L1 and adult *C. elegans*. RNA_i methodology was applied to elucidate PKC4 functions. Nematodes that received dsRNA encoding the kinase exhibited a 90% decline in the level of PKC4 protein. Normal sinusoidal movement of *C. elegans* on agar was disrupted in PKC4-deficient animals and replaced with a "zig-zag"motility pattern of variable amplitude. Electron microscopy revealed that
PKC4-depletion elicited substantial changes in structural features of adult alae. The most striking phenotype involved a near-complete loss of alae. Other animals had alae of reduced size associated with distorted lateral epidermis or misshapen alae of normal size juxtaposed with swollen epidermis. Thus, PKC4 appears to be a central component in a signaling pathway that governs developmental stage-specific generation, organization and/or assembly of a cuticle structure that guides worm movement. Future identification of downstream targets for PKC4 will be necessary to determine whether the kinase regulates expression, subunit assembly, intracellular trafficking or secretion of specialized proteins that create alae or their underlying "strut-like" supporting structures.

652. *jkk-1* and *mek-1* regulate body movement coordination and response to heavy metals through *jnk-1* in *Caenorhabditis elegans*

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Although *in vitro* evidence suggests two c-Jun N-terminal kinase (JNK) kinases, MKK4 and MKK7, transactivate JNK, *in vivo* evidence confirming this paradigm is incomplete. In fact, data from *Drosophila* and murine knockouts indicate JNK deficiency may differ from the composite deficiency of MKK4 and MKK7. Recently, the *Caenorhabditis elegans* homologs of human JNK, *jnk-1* (1), and two MKK-7s, *mek-1* (2) and *jkk-1* (1), were cloned.

Here we characterize *jnk-1*, which encodes two isoforms JNK-1α and JNK-1β. A null allele, *jnk-1(gk7)*, resulted in worms with defective body movement coordination and modest mechanosensory deficits. Similar to *jkk-1* mutants (1), elimination of GABAergic signals suppressed the *jnk-1(gk7)* locomotion defect. *jnk-1(gk7)*, like *mek-1* nulls (2), also showed copper and cadmium hypersensitivity.
Conditional expression of JNK-1α or JNK-1β rescued both phenotypes, suggesting they are not due to developmental errors. While *jkk-1* or *mek-1* inactivation by RNA-mediated interference mimicked *jnk-1(gk7)* locomotion and heavy metal stress defects, respectively, *mkk-4*/*sek-1* inactivation did not, but rather yielded defective egg-laying.
jnk-1(gk7);jkk-1(km2) and *jnk-1(gk7);mek-1(ks54)* double mutants displayed non-additivity regarding amplitude and heavy metal sensitivity, respectively, consistent with these MKK-7s being on separate JNK-1 pathways. Interestingly, both double mutants continued to manifest the modest mechanosensory deficits detected in *jnk-1(gk7)* mutant worms, suggesting the existence of other JNK-1 regulators.

Our results delineate at least two different JNK pathways through *jkk-1* and *mek-1* in *C. elegans*. Further, three distinct genetic models, *C. elegans*, *Drosophila* and mice, now define interaction between MKK7, but not MKK4, and JNK.

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653. Lov neurotransmission

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The nervous system of the adult *C. elegans* male possesses 381 neurons to the hermaphrodite's 302. These additional sex-specific neurons are required for male mating behaviors, namely, response to hermaphrodite contact, backing, turning, location of vulva (Lov), spicule insertion, and sperm transfer. We are specifically studying Lov behavior at the cellular, genetic, and molecular levels.

The HOA and HOB hook neurons are required for Lov behavior (1) and the putative sensory receptor *lov-1* is expressed in HOB (2). How does signaling initiated by the LOV-1 receptor culminate in Lov behavior? We are interested in identifying the neurotransmitters and neuropeptides used by the HOA and HOB mechano- and chemosensory neurons, respectively (3). Anne Hart and Arif Nathoo have identified 21 putative *neuropeptide like protein* (***nlp***) genes in *C. elegans* and made transcriptional GFP fusions (4) which we have screened for expression in the adult male tail.

An *nlp-8::gfp* transcriptional fusion is expressed uniformly throughout the HOB neuron. Expression is also observed in other non-sex, non-stage specific cells. We have constructed a full length *nlp-8::gfp* translational fusion and observed expression only in HOB, suggesting *nlp-8* may be a Lov neuropeptide. We are exploring the role of *nlp-8* in Lov behavior using genetic and molecular approaches.

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654. Characterization of the sperm transfer step of male mating behavior of C. elegans : Two sides to the story.

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C. elegans male mating behavior involves the proper execution of a series of sub-behaviors culminating in the transfer of sperm to the hermaphrodite (Liu, KS and Sternberg, PW. 1995. Neuron 14:79-89). Prior to sperm transfer, the male must insert his copulatory spicules through the vulval opening into the uterus. Ablation of the SPV spicule neurons leads to premature sperm transfer at the vulva, thus the SPV neurons may regulate sperm transfer by inhibiting release (ibid.). Once inserted into the uterus the male will not release sperm until he receives a cue from the hermaphrodite (Liu, KS. 1995. Ph.D. thesis, California institute of Technology). This cue appears to originate in the hermaphrodite gonad, as its ablation (leaving the anchor cell intact) suppresses sperm transfer (ibid.). This aspect of mating behavior therefore requires the perception and integration of a signal between cells of two separate organisms.

We are interested in understanding this communication process. To that end we are currently doing a genetic screen designed to isolate both males and hermaphrodites defective for sperm transfer. Isolating and characterizing these mutants will help to elucidate the signaling process involved, and its regulation. Results from this screen, as well as further characterization of the wild-type process of sperm transfer will be discussed.

655. Structural and topological organization of the *C. elegans polycystin LOV-1*

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lov-1 and *pkd-2* are required for *C. elegans* male mating behavior. These genes act in the same pathway and are required for response and location of vulva (Lov) aspects of mating [1,2]. LOV-1 and PKD-2 are both found exclusively in the male chemosensory neurons (CEMs, rays, hook) where they localize to sensory cilia, suggesting a role in sensory signal transduction. *lov-1* and *pkd-2* are similar to the human polycystic kidney disease (PKD) genes PKD1 and PKD2, respectively. Mutations in either PKD1 or PKD2 account for 95% of all autosomal dominant PKD cases (ADPKD). ADPKD is an extremely prevalent genetic disease, occurring in roughly 1 of every 800 persons.

LOV-1, like polycystin-1 (encoded by PKD1), is a large and complex protein. LOV-1 is composed of 3178 amino acids and is predicted to contain a variety of functional domains. The extracellular amino terminus contains a mucin-like serine/threonine rich region, a number of glycosylation sites, and a G-protein coupled receptor proteolysis site (GPS) near the first putative transmembrane domain (TM1). Just after TM1 is a region of conserved homology with the lipoxygenase and alpha-toxin proteins called the PLAT/LH2 domain. Typically these domains regulate the interaction between the enzyme and plasma membrane associated proteins and lipids [3]. Following the LOV-1 PLAT/LH2 domain are 10 hydrophobic regions. The last six of these comprise a region of homology conserved in all polycystins. These six putative TM domains are related to those found in a variety of channel proteins. Polycystin-2 is comprised primarily of these 6 conserved TM domains and appears to be a calcium permeable channel [4,5]. A physical interaction between the carboxy termini of polycystin-1 and polycystin-2 is required to produce a functional channel [6]. The current model for polycystin function is that polycystin-1 is an extracellular sensor that mediates calcium flux through the polycystin-2

channel[7,8]. Likewise, we speculate that LOV-1 acts as a receptor that regulates PKD-2.

Our main objective is to use *in vivo* experimental approaches to test if these structural and functional predictions for the polycystins are true. My first goal is to determine the overall membrane topology of LOV-1. That is, I will determine where the amino and carboxyl tail of LOV-1 is found relative to the plasma membrane. I will also determine which of the 11 hydrophobic regions found in the LOV-1 sequence are functional transmembrane spanning domains. My second goal is to determine whether the LOV-1 protein is cleaved at the GPS and to test whether the PLAT/LH2 domain is required for LOV-1 membrane localizaton and protein function. Ultimately, I will characterize calcium channel activity *in vivo*. By using *C. elegans* as a model system for ADPKD, we may begin to understand the physiological function(s) of the polycystins.

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656. Control of the backing and turning steps of male mating behavior of *C. elegans*.

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Behavior in its simplest form consists of an organism sensing the environment and executing a motor output based on those signals. The next level is coordination of distinct behaviors, that is, processing a number of signals from the environment and triggering the appropriate response. *C. elegans* male mating behavior provides an excellent model for studying how behavior is coordinated and also for furthering our understanding of sensory perception. Mating behavior in *C. elegans* consists of a series of sub-behaviors with the initiation of one step correlating with the repression of the previous step. Understanding coordination of these behaviors will require furthering our knowledge of the molecular and neural pathways through which the different sub-steps are controlled. We are currently focusing on two of the earliest steps in mating behavior, backing and turning. Efforts are being made to address how these steps are triggered and executed and how one step is repressed when the next step is initiated. Previous research has provided information about the sensory, inter and motorneurons as well as the neurotransmitters required for the execution of these steps (1,2,3). We are currently doing a genetic screen to isolate new mutations that affect male mating behavior. The design of the screen will also allow us to identify mutations in the hermaphrodite which disrupt mating behavior enabling us to determine the hermaphrodite signals which trigger male responses. Further characterization of previously isolated mutations affecting backing and turning will also be described.

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657. Caenorhabditis elegans hermaphrodites shift mating behavior at self-sperm exhaustion

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The Nematode Caenorhabditis elegans is a major model organism, however many aspects of its reproductive biology are poorly understood. It is often asserted that it reproduces primarily thorough selfing and that outcrossing only plays only a small role in its life history. Here we present evidence suggesting that the reproductive biology of this nematode is more complex, and that outcrossing may play a large role in the second half of a hermaphrodite's life. The courtship behavior of C. elegans hermaphrodites is shown to shift in a way that increases their likelihood of outcrossing when a hermaphrodite runs out of self-sperm and becomes limited by access to sperm donated by rare males.

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658. NOVEL ALLELES OF *SUP-9*, WHICH ENCODE A PUTATIVE K+ CHANNEL, REVEAL DEFECTS IN MALE MATING

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Animals carrying the mutation *unc-93(e1500)* are defective in egg-laying, motility, and spicule retraction and display the 'rubber band' phenotype, in which a touch to the head elicits contraction of the body with no backward movement. All of these abnormalities are suppressed by loss-of-function alleles of *sup-9*. *unc-93* and *sup-9* encode transmembrane proteins that, along with the products of *sup-10*, *sup-11*, and *sup-18*, regulate muscle contraction. *sup-9* encodes a presumptive potassium channel. Two novel ENU-induced alleles of *sup-9* were isolated as suppressors of the motility defect of *unc-93(e1500)* mutants. Like *sup-9* null alleles, the novel alleles *sup-9(lr164)* and *sup-9(lr165)* are recessive suppressors of *unc-93(e1500)* homozygotes, restoring motility and egg-laying to wild-type levels. However, unlike previously characterized *sup-9* alleles, *sup-9(lr164)* and *sup-9(r165)* are dominant suppressors of partially uncoordinated *unc-93(e1500)* heterozygotes, suggesting that these alleles encode a SUP-9 protein with an altered or dominant-negative activity. Also, while homozygous null alleles of *sup-9* restore wild-type spicule function to *unc-93(e1500)* males, *sup-9(lr164)* and *sup-9(lr165)* fail to do so. In addition, these novel suppressor alleles complement *sup-9(n180)*, which we found to have a mutation in the first splice-acceptor sequence, but they fail to complement a nonsense allele, *sup-9(n1913)*. We are currently investigating the mechanism of *sup-9(n180)* complementation and are testing other *sup-9* alleles for similar complementation. Both *sup-9(lr164)* and *sup-9(lr165)* are T-to-G transversion mutations. *sup-9(lr165)* results in an L-to-R change in a putative cytoplasmic loop between the second and third membrane-spanning domains of SUP-9, while *sup-9(lr164)* results in an M-to-R change near the end of the third membrane-spanning domain.

Strains containing both *unc-93(e1500)* and either *sup-9(lr164)* or *sup-9(lr165)* exhibit a severe male-mating defect in spite of their normal motility. This defect is dependent on the presence of *unc-93(e1500)*; males containing only *sup-9(lr165)* mate as well as wild-type males while *sup-9(lr164)* males have slightly lower male mating efficiency. *unc-93(e1500)* males are also unable to mate, but the underlying cause of this defect has not been elucidated. Direct observation and use of the male leaving assay of Lipton and Emmons (1999, WBG 16(1):56) suggest that the male-mating defect of suppressed strains is primarily behavioral. In leaving assays, single males are placed in a small spot of food with five paralyzed hermaphrodites. The rate at which males leave a circle of 7 cm diameter is determined. Wild-type males tend to stay with the hermaphrodites, and to leave the circle when hermaphrodites are absent. We found that *sup-9(lr164 or lr165); unc-93(e1500)* males have a higher rate of leaving the circle in the presence of hermaphrodites than do wild-type males. Male behavior in the absence of hermaphrodites is unaffected. Mating experiments demonstrate that males containing *unc-93(e1500)* and certain other *sup-9* missense alleles also have a decreased ability to mate. Experiments are underway to determine whether the male-mating defects are a consequence of interactions between the *sup-9* lesions and *unc-93(e1500)* or of an incomplete suppression of a heretofore uncharacterized male behavioral defect conferred by *unc-93(e1500)*.

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659. Imaging of neuroal activity of touch neurons in C.elegans

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We are attempting to image neuronal activity using yellow cemeleon[1], a GFP-based fluorescent calcium indicator. Yellow cameleon consists of tandem fusions of CFP(cyan fluorescent protein), calmodulin, a flexible linker peptide, M13 (a calmodulin-binding peptide) and YFP(yellow fluorescent protein). Binding of Ca^{++} makes calmodulin wrap around the M13 domain, bringing CFP and YFP closer. The closer the two fluorescent proteins are, the more energy is transferred from excited CFP to YFP, resulting in the decrease of cyan emission and the increase of yellow emission. Thus, Ca^{++} increase can be detected as an increase in the YFP/CFP emission ratio. We have already succeeded in detecting the calcium influx accompanying pharyngeal muscle contractions using this system[2].

We have expressed yellow cameleon (YC2.1) in touch neurons using the *mec-7* promoter. We chose touch neurons because 1) touch stimulation is instantaneous, reversible and reproducible 2) the behavioral response is readily detectable 3) a large volume of detailed studies have been accumulated on the neuronal circuit and the touch response. Transgenic animals expressing YC2.1 under the control of the mec-7 promoter showed strong fluoresence in the ALM, AVM, PLM, PVM neurons, and their touch response was indistinguishable from wild animals. Intact animals from these lines were glued to hydrated agarose pads, and an automatic mechanical stimulator was used to deliver a timed, relatively uniform touch stimulus to the animal. Optical traces of animals treated in this manner revealed YFP/CFP ratio changes that were temporally correlated with the sensory stimulus. At least some of these ratio changes were clearly accompanied by an increase in YFP emission and a reciprocal decrease in CFP emission, indicating increased energy transfer between the fluorophores; however, in other cases sample motion made the individual wavelength traces difficult to interpret. To improve the signal-to-noise ration in our recordings, we are engineering new cameleon derivatives with added introns and different codon usage for worm-optimized expression. The ability of these molecules to function as Ca^{++}-indicators has been confirmed in pharyngeal muscle; we are currently testing whether these new cameleons will improve the sensitivity and reproducibility of signals in our neuronal recordings.

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660. MEC-6, a protein needed for degenerin channel activity, is expressed at the cell surface

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The response to gentle touch in *Caenorhabditis elegans* requires the activity of at least twelve touch function genes. Among them, *mec-4* and *mec-10* are likely to encode subunits of a mechanosensitive channel; they belong to the DEG/ENaC ion-channel superfamily, and dominant mutations in them cause degeneration of the touch cells. Since *mec-4* and *mec-10*-induced degenerations are completely suppressed by mutations in *mec-6,* wild type MEC-6 protein is essential for this degenerin channel activity. Also, mutations in *mec-6* suppress the degenerations caused by *deg-1, unc-8* and partially suppress the hypercontraced phenotype of *unc-105* dominant mutation. Thus, *mec-6* is more generally required for degenerin channel function.

We have cloned *mec-6* gene and had earlier reported that it encodes a predicted protein of 377 amino acids with limited sequence similarity to the mammalian enzyme paraoxonase. *mec-6* is widely expressed in many cell types including the touch receptor neurons consistent with its requirement for functioning of different degenerins. MEC-6 is a type II transmembrane protein with a single N-terminal transmembrane domain and a large extracellular C-terminal tail. MEC-6 is glycosylated when synthesized *in vitro* in presence of microsomes.

We have now confirmed the predicted topology and subcellular localization of MEC-6 by expressing it in cultured cells. MEC-6 was tagged with HA epitope at the C-terminal end and was transiently transfected into CHO cells. Immunostaining of the non-permeablized cells with anti-HA antibodies revealed surface expression of MEC-6, which appears as discrete dots that are reminiscent of lipid rafts/caveolae. Punctate pattern of expression was also seen in *C. elegans* when the rescuing *mec-6::gfp* or the full-length *mec-4::gfp* constructs were injected. In case of *mec-6::gfp,* expression in neuronal processes is weak and hence only a few dots could be discerned, but the punctate expression was very clear in body wall muscle cells. Using the two color variants of GFP, we have shown that MEC-6 and MEC-4 are colocalized; coinjection of ectopically expressing *mec-4::cfp* fusion construct under the control of *myo-3* promoter along with *mec-6::yfp* resulted in overlapping punctate expression in body wall muscle. Furthermore, coinjection of full-length *mec-4::yfp* with P_{mec-4} *cfp* into wild type worms resulted in uniform expression of CFP and punctate expression of YFP along the entire length of all the touch cell processes. However, when the two constructs were introduced into *mec-6* mutants, the punctate expression of YFP was totally abolished whereas the CFP expression from *mec-4* promoter was unaffected. Thus, MEC-6 affects the stability and/or localization of MEC-4 protein, but not the transcription of the *mec-4* gene. Since *u3* allele of *mec-6* also partially suppresses the hypercontracted phenotype of dominant mutation in *unc-105* (a muscle-specific degenerin), we are testing to see if MEC-6 colocalizes with UNC-105.

We have expressed epitope tagged MEC-4 (with FLAG), MEC-6 (with HA) and MEC-10 (with MYC) in CHO cells to study the interactions among these proteins. Immunoprecipitation of MEC-6 pulls down both MEC-4 and MEC-10. Similarly, immunoprecipitation of MEC-10 coprecipitates both MEC-4 and MEC-6 thereby demonstrating that all the three proteins physically interact with one another. In addition, MEC-4 and MEC-10 form amiloride-sensitive Na^+ channels in *Xenopus* oocytes only when coexpressed with either MEC-6 or MEC-2, a stomatin-like protein (see the abstract by Goodman et al.). These results taken together suggest that MEC-6 either function as a channel subunit and/or is required for clustering of the channel complexes into discrete microdomains of the membrane.

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661. Making the Matrix: MEC-1, a Multifunctional Extracellular Protein

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In electron micrographs, the *C. elegans* touch receptor neurons have two distinguishing characteristics: a bundle of 15-protofilament microtubules and extensive extracellular matrix. In the current molecular model for touch sensitivity both of these feature are thought to provide tethering points for the mechanosensory channel. *mec-1* is needed for the elaboration of the extracellular matrix. The *mec-1* gene was first identified by mutations that cause touch insensitivity. In the initial characterization of *mec-1* mutants (Chalfie and Sulston, 1981), loss of *mec-1* was found to affect the touch cells in two ways: it caused the loss of most of the extracellular matrix and it prevented the attachment of touch cell processes near the cuticle and the engulfment of lateral touch cell processes by the surrounding hypodermis that normally occurs during development. These observations suggested that the attachment of the touch cells was important for their function. Since *him-4* mutations prevent the attachment and engulfment without affecting touch sensitivity (Vogel and Hedgecock, 2001), attachment, per se, is not essential for touch sensitivity. We have now cloned *mec-1* and find that the touch sensitivity and attachment functions map to different portions of the molecule.

mec-1 encodes a 1999 amino acid polypeptide with an N-terminal signal sequence followed by a Kunitz-type domain, two EGF domains, 14 additional Kunitz-type domains, and a C-terminus of 160 amino acids. Nine of the Kunitz domains form a large cluster just prior to the C-terminus. The Kunitz and EGF domains are likely to be protein interaction domains. The only other protein with both of these domains is MEC-9, another extracellular protein needed for touch sensitivity, although a substantial class of *C. elegans* proteins have Kunitz domains interspersed with other extracellular matrix motifs. A *mec-1::gfp* fusion is expressed in the touch cells, several other lateral neurons, and the intestinal muscles.

We have characterized eleven *mec-1* alleles: eight are nonsense mutations or mutations at splice junctions that would lead to premature termination, two are missense mutations affecting cysteines in the last Kunitz domain, and one is a Tc1 insertion 100 or so amino acids from the C terminus (and after the EGF and Kunitz domains). The last three mutations and two of the nonsense mutations causing termination in the next to last Kunitz domain result in touch insensitive animals whose touch cells are normally engulfed. By looking at the phenotype caused by other nonsense mutations in a *smg-5* background, which presumably allows for normal production of truncated proteins, we have found two additional nonsense mutations that permit normal engulfment of the touch cell processes. These mutations suggest that engulfment requires the N-terminal region through the sixth Kunitz domains, but not the large C-terminal cluster of Kunitz domains. Thus, the MEC-1 protein has a C-terminal region that is essential for mechanosensory function but not for attachment and engulfment of the touch cell processes.

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662. Functional analysis of the complete DEG/ENaC family of ion channel proteins of *C. elegans*

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mec-4 and *mec-10* encode ion channel subunits of the DEG/ENaC family of proteins. Importantly, MEC-4 and MEC-10 are postulated to be the core subunits of the mechanosensory channel in the six touch receptor neurons. This is an exciting working hypothesis since channels specialized for mechanotransduction have eluded cloning efforts for years. Thus analysis of this channel family is of profound interest in a little understood area of signal transduction. Because the genes encoding these proteins can mutate to cause neurodegeneration the proteins were originally named degenerins.

The database compiled by the *C. elegans* Genome Sequencing Consortium includes a total of 23 additional degenerin-related genes. Our characterization of these genes has led the determination of the expression pattern of all previously uncharacterized degenerins. This analysis revealed that members of the degenerin family function in a variety of cell types ranging from neurons to muscles and epithelia. Some of these degenerin-like genes are expressed in nose touch neurons and could thus be candidates for the elusive mechanosensory channel in those cells. In support of this notion, dsRNA mediated interference with the expression of these degenerins largely decreases the response to nose touch. In addition, we have found degenerin-like genes to be expressed in body touch neurons and motorneurons of the ventral nerve cord where they could co-assemble with known degenerins to mediate the mechanosensory properties of these cells. Specific degenerins are expressed in and are needed for the normal function of the excretory canal cell, which, in the nematode, is the functional equivalent of the kidney. It is intriguing that in mammals, the degenerin-homologous ENaCs function in this organ to regulate electrolyte balance. We will present detailed expression patterns and functional characterization at the meeting.

Our observations indicate, that contrary to what might be expected for such a multi-gene family, members of the degenerin group, closely related in sequence, are not functionally redundant. In an effort to assign degenerin genes to known genetic loci, we are currently attempting to complement closely linked candidate mutations and we are also screening deletion libraries for null alleles.

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663. Elaborating the composition and structure of a touch-transducing complex: towards the determination of the structure of the MEC-4 N-terminal intracellular domain and characterization of 4 proteins that interact with this domain

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One of the looming mysteries in signal transduction today is the question of how mechanical signals, such as pressure or force delivered to a cell, are interpreted to direct biological responses. Elegant genetic and molecular studies by Chalfie and colleagues have identified several proteins thought to form a touch-transducing complex that mediates the response to body touch. At the core of this complex is a mechanically-gated ion channel made up of MEC-4 and MEC-10 subunits. This channel is homologous to the epithelial amiloride Na+ channel (ENaCs) and the acid sensing channels (ASICS)--the DEG/ENaC superfamily. The C. elegans channel subunits have been called degenerins because specific mutant forms of the channel can induce neurodegeneration.

Understanding how the MEC-4/MEC-10 channel functions in touch transduction is a major question in the field of mechanical signaling. These channel subunits are positioned in the plasma membrane with N and C terminal domains inside the cell and a large extracellular domain projecting into a specialized extrcellular matrix. It is thought that specific proteins interact with the intracellular and extracellular domains to exert gating tension on the channel. We are currently focusing on the N-terminal domain to gain insight into mechanisms of channel function.

The MEC-4 N-terminal domain includes an 87 amino acid stretch that is not highly conserved and a highly conserved domain close to the first transmembrane domain that has similarity to a half-site of thiol protease active sites. The conserved domain has been implicated in protein interactions and all known N-terminal mutations affect this subdomain. In order to gain greater insight into the nature of the MEC-4 amino terminal domain, we have created a homology model of this domain MEC-4{N}. Our model is based on sequence identity and similarity to the pro domains of proteases of the cathepsin protease family. Our homology model depicts MEC-4 {N} as a globular domain which is predominantly alpha helical in nature aside from an extended span of residues which comprises the hydrophobic core of the model. The model presents several interesting structural features, including small lipophilic surfaces (potential protein binding sites) and exposed tyrosines (potential phosphorylation site). Efforts are currently underway to solve the structure of MEC-4 {N}and MEC-10 {N} via NMR spectroscopy. We are testing highlighted residues for importance in channel function using site-directed mutagenesis.

To complement genetic studies that identified candidate proteins of the touch transducing complex, we performed a two-hybrid screen for MEC-4 N-interacting proteins. We used the N-terminal domain of MEC-4 as bait to screen a C. elegans yeast two-hybrid cDNA library. We obtained 81 positive clones from a screen of 3 million primary yeast clones. Among these, four candidate interacting proteins were identified that interact in both two-hybrid and GST pulldown experiments. All the candidate interacting proteins are expressed in the touch receptor neurons and can interact with the non-conserved N-terminal domain of MEC-4. Two interacting proteins are probably involved in channel turnover: 2 isolates encode a AAA ATPase homolog located in F23F1.8 (B70326) and 4 isolates are of a them are SINA (seven-in-absentia) homolog located in Y37E.11 (U89792). The AAA ATPase is most likely a subunit of the proteosome required for MEC-4 degradation. RNAi directed against both genes suggests an essential role very early in embryogenesis. Two other candidates identify novel proteins. We obtained 61 isolates corresponding to an ORF located in C15G7.4 (U08022), and 3 isolates corresponding to ORF Y11D7A.12 (A215876). RNAi against these

two proteins induces touch abnormalities and thus these are candidate proteins for regulation or function of the MEC-4 channel.

664. Developmental regulation of a novel outwardly rectifying mechanosensitive anion current, $I_{Cl,mec}$, in embryonic cells of *C. elegans*

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Anion channels perform important roles in a variety of fundamental physiological processes including whole animal salt and water balance, germ cell development, and control of muscle contraction. *C. elegans* offers unique experimental advantages for defining the molecular basis of anion channel function and regulation. To begin exploiting *C. elegans* as a model system for characterizing anion channel biology, we have carried out electrophysiological studies on oocytes, intact and dissociated early embryos, and various cultured differentiated cell types.

Undifferentiated, dissociated embryonic cells express a robust outwardly rectifying mechanosensitive anion current, $I_{Cl,mec}$, that is activated by membrane stretch and depolarization. The characteristics of $I_{Cl,mec}$ include an anion-to-cation selectivity of 4:1, an Eisenman type I anion selectivity and sensitivity to inhibitors of anion and mechanosensitive channels. $I_{Cl,mec}$ is detected in >80% of membrane patches. The unitary conductance of the $I_{Cl,mec}$ channel is 6 pS at ±100 mV. Macroscopic currents of 40-120 pA at +100 mV are typically observed in inside-out membrane patches formed using low resistance patch pipettes. Isolated membrane patches of early embryonic cells therefore contain 60-200 $I_{Cl,mec}$ channels.

$I_{Cl,mec}$ was not detected in unfertilized oocytes. To ascertain when $I_{Cl,mec}$ is activated, we isolated staged embryos from single worms. Robust channel activity was observed in all cells from intact 1-8 cell stage embryos and in dissociated embryonic cells with diameters ranging from 5 to 25 μm. These observations indicate that $I_{Cl,mec}$ is activated shortly after fertilization and that channel activity persists up

to at least the ~100 cell stage of development.

Cells of early embryos exhibit little or no obvious morphological differentiation. To determine whether $I_{Cl,mec}$ is expressed in differentiated cell types, we cultured dissociated embryonic cells on glass cover slips. Culturing freshly isolated blastomeres on cover slips coated with agents that promote cell adhesion stimulates differentiation within 24 h into the major cell types that comprise the newly hatched L1 larva. Cells cultured on uncoated cover slips do not adhere to the growth substrate and remain viable for many days, but do not appear to differentiate morphologically.

$I_{Cl,mec}$ was not detected in cells with well-defined neuronal morphology (n=4), muscle morphology (n=12) or in ASER neurons (n=10). Because $I_{Cl,mec}$ is regulated by membrane stretch, we also patch clamped cultured neurons expressing *mec-7*::GFP. *mec-7* encodes a β-tubulin expressed largely in mechanosensory neurons (Hamelin et al., *EMBO J*. 11:2885-2893, 1992). $I_{Cl,mec}$ was not detected in cell-attached patches on cultured *mec-7*::GFP-expressing neurons (n=15). We also failed to detect $I_{Cl,mec}$ in whole-cell current recordings from mechanosensory neurons under conditions designed to isolate anion channel currents. Robust $I_{Cl,mec}$ channel activity was, however, detected in morphologically undifferentiated embryonic cells maintained in culture for six days. These results indicate that $I_{Cl,mec}$ activity is dramatically reduced in differentiated cells. Reduction of channel activity is not due to maintenance of cells in culture, but instead appears to be due to attainment of a differentiated state.

The abundance of $I_{Cl,mec}$ expression in early embryos, its activation shortly after fertilization, and the significant reduction of channel activity in differentiated cell types suggest that $I_{Cl,mec}$ may play roles in early embryonic development. We are currently using reverse genetic strategies to identify the genes that encode the $I_{Cl,mec}$ channel and its associated regulatory machinery.

665. The ClC Chloride Channel CLH-5 Plays a Role in Mechanosensation

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Voltage-dependent chloride channels of the ClC family are important components in key cellular processes such as cell-membrane excitability, cell-volume regulation, transepithelial transport, and acidification of intracellular organelles. ClC channels function as dimers, although the precise membrane topology of the subunits has not been clearly defined. Specific members of this family have been found localized to either the cell membrane or intracellular vesicles. The importance of these channels is underscored in humans, where mutations in three ClC genes cause distinct diseases affecting either muscle or renal function. We are using a reverse genetic approach to study the functional properties of ClC channels in *C. elegans*. Here we present our results concerning one isoform, CLH-5.

In *C. elegans*, six genes encode ClC channels (*clh* genes). cDNAs for all isoforms have been isolated, and preliminary expression patterns have been defined using reporter gene fusions to green fluorescent protein (gfp) and *lacZ* [1, 2, 3, 4]. Our data indicate that CLH-5 is expressed in touch-sensitive neurons and that it contributes to their function.

To determine the expression pattern for *clh-5*, we used two GFP reporter constructs: *Pclh-5::gfp*, in which GFP expression is under the control of the *clh-5* promoter, and *clh-5D4::gfp*, in which GFP is fused in-frame to the 3' end of a partial *clh-5* genomic fragment that includes the promoter, the N-terminus and transmembrane segments D1 trough D4. In both cases, transgenic animals express GFP in the mechanosensory neurons that mediate response to light touch (AVM, ALML/R, PVM, PVML/R) and in tail interneurons. Transgenic animals expressing the truncated CLH-5 protein tagged with GFP were insensitive to light touch. We hypothesize that this phenotype results from a dominant negative interaction between CLH-5::D4::GFP and wild-type CLH-5.

Consistent with this model, we have obtained a Mec phenotype by expressing *clh-5* dsRNA under control of the *clh-5* promoter.

CLH-5 may be required in a general way for the function of mechanosensory neurons, for example by contributing to the membrane potential. Alternatively, CLH-5 may directly mediate touch sensation through a mechanism involving stretch-sensitive gating. Other members of this ion channel family are volume regulated (*C. elegans* CLH-3 [5] and human ClC-2 [6]), and, although the detailed activation mechanisms are unknown, mechanical forces may be important in regulating these ClC channels. In the future, we are interested in determining the important factors that regulate CLH-5 activity, as well as how CLH-5 may interact with known components of the mechanosensory pathway.

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666. Long-Term Habituation is Dependent of Both Glutamate Availability and AMPA Receptor Function in *C. elegans*.

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C. elegans is capable of showing long-term memory for habituation training of the tap withdrawal response. As in other organisms this training is best produced by distributed or spaced training and lasts at least 24 hours. The neural circuit underlying the tap withdrawal response has been described and there is increasing evidence that the sensory neurons use glutamate as their neurotransmitter (Rankin and Wicks, 2000).

Long-term habituation training for the current experiments consisted of groups of approximately 20 worms that received 4 blocks of 20 taps at a 60s interstimulus interval (ISI) with blocks separated by one-hour rest periods. Testing was conducted 24 hours after training with individual worms given one block of 10 taps also at a 60s ISI. Trained groups were compared to a control group that received a single tap on training day and the same individual testing on day two. The results showed that wild-type worms that received distributed training show significantly smaller responses compared to single-tap controls ($p<0.01$). This retention has been observed at 24, 48 and 72 hours following training.

eat-4(ky5) mutants also received distributed habituation training and although previous studies have shown that *eat-4*(ky5) mutants display enhanced short-term habituation the *eat-4*(ky5) mutant did not show long-term memory for the habituation training. In a follow-up study, *eat-4*(ky5) mutants were given the same distributed habituation training protocol but using a stronger stimulus (trains of 7 taps/sec) rather than the single tap. This resulted in trained *eat-4*(ky5) mutant worms showing decreased reversal magnitude on Day 2 compared to untrained *eat-4*(ky5) controls. This suggests that stronger stimulation likely results in increased glutamate release from the sensory neuron in these mutants.

The alpha-amino-3-hydroxy-5-methyl-4-isoxazole-proprionate (AMPA)-receptor subtype mutant *glr-1*(n2461) is capable of short-term habituation, however, when long-term habituation training was administered to the *glr-1*(n2461) mutant this resulted in no long-term retention of the training on Day 2. When *glr-1*(n2461) worms were given the stronger stimulation (trains of taps) they still did not show long-term habituation. Thus the AMPA receptor does appear to be necessary for long-term memory regardless of level of stimulation. To further elucidate the role of glutamate in long-term memory wild-type worms were placed on plates streaked with either 10 mM 6,7-dinitrquinoxaline-2,3-dione (DNQX; a competitive AMPA-receptor blocker) or vehicle (35 mM sodium hydroxide; NaOH) during training on Day 1. Day 2 testing was conducted drug free. The vehicle-administered worms displayed long-term retention while the worm exposed to DNQX did not. This finding, coupled with the long-term memory deficits observed in the glr-1 mutant strain, lead to the conclusion that activation of the glutamate receptor subtype AMPA is required for the formation of long-term retention.

The role of the N-methyl-D-aspartate (NMDA) glutamate receptor in long-term habituation training was also investigated. Wild-type worms were trained with the non-competitive NMDA antagonists (5R,10S)-(+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]-cyclohepten-5,10-imine hydrogen maleate (MK801). When worms were trained in the presence of MK801 there was no evidence for long-term memory of habituation training. This supports what has been theorized in mammalian models of long-term synaptic plasticity in that activation of AMPA-type receptors may be required for NMDA-type receptor activation and therefore when either receptor is blocked, it effectively attenuates long-term memory.

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667. GENETIC AND PHENOTYPIC ANALYSES OF HABITUATION ABNORMAL MUTANTS

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C. elegans shows habituation response when given repeated mild mechanical stimuli such as touch or tap. The behavior was originally noticed and extensively characterized by C. Rankin. [1] However, genes contributing to the behavior have not been considered. We undertook genetic analyses of the habituation behavior abnormal mutants that were isolated more than ten years ago. The habituation behaviors of these mutants were classified into three groups based on the response patterns: slow (*hab-1, hab-3*), rapid(*hab-2, hab-5*) and normal but incomplete(*hab-4*) habituations. These mutants are also different in patterns of recovery from the habituation . We focused here on analyses of *hab-1*.

The *hab-1* mutant is normal in chemotaxis and adaptation response to volatile chemicals tested. The mutants normally respond to the single tap stimulus. However, the mutant is slowly habituated and rapidly recovered from the habituated state. To identify neurons in which the *hab-1* gene functions, we ablated neurons constituting neural circuit for the mechanical response with a laser microbeam and tested habituation response. From these results we conclude that the gene *hab-1* does not function at the specific neuron. The gene was positioned on the linkage group I. From the three- and two-factor crosses, the gene is located to the right of *aex-6* and left of *lev-10*. By more detailed three-factor crosses with SNP markers the *hab-1* gene is located between 22.3 and 22.7 map units*. We are now sequencing the chromosomal region to identify the mutation site of *hab-1* (*cn308*).

* Informations on SNPs between N2 and CB4856 were provided by S.R.Wicks (Dept, Mol, Biol, Netherland Cancer Institude) and Genome Sequence Center (Washington University).

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668. IDENTIFICATION OF DIFFERENTIALLY EXPRESSED GENES ACCOMPANIED BY HABITUATION

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C.elegans locomotes backward when given mild mechanical stimuli such as tap or head touch. When the stimulus was repeated, the animal enters into a habituated state. For the understanding the habituation mechanism, it is useful to identify genes responsible for the stimulation. In order to identify genes that are changed during the tap stimulation, We adopted the cDNA microarrays method established by Y. KOHARA. The cDNA microarray allow to monitor simultaneously expression of *C.elegans* ten thousand genes.

We isolated mRNAs from the L1 larvae with or without training of 180 times 30-s interstimulus interval tap stimuli. We compared the hybridization density with the mRNAs as probe and found 105 genes that are up-regulated at two to six times higher levels and 143 genes that are down-regulated below one-half during the tap stimulation. Of the up-regulated we noticed ten genes which encoding protein kinase (two genes), Ca-binding protein (one gene), transcription factor (ten genes) and functionally unknown protein (two genes). Of the down-regulated genes, we noticed two genes encoding transcription factor and protein tyrosine phosphatase respectively. Based on sequence information of each yk clone, we amplified the cDNAs by designing oligonucleotide primers. We are currently determining by *in situ* hybridization which neuron expresses each gene.

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669. GENES SHOWING ALTERED EXPRESSION ACCOMPANIED BY HABITUATION

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Habituation to mechanical tap stimuli in *C.elegans* belongs to short term memory. For understanding the habituation, it is useful to identify the neural circuit and genes responsible for the stimulation. Wicks et al., determined the neural circuit for tap-mediated stimulation consisting of mechanosensory neurons, interneurons, and motorneurons [(1)]. However, the exact neuron contributing to habituation was not identified. We therefore addressed this issue by systematically ablating each neuron constituting the network for the mechanical stimuli. We found that the AVDL and AVDR interneurons play a critical role in the habituation in intact animal. And the AVM and ALMLR mechanosensry neurons are important for habituation of the AVDLR ablated animals [(2)]. For further investigation of the habituation mechanism, it is essential to identify genes contributing to the behavior. In order to identify genes that are differentially expressed as a consequence of repeated tap stimulations, we used the differential display technique to compare mRNA expression patterns. DNA bands of interest, those with signals showing the difference in intensity in control lanes relative to the test lanes, were eluted from the gel, re-amplified, ligated into a plasmid vector and their double stranded sequences were determined. Twenty up-regulated and 30 down-regulated clones were identified. Each DNA band on the DD gel plates was isolated, cloned and its DNA sequence was determined. We found that they are functionally classified into four groups : metabolism, neural function,signal transduction and transcription. Of these following four clones were especially interested. Of up regulated genes, two clones encoding MAPKKK, and GTP-binding protein, and of down regulated two clones encoding transcription factor, and ionotropic grutamate receptor GLR-6 were noticed. As a current work we are studying changes in the expression pattern during the tap stimulation. And further we are investigating cells expressing these genes by the *in situ* hybridization.

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670. Mechanosensory Signaling from the ASH Sensory Neurons: The Role of the GLR-2 Ionotropic Glutamate Receptor Subunit

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The *C. elegans* polymodal ASH sensory neurons transmit modality-specific sensory information and are required for a backing avoidance response to both tactile inputs to the nose and osmotic signals. It has been shown that the GLR-1 glutamate receptor subunit is required for the nose touch response, but not for the avoidance of high osmolarity (Maricq et al., 1995; Hart et al., 1995). This raised an interesting question: how are two sensory inputs detected by a single pair of sensory neurons independently regulated? To investigate the mechanism of ASH signaling that mediates these responses, we have undertaken a genetic and electrophysiological analysis of the GLR-1 and GLR-2 glutamate receptor subunits.

GLR-1 and GLR-2 are co-expressed in many neurons, including four of the five pairs of command interneurons of the locomotory control circuit (Brockie et al., 2001). These interneurons receive synaptic inputs from ASH and are required for both the co-ordination and modulation of directed movement. By generating a null mutation in the *glr-2* gene, we have determined that *glr-2*, like *glr-1*, is required for the nose touch response, but not for osmotic avoidance. Interestingly, the nose touch defect of *glr-2* mutants is not as severe as that observed in *glr-1* mutants. Together, these data suggest that GLR-1 and GLR-2 co-assemble to form a heteromeric glutamate receptor.

To further investigate the properties of GLR-1 and GLR-2, an electrophysiological approach was used to characterize glutamate-gated currents in the AVA command interneurons of the locomotory control circuit (See abstract by Mellem et al.). This analysis has shown that glutamate-gated currents are dependent on both GLR-1 and GLR-2, further supporting the hypothesis that the subunits co-assemble. To this end, we have begun to characterize the sub-cellular distribution of GLR-1 and GLR-2. By generating functional GLR-1::CFP and GLR-2::YFP full-length fusions, we hope to identify co-localization of the subunits at postsynaptic sites. This analysis will further our understanding of glutamate signaling from the ASH sensory neurons that mediates the nose touch response.

Brockie et al., 2001. J. Neurosci 21:1510-1522

Hart et al., 1995. Nature 378:82-85

Maricq et al., 1995. Nature 378:78-81

671. EVIDENCE FOR A *CHA-1*-SPECIFIC PROMOTER IN *C. ELEGANS*

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The *cha-1* gene, encoding choline acetyltransferase (ChAT), is required for acetylcholine biosynthesis. Along with the *unc-17* gene, which encodes the vesicular acetylcholine transporter (VAChT), *cha-1* is known to be expressed from a shared promoter as a member of the cholinergic "operon". The overall structure of this operon is conserved in insects and mammals. We now have evidence for a *cha-1*-specific promoter in *C. elegans*. A putative, alternative 5' exon was identified between exons 2 and 3 of *cha-1* by phylogenetic scanning against *C. remanei* and *C. briggsae*. It shares 69% identity and 92% similarity at the amino acid sequence level with both species. The structure of this putative exon (referred to as 2.5) is reminiscent of a 5' exon: it begins with an ATG, it lacks an upstream splice acceptor site, but it contains a consensus splice donor site at its 3' boundary. Furthermore, comparison of the three species revealed two conserved, apparently regulatory regions of 22 bp and 37 bp located approximately 300 bp upstream of the apparent start codon. To test whether exon 2.5 was expressed from a *cha-1*-specific promoter, a 521 bp fragment from the middle of exon 2 to the middle of exon 3 was cloned into the GFP expression vector, pPD95.67, creating a translational fusion. Neuronal expression of this construct was observed in transgenic *C. elegans*, supporting the existence of a *cha-1*-specific promoter. Interestingly, expression appears to be targeted to the secretory pathway, suggesting that exon 2.5 encodes an ER signal sequence.

672. A *mod-5* SUPPRESSION SCREEN FOR GENES INVOLVED IN SEROTONERGIC NEUROTRANSMISSION

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Wild-type animals that have been food deprived slow their locomotory rate in response to bacteria (the enhanced slowing response). Food-deprived *mod-5(n3314)* mutants encountering bacteria slow even more than food-deprived wild-type animals, exhibiting a hyperenhanced slowing response. *mod-5* mutants were originally identified as defective in serotonin (5-HT) reuptake, and the *mod-5* gene has recently been shown to encode a 5-HT reuptake transporter. To identify additional genes involved in serotonergic neurotransmission, we designed a screen for suppressors of the *mod-5(n3314)* mutation. The screen took advantage of a second characteristic of *mod-5(n3314)* mutants: hypersensitivity to exogenous 5-HT. When placed in M9 containing 5-HT, *mod-5(n3314)* mutants stop swimming sooner than wild-type animals. Suppressors of *mod-5(n3314)* can be identified as animals that continue to swim after *mod-5(n3314)* mutants would have stopped.

We screened 18,350 haploid genomes and obtained 61 independent *mod-5* suppressors. Eighteen of these isolates were found also to suppress the hyperenhanced slowing response exhibited by *mod-5(n3314)* mutants. Interestingly, the strength of suppression of the hyperenhanced slowing response did not strictly correlate with the strength of suppression of hypersensitivity to exogenous 5-HT. This observation may indicate that the exogenous 5-HT and locomotion assays assess different pathways that involve 5-HT neurotransmission. To date, six suppressors have been mapped to linkage groups: one to LG I, three to LG II, one to LG V, and one to LG X. Further mapping experiments are underway to determine the identities of these genes.

Our suppressors may define genes that act downstream of the synapses at which *mod-5* acts, *i.e.,* genes involved in transducing the signal in postsynaptic neurons or muscle cells responsible for slowing the locomotory rate of the animal. We also expect to find genes acting upstream of these synapses, because a mutation in the gene *cat-4,* which is involved in 5-HT biosynthesis, suppresses the exogenous 5-HT hypersensitivity of *mod-5(n3314)* mutants.

673. Measuring neurotransmitter levels in isolated *C.elegans* vesicles by HPLC.

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We are in the process of developing an assay to measure transmitter content in synaptic vesicles from *C.elegans* in order to study the function of various vesicular neurotransmitter transporters. We circumvented the problem of obtaining large amounts of tissue sample by measuring transmitter levels using HPLC. Starting with about half a gram of worms, we can by differential centrifugation isolate a crude vesicle fraction. We can demonstrate enrichment of synaptic vesicles in this fraction by the detection of full length *unc-47*::GFP (the GABA vesicular transporter) using Western blot analysis. The presence of *unc-47*::GFP in this fraction suggests it contains synaptic vesicles because this construct is localized to synaptic vesicles and can rescue the *unc-47* mutant phenotype (McIntire *et al.* (1997), Nature 389: p 870-879). We measured amino acid neurotransmitter levels in these fractions by HPLC which is sensitive enough to allow small concentrations (ng/ml) to be reliably quantified. Although we used this procedure to primarily examine the neurotransmitter GABA, this method could also be used to measure quantities of other neurotransmitters such as dopamine, serotonin, octopamine and acetylcholine. In vesicle preparations from N2 animals we demonstrated that GABA is contained in this fraction. As a control for the purity of the synaptic vesicle preparation, we measured GABA levels in *unc-47* mutants which should contain no GABA in their vesicles and observed a 3-4 fold reduction compared to N2 animals. We also examined GABA levels in synaptic vesicles of *unc-46* mutant animals. *unc-46* encodes a novel protein and *unc-46* mutants have a similar phenotype to *unc-47* animals (K. Schuske and E. Jorgensen, personal communication). Since overexpression of UNC-47 is capable of rescuing the *unc-46* mutant phenotype, UNC-46 may function together with UNC-47 to regulate vesicular transport of GABA (K. Schuske and E. Jorgensen, personal communication). We found GABA levels were also 3-4 fold lower in

these mutants further supporting UNC-46's role in regulating transport of GABA into synaptic vesicles. This technique can also be used to quantitate neurotransmitter levels in whole worm extracts. For example, *unc-25* animals, which are missing the enzyme glutamic acid decarboxylase, have significantly decreased GABA levels in their extracts compared to N2 animals. We currently are focusing our attention on obtaining a purer vesicle preparation to reduce the levels of GABA we see in the *unc-47* mutants. Once established, this technique can be used to characterize and identify genes involved in the function and regulation of vesicular transporters in *C.elegans*.

674. Analysis of Fluoxetine Resistant Mutants of *C. elegans*

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Major depressive disorder is a significant public health problem, affecting millions of individuals worldwide. Only since the late 1950s have chemotherapeutic agents been available for treatment. One class of these drugs, serotonin specific reuptake inhibitors (SSRIs), has been particularly effective. Fluoxetine (Prozac) is a representative SSRI that has been widely prescribed. However, in spite of its rational design, the clinically relevant mode of action and basis of side effects of this drug remain unclear.

We have documented a fluoxetine-induced behavior in *C. elegans*, nose contraction, that appears to be independent of 5-HT (nose resistant to fluoxetine, *nrf*) (Choy & Thomas 1999). *nrf* genes fall broadly into two classes based on the abundance of yolk in eggs. *nrf-4*, *nrf-5*, *nrf-6*, and *ndg-4* share a Peg (pale egg) phenotype which the other *nrf* genes do not. NRF-6 and NDG-4 are related multipass membrane proteins; their expression in the intestine is needed for fluoxetine-induced nose contraction and is consistent with a model of hydrophobic molecule transport. Additionally, mRNA for a human NRF-6-related protein is expressed in the prostate, a highly secretory organ. This finding could not have been predicted based on prior understanding of SSRIs.

Additional information can be gained by analysis of other classes of *nrf* mutants. None of the *nrf* mutants isolated in our screen are fully resistant to fluoxetine; higher concentrations of drug and longer incubation times eventually lead to nose contraction. Strong enhancement of the Nrf phenotype is seen only in double mutants between Peg and non-Peg genes, which indicates the presence of two or more separately functioning pathways of fluoxetine response in *C. elegans*. Through mapping and microinjection we have accomplished single cosmid transgenic rescue of two non-Peg mutants, *nrf-2* and *nrf-3*. Also, three new non-Peg *nrf* alleles including at least one new gene, *nrf-7*, have been isolated. The molecular

identities of these genes may reveal previously unknown mechanisms of fluoxetine action in *C. elegans* and humans.

675. Calcium imaging of vulval muscle activity in egg-laying behavior

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Serotonin (5HT) is a neurotransmitter which often functions as a modulator of tissue excitability and behavioral states. In *C. elegans*, one behavioral effect it produces is the stimulation of egg-laying.[1] One mechanism by which it appears to do this is by shifting the worm's vulval muscles from a quiescent or inactive mode to a more active one during which eggs are laid in a cluster. Through a combination of genetic and pharmacologic approaches, we have identified mutants in several genes to be 5HT-resistant for this behavior: *egl-19* (L-type voltage gated Ca^{2+} channel alpha-1 subunit), *tpa-1* (PKC homolog), *acy-1* (adenylate cyclase), & *gpa-14* (novel G-protein).[2,3] While implicated in 5HT-signalling, these molecules may also constitute effectors from other neurotransmitter systems known to interact with 5HT in egg-laying, including neuropeptide signalling: Neuropeptides derived from the *flp-1* gene, for example, are known to potentiate 5HT-response.[4,5]

To gain more insight into these issues, we have been utilizing Cameleon, a genetically encodable and ratiometric Ca^{2+}-sensor[6,7] in both intact & cut-worm preparations to study how vulval muscle physiology changes in the presence or absence of 5HT & and how it responds to agents like forskolin or FLP peptides in N2s and in various mutant backgrounds. Preliminary results indicate that, in the absence of 5HT, vulval muscles in N2 worms typically show sporadic but often clustered Ca^{2+} transients. Upon application of exogenous 5HT (5 mg/ml), this pattern gives way to a more rhythmic train of small transient events (~0.5 Hz).

This data, along with initial mutant characterization, will be presented at the June meeting.

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[2] Waggoner LE *et al*, *Neuron* **21**:203-214

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[4] Schinkmann K & Li C, *J Comp Neurol* **316**:251-260

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[7] Kerr R *et al*, *Neuron* **26**:583-594

676. Suppressors that recover daf-7::gfp expression in tph-1(mg280)

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tph-1 encodes a tryptophan hydroxylase, the key enzyme for serotonin biosynthesis. Our previous studies show that daf-7::gfp expression is reduced 6-fold in the deletion mutant tph-1(mg280). This suggests that serotonin may normally up-regulate daf-7 expression. To isolate genes that upregulate daf-7 expression in response to serotonin signaling, we have been screening mutagenized tph-1(mg280) worms for suppressors that recover daf-7::gfp expression.

We have clonally screened 3,870 haploid genomes and isolated 41 suppressors, 15 of them are being back-crossed. DiI staining of back-crossed suppressors showed a normal dye filling pattern, suggesting that the mutations do not grossly compromise the integrity of the chemosensory neurons. Further examination of the back-crossed suppressors has led us to classify the suppressors into two classes. The first class of the suppressors shows a strong daf-7::gfp expression only in L2 and L3 tph-1(mg280). The second class of the suppressors shows a strong daf-7::gfp expression even in the adults. The results of genetic and molecular characterization of the suppressors will be discussed.

677. Characterization of Several G protein-coupled Receptors for the Secretin Family of Neuropeptides

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The secretin family of neuropeptides includes several physiologically essential molecules: secretin, calcitonin, corticotrophin releasing factor, and glucagon. In humans, defects in receptors of the secretin family cause abnormal function in the liver and pancreas, leading to several gastrointestinal diseases. To better understand the molecular biology of these receptors, we have identified three novel genes in C. elegans that are possible orthologues to the human secretin family receptors using BLAST and ESTs: ZK643.3, C18B12.2, and C13B9.4. These genes exhibit a high level of conservation with mammalian and fly secretin family receptors. Amino acid alignments suggest ZK643.3 is homologous to human corticotrophin releasing factor receptor, C13B9.4 is homologous to human calcitonin receptor, and C18B12.2 is homologous to human secretin receptor (see poster by Mastwal and Hedgecock).

Previous work has shown that ZK643.3 is expressed in the muscle cells of the head and in muscle cells that control opening and closing of the vulva (Coates, 1995). To further characterize this gene, we generated a ZK643.3 promoter::GFP fusion construct. We observed expression in head muscle cells and pharyngeal and ventral nerve cord neurons. RNAi and co-immunoprecipitation of ZK643.3, as well as C13B9.4 and C18B12.2 will provide further insight to the function of this secretin receptor family and identify interacting proteins.

678. A screen for new Hic mutants

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ABSTRACT

International Worm Meeting 2001

A screen for new Hic mutants

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Previous studies by our lab and others have shown that release of acetylcholine at the *C. elegans* neuromuscular junction (NMJ) is regulated by a G-protein signaling network. These studies have shown that serotoninergic signaling through *goa-1* $G\alpha_o$ and *dgk-1* DAG kinase inhibits acetylcholine release. An opposing muscarinic signaling pathway through *egl-30* $G\alpha_q$ *and egl-8 PLC*β enhances acetylcholine release via the production of diacylglycerol (DAG). Two RGS proteins, *egl-10* RGS and *eat-16* RGS, further regulate this pathway by stimulating the GTPase activity of *goa-1* $G\alpha_o$ (*eat-16* RGS) and *egl-30* $G\alpha_q$ (*egl-10* RGS). Mutations in the inhibitory pathway (*goa-1, dgk-1*) make animals hypersensitive to paralysis by the acetylcholine esterase inhibitor, aldicarb (Hic) Mutations in the muscarinic pathway (*egl-30, egl-8*) make animals resistant to aldicarb (Ric).

In order to identify additional molecules which negatively regulate release, we are conducting a broad screen for Hic mutants. Aldicarb sensitivity could be a result of either mutations that effect *presynaptic*neurotransmitter release or *postsynaptic* response to neurotransmitter in the muscle. We are distinguishing these effects using levamisole, a cholinergic agonist that acts directly on the acetylcholine receptors in the muscle. We are focusing on mutants that display a wild-type muscle sensitivity and are thus acting presynaptically. Mutants isolated in our pilots screens will be described.

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679. Specificity of G-protein signaling in taste perception by the nematode *Caenorhabditis elegans*

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We use *C.elegans* to study heterotrimeric G-protein mediated signal transduction. Only seven pairs of chemosensory cells are involved in the detection of tastants (reviewed by Bargmann and Mori 1997, In C.elegans II, CSHP). Five pairs of cells were found to be important for attraction, ADF, ASE ASG, ASI and ASK and two candidate pairs for aversion, ADL and ASH. Despite this limited number of chemosensory cells, the nematode is able to detect and distinguish different tastants. This is most likely accomplished by the expression of multiple chemosensory receptors per cell. Furthermore the observation that these cells express multiple different G protein alpha subunits implies that multiple signal transduction pathways for perception per cell exist. This is in fact the case in olfaction, where one G alpha subunit forms the main stimulatory pathway, which can be modulated by 1 to 3 different stimulatory or inhibitory alpha subunits.

In this project we will determine which G alpha subunits are involved in taste, and whether these signals are modulated. We use a soluble compound chemotaxis assay to determine the effect of loss-or gain-of-function of the various alpha subunits (Wicks et al 2000, Dev Biol 221, 295-307). In this assay nematodes can choose between four quadrants filled with buffered agar. Two opposite quadrants contain the soluble compound and the two other quadrants do not. We are currently searching for suitable compounds, of the taste modalities, salt, sweet and bitter. NaCl was clearly recognized by *C. elegans*. It is thought that NaCl perception relies on ion channels and that G proteins are not involved. However the knock out mutant of *gpa-3* (a G alpha subunit) gives a weaker response to salt compared to the wild type, suggesting that G-protein signaling is involved. Another suitable tastant was the bitter compound denatonium benzoate. At first denatonium benzoate is a strong attractant, yet in time it becomes very repulsive to *C. elegans*. At present we have no explanation for this change in preference. However, this behavior gives us ample opportunities to study G protein signaling in chemo-attraction and chemo-aversion.

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680. Neurotransmitter transporter genes in *C. elegans*.

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With the wealth of genomic sequence data now available, it is possible to begin the analysis of biological phenomena on a genome-wide scale. A particularly intriguing observation is that many genes are members of gene families (sets of genes whose products have closely related structures and/or functions). One such gene family encodes the neurotransmitter transporters and structurally related proteins (the sodium: neurotransmitter symporter family (SNF)). These proteins are required for the efficient clearance of neurotransmitters (and other bioactive molecules) from synaptic clefts. Several of these transporters have well-established roles in behavior and/or neurological disorders. In addition, they are known targets for drugs of psychiatric importance, such as antidepressants, or drugs of abuse, such as cocaine and amphetamines. However, for many, the endogenous substrate and/or cellular functions are unknown. There are at least 18 members of the SNF family in humans and 14 each in *D. melanogaster* and *C. elegans*. Sequence comparisons suggest that humans, fruitflies, and nematodes share a core set of SNF proteins, which include the dopamine, serotonin, and GABA transporters. In addition, they each have unique set of SNF proteins that is distinct for each organism. In *C. elegans*, there are genes that appear to correspond directly to mammalian dopamine (*dat-1*; see abstract by Duerr *et al*.), serotonin (*mod-5*; Ranganathan *et al*., 2000) and GABA transporters, as well as a number of orphan transporters (MacGregor *et al*., 1997 IWM:377; James *et al*., 1999 IWM:436). We now have gene knockouts in five SNF genes (in addition to *mod-5* and *dat-1*) and have begun a systematic analysis of their behavioral phenotypes. Thus far, our observations indicate that transporter gene knockouts generally result in mild behavioral phenotypes. We speculate that the mild phenotypes associated with these mutations reflect functional overlaps between genes ("redundancy") or compensatory mechanisms for the degradation/recycling of neurotransmitters.

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681. A Genetic Interaction Between a Neurotransmitter Transporter and Synaptobrevin

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Motor function in *C. elegans* is controlled primarily by the excitatory neurotransmitter acetylcholine. Choline aceyltransferase (ChAT) encoded by the *cha-1* gene, synthesizes acetylcholine which is transported into synaptic vesicles by the vesicular acetylcholine transporter (vAChT) encoded by *unc-17* (Alfonso, A. et al. Science 261, 1993). Function of these genes is essential for synaptic transmission as null mutations in either gene is lethal. Mutants with residual *unc-17* gene function are uncoordinated, slow growing, small, and are resistant to cholinesterase inhibitors. Acetylcholine is only released into the synaptic cleft after vesicles packaged with the transmitter fuse with the pre-synaptic terminal. To study the role of vAChT in regulating neurotransmitter release *in C. elegans, unc-17* was overexpressed in animals. Transgenic animals exhibited hypersensitivity to the cholinesterase inhibitor aldicarb. This suggests acetylcholine release into the synaptic cleft was increased and that neurotransmission can be modulated by changes in the level of expression of transporter.

Suppressors of the locomotory defect of non null mutant *unc-17 (e245)* may elucidate transport function, isolate components that interact with vesicular transport and may participate in the regulation of transmitter release. The mutation in *unc-17 (e245)* converts a glycine to an arginine in transmembrane domain nine of vAChT (Alfonso, A. et al. Science 261, 1993). We have identified an intragenic suppressor of *unc-17 (e245), unc-17(mg301),* that changes this arginine to lysine. Extragenic expression *of unc-17(mg301)* rescues the locomotory defect of *unc-17 (e245).* This suggests that the arginine interferes with gene function but that glycine at that position is not essential even though this glycine is conserved in vertebrate acetylcholine and serotonin transporters. For example, the pH at this position in the membrane may be key.

sup-8 (e1563) suppresses the movement defects of *unc-17(e245)* but not *other unc-17* alleles. We found that *sup-8* corresponds to a dominant allele of synaptobrevin *(snb-1). sup-8(e1563)* is an isoleucine to aspartate substitution in the single transmembrane domain of synaptobrevin. *unc-17 (e245)* animals expressing the *snb-1 (e1563)* from a transgene in a snb-1(+) chromosomal background show wild-type movement and normal aldicarb sensitivity. Thus, *snb-1 (e1563)* is a dominant suppressor of *unc-17(e245),* restoring normal movement and sensitivity to aldicarb. *snb-1(1563)* animals exhibit no phenotype; for example they are not aldicarb sensitive, suggesting that this *snb-1* allele does not facilitate synaptic signaling. The location of the transporter and the synaptobrevin mutations in the transmembrane domains suggests a possible interaction between neurotransmitter packaging and vesicle fusion which we are trying to show by engineering mutations in these transmembrane segments, as well as biochemically.

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682. Deletion of the dopamine plasma membrane transporter and its effect on behavior

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Dopamine is a neurotransmitter in 8 cells in *C. elegans*, the sensory neurons ADEL/R, PDEL/R, CEPDL/R and CEPVL/R. Function of the dopaminergic neurons can be altered by mutations in *cat-2*, the gene for the dopamine synthetic enzyme tyrosine hydroxylase (Lints and Emmons, 1999). *cat-2* mutants show specific deficits in movement in viscous media; ablations of specific dopamine-containing neurons cause similar deficits (Sawin et al., 2000). Another protein that is important for the function of dopaminergic neurons is the vesicular monoamine transporter, VMAT. VMAT is responsible for loading synaptic vesicles with dopamine (or other monoamines) and is found in the dopamine-containing cells (as well as other aminergic neurons). Deletion of *cat-1*, the gene that encodes VMAT, leads to a variety of behavioral abnormalities, including defects in locomotion that are indicative of a loss of dopaminergic function (Duerr et al., 1999). Recently, we have begun to examine the function of another protein important for normal dopaminergic neurotransmission, the plasma membrane transporter for dopamine, DAT-1 (Jayanthi et al., 1998). In vertebrates, the dopamine transporter allows rapid re-uptake of released dopamine; interference with this transporter leads to increased concentrations of synaptic dopamine and abnormally prolonged post-synaptic responses. DAT-1 is a target of several psychoactive drugs (including cocaine and amphetamine) whose effects are due in part to overstimulation by aminergic neurotransmitters.

DAT-1 is expressed in the eight dopaminergic neurons in *C. elegans* (MacGregor et al. 1997; Nass et al., 1999, 2000). We have isolated a mutant with a deletion in DAT-1 that removes most of the transmembrane domains of the protein. Deletion of this transporter might have multiple effects on dopaminergic neurotransmission. As in vertebrates, deletion of DAT might cause prolonged postsynaptic stimulation by dopamine and enhanced dopamine-dependent behaviors. On the other hand, the reduced re-uptake of dopamine into the presynaptic neuron might decrease the local concentration of dopamine and might lead to less dopamine being loaded into pre-synaptic vesicles by VMAT. This might lead to less dopamine release per stimulus although, again, that dopamine would be expected to persist longer than normal. When we examined behavior in *dat-1* mutants we found no detectable differences in a number of behaviors. However, DAT-1 mutants do show subtle behavioral differences in locomotion that are consistent with an excess of dopamine neurotransmission in at least some dopaminergic synapses.

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683. A Dopamine Receptor in *C. elegans*

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A large body of evidence suggests that dopamine (DA) might function as a neurotransmitter in *C. elegans*. Neurons containing DA, the enzymes necessary for its synthesis and metabolism, as well as distinct transporters required for its uptake into neurons and synaptic vesicles have all been identified in *C. elegans*. Here we report the first DA receptor to be identified in *C. elegans*. The *C. elegans* DA receptor gene encodes two alternatively spliced forms of DA receptors. The longer form of this receptor ($CeDA1_{long}$) contains a 58 amino acid sequence in its third cytoplasmic loop that is spliced out in the shorter form ($CeDA1_{short}$).

Radioligand binding studies using membrane preparations of COS-7 cells transfected with the cDNA of this gene (long or short forms) show that DA binds to these receptors in a saturable and concentration-dependent manner. Using agonist-mediated GTP-gamma-[^{35}S] stimulation studies we found that DA, epinephrine (E) and norepinephrine (NE) can induce GTP-gamma-[^{35}S] binding to membranes of COS-7 cells transiently expressing either the long or short form of this receptor. DA was found to be about 10-fold more potent in the GTP-gamma-[^{35}S] binding assay as compared to E or NE. Other amines such as tyramine, histamine, octopamine and serotonin were found to be ineffective in this assay. HPLC analysis of worm extracts showed the presence of dopamine, its precursors and metabolites, however norepinephrine and epinephrine could not be detected at significant levels. These findings strongly suggest that dopamine is most likely the endogenous ligand for the $CeDA1_{long}$ and $CeDA1_{short}$ receptors.

Both $CeDA1_{long}$ and $CeDA1_{short}$ displayed sensitivity to cholera toxin (CTX). Cells pretreated with CTX for 24 hours prior to assay failed to stimulate GTP-gamma-[^{35}S] binding following incubation with DA, NE or E. Neither of the receptor subtypes showed pertussis toxin (PTX) sensitivity in the same assay, suggesting that these receptors are likely coupled to Gs but not Gi/o. To test this hypothesis, we transfected CHO-K1 cells with the cDNAs of these receptors and assessed their ability to stimulate intracellular cAMP presumably through Gs activation. Both receptor types increased cAMP in a concentration-dependent manner with DA, NE and E.

The ability of these clones to functionally couple to the human inwardly rectifying potassium channel Kir 3.2 in Xenopus oocyte was also tested following co-expression with Go-/Gi- or Gs-alpha subunit cDNAs. All known mammalian dopamine receptors have been found to couple to Kir 3.2. Neither receptor subtype could stimulate Kir 3.2 using the endogenous oocyte G proteins or when co-expressed with *C. elegans* Go-alpha subunits in response to DA or NE; suggesting that these receptors were not coupled to PTX-sensitive G proteins. However, co-expression of bovine Gs-alpha resulted in significant stimulation of Kir 3.2 current by both DA and NE for both receptor subtypes.

We have generated GFP transcriptional reporter constructs with the promoter region of this receptor gene fused to GFP so as to identify the distribution and localization of this protein following injections of this construct into the worm. We have also isolated a *C.elegans* mutant in which this gene has been deleted. Dopamine is known to modulate a variety of behaviors including egg laying, food sensing and motility. Both, analysis of gene expression and behavioral studies are still under progress

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684. Identification and cloning of two putative novel excitatory GABA receptor subunits in *C. elegans*.

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γ-amino-butyric acid (GABA) is the major fast synaptic inhibitory neurotransmitter and serves as the ligand for GABA receptors. Ligand binding causes a conformational change in GABA receptors forming an ion-channel that is selectively permeable to chloride. In *C. elegans,* GABA functions as both an inhibitory and excitatory neurotransmitter. The inhibitory action of GABA is mediated through UNC-49, which is an inhibitory GABA receptor that is localized to body wall muscles. UNC-49 provides contralateral inhibition to antagonistic muscle groups ensuring coordinated locomotion. However, GABA also mediates a novel excitatory function. GABA release from the motorneurons AVL and DVB stimulates the contraction of the enteric muscles, causing the expulsion of gut contents. We are interested in the molecular nature and physiology of the GABA receptor that mediates excitatory neurotransmission of the enteric muscles. We have cloned two genes, *exp-1* and Y46G5A.26*, which could potentially encode excitatory GABA receptors subunits in *C. elegans*.

exp-1, (expulsion defective), was originally identified by Jim Thomas in screens for mutants defective in the defecation cycle and subsequently in screens looking for mutants in GABA function. Of the six genes required for GABA function in the worm, only *exp-1* specifically eliminates the excitatory function of GABA. We hypothesize that *exp-1* encodes an excitatory GABA receptor expressed in the enteric muscle. We cloned *exp-1* and determined the expression pattern of the protein. Genetic map data place *exp-1* in a small interval between *lin-4* and *lin-23* on chromosome II. A fosmid in this region contains an open reading frame with sequence related to GABA receptors. Transgenes bearing a subclone of the open reading frame rescue *exp-1* mutants. In addition, we identified point mutations in five independent alleles of *exp-1*. Rescuing constructs in which the EXP-1 protein is tagged with GFP indicate that EXP-1 is expressed in the enteric muscles. Consistent with our hypothesis that EXP-1 will form an excitatory GABA receptor, we isolated cDNAs that demonstrate EXP-1 has a completely divergent transmembrane domain 2 (TM2). TM2 is known to confer ion selectivity for the ligand-gated ion channel superfamily, and the numerous negatively charged residues present in TM2 suggest that EXP-1 is a cation selective channel.

BLAST searches revealed a second putative excitatory GABA receptor subunit in *C. elegans*. Y46G5A.26* is 58% identical to EXP-1. Specifically, TM2 of Y46G5A.26* is 85% identical to EXP-1, suggesting that this putative GABA subunit may also form an excitatory receptor. We isolated full-length cDNAs of Y46G5A.26* and have created GFP reporter constructs. Both translational and transcriptional GFP fusions indicate that Y46G5A.26* is expressed in GABA neurons. This putative subunit is not expressed in the enteric muscles as expected, which suggests that GABA may play roles in excitation beyond the enteric muscles. We hypothesize that it might be an excitatory autoreceptor.

We are currently characterizing EXP-1 and Y46G5A.26* in *Xenopus oocytes*. We will determine GABA gating, ion selectivity and primary pharmacology for each putative subunit. Both EXP-1 and Y46G5A.26* appear to be members of a novel class of GABA receptor subunits which may lead to the identification of homologous proteins in higher vertebrates.

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685. Characterizing the *C. elegans* Ionotropic Glutamate Receptor GLR-5

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We are interested in the molecular and neuronal determinants of locomotion. In *Caenorhabditis elegans*, a neuronal circuit has been identified that is required for coordinated locomotion. Previously, our lab has shown that two NMDA type ionotropic glutamate receptor subunits, *nmr-1*, and *nmr-2*, and several non-NMDA type receptor subunits, including *glr-1*, *glr-2*, and *glr-5*, are expressed in the command interneurons of this circuit. One cell in particular, AVA, expresses all of these receptor subunits.

In order to understand the contribution of the ionotropic glutamate receptors to the control of locomotion, we have undertaken a combined molecular, genetic, and electrophysiological analysis of the receptors expressed in AVA.

Genetic analysis has shown that disrupting the function of *glr-1* or *glr-2* alters the normal avoidance response to tactile stimulation of the nose of the worm (see abstract by Brockie *et al*). Using patch-clamp electrophysiological techniques we have characterized several properties of the glutamate-gated currents in AVA. In wild type animals, application of glutamate elicits a current with at least two distinct kinetic components; the first current rapidly activates and quickly desensitizes, while the second is slower activating and long lasting. We have shown that a null mutation in *nmr-1* eliminates the slower, long lasting current, whereas null mutations in either *glr-1* or *glr-2* eliminate almost all of the rapidly activating and inactivating current. This suggests that the GLR-1 and GLR-2 receptor subunits may form a heteromeric receptor that is required for the fast glutamate-gated current. Are GLR-1 and GLR-2 sufficient for this rapid glutamate-gated current or are other receptor subunits, such as *glr-5*, required as well?

To test the hypothesis that the rapidly activating and inactivating glutamate-gated current that mediates nose touch requires the coassembly of GLR-1, GLR-2, and GLR-5, we have created a deletion mutation in the *glr-5* gene. We are currently investigating the electrophysiological and behavioral defects associated with this predicted null mutation.

686. Characterization of Ionotropic Glutamate Receptor Subunits GLR-3 and GLR-6

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In *C. elegans* ten putative ionotropic glutamate receptor subunits have been identified(Brockie et al, J Neurosci. 2001 Mar 1;21(5):1510-22) Of these ten, the non-NMDA glutamate receptor subunits GLR-3 and GLR-6 have been shown by transcriptional and translational fusions with GFP to be expressed in a single cell, the interneuron RIA. Another glutamate receptor subunit GLR-2 also shows expression in RIA as well as other cells. Prior laser ablation studies of RIA and its presynaptic partners AIY and AIZ have defined this group of cells to be part of the thermotaxic circuit (Mori I, Ohshima , Nature 1995 Jul 27;376(6538):344-8).

We are interested in understanding the assembly and localization of glutamate receptor complexes containing GLR-2, GLR-3 and GLR-6 within RIA and how they contribute to behavior. To help understand which glutamate receptors may be functioning together we have created CFP and YFP fusions to the amino terminal of the receptor subunits. We are using confocal microscopy to obtain information about the localization of the subunits within RIA. This information may allow us to determine which of the subunits co-assemble to form channels. It also may allow us to use the confocal data along with information about the cell connectivity from electron microscopy(White and Sulston), to identify cells that use glutamate to communicate with RIA.

In order to characterize the contribution of the glutamate receptor subunits GLR-3 and GLR-6 to behavior, we have generated null mutations of the genes that encode each subunit. We are currently characterizing the phenotype of the strains using thermotaxis and a variety of other standard behavioral assays.

687. Identification of functional ion channel domains of *C. elegans* glutamate receptor subunits

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Glutamate receptors are not only abundant in the nervous system of vertebrates but also of invertebrates. In *Caenorhabditis elegans,* 10 putative ionotropic glutamate receptor subunits have been identified so far (Brockie et al. 2001, J. Neurosci. 21, 1510). Two of these, GLR-1 and GLR-2, have been expressed in *Xenopus* oocytes and failed to show ligand-activated currents. Therefore, we set out to investigate if the pore-forming domains of these two subunits are intrinsically functional ion conducting domains. We have previously shown that domain transplantation between different subtypes of glutamate receptors can generate functional chimeras that allow the characterization of those domains.

To test for pore function, we transplanted the pore-forming region of GLR-1 or GLR-2 into either rat GluR1, as a representative of the AMPA receptor family, or rat GluR6, as a representative of the KA receptor family. The resulting chimeras GluR1-PGLR1, GluR1-PGLR2, GluR6-PGLR1, and GluR6-PGLR2, as well as the respective wildtypes GluR1, GluR6, GLR-1, and GLR-2 were expressed and characterized in *Xenopus* oocytes. Surprisingly, GluR1-PGLR1, GluR1-PGLR2, and GluR6-PGLR2 produced ligand-activated currents, despite the GLR-1 and GLR-2 wildtype receptor's apparent lack of ion channel function. The maximal current amplitudes of GluR6-PGLR2 were comparable to wildtype GluR6. GluR1-PGLR1 and GluR1-PGLR2 showed reduced current amplitudes in comparison to wildtype GluR1.

The ion channel properties of the chimeras classify GLR-1 and GLR-2 into the group of non-NMDA receptor subunits, consistent with conclusions derived from sequence homology data.

688. Alternative isoforms and mutants of the *glr-2* ionotropic glutamate receptor

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Ionotropic glutamate receptors are essential mediators of excitation in the vertebrate brain. The iGluR family in vertebrates is quite large, with at least 16 genes and complex post-transcriptional alterations in gene structure. In order to initiate complementary studies in a simpler genetic system, we began to examine predicted iGluRs in *C. elegans*. Among these, the *glr-2* gene from the cosmid K04G7 is particularly interesting. Its predicted sequence contains the key M3 gating determinant (the lurcher domain, TANLAAF) and residues critical in forming the M2 pore, including the Q/R site, which limits channel Ca^{++} conductance when the genomic glutamine codon is edited to arginine. It also carries a carboxy terminus which could be a PDZ interaction sequence (STLF). These features make the gene, like *glr-1*, most analogous to the AMPA iGluRs from vertebrates.

We have previously found that, although the Q/R site is not detectably edited in RNA expressed from this gene, alternative splicing of a region encoding a conserved amino terminal domain does occur. Now we present results from our analysis of the expression of these isoforms. We have begun to examine the localization of expressed transgenes in not only wt, but *glr-2* knockout animals. Behavioural assays are currently in progress, to determine whether the localization of the GFP-tagged GLR-2 isoforms truly reflects functional activity of these iGluRs in the knockout background. At least nose touch sensitivity can apparently be rescued in the knockout by either isoform. The consequences of mutagenesis of the transgenes and of expression in different mutant backgrounds are also being assessed.

689. Identification of New Components in the *C. Elegans* PKD Pathway

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Autosomal Dominant Polycystic Kidney Disease (ADPKD) strikes about 1 in every thousand people. This systemic disease can cause the growth of kidney cysts and affect the liver, pancreas and cardiovascular system. Half of affected individuals will suffer from end stage renal failure by the time they are 60. Approximately 95% of all cases of ADPKD are caused by mutations in either PKD1 or PKD2. The *C. elegans* homologs of PKD1 and PKD2 (*lov-1* and *pkd-2*) are expressed in male sensory neurons in the head, hook and rays(1). Mutations in *lov-1* and *pkd-2* result in defects in two male mating behaviors: response and vulva location (1). Localization and mutant phenotypes suggest the *C. elegans* polycystins LOV-1 and PKD-2 act in the same pathway. The cellular role of the polycystins is still unknown.

The cytoplasmic tails of polycystin-1 and polycystin-2 (encoded by PKD1 and PKD2 respectively) have been shown to interact using the yeast two-hybrid system (2,3). Polycystin-2 has also been shown to interact with itself through its probable coiled-coil domain. Recent studies indicate polycystin-1 and polycystin-2 form a cation channel. We are using the two-hybrid system to examine interactions between LOV-1 and PKD-2 and to identify new proteins in the polycystin pathway.

We have created two cDNA libraries (one directionally cloned, the other random) using mRNA from a male-enriched *him-5(e1490)* population. Initially we will use as bait the intracellular carboxy termini of LOV-1 and PKD-2. These regions are of interest because of the demonstrated interaction between the human polycystins and because a 58 amino acid c-terminal truncation of LOV-1 results in a nonfunctional protein. Another bait to be used in screens is the PLAT domain in LOV-1 because this region is conserved in all polycystin-1 family members.

At the meeting we will present potential candidates isolated and characterized thus far.

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2. Qian, F., et al. *Nature Genetics*. June 1997; 16:179-183

3. Tsiakas, L., et al. *Proc. Natl. Acad. Sci*. June 1997; 94:6965-6970

690. The physiological roles of AVR-14 in *C. elegans* and the parasite, *Haemonchus contortus*.

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avr-14 encodes two subunits of the glutamate-gated chloride channels (GluCls) that are the targets for the important anthelmintic, ivermectin. The gene, the pattern of alternative splicing that results in the production of the two subunits, and the expression patterns are all highly conserved between *C. elegans* and the closely related parasitic nematode, *Haemonchus contortus*. Intracellular recordings from dissected pharynxes showed that *avr-14* mutants retained a wild-type response to applied ivermectin. However, the response to L-glutamate was slightly, but significantly reduced (2-fold lower affinity; n=8), suggesting that there may be a role for AVR-14 in the pharyngeal nervous system. Consistent with this, antibody staining of the dissected pharynxes showed that AVR-14 was expressed on a subset of pharyngeal neurones, probably M1 and M4. Since this antibody did not differentiate between the two splice isoforms, we raised specific antisera against the intracellular loops of the AVR-14A and -14B subunits. In *H. contortus*, the anti AVR-14A antiserum reacted with a few motor neurone commisures and with a pair of sensory neurones in the head. As observed previously, there was no evidence of synaptic localisation of the receptor, which was detected throughout the cell, including the cell bodies. The anti AVR-14B antiserum reacted with three cell bodies, possibly of pharyngeal neurones, in the head region. These antibodies are currently being used on the *C. elegans* preparation. Our data suggest a role for AVR-14-containing GluCls in the regulation of pharyngeal pumping and chemosensation in both species, in addition to their previously identified role in locomotion. Currently, we are attempting to rescue the *avr-14* phenotype by expression of the *H.*

contortus subunits.

691. Expression of neuronal alpha-7-like nicotinic acetylcholine receptors in *C. elegans*

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Neuronal nicotinic acetylcholine receptors (nAChRs) are members of a diverse superfamily of neurotransmitter receptor proteins. nAChRs are highly expressed in the vertebrate brain where they mediate critical physiological functions such as learning and memory. nAChR subunits assemble as pentameric receptors which act as selective cation (Na+, Ca++) channels in response to ACh binding. Recent completion of the *C. elegans* genomic sequence reveals that 8 of 33 putative nAChRs are members of the α7-like family of nAChR subunits. To date, only a single α7 nAChR gene has been identified in the human genome. The significance of the apparent greater diversity of the α7-like nAChRs in nematodes relative to humans remains unknown.

The primary goal of this project is to exploit *C. elegans* to define the physiologic and behavioral roles of α7-like nAChR proteins. Transgenic lines expressing partial translational/GFP reporter fusion constructs have been obtained for four of the *C. elegans* α7-like nAChRs: *acr-7*, *acr-9*, *acr-11*, and *acr-16*. *acr-7* and *acr-11* GFP expression is present in various tail neurons distal to the anus. In addition, *acr-7*::GFP is observed in the pharyngeal muscle. *acr-9*::GFP is expressed in ventral cord neurons of L3s through adults. *acr-16*::GFP is detected in both proximal and distal intestinal cells. Present efforts are directed at enhancing detection of GFP expression, which is relatively weak for all of these α7-like GFP reporters.

Once the expression patterns of the α7-like nAChRs have been defined, genetic strategies will be utilized to detect functional interactions between nAChRs and to reveal new components of cholinergic pathways. Ultimately, our approach should provide insight into the mechanisms of nAChR function in the human brain.

692. *eat-18* is required for nicotinic neurotransmission in the pharynx

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MC is the main excitatory motor neuron of the pharynx and is required for rapid pharyngeal pumping. MC is cholinergic and acts through a nicotinic receptor in the pharyngeal muscle. *eat-2* encodes a subunit of the receptor and is required for rapid pharyngeal pumping.

eat-18 is also required for rapid pharyngeal pumping. Two pieces of evidence indicate that *eat-18* is required for pharyngeal nicotinic channel function: 1.) *eat-18* pharynxes do not respond to nicotine. 2.) We observed allele specific genetic interaction between *eat-2* and *eat-18* indicating that EAT-18 could directly interact with the receptor. Alternatively, *eat-18* could be required for some aspect of MC development or function. We are using a GFP reporter that is expressed in MC to determine the location of the cell body and to assess MC process formation in *eat-18* mutants.

eat-18 is located on the right arm of chromosome I, close to the breakpoint of *eDf7*. We cloned the *eDf7* breakpoint and found that it breaks in Y105E8B.f. A 5kb piece of DNA just to the left of the breakpoint, containing the 5' half of Y105E8B.f, can rescue an *eat-18* mutant. One allele of *eat-18*, *ad820sd*, contains a single base pair change in the first exon of this gene, and a translational GFP fusion to Y105E8B.f is expressed in pharyngeal muscle. These data indicate that Y105E8B.f encodes *eat-18*. We are now sequencing Y105E8B.f from two additional *eat-18* alleles and are attempting to rescue the mutant phenotype by expressing a cDNA from this gene in pharyngeal muscle.

693. Direct interaction between IP3 receptors and myosin II heavy chains in *C. elegans*: contribution to the regulation of pharyngeal pumping.

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Inositol 1,4,5 triphosphate receptors (IP3Rs) are ligand-gated Ca2+ channels that control the release of Ca2+ from intracellular stores, and are therefore central to a wide range of cellular responses. IP3Rs in *C. elegans* are encoded by a single gene, *itr-1* and are widely expressed(1,2). Signalling through IP3 and IP3Rs is known to be important in ovulation(3), control of the defecation cycle(2), modulation of pharyngeal pumping rate and embryogenesis(4). To elucidate the molecular basis of the diversity of IP3R function, we have used a yeast two-hybrid screen to search for novel interactions between ITR-1 and other proteins. This approach identified interactions with two myosin II heavy chains (MYO-1 and UNC-54). We have used co-immunoprecipitation from worm extracts, and interaction between recombinant proteins *in vitro*, to confirm the existence of these interactions. The precise sites of interaction have been localised in IP3R and MYO-1. This enabled us to interfere with the interaction *in vivo* via high-level, heat shock-induced expression of competing peptides and examine the functional consequences. We conclude that this interaction functions in the behavioural modulation of pharyngeal pumping.

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694. The *unc-63* gene encodes a novel nicotinic acetylcholine receptor subunit expressed at the *C. elegans* neuromuscular junction.

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Levamisole is a powerful antiparasitic drug that results in hypercontraction, paralysis and eventually death in *C. elegans*. It acts both as a agonist and open channel blocker on nicotinic acetylcholine receptors. The *C. elegans* body wall muscle cells express two types of nicotininic acetylcholine receptor. One is sensitive to levamisole the other is sensitive to dihydro beta-erythroidine (1). Two previously characterized nicotinic receptor subunits UNC-38 and UNC-29 are part of the body wall muscle levamisole-sensitive receptor (1, 2). Several *C. elegans* mutants resistant to the paralysing action of levamisole have been isolated. We describe the molecular and functional characterization of the *unc-63* gene that defines a levamisole resistance locus on chromosome I. Firstly, we isolated a full length cDNA encoding a novel nicotinic receptor subunit using cross-hybridization with *unc-38* and *unc-29* cDNAs and RT-PCR. The novel cDNA mapped very close to the *unc-63* locus. The *unc-63* mutant shows a variety of phenotypes ranging from strong uncoordinated locomotion and strong levamisole resistance (alleles *x37, x13, x18*) to nearly wild type locomotion and mild resistance to the drug (alleles *b404, x26*). We tested whether the *unc-63* gene is the structural gene for this novel subunit, by sequencing mutant alleles and by mutant rescue transformation experiments. We sequenced the novel cDNA from five *unc-63* mutant alleles (obtained by EMS mutagenesis). We found one missense mutation (*x26*), one nonsense (*x13*), one deletion (*b404*) and two mutations that disrupt splice junction consensus sites. The functional complementation of the *unc-63* (*x37*) mutant gave transformants that exhibited a return to wild type locomotion and recovered sensitivity to levamisole. The unc-63::GFP transgene is expressed in all body wall muscle cells and in a large number of neurones. We also analysed the expression pattern of the non-alpha subunit encoded by the *lev-1* gene. The *lev-1* mutant is slightly uncoordinated and resistant to levamisole. The lev-1::GFP transgene is expressed in the body wall muscle cell. On the other hand, *in vivo* patch-clamp recordings show that the levamisole sensitive nicotinic receptor response is abolished in both *unc-63* and *lev-1* mutants. A hypothesis to account for the findings to date is that the levamisole sensitive nicotinic receptor expressed in body wall muscle cells may contain two alpha-like subunits encoded by *unc-38* and *unc-63* and two non-alpha subunits encoded by *lev-1* and *unc-29* genes.

(1) J.E. Richmond and E.M. Jorgensen (1999)

(2) J.T. Fleming et al. (1997)

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695. Towards understanding the cell biology of nicotine adaptation in Caenorhabditis elegans

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We are studying the cell biology of nicotine adaptation in *C. elegans*. Previously, we have shown that the levels of the UNC-29 nicotinic acetylcholine receptor (nAChR) subunit, expressed in the vulval muscles, are reduced in response to elevated doses of nicotine. This process involves the function of the *C. elegans* PKC homolog TPA-1 [1]. We have now extended our studies towards three additional nAChR subunits, namely UNC-38, LEV-1 and UNC-63, all of which, together with UNC-29, are involved in the control of egg-laying [2].

We have created translational fusions of the nAChR subunits with GFP and studied their expression patterns *in vivo*. LEV-1 is strongly expressed in head and body muscles, including muscle arms projecting to the nerve ring, and in several as yet unidentified head, tail and ventral cord neurons (including VC 4 and 5 that innervate the vulval muscles). LEV-1 may also be expressed in vulval muscles, however, this could only be detected in animals expressing GFP driven by the *lev-1* promoter. LEV-1 expression is already detected in embryos and remains throughout the whole lifespan, although it is strong only in young and well-fed worms.

The expression levels of UNC-63 and UNC-38 are lower and we detect these proteins in head and body muscles, various head and tail neurons, the nerve ring, vulval muscles, ventral cord neurons (including VC 4 and 5), and also in post-synaptic specializations of the nerve cord. It is currently unclear, whether these post-synaptic sites represent neuromuscular junctions or interneuronal synapses (or both). The expression patterns of all four receptor subunits under study are in agreement with previous results by other labs showing that UNC-29, UNC-38 and LEV-1 can form a functional receptor [3,4], since they are (at least in part) present in the same cells and tissues. However, UNC-38 is expressed in many more head neurons than the other nAChR subunits, suggesting that this α-subunit may form functional receptors also with other, as yet unidentified, non-α-subunits. We now try to express epitope-tagged versions of all four nAChR subunits in a single worm, in order to allow for co-localization and co-immunoprecipitation studies.

Furthermore, we studied the effects of nicotine treatment on the expression of LEV-1. Similar as UNC-29, LEV-1::GFP expression levels can be dramatically and reversibly reduced by incubating the worms on 0.25 - 0.5 % nicotine. Initial results suggest that this regulation may be achieved, at least in part, on the transcriptional level, since also GFP expression driven by the *lev-1* promoter alone is reduced by nicotine treatment. In contrast, GFP driven by the *unc-29* promoter is not affected by nicotine, consistent with our results for the translational fusion of UNC-29::GFP, which is regulated in a promoter-independent fashion [1]. We are currently studying the effect of nicotine on the expression of *unc-38* and *unc-63* and we are investigating whether mutations in *dyn-1* [5] (*C. elegans* dynamin) and in *nic-1* (isolated in our lab; nic-1 mutants are impaired in nicotine-adaptation, see abstract by Kim et al.) affect the nicotine-induced downregulation of LEV-1::GFP.

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1 Waggoner et al. (2000) *J. Neurosci.* **20** p. 8802-8811

2 Kim et al. (2001) *Genetics* **154**(4)

3 Fleming et al. (1997) *J. Neurosci.* **17** p. 5843-5857

4 Richmond and Jorgensen (1999) *Nature Neurosci.* **2** p. 791-797

5 Clark et al. *PNAS* **94** p. 10438-10443

696. Identifying Modulators of Voltage-Gated Calcium Channels

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Voltage gated calcium channels (VGCCs) play a number of important physiological roles and have also been implicated in several neuropathologies. The channels are composed of a multisubunit complex of which the alpha-1 subunit forms the channel proper. Structural differences between the alpha-1 gene products account for many of the unique physiological and pharmacological properties of the VGCCs. In addition, calcium entry through these channels is controlled via several modes of regulation including the state of channel phosphorylation, and the binding of effector molecules such as G-proteins. To further our understanding of how VGCC activity is modulated we are using a molecular genetic approach using *C. elegans* as a model animal system. In contrast to the many alpha-1 channels found in vertebrates, *C. elegans* contains only three genes including the L-type *egl-19* gene, the N- and P/Q-type gene *unc-2* and the T-type channel encoded by *cca-1*. Our efforts have focused on characterizing the functional properties of the UNC-2 and CCA-1 gene products. The approach we have taken is to characterize both the phenotypic and physiological properties of various mutants in *unc-2* and *cca-1*, and to use these alleles to screen for candidate genes which may act as VGCC modulators. We are also currently expressing the nematode channels in tissue culture cells in order to establish and compare their electrophysiological and pharmacological properties with their respective vertebrate counterparts.

697. PHYSIOLOGICAL ROLE OF K-CL COTRANSPORTERS IN C. ELEGANS

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In vertebrate cells the K-Cl cotransporter has the capacity to reduce cellular concentration of K and Cl. This plays several documented roles: regulation of cytoplasmic chloride concentration, reduction of cell size in development, defense against cell swelling under hypoosmotic and isoosmotic conditions. The last, which includes the defense against swelling during moderate warming due to hyperactivity of the Na-K pump, has received the least attention but may be the most physiologically relevant. The gene sequences for two mammalian isoforms of this cotransporter were identified in 1996 and at that time two similar sequences were recognized in the C. elegans database (KO2A2.3, "KO2", on chromosome 2, and R13A1.2, "R13", on chromosome 4). Both C. elegans isoforms are expressed,as demonstrated by their presence in EST’s and cDNA libraries. K02 has been shown to have K-Cl cotransport activity when transfected into mammalian cells.

We have carried out dsRNAi blockages against both of these genes, singly and in combination, and found that about a third of the injected parents produced progeny that exhibit reduced survival at elevated temperature (33°C and 37°C) and increased sensitivity (seen as immobilization) to isoosmotic challenge induced by exposure to 100 or 200 mM ammonium chloride in M9 medium. A deletion mutant of K02 obtained from Gary Moulder at U. Oklahoma has not shown a phenotype by these tests and is now being used as a target for RNAi knockout of the R13 gene. GFP’s expressed from upstream sequences of the two genes indicate that both isoforms are consistently expressed in pharyngeal muscle. The extent to which either is expressed elsewhere is still being determined. These studies may lead to establishing the role of K-Cl cotransporters in the whole organism and lay the foundation for a genetic analysis of their control

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698. CHARACTERIZING THE NEURONAL FUNCTION OF THE SUP-9/SUP-10/UNC-93 TWO-PORE K+ CHANNEL COMPLEX

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Rare altered-function mutations in the genes *unc-93*, *sup-9*, and *sup-10* result in the abnormal regulation of muscle contraction. These mutants move sluggishly, are unable to lay eggs, and exhibit the rubberband phenotype: when worms are prodded on the head, they contract and relax along their entire bodies without moving backwards. Genetic studies suggest that these three genes act at the same step and likely encode subunits of a protein complex. We have shown that *sup-9* encodes a two-pore K^+ channel subunit with similarity to the mammalian Two-pore Acid Sensitive K^+ channels TASK-1 and TASK-3. *unc-93* and *sup-10* encode novel putative transmembrane proteins.

We have found that in addition to being expressed in muscle, *sup-9::gfp* and *unc-93::gfp* are both expressed in 10 to 15 neurons in the head, including the four SIA neurons. The SIA neurons form very few synapses with other neurons, and their function remains unknown. To identify neuronal defects caused by *sup-9(gf)* mutations but masked by its muscle-induced paralysis, we are expressing *sup-9(gf)* under global neuronal promoters. Since in muscle the *sup-9(gf)* defect requires *unc-93* function, we hypothesize that a broadly expressed SUP-9(gf) K^+ channel will affect only those neurons in which endogenous *unc-93* is expressed.

We are also continuing our efforts to identify biophysically K^+ currents produced by the putative SUP-9/UNC-93/SUP-10 channel complex. We have generated stable HEK293 cell lines transfected with cDNAs encoding these proteins. Using whole-cell voltage clamp techniques we are characterizing the currents present in these cells. Mammalian two-pore K^+ channels are regulated by multiple factors, including pH, membrane stretch, arachidonic acid, local and volatile anesthetics, and temperature. We hope to identify the factors that regulate the activity of the SUP-9 channel complex and the roles that UNC-93 and SUP-10 play in this regulation.

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699. CARGO RECOGNITION BY SYNAPTIC VESICLE KINESIN

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Kinesins constitute a large family of motor proteins responsible for transport of a variety of cellular orgenelles. *unc-104 encodes* a *C. elegans* kinesin that is involved in axonal transport of synaptic vesicles [Hall and Hedgecock, 1991]. The UNC-104 protein is essential: null mutations result in early larval lethality. However, a few hypomorphic mutations of *unc-104* have been isolated. These mutant animals are paralyzed, and EM analysis revealed that their synaptic terminals are severely depleted of synaptic vesicles; vesicles are instead retained in the cell body [Hall and Hedgecock, 1991].

Although UNC-104 and its mammalian homolog KIF1A have been shown to transport synaptic vesicles to the nerve terminal little is known how these motor proteins recognize their cargoes. What is the molecular basis of cargo recognition? One theory proposes that UNC-104 recognizes proteins associated with the synaptic vesicle membrane. This is supported by the fact that protease treatment of synaptic vesicles disrupts their transport to the nerve terminal [Muresan et al., 1996]. Protein-protein interaction has been also shown to determine cargo recognition for two other kinesins: conventional kinesin and KIF3 [Verhey et al., 2001; Setou et al., 2000]. Alternatively, UNC-104 recognizes synaptic vesicles by binding specific lipids. This hypothesis is supported by the fact that UNC-104 has a pleckstrin homology (PH) domain, which is a lipid-binding moiety. Furthermore, mutations disrupting lipid metabolism affect synaptic vesicle transport [Harris et al., 2000].

We propose that UNC-104 identifies its cargo by binding a lipid component of the synaptic vesicle membrane via its PH domain. If this hypothesis were true, the following predictions could be made: the UNC-104 PH domain would bind lipids specific to synaptic vesicle membrane, and lipid binding by the PH domain would be required for cargo recognition. We have demonstrated that UNC-104 has a functional PH domain which specifically binds $PI(4,5)P_2$. We have also identified an *unc-104* partial loss-of-function mutant that results from a mutation of a conserved amino acid in the PH domain. We are currently testing whether abolishment of UNC-104-lipid binding affects synaptic vesicle transport *in vivo*. We also plan to determine if PH domain- $PI(4,5)P_2$ binding is sufficient for cargo recognition by UNC-104.

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700. Movement of IDA-1::GFP-tagged vesicles in *C. elegans* nerve processes

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In mammals, the closely related protein tyrosine phosphatase-like type 1 transmembrane proteins ICA512 (IA-2) and phogrin (IA-2beta) are restricted to neuroendocrine tissues including brain, pituitary and pancreatic beta-cells, where they are localized to dense core vesicles (DCVs) of the regulated secretory pathway. Phogrin has been shown to undergo Ca^{2+}-dependent phosporylation in response to secretagogues and ICA512 to interact with betaIV spectrin and beta2-syntrophin. These findings together with the restricted expression pattern, intracellular localization and the strong conservation between species suggest a role of these proteins in regulated secretion. They are of additional interest as major targets of autoimmunity in type 1 diabetes.

We recently reported the existence of a single ortholog of phogrin and ICA512 in *C. elegans*, IDA-1 (islet cell diabetic autoantigen). Using GFP reporter constructs, we localized *ida-1* gene expression to a subset of about 30 mostly sensory neurons and the vulval neurosecretory-like uv1 cells. Expression was sex-specific and included the hermaphrodite-specific vulval motoneurons and many male-specific neurons.

In contrast to synaptic vesicles (SVs) in *C. elegans*, very little is known about the larger dark-cored vesicles which have been seen in the electron-microscopic analysis of the worms nervous system. We hypothesize that IDA-1 in *C. elegans* like ICA512 and phogrin in mammals is specific to DCVs which are distinct from synaptic vesicles and secrete neuropeptides or hormones in a regulated fashion.

SNB-1::GFP-tagged SVs have proven very useful tools, and more recently Scholey and colleagues showed that in live *C. elegans* animals movement of GFP-tagged molecular rafts in amphid dendrites can be observed with a fluorescent light microscope. We generated transgenic *C. elegans* strains expressing an IDA-1::GFP fusion protein, showing punctate staining within neuronal cell bodies and their processes. The distribution of IDA-1::GFP in nerve processes is reminiscent of the bouton-like staining of SV markers. In contrast to the reported subcellular distribution of SNB-1::GFP, IDA-1::GFP strongly stains cell bodies though is excluded from the nucleus consistent with DCV biogenesis and recycling at the trans-Golgi network and endosomal compartments. Small IDA-1::GFP punctae shuttle between larger immobile GFP accumulations distributed periodically along axons and dendrites. Our initial analysis of vesicle movement in PHC dendrites reveals rates of transport of ~ 1.2 µm/s in retrograde and more variable ~ 2.6 µm/s in anterograde direction indicative of fast microtubule-based transport.

We generated IDA-1::GFP transgenic mutants defective in motor proteins including *unc-104*, *osm-3*, and *che-3* to directly test a requirement of IDA-1::GFP-tagged vesicle transport by those molecular motors. In an attempt to define similarities and differences between *C. elegans* SVs and the IDA-1::GFP-tagged structures, we introduced the IDA-1::GFP transgene into a variety of mutant backgrounds that are known to severely disrupt the localization of synaptic vesicles and proper formation of synapses, such as *unc-11*, *syd-2*, and *rpm-1*.

The observed subcellular localization and movements of IDA-1::GFP are consistent with a vesicular localization of IDA-1 and indicate that the nematode will be a useful model to study the molecular mechanisms involved in DCV transport and recycling.

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701. Cloning and characterization of a 'putative' transport defective mutant that mis-accumulates synaptic vesicle proteins in *C. elegans* neurons

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Axonal transport is a microtubule motor-dependent process that carries vesicles and organelles to and from the cell body to the synapse. To understand the regulation of this process we are characterizing and cloning putative transport-defective mutants isolated using tagged cargo.

Synaptic vesicles (SVs) were visualized in mechanosensory neurons using GFP tagged synaptobrevin. Four *sam* mutants (*js121, js319, js320 and js351*) mis-accumulate tagged synaptobrevin at the end of the main process of the mechanosensory neurons where no synapses are described. All other domains of tagged synaptobrevin expression/localization are unaltered in the neurons examined thus far. These mutants fall into three complementation groups where *js121* and *js319* are allelic to each other. Both *js121* and *js351* are sterile and have a protruding vulva, perhaps indicating their role in a common pathway. Two of the four mutants have been further characterized. They mis-accumulate synaptotagmin and synaptobrevin. However, no mis-accumulation of synaptogyrin, rab3, dynamin-1 or Rim is observed. The absence of these proteins suggests that the sites of synaptobrevin mis-accumulation are unlikely to form ectopic synapses. The mis-accumulations are also dependent on microtubules and the kinesin-like protein UNC-104. Taken together these data suggest that the mutants are likely to alter transport of SVs or SV precursors.

The *sam-11* mutations have been mapped to the left arm of chromosome I. Using Hawaiian polymorphisms the gene has been restricted to a 250 kb region. Amongst the predicted ORFs in this region is the dynein heavy chain gene (*dhc-1*). Dynein has been shown in other systems to be involved in retrograde transport of cargo in neurons. Progress in cloning the *sam-11* gene will be presented.

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702. Direct Visualization of the Movement of Motor Proteins and their Cargo within Neurons of Living *C. elegans*

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The neuron is a highly polarized cell that elaborates two processes, a dendrite that is specialized for neuronal signal reception and an axon that is specialized for signal conduction and transmission. The formation and function of these processes depends upon the microtubule-based transport of cellular components from their sites of synthesis in the neuronal cell body to their sites of utilization at the terminus. We have developed a time-lapse fluorescence microscopic assay that allows us to visualize the movement of MT-based motor proteins and their cargo along neurites within living *C. elegans* (Orozco et al., 1999, Nature, 398 (6729):674). We are using this assay to dissect the kinesin-II / IFT raft transport pathway that contributes to the formation and function of chemosensory neurons and sensory cilia (Signor et al., 1999, JCB 147:519), and the monomeric UNC-104 synaptic vesicle transport pathway that is required for proper synaptic transmission (Zhou et al., 2001, J. Neurosci., in press). Using time-lapse fluorescence microscopy we have visualized fluorescent kinesin-II motor (KAP-1) and raft proteins (OSM-1, OSM-6 and CHE-2) moving along dendrites and sensory cilia at 0.7 μm/s in the anterograde direction and 1.1 μm/s in the retrograde direction. Retrograde ciliary transport of these proteins is abolished in the absence of CHE-3 dynein, leading to a plausible model for bi-directional transport in chemosensory neurons. UNC-104 motor proteins move in both directions along neuronal processes, some of which were definitely identified as axons and others as dendrites, suggesting a role for this pathway in synaptic transmission at axo-dendritic and dendro-dendritic synapses. Using kymograph analysis, we observed that UNC-104::gfp particles moved bidirectionally at an average velocity of 1.0 μm/s, which is close to the *in vitro* velocity of microtubule gliding driven by purified monomeric kinesin at high motor density. These data are consistent with the hypothesis that multiple UNC-104 motors move synaptic vesicles along axons and dendrites at 1.0 μm/s, and that these motors are recycled by a retrograde transport system that coincidentally moves at the same velocity.

703. *zd8* causes cell migration and axon fasciculation defects

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Neurons project axons during development that migrate long distances along stereotypic pathways to find their appropriate targets and establish the initial connectivity of the nervous system. The trajectory of each axon is determined by the response of its growth cone to spatial signals along its route. These signals include cell surface and extracellular matrix molecules that provide short-range cues as well as diffusible molecules that provide long-range guidance information. These signals can either attract or repel a migrating growth cone and, through their combined action, these signals orchestrate correct axon outgrowth and pathfinding. To identify genes involved in axon growth and guidance, we have performed several screens for mutants with axon pathfinding defects (see abstract by Chiu et al.).

One mutant identified in our screens, *zd8*, has a variety of cell migration and axon guidance defects. *zd8* mutants are uncoordinated and have a notched head and a withered tail phenotype. The PVQ neurons are located in the tail and each projects a single axon that runs in the ventral nerve cord to the nerve ring in wild type animals. The PVQ axons often defasciculate from the nerve cord and wander anteriorly in lateral positions in *zd8* mutants. We also found defects in the migration and axon outgrowth of the HSNs. The HSNs are often found either anterior or posterior of their normal position in the midbody and their axons often fail to follow their normal trajectory to the nerve ring in *zd8* animals. *zd8* also causes defects in the growth of other axons. Thus, the gene defined by *zd8* is needed for both cell migration and axon fasciculation. To clone and characterize further this gene, we mapped *zd8* to a small interval and are testing cosmids in this region for rescue.

704. Sensory axon guidance defects in *C. elegans*

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Worms depend on taste, touch and smell to sense and explore their environment. Appropriate responses to information received through these different modalities depend on precise connectivity between sensory neurons and downstream effector neurons in the nerve ring. Little is known about how axons migrate through the nerve ring neuropil during development to find their synaptic partners. Chemosensory axon development can be divided into four stages of growth: 1) initial ventral outgrowth in the amphid commissure (which includes outgrowth and ventral guidance), 2) axons join the nerve ring, 3) dorsal extension in nerve ring, 4) target identification and termination.

To study this process we focused on the guidance of the ASI chemosensory neurons to their appropriate positions in the nerve ring. A mutant screen led to the isolation of 16 candidate mutant strains that appeared to have ASI axon guidance and termination phenotypes. These mutations define at least five new genes, which we have named *sax-10-14* (sensory axon guidance). We have isolated five alleles of *sax-10*. The canonical allele, *ky297*, has defects in both sensory axons and interneuron axons in the nerve ring. Many classes of axons reach the nerve ring appropriately, but then terminate or become misguided. Axons in the body and ventral cord appear normal. A subset of sensory axons are misguided or terminated in *sax-12* mutants, suggesting a more specific defect. *sax-13* also affects many but not all sensory axons and some interneurons in the nerve ring. Despite these defects the overall position and morphology of the nerve ring is normal in many *sax-10*, *sax-12* and *sax-13* animals. *sax-14*, however, has more severe nerve ring phenotypes that are being characterized further.

Mutations in *sax-10*, *sax-12*, *sax-13*, *sax(ky353)*, *sax-5*, *sax-3*, *unc-6* and *unc-40* affect the dorsal extension of axons and may play a role in pathfinding. Mutations in *sax-12*, *sax-13*, *sax-5*,

sax-3, *unc-6* and *vab-1* also affect the first guidance step, initial ventral guidance, of some chemosensory neurons. To facilitate genetic analysis and cloning of these new *sax* genes, we are mapping the genes and currently trying to rescue the *sax-10* phenotype by cosmid injections and germline transformation. We will present our latest progress in these endeavors.

705. *C. elegans* zfh-1, a zinc finger homeobox transcription factor involved in axon guidance

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Correct wiring of the nervous system requires that in the developing embryo each axon finds its proper position and interaction partners.

A genetic screen designed to identify new genes involved in axonal guidance and fasciculation utilized worms expressing GFP in interneurons under the control of the glr-1 promotor. F2 progeny of animals mutagenized with EMS were scored under a fluorescence microscope for fasciculation defects in the ventral cord. Mutant animals were recovered from the slides.

One of the mutations isolated was rh315. Defasciculation, i.e. crossing of ventral cord axons from right to left side is only observed in a fraction of mutant worms. In almost every animal however, a misguided axon does not reach the ventral cord at all and is running dorsally along the full body length. In addition, laterally extending axons are found occasionally. Axons of motorneurons and of GABAergic neurons are also affected, showing defasciculation of dorsal and ventral bundles. Commissures sometimes fail to reach the dorsal cord and run laterally. Other tissues and cell migrations appear normal. Mutant worms are fairly active but tend to curl and change direction of movement quite often.

The gene mapped to chromosome IV between unc-17 and an SNP-marker on cosmid W03D2. Rescue of the mutant phenotype was obtained with both of the overlapping cosmids T08C8 and F28F9 and with a 10.4 kB PCR fragment containing the predicted gene F28F9.1. This gene encodes a transcription factor with a central homeodomain and two clusters of C2H2-type zinc-fingers (ZFs) at either end. Sequencing of the mutant revealed a stop codon in intron five which truncates the protein in the non-conserved region following the

homeodomain and thus deletes the C-terminal ZFs. It is homologous to zfh-1 (**z**inc **f**inger **h**omeodomain protein **1**) in *D. melanogaster*, with 89% identity in the C-terminal cluster of three ZFs, and 87% identity between the two N-terminal ZFs of the worm and two of the fly's six ZFs on this side. The homeodomains are 60% similar. Homologs are also found in vertebrates where they are expressed in the central nervous system, heart, skeletal muscle, and T lymphocytes and act as transcriptional repressors.

706. RNAi screen to identify genes involved in axon guidance

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Our aim is to understand the molecular basis of axon guidance and fasciculation. Signaling molecules and receptors important for axon guidance and fasciculation are likely to be found among extracellular and cell surface molecules. As a first step to identify novel axon guidance genes the *C. elegans* genome was screened for putative extracellular and cell surface proteins, using representative insect or mammalian proteins or their fragments resulting in a list of several hundred genes. Among them were homologs of known extracellular matrix (ECM) and cell adhesion (CAM) molecules as well as a number of novel proteins forming new families. As a next step to identify axon guidance genes the function for every predicted gene on the list will be investigated by generating transient knockouts using RNAi. In a first approach the quick procedure of direct injection of dsRNAi was performed. The results after dsRNA injection of seven already known axon guidance genes (*unc-5, unc-6, unc-40, unc-44, unc-51, unc-129 and sax-3*) revealed that direct injection of dsRNA does not work reliably with genes involved in neuronal development. Nevertheless about hundred genes among them members of several CAM families including all IgCAMs and cadherins were injected as dsRNA but the previous results were confirmed as no dramatic effects in the nervous system nor in other tissues could be detected. Therefore we will test different ways of dsRNA introduction into worms to find out the most efficient one.

Currently we investigate two methods of RNAi, that have already been successfully used by other labs: feeding worms with dsRNA expressing bacteria and transgenic worms which express dsRNA themselves. To reduce cloning steps we are constructing a vector which can be used for feeding and at the same time for making transgenic worms. The gene of interest is flanked on both sides by a T7 promoter and regulated by the *C. elegans hsp-16* promoter. The T7 promoter gives rise to dsRNA

production in the feeding bacteria. If feeding is not efficient the *hsp-16* promoter allows us to induce ectopic transcription of the target gene in transgenic worms. As axon development takes place in a time window of only a few hours during the second have of embryogenesis the *hsp16* promoter enables us to inactivate a gene in a specific developmental stage so that additional phenotypes due to further functions of the investigated genes are minimized. Genes of interest can be inserted into the vector as PCR fragments in sense and antisense orientation via TA-cloning.Transgenic worms carrying the vector produce after heat shock both single stranded RNAs, which anneal to dsRNA causing transient knock out.

707. The *C. elegans* neurogenin-like gene *ngn-1* has roles in axon outgrowth and guidance

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Neurogenins are a class of *atonal*-like bHLH transcription factors originally identified as vertebrate neuronal commitment factors. In some lineages, neurogenins act at the top of a cascade of bHLH factors required for neuronal development, and knockout mice lack certain classes of neurons. It has recently been proposed that neurogenins have roles in specifying both general neuronal properties and subtype-specific characteristics. We identified a worm neurogenin family member, *ngn-1* (Y69A2AR.d), in a search for *atonal*-like genes that might function with *lin-32* in male tail ray development. Inhibition of *ngn-1* function by RNAi does not cause a ray-missing phenotype in males, as might be expected if *ngn-1* functioned at the top of a ray-determination cascade. Interestingly, however, *ngn-1* function seems to be required for the axonal guidance and connectivity of a subset of neurons in the worm.

Interference with *ngn-1* function by RNAi leads to a weak and variable Unc phenotype. To look more specifically at neuronal development, we have examined the effects of *ngn-1(RNAi)*on the expression of a variety of neuronal reporters. The most pronounced effect was seen with *ttx-3::gfp*, which is expressed in the AIY interneurons: *ngn-1(RNAi)* caused a highly-penetrant short-stop phenotype, in which the AIY axon extends anteriorly, but fails to turn dorsally to enter the nerve ring. Lower frequency defects have also been observed in *glr-1::gfp*-expressing neurons, in which ventral and ring axons sometimes wander or follow lateral or dorsal paths. Defects have also been observed in the RnB axons in the male tail, which show disorganization in the preanal ganglion region, where they synapse onto their

target(s). We have seen no noticeable defects in other classes of neurons, including sensory neurons (as visualized by DiO staining) and GABAergic neurons (by *unc-47::gfp*expression). Moreover, we have not seen a reduction in the number of gfp-expressing cells with any of these markers, as might be expected if *ngn-1* has a role in specifying the identity of neuronal precursors.

To better understand how *ngn-1*might function to specify axonal properties, we have constructed a full-length *ngn-1::gfp* reporter. With this construct, we have seen embryonic expression starting at about 120 min; by late gastrulation, expression is seen in 25-30 cells, at least some of which are likely to be AB descendants. Expression becomes restricted to ~10 head cells by the time of ventral enclosure; this expression persists until the three-fold stage. By hatching, no expression can be detected. A more complete characterization of this expression pattern is underway. The expression we have seen is consistent with two possibilities: *ngn-1* could be acting cell-autonomously to promote neuronal characteristics in a restricted subset of neurons, including AIY, or it could act to specify earlier aspects of neuronal development in a pioneer cell(s) upon which the outgrowth and guidance of other axons depends. Further characterization of RNAi phenotypes and reporter expression should allow us to distinguish between these models.

708. *max-3(ju255)*, a new gene required for motoneuron axon guidance

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Axons are guided to their target area by recognizing specific cues. Both long range guidance cues such as netrins, and short range guidance cues, such as semaphorins and ephrins, have been extensively studied in mammals, flies and worms. However, the signal transduction pathways of these cues are poorly understood. We have isolated a mutation, *max-3 (ju255)*, during the integration of an extrachromosomal array [P*unc-25*::GFP] that is expressed in the RME, VD, and DD motoneurons. In wild type animals, the nerve processes of VD and DD motoneurons are connected by commissures that run from the ventral to the dorsal side of the animal. *max-3 (ju255)* animals show variable, low percentage of commissural axon guidance defects (ranging from wild type to 10%). However *max-3 (ju255)* enhances the axon guidance defects of *max-1 (ju39)* which encodes a novel, conserved intracellular protein (see abstract by Huang et al.). *max-1 (ju39)* alone exhibit 20% commissure guidance defects while *max-1 (ju39); max-3 (ju255)* double mutants show 46% defects. *max-3 (ju255)* is linked to chromosome II. Further mapping and cloning of *max-3 (ju255)* will be presented.

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709. AHR-1 and netrins: Examining potential interactions during neuronal migration

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ahr-1 is the ortholog of the mammalian aryl hydrocarbon receptor, a ligand activated transcription factor (1). *ahr-1:gfp* is expressed in neurons coincident with their differentiation, and *ahr-1*-defective animals have subtle locomotive defects. To better understand the role of *ahr-1* in the developing *C. elegans* nervous system, we analyzed the expression of the *unc-119:gfp*marker in *ahr-1 (ia03)* worms. We discovered that the SDQR interneuron and the AVM touch receptor neuron migrate inappropriately in *ahr-1 (ia03)*animals. Previous studies have described a role for UNC-6 netrin (2) and voltage-gated calcium channels such as UNC-2 (3) in the migration and differentiation of these neurons. One function of*unc-2* may be to modulate response to UNC-6, as cytoplasmic calcium has been shown to mediate responses to netrin in other systems (4, 5). The role of aryl hydrocarbon receptor signaling during the development of the nervous system is not understood, and we are examining potential interactions between *ahr-1*and *unc-6.*

AVM and SDQR are the daughters of the QR.pa neuroblast. In wild type animals, SDQR is born during the first larval stage and migrates anteriorly and dorsally to a position on the ALMR-associated nerve. The SDQR axon projects dorsally to the dorsal nerve cord before proceeding anteriorly. In the majority of *ahr-1 (ia03) a*nimals, the SDQR cell body is misplaced ventrally, and the axon projects anteriorly. This displacement of the SDQR cell body is similar to that seen in *unc-6*-defective animals. However, while the SDQR axon projects anteriorly in *ahr-1* mutants, it projects ventrally in *unc-6*-defective animals. These data suggest testable hypotheses. *ahr-1* and *unc-6* may function in the same pathway to regulate the migration of the SDQR cell body and growth cone. Alternatively, *ahr-1*function may be required for SDQR to respond to guidance cues other than UNC-6 netrins. Since the migration defects in *ahr-1 (ia03)*or *unc-6 (ev400)* worms are partially penetrant, we should be able to distinguish between these models by analyzing double mutants. We will present our progress in these studies and our analysis of AVM defects in *ahr-1 (ia03)* animals at the meeting.

1. Powell-Coffman et al. (1998) PNAS 95: 2844-2849
2. Kim et al (1999) Development 126: 3881-3890
3. Tam et al (2000) Dev. Biol. 226: 104-117
4. Hong et al (2000) Nature 403: 93-98
5. Zheng (2000) Nature 403: 89-93

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710. THE IDENTIFICATION OF PROTEINS THAT INTERACT WITH UNC-73/TRIO IN *C. ELEGANS*

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C. elegans unc-73, also known as *trio* in mammals and *Drosophila* , was the first gene of this family to be identified with a role in axon pathfinding. The axons of many neurons fail to reach their target tissues and often travel along abnormal pathways in *unc-73* mutant animals. Recently it was also discovered that *trio* is required for the correct formation of photoreceptor, CNS and motor axon pathways in *Drosophila*. Experiments in both *C. elegans* and *Drosophila* reveal that UNC-73/Trio function involves signaling through the Rac GTPase and its downstream effectors to regulate the cytoskeletal rearrangements necessary for growth cone migrations.

unc-73 is a complex gene predicted to encode seven overlapping transcripts. Together the intracellular proteins encoded by these transcripts contain, from N terminus to C terminus, eight spectrin-like repeats, a tandem Dbl homology (DH) and pleckstrin homology (PH) domain combination, an SH3 domain, a second DH/PH domain combination, an immunoglobulin domain and a fibronectin type III domain.

DH domains are known to have RhoGEF activity, which is the ability to activate members of the Rho family of GTPases. The first UNC-73 DH/PH domain specifically activates the Rac GTPase while the second domain is specific to Rho. Interestingly, animals with a mutation that eliminates the activity of the first DH domain are uncoordinated (Unc), presumably as a result of axon guidance defects, while a deletion of the exons encoding the second DH domain results in early larval lethality.

Two approaches are being used to identify proteins that interact with UNC-73. First, a genetic screen is being set up based on the observation that overexpression of a construct encoding a truncated form of UNC-73, containing just the DH-1/PH-1 and SH3 domains, in wild-type animals results in a severe Unc phenotype, similar in character to that of the moderate to severe alleles of *unc-73*. An integrated line containing this construct is fully penetrant for the Unc phenotype and will be used in a screen for nonUnc suppressors. Mutations in genes that might be expected to suppress this dominant phenotype include those that act downstream of the Rac GTPase pathway. Mutations in genes upstream of unc-73 that activate the UNC-73 DH1 domain may also suppress. Second, a yeast two hybrid screen was used to identify several proteins that potentially interact with UNC-73. One of the interacting proteins, also confirmed by pull-down experiments with bacterially expressed proteins, is an actin-binding protein. This is consistent with our hypothesis that UNC-73 is involved in the regulation of the actin cytoskeleton.

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711. A Semaphorin and a VEGF system controling morphogenesis in C.elegans.

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The *SEMAPHORINS* are known as chemorepulsive molecules involved in axon guidance (Raper,Curr.Opian.Neurobiol.10,2000). We took a genetic approach to understand the role of *SEMAPHORIN* in cell migration and signalling in *C.elegans*. A *Ce-SEMAII-A* mutant (*mab-20*) was isolated and showed limited axon guidance abnormalities (Roy et al., Dev.127(2000)). Surprisingly, a null allele (*ev574*) exhibits penetrant epidermal cell migration defects affecting morphogenesis and male tail development. We performed an enhancer screen on a hypomorph of *mab-20* allele (*bx61*) and isolated different mutant genes enhancing its morphogenesis defects. One of these mutations (*ev732*) has been genetically mapped to linkage group IV, where a known *CE-PLEXIN-A* homologue (*Y55F3AL.1)* has been previously identified by the GENOME SEQUENCING CONSORTIUM (GSC). By performing PCR-screening on a frozen chemically mutagenized worm library, we isolated a deletion allele in the *Y55F3AL.1* gene (*ev724*) and found it is allelic to *ev732* for the Mab (male abnormal) phenotype. The *C.elegans* male tail consists of a fan shaped acellular structure in which are encasted two bilaterally symmetric sets of nine sensory rays, each ray consisting of a structural cell sheathing two sensory neurons. For these two alleles, ray 1 is anteriorly positioned to the other rays when compared to wildtype (WT) males. A developmental phenotypic characterization revealed that ray 1 precursor cells are generated normally in *Ce-PLEXIN-A* alleles but later on fail to detach from the SET cell syncitia. Interestingly, the same phenotype has been observed in knockout alleles of *Ce-SEMAI-A* (*ev709*) and *Ce-SEMAI-B* (*ev715*) (Val Ginzburg, personal communication). This striking similarity between *Ce-SEMAI-A/B* and *Ce-PLEXIN-A* mutant phenotype, in contrast to *Ce-SEMAII-A* Mab phenotype which consists of fusions between the different rays, suggests that *Ce-SEMAI-A/B* and *Ce-PLEXIN-A* are part of a common genetic pathway controlling ray 1 position. This hypothesis is being tested by constructing double *Ce-SEMAI-A/B* ; *Ce-PLEXIN-A* mutants.

Little is known about the role of *NEUROPILIN/SEMAPHORIN* complexes and their interactions with the *VEGF* (vascular endothelial growth factor) system in vertebrate cardiovascular development (Soker et al., Cell92(1998) ;Miao et al.,JCB146(1999)). As we did for *SEMAPHORINS*, we undertook a genetic approach to understand *VEGF* signaling and function in *C.elegans*. No *NEUROPILIN* like molecule have yet been identified in the *C.elegans* sequenced genome. However, a *VEGF* like ligand (*Y39A3CL.6*) and two *VEGF* receptor (VEGFr1/Flt-1 ; VEGFr2/Flk-1) candidates (*F59F3.1* ; *F59F3.5*) have been identified by the GSC. Using a reverse genetic approach we isolated deletion alleles in both receptor genes (*F59F3.1-ev760* ; *F59F3.5-ev761*). So far, the phenotypic analysis revealed an anteriorly positioned ray 1 in male tail of *F59F3.1*(*ev760)* mutant males. The future work will include determining the expression patterns of both receptors and a phenotypic characterization of the second homologous receptor mutant *F59F3.5*(*ev761)*. We recently obtained a knockout allele of *Y39A3CL.6* which should help characterizing this pathway.

We have shown that *Ce-SEMAI-A/B* and *Ce-PLEXIN-A* affect ray 1 positioning during *C.elegans* male tail development in contrast to *Ce-SEMAII-A* which likely has a broader role in keeping each ray distinct. This is consistent with the fact that *Ce-SEMAI-A/B* are transmembrane anchored semaphorins and that *Ce-SEMAII-A* is a secreted semaphorin, thus likely having a broader action range. The phenotypic similarity observed so far between the *SEMAPHORIN* ligand/receptor system and the *VEGFr1/Flt-1* receptor homologue in *C.elegans* suggests a role for both systems in ray cell migration and patterning. We think that the *C.elegans* male tail

offers a simple system to study cell migration and we plan to use it in order to genetically understand the interaction between these two systems.

712. Integrin function is required for gonad morphogenesis and ovulation in *Caenorhabditis elegans*

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Extension of the hermaphrodite gonad arms in the nematode *C. elegans* depends on migration of distal tip cells along the body wall extracellular matrix (ECM). The migratory path is defined by UNC-6/netrin, UNC-129/TGF-β, matrix metalloproteases, and intracellular signaling through CED-10/Rac. Integrin receptors, which link the ECM and intracellular signaling, play a significant role in cell migration during vertebrate development. To determine the contribution of integrins to gonad morphogenesis, we inhibited the activity of the endogenous βpat-3 integrin. Transgenic nematodes were generated that express an HA-βtail transgene consisting of the βpat-3 transmembrane and cytoplasmic domains connected to a heterologous extracellular domain. High levels of HA-βtail expression in muscle and gonad caused transdominant inhibition of endogenous integrins as shown by induction of a paralyzed, arrested-at-two fold (Pat) embryonic lethal phenotype similar to the *pat-3* null phenotype. Lower levels of transgene expression caused a small percentage of animals to exhibit uncoordinated (Unc) movement, disorganized muscle filaments, reduced egg-laying, aberrant migration of gonadal distal tip cells along the dorsal ECM, and accumulation of oocytes in the ovary. Conserved tyrosine residues in the beta integrin cytoplasmic domain regulate fibroblast chemotaxis in vitro. Mutation of these tyrosines did not alter the effects of HA-βtail on gonad migration, but did increase embryonic lethality during early morphogenesis, suggesting a role for these residues in early development. In order to investigate a possible link between integrins and Rac signaling, we constructed double mutants with HA-βtail transgenic animals expressing low levels of the transgene (mwIs25) and nematodes expressing mutant alleles of either ced-5 (DOCK180) or unc-73 (Rac-GEF), both of which normally activate Rac. *Ced-5* mutant animals are defective in cell corpse engulfment and migration of the distal tip cells.

Weak *unc-73* mutant alleles result in defects in axon guidance and pathfinding. These phenotypes suggest a possible interaction of cell adhesion receptors and Rac signaling. Ced-5 (n1812); mwIs25 hermaphrodites have a dramatic increase in ovulation defects including prevalent endomitotic nuclei, whereas unc-73 (e936); mwIs25 hermaphrodites show defective muscle filament organization, including defects in vulval muscle. These results demonstrate an important role for integrins in gonadogenesis and early morphogenesis and suggest that integrin signaling through Rac contributes to gonad development and fertility in *C. elegans*.

713. *mig-23* encodes an apyrase similar to the mammalian Golgi UDPases

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The migration of gonadal distal tip cells (DTCs) is coupled with the elongation of gonadal tubes and requires an appropriate interaction between the gonadal and body-wall basement membranes. To understand the molecular mechanisms operating in the directional migration of DTCs, we have isolated *mig* mutations affecting DTC migration. The DTCs in *mig-23* mutants often fail to migrate dorsally as in *unc-5*, *-6* or *-40*, or they meander on the lateral hypodermis during dorsal migration as in *mig-17*. In addition to the DTC defects, *mig-23* mutants also show defects in fasciculation of the ventral nerve cord. We cloned *mig-23* and found that it encode a double transmembrane protein of the apyrase family. Apyrases are known as enzymes that hydrolyze both di- and triphosphate nucleotides. MIG-23 is most similar to the Golgi-UDPases which are involved in protein glycosylation in the Golgi apparatus during secretion. Disruption of yeast apyrases *YND1* or *GDA1* is known to abolish *N*- and *O*-glycosylation. In order to determine which cells must express *mig-23* for correct DTC migration, we are conducting mosaic analysis using *sur-5::GFP* (gift from Min Han) as a marker. So far, our results suggest that *mig-23* is required in the C lineage from which some of the body wall muscles, hypodermal cells, and neurons are segregated. Therefore, the DTC migration defects are likely to be non-cell

autonomous. Since the MIG-17 metalloprotease and the UNC-6 Netrin are potential glycoproteins and function non-cell autonomously for DTC migration, they could be the candidates for MIG-23 targets.

714. The role of the egg shell in cell migrations in the early *C. elegans* embryo

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Nematodes were considered to be important paradigms for determinate development where fates are assigned (autonomously) by descent and cells are placed by cell cleavages. In the last 15 years or so it was shown that many inductions occur during early embryogenesis of *C. elegans* demonstrating that a determinate cell lineage can be specified by cell-cell interactions. Recently it was also shown that cells in the worm embryo are not placed by cell cleavages but cell migrations into their terminal positions. Thus the worm may be suited to generally study the mechanisms of cell migration. In initial experiments we tested the role of guiding posts or local cell-cell interactions for cell guidance. Surprisingly neither mechanism alone nor the redundancy of both appears to be involved in navigating cells. To test wether the egg shell confers positional information we analysed the development of egg shell-less embryos. Egg shell and vitelline membrane were removed and embryos were cultured under a 4D-microscope. Since cells can still migrate the shell does not appear to supply any essential information.

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715. A Screen for Suppressors of Distal Tip Cell Migration Defects Induced by Ectopic *unc-129* Expression

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unc-129 was first identified as a gene influencing axon guidance in *C. elegans*. The uncoordinated phenotype observed in *unc-129* mutant animals results from defects in axon guidance of the DA, DB, VD and DD commissural classes of motorneurons. Cloning of the *unc-129* gene showed that it encodes a TGF-beta ligand that functions in dorsal muscles to mediate axon guidance. Biochemical characterization confirmed that *unc-129* is processed and dimerized, consistent with its identification as a TGF-beta ligand. TGF-beta ligands act through heterodimers of type I and type II serine threonine kinase receptors. In *C. elegans* there are two characterized TGF-beta pathways, one affecting body size and male tail formation, the other regulating dauer formation. These pathways use distinct type I receptors, SMA-6 and DAF-1, but share a common type II receptor (DAF-4). No other TGF-beta receptors have been identified in the genome sequence. Mutations in these receptors as well as their downstream mediators, the SMADs, have not been reported to result in uncoordinated phenotypes. In order to explore the possibility that *unc-129* acts through known TGF-beta receptors, we examined receptor mutants for the presence of axon guidance defects. Mutations in the TGF-beta receptors did not cause axon guidance defects. Furthermore, a double mutant of the type I receptors, sma-6 and daf-1, did not display defects in axon guidance. These data suggest that UNC-129 does not act through the traditional type I and II receptors.

In order to identify downstream mediators of *unc-129*, we began a mutagenesis screen for suppressors of ectopic *unc-129*. Ectopic expression of *unc-129* in both dorsal and ventral muscles, which occurs in an *unc-130* mutant, results in distal tip cell (DTC) migration defects. These defects are suppressed by mutations in *unc-129*, which, on their own do not cause DTC defects. This suggests that defects in components of the *unc-129* signaling pathway should suppress the DTC defects induced by ectopic *unc-129* expression. Expression of unc-129 in both dorsal and ventral muscles using the *myo3* promoter to drive expression also results in defects in distal tip cell (DTC) migration, mimicking the unc-130 mutant phenotype. In principle, defects in components of the *unc-129* signaling pathway should suppress the DTC defects of *myo-3::unc-129* transgenic animals. Using a small scale F2 screen, we have isolated 7 mutations that suppress the DTC migration defects of *myo-3::unc-129* transgenic animals. These mutants do not display defects in body size, male tail formation or dauer formation.

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716. ADM-1 AND ADM-2: DISINTEGRIN-METALLOPROTEASES IN CELL MIGRATION

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The ADAM gene family encodes multidomain proteins, which contain a transmembrane domain and both **A D**isintegrin **A**nd **M**etalloprotease domains. Members of the ADAM family were previously shown to be involved in cell-cell adhesion, cell migration and organ formation.

ADM-1 was the first reported ADAM member in *C. elegans*, and was shown to express in several tissues including sperm, plasma membrane of embryonic cells and sheath cells of sensory organs. Western blots using the monoclonal Ab against the cytoplasmic tail of ADM-1 (17B7) and polyclonal Abs against the extracellular domain have previously shown the proteolytical processing of ADM-1 from a precursor to a mature form (1).

Molecular analysis of *unc-71* mutants has shown molecular defects in the *adm-1* locus (Yishi Jin and Xun Huang personal communication*). *unc-71* is involved in one of the pathways of sex myoblast migration and in axon outgrowth (2). Immunoflourescence, immunopercipitation and western blots are being used to analyze the specific protein expression of *adm-1/unc-71* mutants.

The *adm-2* gene was cloned and sequenced in our lab (3). An *adm-2::gfp* construct was shown to be expressed in the whole embryo at early stages. *adm-2::GFP* expression is limited to the nerve ring, ventral and dorsal nerve cord and the CAN neurons in larvae and adults. Recently, a mutant strain carrying a deletion of the *adm-2* gene has become available to us (tm347*). We are conducting genetic crosses with strains carrying a *mec-2::gfp unc25::gfp* and *adm-2::gfp* constructs in order to reveal differences in the pattern of neuron morphology, migration and organization in this mutant strain that appears morphologically wild-type.

We are also using RNAi of *adm-1* and *adm-2* on N2 animals, as well as on both mutants in order to reveal any interaction between these genes. We will discuss our biochemical and genetic studies on the characterization of *adm-1* and *adm-2*.

* We gratefully acknowledge Yishi Jin, Xun Huang and Michael Stern for *unc-71* alleles and Shohei Mitani and Yuji Kohara for the *adm-2* deletion mutant.

(1) Podbilewicz (1996) MBC 7, 1877

(2) Chen and Stern (1998) TIG 14, 322

(3) Adir (1999) 12th International *C. elegans* Meeting: 140

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717. Roles of the ephrin EFN-4 and the semaphorin MAB-20 in embryonic morphogenesis

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We are interested in the process of epidermal morphogenesis. Mutations in the Eph receptor tyrosine kinase *vab-1* and the ephrin ligand *mab-26/efn-4* cause variable defects in gastrulation cleft formation and epidermal enclosure. The most striking embryonic phenotype of *efn-4* mutations is the persistence of an aberrant gastrulation cleft due to defective migrations of neuroblasts following gastrulation. *efn-4* mutations are synthetically lethal with all *vab-1* mutations tested suggesting that they do not function in a simple receptor-ligand linear pathway (see abstract by Chin-sang and Chisholm).

To identify genes potentially functioning in the *efn-4* pathway we examined mutants with phenotypes similar to those of *efn-4*. *mab-20* mutations cause defects in male tail morphogenesis similar to those of *efn-4* mutants (Baird et al, Development 113: 515). *mab-20* encodes a homologue of the vertebrate semaphorin 2a and is required for the correct formation of P cell contacts during epidermal morphogenesis (Roy et al, Development 127: 755). We have analyzed the early embryonic phenotypes of the null allele *mab-20(ev574)* and a weaker allele *e819* using 4D microscopy. Like *efn-4* mutants, *mab-20* mutants display variable defects during gastrulation cleft formation. 34% of embryos displayed gastrulation clefts that persisted until ventral enclosure, followed by failure of epdiermal enclosure. A small number of animals had disorganized gastrulation clefts, were able to enclose ventrally, then ruptured at the 2 or 3-fold stage or were unable to elongate past 2-fold. 23% of embryos displayed a larger than normal gastrulation cleft but were able to enclose and hatch.

Phenotypic analysis of *mab-20; efn-4* double mutants suggests that the *efn-4* and *mab-20* mutations are additive for later defects. However, defects in gastrulation cleft closure and epidermal enclosure are not additive in the double mutants. Taken together, our data suggest that *mab-20* and *efn-4* might function in a common or antagonistic pathway to regulate neuron migrations during gastrulation cleft closure but that they have different functions later in embryogenesis.

The additive effects of *mab-20; efn-4* double mutants show that the synthetic lethality observed between *efn-4* and *vab-1* mutations is specific. We therefore screened for mutations that synergize with a *vab-1* kinase allele. From a pilot screen we identified a mutation defining a new locus, *vab(ju105) X*. *ju105* mutant larvae have posterior morphological defects and are movement impaired. *ju105* mutant embryos have a low penetrance of gastrulation cleft defects and often fail to elongate past 2-fold. The characterization and mapping of *vab(ju105)* will be presented.

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718. A SCREEN FOR REGULATORS OF BAR-1 MEDIATED WNT SIGNALING IN THE Q NEUROBLAST LINEAGE

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The Wnt signaling cascade plays an important role in many developmental processes by regulating the expression of specific Wnt-responsive target genes. Furthermore, inappropriate activation of the pathway is found in many types of cancer. The interaction of Wnt with its receptor Frizzled (Fz) leads to the inhibition of a complex consisting of the tumor suppressor protein APC, the scaffolding protein Axin and the kinase GSK3beta. The effector beta-catenin, which is normally targeted for degradation by this complex, translocates into the nucleus where it interacts with Tcf transcription factors to activate Wnt target gene expression. In *C. elegans*, a canonical Wnt pathway consisting of EGL-20/Wnt, LIN-17/Fz, BAR-1/beta-catenin and POP-1/Tcf activates the expression of target genes like the homeobox gene *mab-5* in the Q neuroblast lineage (1). *mab-5* specifies the direction of the migration of the Q daughter cells. Thus, *mab-5* is expressed in the Q cell lineage on the left side of the animal (QL) and induces posterior migration of these cells. *mab-5* is however not expressed in the right Q cell lineage (QR), which results in migration in the opposite, anterior direction. This asymmetric *mab-5* expression is the result of a difference in sensitivity to the EGL-20/Wnt signal between the QL and QR cells (2). We are interested in the mechanism and regulation of the Wnt pathway. In a yeast-two-hybrid screen we have isolated a protein, C37A5.9, which specifically interacts with BAR-1. C37A5.9(RNAi) results in the posterior migration of both the QL and QR daughters, a phenotype that is also seen in *mab-5* gain-of-function mutants and when a constitutively active form of BAR-1 is overexpressed. In addition, this phenotype is similar to the phenotype of the known negative regulator *pry-1* (3). We have shown that C37A5.9 is mutated in the *pry-1* allele mu38 (see abstract Rik Korswagen). C37A5.9 contains a DIX and RGS domain, and is similar to fly and vertebrate Axin. We propose that C37A5.9 is a functional Axin homologue and that a conserved APC/Axin/GSK3beta-like complex may regulate BAR-1 stability in *C.elegans*.

To isolate additional regulatory components of the Wnt pathway, we screened for mutations that show a phenotype which is similar to that of C37A5.9/*pry-1*. We used the final positions of the Q daughter cells as a readout. To rapidly assess the positions of the Q cells, we used an integrated *mec-7::gfp* reporter transgene which is specifically expressed in the touch receptor neurons (including the Q daughter cells AVM and PVM). We isolated 10 independent mutations that show posterior migration of both the QL and QR daughter cells in an *egl-20*/Wnt mutant background. Complementation tests and preliminary mapping results suggest that we have hit at least 5 genes, which we are currently mapping. This approach should identify new repressors of the Wnt pathway and provide us with further insight in the mechanisms of Wnt target gene regulation.

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719. How does *mig-13* guide anterior migrations?

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mig-13 is a guidance factor that promotes cell migrations in the anterior direction (Sym et. al., 1999). Previous work demonstrated that *mig-13* is required for the anterior migrations of the QR descendants and the BDU neurons (Sym et. al., 1999). Consistent with the role of *mig-13* in anterior migrations, we have also found that *mig-13* also directs the anterior migration of the distal tip cell (DTC) in the posterior gonad arm during late L3.

We are taking several approaches to understand how *mig-13* can guide many anterior migrations. *mig-13* encodes a novel transmembrane protein containing putative protein-protein interaction domains: a CUB domain and a LDL-receptor repeat in the extracellular region as well as a proline-rich domain in the intracellular region (Sym et. al., 1999). We have examined the function of these domains in MIG-13 by deleting them and assaying the *in vivo* activity of the resulting MIG-13 construct. Our data suggests that a MIG-13 construct lacking the intracellular domain can confer partial function in directing the QR descendants to the anterior.

Previous mosaic analysis revealed that *mig-13* acts non-autonomously to direct the migrations of the QR lineage (Sym et. al., 1999). To determine where *mig-13* expression is sufficient to guide the migrating cells, we have expressed *mig-13* in different sets of tissues, as well as in specific subsets of cells. Expression of *mig-13* in all neurons but not any of the other tissues we have tested rescues the QR descendant and DTC migrations in *mig-13* mutant animals. This suggests that *mig-13* might function in neurons.

To pinpoint the cells in which *mig-13* acts, we are also refining previous mosaic analysis. For the guidance of the QR descendants, we have narrowed the focus of *mig-13* activity to the AB.pr (and possibly the AB.pra) lineage. We have also isolated several mosaic animals that have wild-type QR descendant migrations but mutant DTC migration in the posterior gonad arm, raising the possibility that the migrations of the QR descendants and the posterior DTC are guided by different cells.

Reference:

Sym M, Robinson N and Kenyon C. Cell, 1999 Jul 9, 98(1):25-36.

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720. An *in vitro* System for Studying Cell Movements in Early Embryos

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The proper migration of cells during embryogenesis is crucial for development. Gastrulation is the first major cell movement of the developing nematode *C. elegans*, yet surprisingly little is known about the mechanisms of this movement. We wished to use a blastomere culture system (1) to study gastrulation movements *in vitro*, since isolated cells can be used to address questions that cannot be addressed in intact embryos. As a first step, we asked whether or not the physical constraint of the eggshell and the underlying vitelline membrane were necessary for gastrulation. It was previously observed that gastrulation does not require the eggshell and that gastrulation-like movements may also occur in devitellinized embryos (1,2). We confirmed these observations by removing both the eggshell and the vitelline membrane and filming the embryos using a 4D microscope. Gastrulation begins with the ingression of the two E daughters, Ea and Ep, toward the center of the embryo, followed by MSpp, P_4, MSpa, and D. We found that ingression of Ea and Ep occurs *in vitro* consistently (6/6 embryos), and at the time of normal gastrulation, suggesting that the *in vitro* system can be used to study normal gastrulation. Previous cell lysis experiments showed that an intact AB cell was not required for gastrulation (3). We wanted to further delimit the requirements for gastrulation by determining whether the progeny of an isolated, cultured P_1 cell could gastrulate. By isolating the P_1 blastomere and filming its development, we saw that gastrulation movements occurred even when only P_4 and MSpp contacted the E_2 cells. In addition, these results indicated that the normal geometry of the embryo was not essential for proper gastrulation. As with the devitellinized embryos, movements in the P_1 isolates occurred at the same time as in normal embryos. Taking advantage of the better optics resulting from reduced cell numbers, we wanted to begin to determine which cells were crawling by tracing and analyzing cell shapes in the P_1 isolates. Preliminary evidence showed that Ea and Ep remained roughly spherical, while P_4 and MSpp cells changed their shape during the time of gastrulation, making extensions towards each other, over Ea and Ep. These results suggest that the E cells do not crawl into the center of the embryo during gastrulation; rather, they appear to be pushed in by P_4 and MSpp. *In vitro* blastomere culture presents a useful system for studying cell migration in early development. We are currently using this system and gastrulation-defective mutants to test hypotheses relevant to how gastrulation is regulated.

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721. CHARACTERISATION OF MUTANT ALLELES INVOLVED IN C.ELEGANS CUTICLE FUNCTION

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C. elegans hypodermal cells synthesise five distinct cuticles through the nematode life cycle: the first one within the egg prior to hatching; the remainder during each moult. The animal form is determined by the cytoskeletal organisation of hypodermal cells during elongation of the embryo. After the elongation process, the cytoskeleton becomes disorganised and the cuticle assumes the role of maintaining the shape of the nematode. We are interested in identifying genes essential for synthesis of the nematode cuticle.

Our lab has performed a forward genetic screen to isolate embryonic lethal mutants that complete the elongation process but then collapse, failing to synthesise the L1 cuticle. We are currently characterising one mutant allele, named ij015, generated from this screen. Homozygous embryos for ij015 express dpy-7_GFP and col-12_GFP constructs suggesting that transcription of collagen genes occurs but an intact cuticle is not assembled. We have positioned this allele on the genetic map and rescued the mutant phenotype by a single cosmid clone. Detection of the coding sequence contained in the positive clone responsible for the observed phenotypic rescue is on progress.

In an alternative approach, we have identified a C. elegans gene predicted to encode a bZIP transcription factor that with RNAi phenocopies the elongation then collapse phenotype we associate with cuticle synthesis failure. This transcription factor appears to be expressed in hypodermis of embryos and later stage animals. We have also found a recessive mutation causing a similar phenotype to that obtained with RNAi. This together with the observed spatial expression pattern indicates this transcription factor may have a role regulating expression of genes involved in the formation of the first cuticle.

722. The structural role and interactions of the DPY-7 cuticular collagen in the exoskeleton of Caenorhabditis elegans.

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The *C.elegans* cuticle (exoskeleton) is a multi-layered extracellular matrix consisting predominantly of small collagen-like proteins that are extensively cross-linked. The cuticle is synthesised by the underlying hypodermis 5 times throughout the lifecycle of the animal. Previous investigations have indicated that the cuticular collagen DPY-7 has an important structural role within the complex architecture of the exoskeleton. Using immuno-fluorescence we have shown that this collagen locates to circumferential stripes within the cuticle. There is one DPY-7 containing stripe per cuticular annulus. We are utilizing a range of techniques (including immuno-electron microscopy and scanning electron microscopy) to further elucidate the precise localisation of this protein within the cuticular matrix.

Using a mono-clonal antibody specific for DPY-7, we can detect the protein on western blots from cuticle extracts. The DPY-7 detected is much larger than monomer. Cuticle collagens are crosslinked by non-reducible bonds during formation of the cuticle. The various high molecular weight species we detect will be cross-linked forms of DPY-7. By a combination of genetic and immuno-fluorescent means we have shown that DPY-7 physically interacts with at least three other cuticle collagens. We are interested to determine if the cross-linking of DPY-7 we detect by westerns is with the same collagen molecules we know by other means DPY-7 interacts with. We are attempting to purify the cross-linked species with the intention of identifying cross-linked partners.

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723. Localization of innexins

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The innexin family of proteins, first identified in *Drosophila* and *C. elegans,* is functionally equivalent to the vertebrate connexin family of gap junction channel proteins, although there is no significant amino acid sequence similarity between innexin and connexin proteins. Members of both families mediate the formation of intercellular channels, with similar properties, when expressed in paired Xenopus ooctyes. Gap junctions allow small molecules, such as ions, metabolites and second messengers to pass directly between coupled cells and thus provide direct intercellular communication. We are particularly interested in the roles that gap junctions may play during embryogenesis and in specifying the pattern of nervous system wiring. As a prelude to investigating their developmental roles, we are examining the expression patterns of the worm innexins, through GFP translational fusions and staining with specific antibodies. In addition, we are investigating mutant, RNAi, and overexpression phenotypes for several innexin genes.

There are 25 innexins that have been identified by the Genome Sequencing Consortium. cDNAs have been reported for 19 of these genes, and 4 genes without corresponding cDNAs are expressed as GFP fusions, indicating that few if any innexin loci represent pseudogenes. We have generated antibodies or made GFP fusions for 17 of the innexins and have observed expression patterns for 13 of the genes. Mutants are available for five innexin genes. *unc-7*, *unc-9* and *eat-5* all appear to have defects in neuronal or muscle electrical conduction. *inx-3* and *inx-13* (see accompanying abstracts) are embryonic lethal and L1 lethal, respectively. To date, one other innexin has a phenotype by RNAi, and three appear to have defects resulting from overexpression.

Some generalities are emerging from the analysis of expression patterns. Most tissue types, and probably individual cells, express more than one innexin. Some innexins are expressed widely, while the expression of others is restricted to a few cells. We have also observed a potential functional pairing of innexins. In one instance, *inx-12* and *inx-13*, which likely arose by a tandem duplication followed by divergence (39% identical), have the same RNAi phenotype. In a second case, INX -2 and INX-3, whose genes are not in close physical proximity, co-localize and interact in some fashion. In *inx-3* mutant embryos INX-2 is difficult to detect and may be unable to form punctate plaques on plasma membranes.

With regard to our interests in the contributions of innexins to embryogenesis and to the specificity of neuronal wiring, we have identified 3 innexins that are expressed as early as the 2-cell stage of embryogenesis. Loss of function of one of these innexins, INX-3, has no obvious effect on early embryogenesis; future experiments will test the possibility that another innexin(s) compensates for its function. In the nervous system at least 6 innexins are expressed, with varying localization patterns. It will be interesting to determine whether the particular innexin(s) expressed by a neuron contributes to the specificity of its wiring.

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724. Gap junction protein INX-13 is essential for excretory cell function.

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In invertebrates gap junction channel proteins are encoded by innexin genes. *In C. elegans* there are 25 innexins present in the genomic sequence. We are investigating the roles that gap junctions may play in embryogenesis and have been analyzing *inx-3*, which is expressed strongly in embryos. Of the remaining 24 innexins, *inx-13* is the most similar to *inx-3* (42% identity, 60% similarity over 371 amino acids), and so we examined the pattern of *inx-13* expression to determine whether it might also be expressed during embryogenesis, possibly in association with *inx-3*. Antibodies raised against a peptide representing the carboxyl terminus of INX-13 were used to examine INX-13 expression. INX-13 is expressed in embryos and is detected at the plasma membranes of contacts between hypodermal cells, in a pattern similar to that observed for the zonula adherens protein JAM-1, except that INX-13 outlines hypodermal cells discontinuously with large puncta. This expression pattern overlaps with that of *inx-3*. Postembryonically INX-13 continues to be expressed in seam cells. Additionally, INX-13 is strongly expressed in the excretory cell throughout all larval and adult stages, and is expressed in the developing vulva and spermatheca.

An *inx-13* deletion mutant was isolated by the Gene Knockout Consortium (Univ. Oklahoma). *inx-13(ok236)* has a 1.6-kb deletion that removes 2 exons, including the predicted fourth transmembrane domain of INX-13. Homozygous *inx-13(ok236)* mutants all arrest at the L1 stage. There is no embryonic lethality associated with this mutation. It appears that the hypodermis and/or body cavity of an L1 mutant animal fills with fluid, and the animal arrests as a dead rod. This phenotype is similar to the description of animals after ablations of the excretory pore, duct, or excretory cell (Nelson and Riddle. 1984. *J. Exp Zool.* 231:45-56.), components of the excretory/secretory system that functions in osmoregulation. Other cell types, such as pharyngeal, intestinal, and neuronal cells, do not appear to be affected. The RNAi phenotype for *inx-13* is similar, though not as severe -- RNAi animals often survive to later larval stages before they assume a dead rod phenotype. Interestingly, the RNAi phenotype for *inx-12*, which lies only 2 kb from *inx-13*, is similar, raising the possibility that these two innexins function together. [*inx-12* has only 39% amino acid identity with *inx-13* and so we do not think that RNAi directed against *inx-12* also affects *inx-13*.] Extensive gap junctions between the excretory canals and the hypodermis have been described (Buechner et al. 1999. *Dev. Biol.* 214: 227-241). We therefore hypothesize that INX-13, and possibly INX-12, contribute to the formation of the gap junctions formed between the canals and hypodermis and play an essential role in osmoregulation. Because homozygous *ok236* embryos show no apparent hypodermal defects, INX-13 likely plays a redundant role during embryogenesis. We know of at least three other innexins expressed in the hypodermis at this time, including *inx-3*, and it is possible that any of them may compensate for loss of INX-13. We have not yet addressed the potential role of INX-13 during vulval and spermathecal development.

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725. Characterization of *C. elegans* claudins

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Homologues of claudin, major integral protein of mammalian tight junction with four span transmembrane structure, were found in the genomic data base of *C. elegans*. Among them, CLC-1(C09F12.1) was discovered by a blast search with mouse claudin-6, it was found later that homology is also present with mouse claudin-7 and human claudin-14. It has in its first loop two Cys residues that are conserved among mammalian claudins. CLC-2 (T05A10.2) was found by a blast search with CLC-1, and has 33% identity (70% homology) with CLC-1, although no significant homology was found with vertebrate claudins so far known. CLC-3 (ZK563.4) has homology with mouse claudin-10 and cattle claudin-16. CLC-4 was found with a blast search with mouse claudin-6, but it was thought to be an eight span transmembrane protein at that time. Recently, it was found that this gene (C01C10.1) encodes two separate proteins, and the down-stream operon (C01C10.1b) was reported to encode a Gas3/PMP-22 homlogue (Agostoni E.et al., Gene, 234, 267-274, 1999). Product of the other operon (C01C10.1a) was not characterized well. Homology with claudin-6, -7, and -9 was found with this product, therefore named as CLC-4. These four claudin homologues have either PMP-22/EMP/MP20 motif or transmembrane four signature or both, and most of them have srg integral membrane protein motif. Furthermore, two conserved Cys residues are present in all *C. elegans* claudins. Interestingly, many of vertebrate claudins have one or two of these motifs or signature. Although most of vertebrate claudins has CLAUDIN3 signature, no such signature was found in the nematode claudins.

All of coding sequences of the nematode claudins were isolated from cDNA libraries, therefore they are all expressed in the worm. Some ESTs were reported for CLC-1 and -3, previously. Expression of these claudins was studied with GFP-tagged molecules. All of claudins is expressed in spermatheca, intestine and hypodermis. CLC-1 and -4 were expressed strongly in pharynx, and sometimes localized at cell-cell junctions. They are also seems to be expressed in excretory-secretory system. CLC-1::GFP is also expressed at cell-cell junction of vulva. Localization of CLC-1 is under study with HA-tagged molecule. Preparation of antibodies against the nematode claudins was very difficult so far. But, affinity purified antibodies raised against loop 1 of CLC-1 seem to be useful for CLC-1 detection in the worm. Results with these antibodies were very similar to those obtaind with GFP-tagged CLC-1. To see if these claudins function as mammalian claudins do, *i.e.* barrier function, penetration of TRITC-dextran (MW=10,000) was checked after injection of dsRNA's (RNAi). Experiments with full length CLC-1 dsRNA showed that barriers for the high molecular weight dye were damaged by RNAi, in other words, penetration of the dye to pharynx and some other tissues were observed. Similar experiments with other claudins did not detect any barrier damage. This is because the dye only goes into entrance of intestine under normal condition, therefore, we tried weak osmotic shock to deliver the dye to entire intestinal lumen and excretory-secretory duct system. Under this condition, control injection of dsGFP did not result in penetration of the dye to other area of the body. On the other hand, RNAi with the combination of CLC-1 and -4 RNA's resulted in penetration of the dye to 72% of the worm from pharynx, intestine and vulva (or from excertory-secretory system) to the body, whereas barrier was damaged only 40% of worm with a combination of CLC-3 and -4. RNAi effects with other combination of CLC's will be reported, and effects of RNAi to the retention of the sperm in spermatheca will be studied. Accordingly, claudins of *C. elegans* seems to function as barrier at least partly. Other functions, if any, will be surveyed. We would like to thank excellent technical assistance of Miss. Akiko Kamamoto, without her help this project could not be completed.

726. Characterization of the leucine-rich repeat protein family in *Caenorhabditis elegans*

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Leucine-rich repeats (LRRs) are short, amino-terminal sequence motifs which characterize a superfamily of proteins. Although the individual members of this protein family vary in function and cellular localization, most seem to be involved in protein-protein interactions. *Caenorhabditis elegans* possess 23 genes which appear to encode proteins containing an extracellular amino-terminal LRR domain. Some of these are apparent orthologs of molecules implicated in activities such as neuronal development. However, the functional significance and expression patterns for most of the predicted LRR gene products have not yet been determined. The simple multicellular architecture of the *C. elegans* nematode will provide an ideal context in which to designate roles for the putative LRR gene products and, additionally, to characterize the apparent orthologs more completely.

727. The *C. elegans* Numb homologue is expressed on the baso-lateral surfaces of polarized epithelia and interacts genetically with *par-3* and *unc-101*.

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We are interested in understanding the role of the *C. elegans* homologue of the Drosophila gene numb. In flies, Numb is required for cell fate specification during development of the peripheral nervous system and the development of certain muscles. The protein is segregated asymmetrically at several cell divisions within the sensory organ precursor cell lineage and this asymmetric segregation is required for correct cell fate specification. The biochemical function of Numb, however, remains largely obscure. Previously we reported that the *C. elegans num-1* locus generates two transcripts, *num-1A* and *num-1B*, that are generated by distinct tissue specific promoters. The transcripts have 3 3' exons in common but differ at the 5' termini. *num-1A* encodes a protein containing a PTB domain that is 70% identical to that in Drosophila Numb. *num-1B* encodes a novel protein. We generated GFP fusion constructs specific for the A and B forms. NUM-1A::GFP was found to be expressed in most epithelial tissues. NUM-1B::GFP was expressed in the excretory cell and four cells in the uterus. At the subcellular level, both proteins localized to the baso-lateral cell surface, its apical most boundary often colocalizating with the adherens junctions (in the uterine cells, however, NUM-1B was cytoplasmic). Hermaphrodites overexpressing NUM-1A display several gross abnormalities, all of which are consistent with defects in epithelial cell function. These include the formation of vesicles in the cytoplasm of intestinal cells, ectopic vulval induction, abnormal outgrowth and morphology of the excretory canals. In addition, animals overexpressing NUM-1A are strongly Egl and weakly Dpy. Worms overexpressing NUM-1B display defects in the outgrowth of the excretory canals. In transgene-mediated RNAi experiments 70% of hermaphrodites carrying an RNAi construct specific for the A form arrested as embryos. However, in RNAi experiments in which feeding or injection protocols were used,

no mutant phenotypes were observed. In hermaphrodites harbouring an RNAi construct specific for the B form, outgrowth of the excretory canals was abnormal. The baso-lateral localiztion of NUM-1 and the presence of vesicles in the intestines of animals overexpressing NUM-1 suggested to us that the protein might be required for correct apical-basal polarity or for correct polarized protein transport in the cells in which it is expressed. To test these ideas we first examined genetic interactions between *num-1A* and *par-3*. *par-3* encodes a PDZ-domain protein that regulates asymmetric cell division in the early *C. elegans* embryo. PAR-3 is also expressed on the apical surfaces of intestinal cells. A Drosophila homologue of PAR-3, Bazooka, is involved in the establishment of apical-basal polarity. In *C. elegans, par-3* null mutants are maternal effect lethal; *par-3* single mutants lacking just zygotic activity [*par-3(zyg)*] become fertile adults. We found that 84% of *par-3(zyg)* hermaphrodites that also overexpressed NUM-1A accumulated vesicles in the hypodermis and arrested at the L4-to-adult moult. Neither *par-3* single mutants nor worms that overexpress NUM-1 showed such defects. In mammalian cells, the protein mu1B, a clathrin-associated protein has been implicated in the transport of proteins to the baso-lateral surfaces of polarized epithelial cells. A homologue of mu1B in *C. elegans, unc-101* has been suggested to function in intracellular protein trafficking in the worm. For example *unc-101* is thought to be required for the correct trafficking of LET-23, a baso-laterally localized protein that functions as the receptor for the inducing signal during vulval development. We found that *unc-101(m1)* hermaphrodites that also overexpressed NUM-1A were completely vulvaless. Vulval induction in *unc-101* single mutants is wild type. We are presently conducting further experiments to investigate possible roles for NUM-1 in the establishment or maintenance of epithelial cell polarity in the worm.

728. Keep Your Insides In and Your Outsides Out: VWFA Domain Proteins in the *daf-2* Cuticle and the Male Intestine

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Caenorhabditis elegans is an ideal model system for the study of extracellular matrix biology as the *C. elegans* genome contains approximately 177 collagen genes, involved in the cuticle, basement membrane and other matrices. However, the proteins responsible for organising these collagens and linking them to cells are not known. In human extracellular matrices, a number of human collagen-binding proteins and collagen receptors contain VWFA domains. These are 200 amino acid modules which are directly responsible for collagen-binding function through a conserved mechanism. VWFA domain proteins of C. elegans are therefore candidates for collagen binding and organising proteins and we have been studying them to determine their role in *C. elegans* extracellular matrix biology. Here we report the findings for two genes, M01E10.2 and R10H10.3

M01E10.2: 5’ and 3’ RACE and RT-PCR showed the gene structure to be as follows; leader sequence, VWFA domain, cuticulin/ZP domain, transmembrane region and cytoplasmic tail. This differes from the predicted sequence, which gives an additional mucin-like domain detween the N-terminus and the VWFA domain. RT-PCR using cDNA from 2 hr intervals across the life cycle showed expression peaking at the inter-moult stages, similar to that seen for cuticle collagens. RNAi in N2 worms gave no apparent phenotype, but RNAi in *daf-2* strain gave dumpy adults. This effect was restricted to *daf-2* as other *daf* strains did not produce dumpy adults. M01E10.2 therefore appears to be a cuticle component. Significantly, untreated *daf-2* adults are longer than N2s, suggesting that the *daf-2* adult may synthesise different cuticle components to the N2 adult. These observations provide a direct link between the *daf* pathway and cuticle synthesis and provide an opportunity to examine this in detail.

R10H10.3: This gene has the following structure; leader sequence, CUB domain and VWFA domain. RT-PCR showed that the gene is expressed constitutively across the life cycle. Transgenic worms carrying a promoter-GFP gene fusion showed that the gene is expressed in the intestine, and that expression is highter in males than hermaphrodites. This may be due to the presence of male-specific gene enhancers or sex-related differences in the intestine. R10H10.3 could be involved either in the assembly of intestinal epithelium basement membrane or in the assembly of the intestinal glycocalyx.

The characterisation of these two genes shows that both probably form part of the extracellular matrix; however, their precise functions are likely to be quite different. This exemplifies the diverse roles that homologous protein modules can evolve to fulfil, and indicates that similarity at the sequence level does not always imply a straightforward similarity of function.

729. Intexin (*itx*), a novel member of the Caspr family delineates the basolateral domain of intestinal cells and is found in ensheathing glia in the head

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Casprs are members of the Neurexins superfamily, a group of transmembrane proteins that mediate cell-cell interactions in the nervous system. The Caspr proteins mediate axon-glia and glia-glia interactions and in vertebrates, they are also found in other tissues in addition to the nervous system. Two Caspr proteins, encodes by WO3D8.6 and F20B10.1 are found in *Caenorhabditis elegans*. Both proteins contain several EGF and Laminin-G domains in their large extracellular region but lack the characteristic discoidin-like domain that is found in all mammalian Casprs. A transgenic line expressing GFP reporter construct fused to the promoter region of W03D8.6 revealed that this gene is expressed exclusively by epithelia of intestinal cells, as well as in socket and sheath cells of the inner labial sensilla. Whole mount immunofluorescence staining confirmed that W03D8.6 gene product is expressed by intestine epithelia, where it is confined to the basolateral membranes. We therefore termed W03D8.6 Intexin (*itx*).

Disruption of Intexin expression by RNA interference (RNAi) was examined in wild type N2 strain, as well as in 2 other transgenic lines: jam-1::GFP (JcIs1) and cdh-3::GFP (arIs51). Preliminary characterization of the RNAi effect revealed that the abrogation of Intexin expression did not disrupts organogenesis or integrity of the intestine at any developmental stage. Nevertheless, in JcIs1 the expression of jam-1::GFP, was dramatically impaired such that no GFP could be detected in these worms. This effect was specific and was exclusively observed in the gut epithelia. The expression of jam-1::GFP in other epithelial tissues such as

the hyodermis and pharynx remained intact. In contrast, the expression of cadherin3 was not affected by Intexin RNAi. These results suggest that Intexin may be required to delineate the apico-lateral domain of gut epithelia where adherence junctions typically assemble. Ultrastructural studies are underway to study the affect of intexin RNAi on the morphology of these junctions, as well as to determine the exact localization of Intexin in ensheathing glial cells.

730. Exploring the role of *zyg-11* in one cell stage embryos

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In *C elegans*, polarity along the antero-posterior (AP) embryonic axis is established soon after fertilization by a sperm component that defines the posterior pole of the animal. It has been shown recently that this sperm component likely corresponds to astral microtubules nucleated from the paternally contributed centrosome. This initial cue then results in the polarized distribution of several of the Par proteins along the AP axis; for instance, PAR-3 is restricted to the anterior cortical domain, PAR-2 to the posterior cortical domain. This will lead in turn to the asymmetric division of the one cell stage embryo into a larger anterior blastomere and a smaller posterior one.

We are exploring whether embryos derived from homozygous *zyg-11* mutant hermaphrodites (hereafter referred to as *zyg-11* mutant embryos) may be defective in setting up polarity along the AP axis. *zyg-11* mutant embryos have a number of abnormalities detectable by DIC microscopy, including cases of equal first division and apparent reversal of polarity, with a smaller anterior blastomere and a larger posterior one. Moreover, meiosis II is significantly prolonged and not completed properly in mutant embryos. ZYG-11 encodes a protein with no obvious functional motifs but with clear orthologues in other metazoans. We are investigating the nature of the *zyg-11* mutant phenotype in more detail using live imaging with several GFP fusion proteins, as well as by determining the distribution of PAR proteins and other markers of AP polarity. In addition, we have raised an antibody against ZYG-11 and are in the process of generating a ZYG-11::GFP fusion to determine ZYG-11 distribution and dynamics. These approaches taken together should help us elucidate whether *zyg-11* plays a role in setting up AP polarity in the one cell stage embryo.

731. Unraveling the role of calcium signaling in the early *C elegans* embryo

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Calcium functions in a multitude of cellular processes as a second messenger. We are interested in determining the possible roles that calcium plays in determining organizational changes in the early embryo. Specifically we are analyzing the effects of manipulating calcium signaling on embryo polarity and cytokinesis. One possible membrane-based signaling pathway is through inositol triphosphate (IP3), a pathway that triggers calcium release. The *C. elegans* genome contains a single IP3 receptor, *itr-1* (Baylis et al., 1999. *JMB* 294:467). Although this receptor has a role in the adult (Santo et al. 1999. *Cell* 98:757) and during morphogensis (C. Thomas, pers. comm.), little has been described about its role in the very early embryo. Analysis of living embryos possessing a weak allele (*jt73* -Santo et al. 1999) of this gene reveals a failure in the completion of cytokinesis in the early cleavage stage. This failure occurs at the final end of cleavage, suggesting that IP3 signaling may play a role in regulating changes in cytoarchitecture necessary for completion of cytokinesis. Additionally, we are investigating the use of caged compounds in *C. elegans* embryos, including caged IP3, to assess the effects of altering calcium signaling on cytokinesis and embryo polarity.

732. THE *C.ELEGANS* PROTEIN PHOSPHATASE 1 HOMOLOGUE(*CE*PP1) IS ESSENTIAL FOR EMBRYOGENESIS AND POST-EMBRYONIC DEVELOPMENT

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Protein dephosphorylation functions as modulator in many biological events. To elucidate the regulatory aspects of protein dephosphorylation, we identified and cloned the gene encoding protein phosphatase 1 homologue(*Ce*PP1), which was found within cosmid F56C9 located LGIII. From the expression pattern of the *Ce*PP1::GFP fusion protein and in situ hybridization, the gene is ubiquitously but especially strongly expressed in gonad, neuron, muscle and intestine. Microinjection of double stranded RNA of this gene induced an embryonic arrest at multicellular stages.

To characterize in more detail the function of gene(*pp1-1*) encoding *Ce*PP1, we isolated a mutant(*tm291*) by TMP/UV method, which had a deletion of exon VII of *pp1-1*. Hermaphrodites heterozygous for the *pp1-1* mutation produce homozygous embryos which can hatch and grow to adulthood. However, progeny produced from hermaphrodites homozygous for its mutation almost (96%) die at multicellular stages during embryogenesis. DAPI staining of embryos revealed that the presence of chromatin bridge and multinucleated cells, suggesting the gene being implicated in chromosome condensation and segregation. However, the staining of the mutant with antisera against

α-tubulin was normal. These observations are reminiscent of the *air-1*/aurora-related kinase gene defective mutants(Schumacher *et al*., Dev. 125 4391, 1998) or nuclear lamin(*lmn-1*) defective embryos(RNAi)(Liu *et al*., Mol.Biol.Cell 11 3937, 2000). The defect of karyokinesis in the *pp1-1* mutants may be elucidated by the following results; *Ce*PP1 expressed in HEK 293 cells catalyzes Pi release from phospho-histone H3 phosphorylated by PKA. The morphological abnormality of homozygous mutant at the post-embryonic stage is also observed including vulva and gonad formation. These phenotypes are also reminiscent of *stu-7/air-2* defective mutants (Woollard and Hodgkin, Mech. Dev., 82 95, 1999). The *pp1-1* gene was strongly expressed in the AFD and ASE amphidal neurons. We therefore are ongoing to elucidate the implication of the gene in both neurons. As mutant showed normal movement, we can examine chemotactic and thermotactic behavior which were sensed by ASE and AFD, respectively. Preliminary studies showed that the mutants displayed normal attraction to NaCl, but abnormal thermotactic behavior.

733. Evidence for cell-cycle regulation of the A/P polarity machinery in the 1-cell embryo

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Polarity along the anterior-posterior (A/P) axis is established in the 1-cell stage shortly after fertilization and is determined by the sperm, whose position specifies the posterior end of the embryo (Goldstein and Hird, 1996). Last year, Matt Wallenfang in our lab and Kevin O'Connell in John White's lab showed that microtubules emanating from the sperm asters provide the asymmetric cue that polarizes the embryo (Wallenfang and Seydoux, 2000, O'Connell et al, 2000). During interphase, these microtubules remain concentrated in the posterior end of the embryo, consistent with their polarizing role. During mitosis, however, the sperm asters are recruited to form the first mitotic spindle and microtubules are no longer confined to the posterior. How then is polarity maintained during mitosis?

To answer this question, we have used time-lapse microscopy to analyse the localization dynamics of GFP:PAR-2 and GFP:PAR-6 fusions in wild-type, par-2(RNAi) and par-6(RNAi) embryos (Thanks to Ken Kemphues for the GFP:PAR-2 integrated line). PAR-6 and PAR-2 localize respectively to the anterior and posterior half of the zygote and are required to exclude each other from their respective domains (Boyd et al., 1996; Hung and Kemphues, 1999). We find that exclusion of PAR-6 from the posterior is independent of PAR-2 during interphase, but becomes fully dependent on PAR-2 during mitosis. In contrast, exclusion of PAR-2 from the anterior depends on PAR-6 at all times. These observations are consistent with previous reports from the Kemphues lab (Boyd et al., 1996; Hung and Kemphues, 1999) and provide evidence for two distinct, cell-cycle-dependent phases in the establishment of A/P polarity: a first PAR-2-independent, microtubule-dependent phase which causes PAR-6 to be excluded from the posterior during interphase, and a second, PAR-2-dependent, microtubule-independent phase which confines PAR-6 to the anterior

during mitosis. The transition between these two phases coincides with the onset of mitosis and a brief period (mitotic prophase) during which PAR-6 accumulates in nuclei. We hypothesize that upon entry into mitosis, PAR-6 (or one of its interactors) becomes modified so that it no longer responds to microtubules and instead becomes responsive to PAR-2. Our observations suggest that polarity is maintained during mitosis by the action of PAR-2, which continues to mark the posterior end of the embryo at a time when microtubules are no longer able to provide positional information. (Many thanks to Sekyung Oh for help in the characterization of GFP:PAR-2 and GFP:PAR-6 dynamics).

734. Early embryogenesis differs even in close relatives of C. elegans

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We have started to study close and more distant relatives of C. elegans for their pattern of embryogenesis. While many differ only marginally from C. elegans, others show considerable differences. These include the sequence of divisions, variations in the spatial arrangement of blastomeres, presence or absence of polarity reversal in the germline, requirement of early zygotic transcription for cleavage, mode of cell specification, gastrulation, and temperature tolerance. Variations in many but not all variations can be understood as different aspects of heterochrony. We find that,depending on the parameter considered, species closer related to C. elegans may nevertheless express stronger differences than species phylogenetically more distant. This suggests that environmental factors influence the pattern of early development.

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735. Computer Simulation of Early Cleavage of *C. elegans* Embryo

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Cellular arrangements are important for development. In *C. elegans*, cell-cell interactions are essential for cell-fate determination in early embryos. The arrangement of the cells in embryo is largely restricted by physical conditions including the force and direction of cell division, the existence of hard egg shell, the properties of cell adhesion, membrane, cytoskeleton, and so on.

Here we present a computer simulation of early cleavage of *C. elegans* embryo, which is constructed to provide a platform to examine the relationships among various physical parameters and early cleavages by comparing the results of simulation with real embryos of wild type and various mutants. Currently this simulator is based solely on dynamic model of cells and, therefore, includes neither chemical reaction networks nor gene-regulated networks within the cells, in order to make the model as simple as possible. For this purpose, we implemented cells, eggshell and cell division, and we expressed a cell by a mesh bag (= membrane) containing soft balls (= cytoplasm). The unit particles of the cell parts are connected by springs and dampers (=shock absorbers). Eggshell is approximated by a quadric function. Timing of cell division was given and cell division was carried out by contraction of a contractile ring in the middle of the cells.

Using the simulator, we performed several simulations of early cleavages up to 4-cell stage. The results were that the movement and arrangement of the cells were almost the same as the real embryo, except that the cell arrangement of 3-cell stage was slightly different. This means that the restriction of cell movements by eggshell and the repulsive forces of cell division were reconstructed successfully but the lack of cytoskeleton may cause the discrepancy at 3-cell stage. In the simulation, the cleavage of P_1 is temporally a bit behind that of AB, which is the normal pattern of wild type. In another simulation, we set the cleavage timing of P_1 ahead that of AB. The results were striking; the resulting 4 cells were arranged not in the usual diamond shape but in T shape in which ABx can not contact with P_2. Interestingly, this T shape, to some extent, looks like the embryo of *A. nanus* in which the cleavage of P_1 is much ahead that of AB (Wiegner and Schierenberg, *Dev. Biol.* 204, 3-14 (1998)). We are currently improving the simulator by adding attractive forces between the particles of cytoplasm and implementing other phenomena in early cleavages of the embryo.

736. *pod-2*, along with *pod-1*, defines a new class of genes required for polarity in the early *Caenorhabditis elegans* embryo

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The asymmetric division of the one-cell *Caenorhabditis elegans* zygote gives rise to two cells of different size and fate, thereby establishing the anterior-posterior (a-p) axis. A number of genes required for this polarity have been characterized, but many components remain unidentified. Recently, our laboratory discovered a mutation in the *pod-1* gene (for polarity and osmotic defective) that uniquely perturbed polarity and osmotic protection. Here, we describe a new polarity gene upon disruption shows a loss of physical and developmental asymmetries in the one-cell embryo, including the mislocalization of PAR proteins required for early polarity. Furthermore, mutant embryos are osmotically sensitive, allowing us to designate this gene *pod-2*. Thus, *pod-2*, along with *pod-1*, defines a new class of *C. elegans* polarity genes. Our data suggest *pod-2* may be required to properly position an a-p polarity cue. The temperature shift studies suggest *pod-2* is required before fertilization suggesting a potential role for polarity generation in the oocyte. *pod-2* gene has been mapped to 500kb region of DNA and the molecular identity is about to be revealed.

737. UNC-45 is an evolutionarily conserved myosin-interacting protein

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unc-45 is essential for normal thick filament development in body wall muscles. The protein product (UNC-45) contains TPR repeats and has similarity to the yeast She4p protein which is involved in asymmetric segregation of specific mRNAs, but its biochemical function is unknown. We have previously shown that UNC-45 differentially co-localizes in body wall muscle thick filaments with myosin heavy chain B (MHC B) but not MHC A. In contrast to other body wall muscle thick filament components, unc-45 is also maternally contributed and the protein and mRNA are present in all cells of the early embryo. However, zygotic unc-45 expression is only detected in the developing muscle cells. Yeast two-hybrid screens show that UNC-45 interacts with at least two non-muscle myosins, including NMY-2, a type II myosin necessary for asymmetric cell division. UNC-45 and NMY-2 also co-localize in vivo, at the cortex of the cell in early embryos, and localization of UNC-45 is dependent on the presence of NMY-2. Rather than being a muscle-specific protein, this would argue that the UNC-45 protein may have a more general role as a myosin chaperone, stabilizer, or assemblase. We have recently found UNC-45 sequence homologues in Drosophila and humans, and a lethal mutation in the Drosophila gene, leading us to propose that this family of proteins may be an evolutionarily conserved class of myosin interacting proteins.

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738. Toward an understanding of *tbx-9*

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tbx-9 is a member of the T-box family of transcription factors. T-box genes have been found in various metazoans, and many of the genes play an important role in pattern formation especially in embryogenesis. *tbx-9* encodes a transcription activator since the *tbx-9* product can induce the transcription of a reporter gene containing the *tbx-9* binding sequence upstream of the gene. The expression of *tbx-9* is first observed in nucleus of the E cell at the 8-cell stage by *in situ* hybridization analysis. The expression lasts up to its daughters, Ea and Ep. At this stage, Ca, Cp and some AB-derived cells that seem to be precursors of hypodermal cells start *tbx-9* expression. The expression of *tbx-9* can also be seen in 4 body wall muscle precursor cells of MS descendants at the 200-cell stage. The latest expression of *tbx-9* is in precursor cells of hypodermis that disappears just before morphogenesis. A *tbx-9* deletion mutation produced by gene disruption causes disorganization in embryogenesis that occurs predominantly in posterior part of the body. At least one reason for the deformity is a defect in the positioning of body wall muscle cells at the comma stage. Penetrance of the phenotype is low, suggesting the presence of another gene that is functionally redundant with *tbx-9*. Indeed, all of the *tbx-9* mutant embryos given dsRNA of *tbx-8*, another T-box gene phylogenetically most related to *tbx-9*, show abnormality in the overall length of the body. In these embryos body wall muscle cells are apparently detached from hypodermis. Although N2 embryos given *tbx-8* dsRNA also reveal disorganization in their bodies, penetrance of the phenotype is low and the deformity is observed predominantly in the central part of the body.

To elucidate the nature of transcription regulation responsible for the *tbx-9* mutant phenotype, I have been trying to identify target genes of *tbx-9* by cDNA microarray analysis. Search for genes whose expression level is altered in embryos of the strains in which the amount of the *tbx-9* product has been modified, and tests of these genes by *in situ* hybridization analysis to know whether the increase or decrease of their expression truly reflects on the change of expression pattern of them are in progress.

739. The *C. elegans* Homothorax/Meis homolog UNC-62 is required for embryonic patterning and viability

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Cofactor homeodomain proteins such as Drosophila Homothorax (Hth) and Extradenticle (Exd) and their respective vertebrate homologs, the Meis/Prep and Pbx proteins, can increase the DNA binding specificity of Hox protein transcription factors and appear to be required for many of their developmental functions. We have shown that *unc-62* encodes the *C. elegans* ortholog of Hth, and that three maternal-effect *unc-62* mutations can cause severe posterior disorganization during embryogenesis (Nob phenotype) superficially similar to that seen in embryos lacking function of the two posterior-group Hox genes *nob-1* and *php-3*. A more severe zygotic allele causes earlier embryonic arrest, and two weaker alleles result in some larval lethality and vulval abnormalities, respectively, suggesting that *unc-62* may play roles at several stages in development. Molecular analysis of the locus has identified five mutational lesions and revealed the presence in wild-type animals of four alternatively spliced transcripts, resulting from assortment of two alternative first exons and an interior alternative splice involving a portion of the homeobox. In contrast to *unc-62*, the *ceh-20* gene, which encodes the *C. elegans* ortholog of Exd, does not appear to have essential embryonic functions based on analysis of strong loss-of-function mutations and RNAi experiments.

740. Genes that Affect Asymmetric Cell Division and Cell Fate in the Nervous System

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The *C. elegans* protein HAM-1 is asymmetrically distributed in several cells in the dividing embryo and participates in the asymmetric divisions of at least five neuroblasts. In particular, we study the role of HAM-1 in the division of the HSN/PHB neuroblast. Antibody staining analyses show that HAM-1 is asymmetrically distributed in this neuroblast and inherited by its posterior daughter, the HSN/PHB precursor. Loss of *ham-1* function can cause the daughter cell that normally does not inherit HAM-1 to transform into an extra HSN/PHB precursor. This transformation results in duplications of the HSN and PHB neurons. Our current model is that HAM-1 acts as a tether for cell fate determinants and restricts the localization of these determinants to the HSN/PHB precursor.

With the overall goal of identifying other players in this process, we conducted three genetic screens for mutants with Ham-1 phenotypes. Each screen was done in a *gmIs12* background, a strain that expresses GFP in the PHB phasmid neuron, one of the cells duplicated in *ham-1* mutants. For each screen, we simply looked for mutants with abnormal numbers of GFP-expressing phasmid neurons. We isolated two main classes of mutants. The first class has extra phasmid neurons; we found 16 of these mutants, at least nine of which are in genes previously characterized: *ham-1*(*gm214*, *gm267*, and *gm279*), *ced-3*(*gm265* and *gm266*), *egl-5*(*gm224* and *gm316*), *lin-32*(*gm239*), and *egl-27*(*gm314*). Three more mutants in this class are allelic: *gm280*, *gm300*, and *gm301*. The second class of mutants has fewer than normal phasmid neurons; we found 16 of these mutants. This class of mutants is interesting because this is the phenotype one would expect for the loss of a determinant that specifies HSN/PHB precursor fate.

We were intrigued to find that *egl-27* mutants have extra phasmid neurons. EGL-27 is a homolog of MTA1, a member of the NURD complex (nucleosome remodeling and histone deacetylase). *egl-27*; *gmIs12* worms have extra phasmid neurons infrequently, but *egl-27*; *gmIs12*; *ham-1*(*n1811* or *gm267*) animals have a significantly enhanced PHB duplication defect. *egl-27* has previously been implicated as a possible transducer of Wnt signaling, a process known to affect cell polarity, and experiments are underway to explore what roles EGL-27 and other members of the NURD complex might be playing in the HSN/PHB lineage in conjunction with HAM-1.

Another mutant we have been characterizing that affects this process is *gm34*. *gm34* mutants are frequently missing HSNs and PHBs, and this phenotype is epistatic to *ham-1*, *ced-3*, and *lin-32* mutations. We cloned the defective gene by conventional mapping and injection rescue; its sequence (C18A3.8) predicts that it is a basic helix-loop-helix (bHLH) transcription factor. LIN-32 is another bHLH that plays a role in the HSN/PHB lineage, and since bHLH proteins are known to regulate transcription of target genes as dimers, we are currently exploring the possibility that this new bHLH and LIN-32 interact to determine the fates of cells in the HSN/PHB neuroblast lineage.

741. *Cki-1* : a link between the timing of cell divisions and the acquisition of proper cell fates in the *C.elegans* somatic gonad

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The formation of a complex mulitcellular organism requires the precise specification of many different cell types at the correct time and position. Signals specifying different cell fates may only act at a specific time during development, so a window of competence to respond to these signals must be tightly regulated. This temporal control can be linked to the progression through a cell cycle.

The characterization of *cki-1* in *C.elegans* has suggested a possible role of this p27KIP homologue in blocking cells from dividing until they are appropriately determined. This has specifically been shown in the vulval precursor cell lineage. Similarly, *cki-1* may also play a role in the correct timing of cell divisions in the somatic gonad. Through laser microsurgery experiments it was shown that the extra distal tip cells (DTCs) arising in *cki-1(RNAi)* animals do not arise from preexisting DTCs.

In order to further delineate the cell lineage giving rise to the extra DTCs, we ablated Z1 and Z4 in *cki-1(RNAi)* and wild type animals. None of the ablated animals formed DTCs indicating that the extra DTCs in *cki-1(RNAi)* animals arise from the somatic gonad lineage. On the other hand, *cki-1(RNAi)* animals which had the Z2 and Z3 germline precursors ablated still showed the formation of extra DTCs, showing that the extra DTCs do not arise from a transformation of a germ cell into a DTC. To understand which somatic gonadoblast daughters can give rise to the extra DTCs, we ablated Z1.a and Z4.p, or alternatively Z1.p and Z4.a in *cki-1(RNAi)* and wild type animals. In both cases, distal tip cells or extra distal tip cells, respectively, were formed after ablation in *cki-1(RNAi)*. This suggests that in *cki-1(RNAi)* animals either of these gonadal cells can give rise to DTCs. Interestingly, the common cell type formed from these two precursors are the sheath/spermatheca cells.

To test the possibility that these cells could be transformed into DTCs, we performed antibody staining for CEH-18, a marker of sheath cell differentiation. In *cki-1(RNAi)* animals we observed gonad arms with aberrant numbers and positions of sheath cells, showing both more and less than the normal complement of sheath cells. In addition, as in other mutants affecting sheath cell number or function, *cki-1(RNAi)* animals display an endomitotic oocyte (Emo) phenotype. The possibility that sheath cells may transform is also supported by branched gonad arms in *cki-1(RNAi)* animals. To fully understand whether the sheath cell lineage alterations are due to a transformation of a sheath cell into a DTC, we are currently performing lineage analysis on the somatic gonadal cells in *cki-1(RNAi)* animals. Ablation of SS precursors in *cki-1(RNAi)* animals will also show whether these are the cells that give rise to the extra DTCs. Our goal is to understand the link between the regulation of the correct timing of cell divisions by a cyclin-dependent kinase inhibitor and the acquisition of specific cell fates.

742. Genetic Analysis of Cell Cycle Control in the Germ Line

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Sexual reproduction depends on the organized proliferation and specification of germ cells. In addition, reliable mechanisms are needed to ensure the faithful replication and segregation of the genetic material during mitosis and meiosis. Checkpoints monitor genetic damage, the alignment of sister chromatids on the metaphase plate, and spindle structure. In order to address how cell cycle control is integrated into the developmental context of germline development, we are undertaking genetic screens for sterile mutations with cell cycle defects. This ongoing screen is focusing on two mutant classes: 1) mutations affecting anaphase onset and M-phase exit; and 2) mutations affecting early aspects of meiotic cell cycle progression. Here we briefly summarize our progress.

To begin to address mechanisms regulating chromosome segregation during meiosis and mitosis, we have focused on genes in the *emb-30* pathway. Genetic and phenotypic analysis revealed that *emb-30* is required for metaphase-to-anaphase transitions during meiosis and mitosis (1). *emb-30* encodes the likely ortholog of APC4/Lid1, a component of the anaphase-promoting complex/cyclosome that targets anaphase inhibitors and mitotic cyclins for ubiquitin-mediated protein degradation. To define additional genes that function together with *emb-30*, we undertook a genetic screen for mutations that phenocopy zygotic sterile severe-reduction-of-function *emb-30* alleles (class I alleles). In *emb-30*(class I) alleles, germ cells in the distal mitotic zone begin to block in mitosis in the late L3 stage. We conducted a genome-wide screen for Sterile mutations that accumulate mitotic germ cell nuclei in the distal mitotic zone, as revealed by staining with anti-phosphohistone H3 antibodies. To date, we have screened approximately 23,000 haploid genomes and recovered 16 mutations in at least 5 genes. These include *emb-30* (1 allele) and *emb-27* (1 allele), two likely APC components (1, 2). Five

alleles fail to complement *evl-22(ar104*) (3) and *mat-2(ax102*ts*)* (2). We mapped *evl-22/mat-2* to ~0.02 mu right of *unc-53*, and thus *evl-22/mat-2* may encode APC1 (W10C6.1). Two mutations fail to complement *mat-3(or180*ts*)* (2). Two mutations appear to define a new locus we are tentatively calling *bim-1*, for blocked in M-phase. We hope to have the remaining mutants characterized by the meeting.

In the course of this genetic screen, we recovered a Sterile mutation, *glp(tn1206)*, which may define a new locus required for normal germline proliferation and meiotic progression. *glp(tn1206)* hemaphrodites produce approximately 100 germ cells in each gonad arm and some sperm, but no oocytes. The somatic gonad appears normal, as detected by *lim-7::gf*p and *lag-2::gfp* staining. In the adult stage, no mitotic germ cells are observed. Rather, the germ cells appear to be in an early meiotic stage as revealed by staining with anti-HIM-3 antibodies (4). Males also have reduced germline proliferation. *glp(tn1206)* maps between *unc-22* and *dpy-26* on *LGIV*. Progress in genetic and phenotypic analysis will be reported.

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743. A mutant which enters cell cycle during dauer

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The biology underlying the cell cycle at the unicellular level is relatively well understood. However, multicellular organisms require that the cell cycle be perfectly synchronized with developmental and environmental cues in order to form a fertile adult. *C. elegans* is an excellent model organism to study the developmental regulation of the cell cycle due to its simple life cycle, its invariant cell lineage and the battery of molecular tools available.

Harsh conditions during post-embryonic development cause the worm to execute an alternative diapause stage called dauer. The dauer stage is characterized by morphological and metabolic changes accompanied with a global cell cycle arrest mediated at least in part by *cki-1*, a member of the $p27^{cip/kip}$ family. The role of *cki-1* in mediating the dauer cell cycle arrest is further supported by *RNAi* experiments wherein seam cells abnormally enter the cell cycle during this stage. This makes this stage a suitable background to study developmental regulation of the cell cycle.

In order to visualize cells that enter the cell cycle abnormally during the dauer stage, we crossed an S phase reporter(*rnr::*GFP) into a *daf-7 (e1372)* background. We screened 3000 haploid genomes and we recovered one mutation that causes lateral hypodermal cells to enter the cell cycle abnormally during the dauer stage. However, unlike *cki-1*(*RNAi)* animals, somatic gonadal cells also strongly express the S phase reporter during dauer. We also noticed that some worms had extra intestinal nuclei in the posterior region. Since this phenotype is easy to score, we therefore decided to focus on this phenotype to further characterize this mutant.

In order to visualize intestinal nuclei *in vivo*, we crossed our mutant into an *elt-2::*GFP background to mark intestinal nuclei. We observed as many as 50 nuclei in a *rr33* mutant adult. The mutant worms with extra nuclei are fertile but have a reduced brood size. In the

wild type, intestinal nuclei undergo karyokinesis in the L1 to give rise to a binucleate cell. However in *rr33* the intestinal cells are often tetranucleate(56%, n=25). The extra nuclei observed in *rr33* do not occur embryonically since all newly hatched L1's possess 20 nuclei as in the wild type. We propose that these cells execute a second nuclear division in the L1, perhaps at the expense of one round of endoreplication, which could account for the final number of intestinal nuclei we observe. To confirm this hypothesis, we are presently performing DNA quantification to determine if nuclei in the tetranucleate intestinal cells in *rr33* contain less than 32N DNA in addition to lineage analysis to identify when this defect occurs. Mapping of this mutant is currently in progress.

744. Distinct developmental functions of the two *C. elegans* homologs of a cohesin subunit Rad21

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Cohesin maintains cohesion of the sister chromatids after replication, and is essential for proper segregation of chromosomes. Recent studies have shown that progression of anaphase depends on the cleavage of cohesin subunit Rad21/Scc1 by separin protease in yeast. Yeast cohesins are bound between sisters along the entire chromosomes and are cleaved simultaneously at the onset of anaphase, whereas in vertebrates cohesins on the arm region dissociate without cleavage and are replaced by condensins in prophase, and only cohesins at the centromeric regions hold sister chromotids together until they are cleaved.

The cohesin complex contains either Rad21/Scc1or Rec8, Scc3, Smc1, and Smc3. This subunit composition is highly conserved from yeast to humans. Almost all the organisms have at least two distinct cohesin complexes, in which different types of subunits are contained. For instance, yeast has two complexes, one including Rad21/Scc1 for mitosis and the other including Rec8 for meiosis. In the nematode *C. elegans* we identified two genes homologous to *rad21* by genome-wide database search. Only *C. elegans* has two genes homologous to *rad21* so far. To see whether the two types of cohesin complexes function differently and how they are developmentally regulated, we initiated molecular and functional characterization of the two RAD-21 homologs in *C. elegans*.

One of the two genes (F10G7.4; hereafter *rad-21.1*) turned out to be essential for embryogenesis, because we observed 100% embryonic lethality by RNAi-by-soaking of this gene. When closely observed, each cell division in *rad-21.1*-depleted embryos took longer than in wild type, although the patterns of cell lineage seemed unaffected. The appearance of nuclei was abnormal, but the amount of DNA in

each nucleus seemed equal and not affected judging from DAPI staining. Whether there are any minor defects of chromosome segregation in *rad-21.1* (RNAi) embryos is under investigation by FISH analysis. *rad-21.1* was also essential for fertility and larval development, because complete sterility (by RNAi-by-L1-soaking) and larval arrest as escaper (by dsRNA injection) were observed. We produced polyclonal antibodies against RAD-21.1 protein, and found that in early-satge embryos RAD-21.1 protein localize to the chromosomes in a cell cycle dependent manner, as has been seen in vertebrates. RAD-21.1 could also be detected in the germ cell nuclei in larval-stage worms and in condensed germ cell nuclei in adult gonads.

The other gene (K08A8.3; *rad-21.2*) appeared to function in larval development, because we observed larval arrest by RNAi, although the penetrance was not high (aproximately 50%). The level of transcription of *rad-21.2* was lower than *rad-21.1,* judged by nothern blotting and whole mount *in situ* hybridization. We found that RAD-21.2 protein was localized to almost all nuclei in late embryos and larvae, also suggesting the function of RAD-21.2 in the larval development. Unlike RAD-21.1, RAD-21.2 was not detected in nuclei in early embryos, nor in germ cell nuclei in larvae.

From these results, we speculate that the two *rad-21* genes perform different functions and are regulated developmentally in *C.elegans*.

745. A deletion of *C. elegans hcf-1* causes a cold - sensitive early embryonic cell division defect

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C. elegans hcf-1 was found because of its similarity with human *HCF-1*. Human *HCF-1* encodes a cellular component of a viral transcription activation complex required for herpes simplex virus (HSV) gene expression. A mutation in HCF-1 can arrest cell cycle progression in a mammalian cell line, but the mechanism of HCF-1 function in mammalian cells has not been elucidated. The product of *C. elegans hcf-1* (CeHCF) shares functional properties of HCF-1 including association with the HSV viral protein VP16. The goal of our research is to determine the cellular function of HCF proteins and we are using *C. elegans* as a model organism for this purpose.

Previously, we have reported the isolation of an *hcf-1* deletion mutant from a chemically mutagenized *C. elegans* deletion library (R. Plasterk, personal communication). Homozygous deletion mutant worms are viable and do not show any gross morphological or developmental defect at 20°C. Analysis of the brood size at 15, 20, and 25°C showed that the mutant brood size is about 50% of that of the WT at all three temperatures. At 15°C, however, about 10% of mutant embryos also do not hatch. A further temperature decrease to 12°C resulted in a further reduced mutant brood size (25% of WT) and only a 25-50% hatching rate. Thus, the *hcf-1* deletion mutant has a small brood size and a cold - sensitive embryonic lethal phenotype. This cold sensitive phenotype can be enhanced by propagating worms at low temperature (12°C) for more than two generations. Time course analysis of mutant embryos at 12°C showed that most of the non-hatching embryos arrest before the comma stage, and have early embryonic cell division defects, including defective nuclear segregation, spindle assembly, and cytokinesis.

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746. A GENETIC APPROACH FOR STUDYING THE FUNCTION OF THE *AIR-1* KINASE IN C. elegans

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Mitotic division of eukaryotic cells is a highly regulated sequence of cellular events, including chromosomal separation and cytokinesis. Deregulation of these processes can cause catastrophic defects in cell division, leading to various diseases including cancer in humans. We are using the nematode *C. elegans* as a genetic model system to study the molecular mechanisms that underlie proper chromosome segregation and cytokinesis.

A *C. elegans* member of the Aurora/Ipl1 family of protein kinases, AIR-1, is localized to mitotic centrosomes. Depletion of AIR-1 function by RNA interference (RNAi) results in embryonic lethality that is characterized by abnormal spindle/centrosome formation and anueploidy. To elucidate *air-1* function at a molecular level, we are isolating and analyzing genetic mutants of this locus. By PCR screening a library of *C. elegans* deletion mutants, we identified a strain that lacks the *air-1* locus (*air-1* (Δ). This deletion removes exons 1 to 6 of *air-1* gene as well as a single exon from the adjacent gene. In contrast to the *air-1*(RNAi) phenotype, homozygous *air-1*(Δ) animals complete embryogenesis. This is likely due to embryonic rescue by the maternal *air-1* gene product. RNAi depletes both maternal and zygotic gene products, thus the *air-1*(Δ) mutant likely represents the zygotic loss-of-function phenotype. Like many other cell cycle-deficient mutants in *C. elegans*, *air-1(*Δ) homozygotes are uncoordinated (Unc) and sterile. The ventral nerve cord and the germ line divide post-embryonically, and thus are not rescued by maternal gene products. Subsequent genetic complementation tests with other mutants in the *air-1* interval found that two alleles of a previously characterized lethal gene, *let-412*, fail to complement the *air-1*(Δ) allele. All three alleles are likely to eliminate gene function, and we are currently analyzing the mutant phenotypes in greater detail.

747. STU-4, the C. elegans homolog of the S. cerevisiae separin ESP1, is required for meiotic and mitotic chromosome segregation during embryonic and post-embryonic cell divisions.

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How do cells coordinate the processes of chromosome segregation and cell division? To better understand this issue we have been studying the classic temperature-sensitive sterile-uncoordinated mutant, stu-4.

In *stu-4* (e2406) mutants, cells undergo multiple rounds of normal DNA replication and centrosome duplication, but are aberrant in chromosome segregation and cell division resulting in the formation of polyploid cells. However, there is no apparent delay in the progress of subsequent cell cycles. Furthermore, the defect is not restricted to mitotic cells since meiosis is affected as well.

The *stu-4* gene encodes a protein with homology to the separins, an emerging class of proteases, which act at the Metaphase to Anaphase transition to cleave the cohesins that hold sister-chromatids together. A second function in Anaphase B spindle movement was also shown recently in S. cerevisiae. We are interested in understanding the function of this protein in a higher metazoan since there are subtle but important differences in the mechanisms of chromosome segregation and cell division between them and yeast.

748. A novel synthetic approach to identify additional roles for lin-35/Rb in cell growth and proliferation.

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The Retinoblatoma gene product (Rb) is commonly regarded as the prototype for tumor suppressor genes. Numerous studies have indicated roles for Rb in cell-cycle regulation, transcriptional control, differentiation, and apoptosis. The *C. elegans* genome encodes for a single Rb-like protein, LIN-35 (Lu and Horvitz, 1998). *lin-35*/Rb was identified as a class B synthetic multivulval (SynMuv) gene . Loss-of-function mutations in SynMuv genes lead to a "multivulval" phenotype whereby affected animals contain multiple ectopic egg-laying structures. However, this phenotype is only obtained with specific combinations of SynMuv mutations, and the majority of SynMuv mutants, including *lin-35*/Rb, show no obvious defects on their own.

This finding is surprising as it would seem to indicate that *lin-35*/Rb carries out no essential function in worms, at least on its own. Such results contrast greatly with the known functions of Rb in other systems including Rb knockout studies in flies and mice where loss of Rb is lethal. In addition, because *lin-35*/Rb was found to be expressed in essentially all cell-types during *C. elegans* development (Lu and Horvitz, 1998), the question becomes, what is the function of *lin-35*/Rb in all these cells and how can such functions be identified? This question can be broadened to ask: How can functions be ascribed to genes where mutations show no obvious phenotype? This issue will likely arise with increased frequency as the genome becomes saturated for mutations with clear plate phenotypes.

We have designed and initiated a novel genetic screen with two basic goals: 1) to determine what additional biological functions *lin-35*/Rb may carry out during nematode development; and 2) to identify genes that may cooperate with Rb in carrying out these functions. Such genes may have conserved functions in higher eukaryotes and could conceivably play a role in

Rb-mediated carcinogenesis. The screen is based on the premise that any additional functions of *lin-35*/Rb will require cooperation with a second site mutation (a synthetic interaction), similar to its known role in the SynMuv pathway. The screen utilizes a strain that is homozygous for a strong LOF mutation in *lin-35* (*n745*) but carries an extrachomosomal array containing both *lin-35* rescuing sequences and *sur-5::*GFP. In this way, green *lin-35+* animals can be readily distinguished from non-green *lin-35* animals. The screen seeks to identify phenotypes that are distinct to the non-green (*lin-35*) population of animals, thereby implying a phenotype that is specifically synthetic with *lin-35*/Rb.

Using the above approach we have identified at least seven *slr* mutations (for Synthetic with *lin-35*/Rb) defining at least six different genes. These mutations in conjunction with *lin-35*/Rb lead to pleiotrpic defects including lethality, slowed growth rates, decreased size, and infertility. One allele, *slr-1(ku298),* shows a striking synthetic lineage defect with *lin-35*/Rb, resulting in generalized excess cell divisions. Importantly, this result indicates that hyperproliferation in *C. elegans* can follow the same genetic pattern as multi-step carcinogenesis in humans. Interestingly, a hyperproliferation phenotype is not observed when *slr-1* is combined with a number of other SynMuv genes tested, indicating a specific role for *lin-35*/Rb in this process.

We will describe our substantial progress towards mapping and identifying the affected gene products; six mutations have been mapped to chromosomal sub-regions and cosmid rescue experiments are underway for several of these. In addition, details of our screen will also be presented. We are encouraged that our technical approach will prove to be of general use for those wishing to identify mutations that may interact synthetically with their gene of interest.

Lu and Horvitz (1998) *Cell* ***95,*** 981-91.

749. A proteomics approach to the identification of CUL-2 substrates

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Cell cycle transitions are potentiated by the degradation of positive and negative cell cycle regulators through the ubiquitin-mediated proteolytic pathway.

Cul-2, a component of a ubiquitin-ligase complex, regulates diverse aspects of cell cycle progression. Human Cul2 functions with the VHL tumor suppressor protein in a CUL2/VCB complex. Germline mutations in VHL cause a hereditary cancer syndrome. In vertebrates, the CUL2/VCB complex contains four protein subunits, CUL2, Rbx1, Elongin C, and VHL, and has been shown to function as a ubiquitin ligase in vitro. In C. elegans, CUL-2 negatively regulates CKI-1 and is required for the G1-to-S phase transition in germ cells. Inactivation of maternal cul-2 product results in an early embryonic arrest with only 24 cells on average. There are three major phenotypes in the cul-2 mutant embryos: a defect in chromosome condensation, leading to the formation of multiple nuclei; a delay in mitotic progression, with six fold longer mitosis than in wild type; and a defect in cytoskeletal movements.

Inactivation of a ubiquitin-ligase is expected to result in the accumulation of substrates. As CUL-2 is predicted to function as a ubiquitin ligase, cul-2 mutants should have an accumulation of substrates that contribute to the observed phenotypes.

We have performed a proteomic survey using two-dimensional gel electrophoresis to identify proteins whose levels change in cul-2 RNAi animals relative to wild type. In addition to the expected increase in substrate proteins, the levels of certain other proteins may also increase or decrease as a secondary consequence of an increase in the level of transcriptional regulators. These secondary changes can also be observed in a proteomic screen and can give us information about the physiological role of cul-2. We have optimized conditions for protein

preparation and 2D-gel electrophoresis. We have found potential protein candidates that change in cul-2 mutants relative to wild type. These proteins will be identified by mass spectrometry, and we hope to be able to present this data at the meeting.

750. Interfering with DNA replication components delays embryonic cell divisions

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One important aspect of development is the acquisition of cell cycle durations specific for each lineage. In wild-type *C. elegans* embryos, this is already apparent at the two cell stage, as the smaller posterior blastomere P1 divides~2min after the larger anterior blastomere AB.

In a large scale RNAi based screen (1), we identified 8 genes whose RNAi phenotype results in the P1 blastomere dividing >5 min after the AB blastomere. Strikingly, all 8 genes turned out to be components important for DNA replication. We analyzed the timing of divisions in such RNAi embryos in more detail using time-lapse DIC microscopy. We found that the cell cycle was slowed down in both AB and P1, as well as in the one cell stage embryo. The cell cycle was slowed down to a larger extent in P1 than in AB, thus explaining the increased delay observed between division of the two blastomeres. Similar results were reported recently by others examining the timing of division in embryos lacking the function of a subunit of the DNA polymerase alpha-primase complex (2).

We tested whether we could induce similar phenotypes by inhibiting DNA replication with hydroxyurea (HU). We found that HU-treated animals also display slowed down early embryonic cell cycles. We are currently addressing whether this may result from the activation of a DNA replication checkpoint.

1 Gönczy et al. Nature 408: 331-336 (2000)

2 Encalada et al. Dev Biol 228: 225-238 (2000)

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751. The *C. elegans* homologues of eIF5A and the ATP synthase b subunit are required for germline development

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We have previously reported a reverse genetic screen to identify genes functioning in germline development (Midwest *C. elegans* meeting 2000). Briefly, subtracted cDNA probes either enriched for or deprived of the germline-specific transcripts were hybridized to the high-density cDNA grid containing ~7600 non-overlapping EST clones (~40% of the predicted genes in the genome). One hundred and sixty eight candidate clones were then subjected to RNAi. Of these, 21 resulted in embryonic or larval lethality and 15 showed sterility with a variety of different defects in germline development, which included those that caused unfertilized eggs, masculinization of the germline, underproliferative germ nuclei, pachytene arrest, abnormal diakinesis, degeneration of the germ nuclei, and tumorigenesis of the germline (collaboration with M. Mochii, N. Ueno and Y. Kohara).

We focused on two of the clones that showed the sterility phenotype. One was yk445a8, derived from the T05G5.10 gene encoding a homologue of eIF5A. RNAi of this clone caused sterility similar to the *glp-4* mutant; a small gonad with few undifferentiated germ nuclei was observed. The facts that no excess dead cell was observed in the gonad during all larval stages and that germline-tumorigenesis of the *gld-1; gld-2* double mutant was suppressed in RNAi worms indicated that T05G5.10 functions in germ cell proliferation. *C. elegans* has another eIF5A homologue, F54C9.1. RNAi of this gene caused a larval arrest phenotype and escapers showed sterility with the abnormal appearance of spermatheca and uterus. We used *in situ* hybridization and Northern analysis to analyze the germline-specific expression pattern of T05G5.10 and the global expression pattern of F54C9.1. Although eIF5A was originally isolated as a candidate translation initiation factor, recent studies have suggested a role of eIF5A in many aspects of RNA metabolism, including nuclear export, cytoplasmic degradation or translation. We speculate that germline-specific eIF5A is involved in regulation of a set of transcripts essential for the proliferation of germ nuclei. Now we are working to clarify the cellular localization and the functional differences between these two homologues.

The other clone in which we are interested is yk519f1. It corresponds to the F35G12.10 gene, which encodes a homologue of the ATP synthase b subunit. The RNAi of F35G12.10 caused many germ nuclei to arrest at the pachytene stage and spermatocyte-like nuclei were occasionally observed. F35G12.10 shows a strong similarity with another *C. elegans* gene, F02E8.1. RNAi of F02E8.1 caused larval arrest with complete penetrance, consistent with F02E8.1 functioning as a subunit of ATP synthase, a well-known complex involved in mitochondrial energy generation. Northern analysis of staged worms showed that F35G12.10 was expressed in later larval stages and in adults and F02E8.1 was expressed at all stages. Now we intend to determine if the germline-specific ATP synthase b subunit regulates germline fate through the function of mitochondria.

752. SNP Mapping of ego-3, a C. elegans Gene Involved in Germline Development

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The regulation of the decision between proliferation and differentiation in the C. elegans germline has been well characterized. Central to this inductive cell signaling pathway is the glp-1 (germline proliferation defective) gene, which codes for a Notch-type receptor protein. GLP-1 receives a signal (LAG-2) from a pair of somatic cells called the distal tip cells (DTC). If the DTCs are killed, or if GLP-1 is not functioning, all the germ cells enter meiosis, leaving no proliferating cells.

ego-3 was isolated from a screen for genetic enhancers of glp-1. Mutants of ego-3 are characterized by an early germline defect; in L3 and L4 larvae, the germ cells arrest either in mitosis or early meiosis, their cell cycle state being difficult to interpret. The later germline phenotype is more variable and includes proximal proliferation and defects in oogenesis.

Previous three-factor mapping places ego-3 between daf-21 and sdc-3, just to the left of unc-61. Through SNP (single nucleotide polymorphism) mapping, we have now localized ego-3 to a region of approximately 118 kb, predicted to include 23 genes. SNP mapping is being continued, while cDNAs of predicted genes in this area are also beginning to be tested by RNA interference.

753. Genetic characterization of *fbf-1*, *fbf-2* and *puf-8*

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In *C. elegans*, the hermaphrodite switch from spermatogenesis to oogenesis is achieved by post-transcriptional regulation of the *fem-3* mRNA. Two nearly identical RNA-binding proteins, FBF-1 and FBF-2, are essential regulators of the sperm-oocyte switch (1). The *C. elegans* FBFs and their homolog *Drosophila* Pumilio are founding members of the Puf family. The *C. elegans* genome encodes ten Puf proteins: FBF-1, FBF-2 and PUF-3-10. RNAi directed against single *puf* family members reveals that *puf-8* also plays a role in controlling the hermaphrodite sperm/oocyte switch.

We have generated deletions in *fbf-1*, *fbf-2* and *puf-8* to facilitate genetic and phenotypic analysis of single mutants (*fbf* RNAi knocks out both *fbf-1* and *fbf-2*). (1) *fbf-1:* Two deletion mutants, *fbf-1(ok91)* and *fbf-1(ok224)* are putative null alleles and have the same phenotype. *fbf-1* homozygotes are generally healthy and fertile, but have minor germline defects. Brood sizes are larger than normal, suggesting more sperm, and a few animals (~1%) make excess sperm and no oocytes. (2) *fbf-2:* One deletion, *fbf-2(q655)*, removes the region encoding the 8th Puf repeat as well as 1.4kb downstream of the gene. This deletion has a low penetrance Fog (feminization of the germline) phenotype, enhances *fem-1* and *fem-2*, and has a higher penetrance Fog phenotype in a *smg* background. We are screening for more *fbf-2* deletions, since this one appears unusual.

(3) *puf-8: puf-8(q725)* appears to be a null allele. It was only recently obtained, and its characterization is in progress.

We have generated double mutants by isolating *fbf-1(q662)* and *fbf-1(q678)* in an *fbf-2(q655)* background, and by isolating *fbf-2(q704)* in an *fbf-1(ok91)* background. The phenotypes of these double mutants are similar in that all three *fbf-1 fbf-2* double mutants generated to date have both germline proliferation and sperm/oocyte switch phenotypes.

We are currently varying the doses of *fbf-1* and *fbf-2* genetically to ask whether FBF-1 and FBF-2 have distinct roles in germline development and whether their overall dose is important for determining the number of sperm. In addition, we are using the mutants to look for interactions with other germline fate regulators using RNAi, antibody staining and germline reporters.

1. Zhang et al. (1997) *Nature* 390, 477-484.

754. Three 3'UTR elements function together to regulate the translation of *nos-2*, a maternal RNA associated with P granules in early embryos.

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P granules are germline-specific organelles rich in messenger RNAs and RNA-binding proteins. Their function remains poorly understood but may involve regulation of mRNAs important for germline development. One RNA associated with P granules is *nos-2* (Subramaniam and Seydoux, 1999; Schisa et al, 2001). *nos-2* encodes a homologue of *Drosophila nanos* required for primordial germ cell development. *nos-2* RNA is synthesized in the maternal germline and is inherited as a maternal RNA in embryos where it segregates with the germ lineage. Although *nos-2* RNA is abundant in oocytes and early embryos, it is translated only in the 28-cell stage in P4, the precursor to the primordial germ cells Z2 and Z3.

To understand how *nos-2* translation is restricted to P4, we have begun a functional analysis of the *nos-2* 3UTR. We fused different regions of the *nos-2* 3UTR to a GFP reporter driven by the *pie-1* promoter (an efficient promoter for maternal expression of transgenes). Using this approach, we identified a 200-bp sequence in the *nos-2* 3 UTR sufficient to restrict GFP expression to P4. Mutational analysis of this sequence indicates that it is composed of three elements. A first element prevents premature translation in oocytes, a second element prevents expression in somatic lineages, and a third element is required to activate translation in P4. Our data indicate that *nos-2* translation is under both positive and negative regulation. Similar observations have been made with *Drosophila nanos,* suggesting that regulation of this germ plasm mRNA may have been conserved in evolution. Experiments to identify transacting proteins operating on each element are underway.

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755. Functional analysis of two HMG box genes in germline development

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We have analysed function of 2500 *C. elegans* genes by an RNAi-by-soaking method using a non-redundant cDNA set. Among them, some caused sterility but no somatic phenotypes. Two HMG box genes belonging to distinct HMG subfamilies, namely *hmg-3* (C32F10.5) and *hmg-5* (F45E4.9), both of which caused similar underproliferated germline phenotypes in F1 generation.

In order to analyse the function of each HMG gene at the post-embryonic stage, L1 larvae were soaked in dsRNA solution (RNAi^{L1}). About 51% of *hmg-3(RNAi^{L1})* worms showed sterility with few germ cells, but no *hmg-5(RNAi^{L1})* worm showed sterility. These results imply that *hmg-3* is required for germline proliferation in the postembryonic stage, whereas *hmg-5* is required only in embryogenesis.

The *hmg-3* gene has one paralog, *hmg-4* (T20B12.8), which shows 77% identity. Unlike *hmg-3(RNAi)*, *hmg-4(RNAi)* caused larval lethality. The affected F1 progeny were paralyzed and died at the L1-2 stage, but no other morphological abnormalities were observed. In addition, disruption of both *hmg-3* and *hmg-4* by RNAi resulted in synthetic embryonic lethality. The arrested embryos showed gut and muscle differentiation, but they failed to undergo morphogenesis. 4D time-lapse recording showed that cell division started delaying around 2hr after pronuclei fusion, and seemed to arrest before the onset of morphogenesis. They were apparently normal in early embryogenesis. Therefore, these two genes may be functionally redundant in embryogenesis, and they may function independently in germ and soma after embryogenesis.

HMG-3 and HMG-4 are members of the structure-specific DNA recognition protein (SSRP) family, and HMG-5 has a weak similarity to human mitochondrial transcription factor 1. In vertebrates, SSRP family proteins are known to be involved in various processes, such as DNA replication, transcription and V(D)J recombination. For further analysis of the specific function of each gene, we plan to isolate deletion mutants of the hmg genes.

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756. *mep-1* Is Required for Germ/Soma Distinction

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Distinguishing between germline and soma is perhaps the most fundamental developmental process. In *C.elegans* embryos, the germ/soma distinction has been shown to be regulated by several maternal factors including the PIE-1 protein, which appears to function to suppress somatic development in the germline. We have identified a protein, MEP-1, (formerly EPS-1) that appears to provide a nearly opposite function post-embryonically for the maintainance of somatic cell fates. Removal of maternal and zygotic *mep -1* activity by RNAi or by mutation [thanks to Puoti, Moulder and Kimble for the null allele] yields arrested larvae whose somatic cells appear to adopt germ cell characteristics. The intestinal cells and hypodermal cells of these larvae express germ-specific proteins: PGL-1, GLH-2, and GLH-3 which are also known to be components of the P-granules. The ectopic P-granules produced in *mep-1* arrested larvae resemble bonafide P-granules in the germcells. Our genetic studies suggest that MEP-1 functions in somatic cells to suppress the *denovo* synthesis of P-granule components. The following evidence supports the hypothesis that MEP-1 is regulating P-granule components transcriptionally:

1.) *mep-1* encodes a C_2H_2 type Zn-finger protein similar to Su(Hw) and hZFX; Su(Hw) is known to regulate transcription by binding to specific DNA sequences

2.) high levels of the *pgl-1* and *glh-2* transcripts are detected in *eps-1(-)* arrested larvae by *in situ* hybridization.

3.) *mep-1* genetically interacts with C. elegans genes believed to play a role in the organization of higher order chromatin structures.

We hypothesize that MEP-1 may be functioning to maintain germ/soma-specific transcriptional domains. We are currently examining possible response element(s) in the *pgl-1* genomic sequence which confer *mep-1* dependent regulation.

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757. The *C. elegans ptc* and *ptr* genes: A cholesterol connection?

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Patched (PTC) is a multipass membrane protein controlling cell fate and proliferation; in humans, PTCH functions as a tumour suppressor. Biochemical analyses have shown that PTC is a receptor for Hedgehog (Hh). It was postulated that PTC and the serpentine membrane protein Smoothened (SMO) form a complex whereby Patched inhibits SMO; Hh relieves this inhibition by binding to PTC. In turn, SMO activates the transcriptional regulator Ci to express TGF-beta and Wnt family members.

In *C. elegans*, BLAST and Clustal W analyses indicate that there are 3 *ptc* genes and 26 *ptc-related* (*ptr*) genes. Moreover, the hydropathy plots of *ptc* and *ptr* genes are very similar; these proteins are predicted to encode 12-pass membrane proteins with topologies similar to those of Patched proteins identified in other organisms. The PTC and PTR proteins also belong to a larger family of proteins containing sterol sensing domains (SSD); members of the SSD family include PTC, Dispatched (CHE-14), HMG CoA reductase, SCAP, SREBP and NPC1.

We have searched the *C. elegans* genome for other components of the Hh/PTC pathway. There are no obvious Hedgehog or Smoothened homologues encoded by the genome although a large family of Hedgehog-like proteins have been identified (Aspöck et al.1999. Genome Res. 9: 909), some of which may have signalling properties. Moreover, the activity of TRA-1, the single *C. elegans* homologue of Ci, appears to have been usurped by the sex determination pathway. The apparent absence of many of the components of the Hedgehog/Patched signalling pathway in *C. elegans* has led us to examine the role of *Ce-ptc-1* in the development of the worm. From an evolutionary standpoint, a study of the *Ce-ptc* genes might shed light on the ancestral roles of Patched proteins or perhaps uncover new functions.

Results of RNAi and mutational deletion studies will be presented indicating that *ptc-1* is an essential germline gene (Kuwabara et al. 2000, Genes Dev.14:1933). Animals lacking *ptc-1* activity are essentially sterile with multinucleate germ cells arising from a probable defect in germline cytokinesis. The membranes normally separating individual germ cell nuclei are absent in *ptc-1* mutants; the loss of these membranes allow multiple nuclei to cycle synchronously through mitosis. We conclude that these membranes maintain autonomous domains within the germline syncytium. It is unclear whether *ptc-1* mutants display cytokinesis defects because the cleavage furrow is not formed or because it is not stabilized. Anti-PTC-1 polyclonal antibodies indicate that PTC-1 protein is normally enriched at the apices of the membranes separating individual germ cells. One interpretation of the *ptc-1* mutant phenotype is that *ptc-1* normally plays a germline-specific role in membrane trafficking. In addition to our studies on the *ptc* genes, we are undertaking a global analysis of the 26 *C. elegans ptr* genes. Preliminary results of these studies will be presented.

Taken together, our analyses of the *ptc-1* and *ptr* genes combined with studies of other *C. elegans* SSD protein encoding genes, such as *che-14* (Michaux et al. 2000. Curr. Biol. 10:1098) and *npc-1* and *npc-2* (Sym et al. 2000. Curr. Biol. 10:527) indicate that the SSD proteins are involved in a diverse range of developmental processes, which may all share a common link to cholesterol.

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758. Targeting RNAs to P granules: analysis of sequence requirements

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Targeting RNAs to P granules: analysis of sequence requirements

P granules are cytoplasmic structures of unknown function that are associated with germ nuclei in the *C. elegans* gonad, and are localized exclusively to germ cells, or germ cell precursors, throughout the life cycle. All of the known protein components of P granules contain putative RNA-binding motifs, suggesting that RNA is involved in either the structure or function of the granules. We have shown that P granules in the gonad normally contain a low level of RNA, and that certain genetic and physiological conditions result in an increase in RNA levels. Several, diverse mRNAs, including *pos-1, mex-1, par-3, skn-1, nos-2,* and *gld-1* mRNA, are present at least transiently within P granules. In contrast, *actin* and *tubulin* mRNA, and ribosomal RNA are either not present in P granules, or are present at relatively low levels. At present we know of one shared feature of the RNAs that are enriched in P granules; each is a member of a group called class II maternal mRNAs [1]. These mRNAs are rapidly degraded during early embryogenesis in somatic blastomeres while they persist in the germline precursors.

We are currently investigating what sequences are required to target certain mRNAs to P granules. We are taking a microinjection approach to this problem utilizing fluorescently labeled RNAs. Somewhat surprisingly, our preliminary results have indicated that a trimethyl guanosine-capped and in vitro polyadenylated *actin* transcript is targeted to P granules within 90 minutes post-injection. We are now injecting various forms of the *actin* transcript as well as various forms of a *pos-1* transcript in order to determine what sequences target mRNAs to P granules in the gonad.

[1] Seydoux, G. and FIre, A. (1994) *Development* 120, 2823-2834.

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759. PGL-1, PGL-2, and PGL-3, a family of P-granule proteins, function redundantly to ensure fertility in both sexes of *C. elegans*

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P granules are distinctive ribonucleoprotein complexes observed specifically in the cytoplasm of germ cells throughout development. We previously identified PGL-1 as being a constitutive protein component of P granules. The presence of an RGG box predicts that PGL-1 is an RNA-binding component of P granules. *pgl-1*mutants contain defective P granules and are sterile, due to defects in proliferation and gametogenesis. Interestingly, the sterility caused by null alleles of *pgl-1* is highly sensitive to temperature. Our identification and analysis of two additional *pgl-1*-related genes, termed *pgl-2* and *pgl-3*, demonstrate that the PGL proteins function redundantly, and at low temperature, PGL-2 and PGL-3 are sufficient for fertility.

PGL-2 has 34% identity with PGL-1 in its N-terminal region. PGL-3 has 62% identity with PGL-1 throughout its length and contains an RGG box. Based on yeast two-hybrid and GST pull-down results, the three PGL proteins interact with each other. Furthermore, PGL-1 and PGL-3 are co-immunoprecipitated from both embryo and oocyte extracts, indicating that they are indeed in the same protein complex *in vivo*. Immunofluorescence analysis has demonstrated that in wild-type worms PGL-3 is associated with P granules at most stages of development, like PGL-1, but interestingly, PGL-2 is associated with P granules only during postembryonic development. Based on molecular epistasis results, each PGL protein associates with P granules independently of the other two.

To address whether PGL-3 functions redundantly with PGL-1, we isolated a *pgl-3* deletion allele. We found that *pgl-1; pgl-3* double mutant hermaphrodites and males show significantly enhanced (but not 100%) sterility at low temperature, compared to either single mutant. Double mutant hermaphrodites contain a severely underproliferated germline, indicating that the primary defect is in proliferation. Depletion of *pgl-2* by RNAi did not enhance sterility further.

Our findings suggest that both PGL-2 and PGL-3 are components of P granules that interact with PGL-1 and that at least PGL-3 functions redundantly with PGL-1 to ensure fertility in both sexes during *C. elegans* germline development.

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760. The enigma of *(y2):* an XO-specific lethal mutation

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During embryogenesis, a *C. elegans* embryo must first assess its genetic dosage of X chromosomes, and then develop accordingly as male (XO) or hermaphrodite (XX). This development involves not only morphological sexual differentiation, but also implementation of X-chromosome dosage compensation, an essential process. Dosage compensation in worms is achieved by repressing the level of X expression by half in XX animals. Genetic screens for sex-specific lethality have been a successful approach to dissecting the pathway involved in counting the number of X chromosomes (X-signal elements *fox-1* and *sex-1*), and in implementing the consequences of that counting process (the *sdc* genes, and dosage compensation-specific genes). The target of the counting process is the *xol-1* gene, which coordinates sexual morphology with dosage compensation. *xol-1* XO mutants inappropriately activate the hermaphrodite mode of development--they are feminized, and die due to underexpression of X-linked genes. Interestingly, in addition to its essential role in XO animals, *xol-1* has a secondary role in the feminization of XX animals.[1]

The maternal-effect autosomal mutation *y2* was isolated in a screen for sex-specific phenotypes and exhibits XO-specific embryonic lethality. Unlike *xol-1* mutants, *y2* mutants exhibit reduced expression from the X chromosome in both XX and XO karyotypes. XX hermaphrodites have phenotypes that are consistent with perturbed expression from X. Therefore, *y2* XO animals could suffer from reduced gene expression below a critical threshold, and die as a result. However, overexpression of *xol-1* from a ubiquitously expressed promoter can rescue *y2* XO lethality, whereas the XX lethality from overexpression of *xol-1* is not rescued by *y2*. This result suggests that *y2* does impinge on the regulatory pathway for sex determination and dosage compensation. Surprisingly, the rescued animals are feminized, a phenotype not seen when *xol-1* is overexpressed in a wild-type XO background. Paradoxically, staining of dying *y2* XO embryos with antibodies against the dosage compensation complex indicates that the complex is not localized to X, as it is in dying *xol-1* XO embryos.

At this time, no simple explanation can be found for the role of *y2*, but we are intrigued by the possibilities. *y2* may generally regulate gene expression, act as an autosomal signal element, be involved in the coordinate control of dosage compensation and sexual differentiation, or function with *xol-1* to specify sexual differentiation. Currently, efforts are being taken to map and clone *y2*, and genetic and molecular experiments are being performed to address how this mutation may act on known genes in the pathway.

[1]Miller, L.M., Plenefisch, J.D., Casson, L.P., and Meyer, B.J. (1988). *Cell* 55, 167-183.

761. The nuclear receptor SEX-1 in *C. elegans*sex determination

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In *C. elegans*, the primary sex determination signal that specifies the two sexes, XO males and XX hermaphrodites, is the number of X chromosomes relative to the sets of autosomes. The X-signal elements located on the X chromosome function cumulatively to repress the sex determination and dosage compensation switch gene *xol-1*, whose level of expression sets the sexual fates. The X-signal element, *sex-1*, encodes a nuclear receptor that transcriptionally regulates *xol-1*. Previously, we have identified a 2.8 kb region of the *xol-1* promoter that binds SEX-1 *in vivo* (Carmi, 1998). A GFP reporter fused to this 2.8 kb promoter region is derepressed in a *sex-1(gm41)* mutant background, further suggesting that SEX-1 regulatory regions might be contained within this fragment of the *xol-1* promoter. To determine whether SEX-1 binds directly to the *xol-1* promoter, we are undertaking *in vitro* binding experiments with a partially-purified DNA-binding domain of SEX-1 and fragments of the *xol-1* promoter. In these experiments, we are also interested in investigating whether SEX-1 functions as a monomer or dimer.

Carmi I., Kopczynski J.B., and Meyer B.J. (1998). The nuclear hormone receptor SEX-1 is an X-chromosome signal that determines nematode sex. Nature *396*, 168-73.

762. Systematic alteration in the sex determination system of *C. elegans*

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Sex in *C. elegans* is normally determined by X chromosome dosage (XX hermaphrodite, XO male). This primary signal acts through the coordinate *xol-1* and *sdc* regulators to set the state of a cascade of seven regulatory genes located on the autosomes (*her-1, tra-2, tra-3, fem-1, fem-2, fem-3* and *tra-1)* which control both germline and somatic sexual phenotype, without affecting dosage compensation. Previous work has shown that stable strains can be constructed in which sex is determined not by the X chromosome but rather by the state of the autosomal genes *tra-1* (Hodgkin 1983) or *tra-2* (Miller et al. 1988).

A series of further strains have now been constructed in order to demonstrate that any one of the seven sex determination genes, or even suppressors of these genes, can become the sole determinant of sex. As a result, the primary sex determination role can be transferred to each of the five autosomes in turn, thereby converting each one into a sex chromosome. These different male/female or male/hermaphrodite strains mimic various natural sex determination systems found in other animals. For example, strains based on *tra-3* mimic the system in the fly *Chrysomya rufifacies*, with two kinds of female producing either all-male or all-female broods. Stable strains with extrachromosomal determination of sex have also been constructed. Environmental sex determination can be created, by introducing temperature-sensitive mutations into one or more of these genes.

Mutations in at least two genes also exhibit starvation-enhanced feminization phenotypes, although complete sex-reversal by nutritional state (as found in certain other nematode species) has not yet been achieved. Allele-specific responses to starvation in different *her-1* mutant strains suggest a possible function for the hitherto enigmatic small transcript of *her-1*.

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763. Characterization of TRA-3 calpain, a protease involved in sex determination

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The *tra-3* gene promotes female development in XX animals: XX *tra-3(m-z-)* mutants are transformed from hermaphrodite to male, while XO *tra-3* mutants are essentially wild-type males. Barnes and Hodgkin have shown that *tra-3* encodes a predicted homologue of the large subunit of calpain, a calcium-activated regulatory cysteine protease (EMBO J., 15:4477-4484, 1996). Typical calpain large subunits have four domains (I-IV); domain II contains the catalytic active site, and domain IV contains a series of calcium-binding EF-hand motifs. The predicted TRA-3 protein is an atypical calpain because it has extensive sequence similarity to domains I-III of vertebrate calpains but lacks domain IV; instead, TRA-3 contains an unrelated domain T containing a C2 domain, which may bind calcium. Two mammalian calpain homologues that lack domain IV and share extensive sequence similarity with TRA-3 have recently been identified; we propose that TRA-3 is the founding member of a new subfamily of calpain proteases.

We are investigating how TRA-3 functions in the sex determination pathway and, more generally, how the proteolytic activity of TRA-3 is regulated. We have shown that TRA-3 function *in vivo* depends on having an intact active site. Moreover, we have demonstrated that TRA-3 has proteolytic activity *in vitro*. Surprisingly, the proteolytic activity of TRA-3 is calcium-dependent despite the absence of calcium-binding EF hands. Physiological substrates for calpains were previously unknown; however, we have shown that TRA-3 cleaves the sex determination protein TRA-2A when co-expressed in a heterologous system. Furthermore, the *in vivo* activity of *tra-3* is dependent on *tra-2*, suggesting that TRA-2A may represent a physiological substrate for TRA-3 proteolysis.

We examined the basis for sex-specific regulation of *tra-3* in the soma, and also for regulation of *tra-3* in the hermaphrodite germline. We have obtained evidence that *tra-3* mRNA and protein are expressed in both sexes; steady-state levels of *tra-3* gene products appear to be comparable in both sexes. In addition, we have shown that domain T and its associated C2 domain are not required for TRA-3 proteolytic activity. Instead, calcium regulation of TRA-3 and other calpains may be the responsibility of a novel C2 domain that was identified after analysing the crystal structure of domain III of m-calpain. It is likely that TRA-3 also contains this C2 fold because it shares sequence similarity with m-calpain throughout domain III (Hosfield et al., EMBO J., 18:6880-6889, 1999; Z. Jia, pers. comm.). Although the C2 domain contained in domain T is not responsible for conferring calcium dependence on TRA-3 proteolytic activity, we have shown that this C2 domain is capable of binding calcium and may regulate TRA-3 activity in the germline.

764. TRA-1 is a Phosphoprotein and Interacts with FEM-2, a Protein Type 2C Phosphatase

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In *XO* animals the three fem genes, *fem-1*, *fem-2*, and *fem-3*, act to negatively regulate *tra-1*, the terminal regulator of somatic sex determination. To understand the mechanism of *tra-1* regulation by the *fems* we have attempted to detect physical interactions between these proteins.

We have identified an *in vitro* interaction between FEM-2, a Type 2C protein phosphatase, and the product of its genetic downstream target, *tra-1*. The *tra-1* gene encodes two putative zinc finger proteins, TRA-1A and TRA-1B. The zinc fingers of TRA-1 are related to those of the vertebrate GLI proteins. Deletion analysis suggests the phosphatase domain of FEM-2 interacts with the first two zinc fingers of TRA-1 common to both TRA-1A and TRA-1B.

These results are consistent with a model where FEM-2 negatively regulates TRA-1 activity through a dephosphorylation event. A prediction from this model is that TRA-1 must be phosphorylated in a sex specific pattern. Over-expression of Myc-TRA-1 has enabled the detection of at least two MycTRA-1 isoforms that can be collapsed to a single band upon phosphatase treatment.

In order to identify phosphorylated domains of TRA-1, we have expressed several fragments of MycTRA-1 in transgenic animals. We have generated anti-TRA-1 antibodies and are attempting to characterize the phosphorylation state of endogenous TRA-1A. We will report on the phosphorylation state of MycTRA-1 fragments and attempts to purify endogenous TRA-1A.

765. A genetic screen for regulators of sexual development in the male tail

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The DM domain transcription factor *mab-3*, like its *Drosophila* homologue *doublesex (dsx)*, regulates diverse male-specific traits that include expression of yolk in the intestine and the development of male-specific nervous system structures. In the *C. elegans* male tail, *mab-3* is required for differentiation of V rays, male sensory structures that are required for mating. In *mab-3* mutant males, the V5- and V6-derived neuroblasts fail to generate the sensory neurons and support cells that comprise the adult V rays, and instead differentiate primarily as hypodermal cells.

While it has been clearly demonstrated that MAB-3 acts directly to repress yolk transcription in the male intestine[1], less is understood about the role of *mab-3* in V ray differentiation. We have recently shown that *mab-3* is not absolutely required for V ray formation; overexpression of the bHLH gene *lin-32* can direct V ray differentiation in the absence of *mab-3*. We propose that *lin-32* acts as a primary determinant of V ray fate, while *mab-3* acts to potentiate *lin-32* activity[2]. Thus *mab-3* and *lin-32* act in concert to promote differentiation of the V5- and V6-derived neuroblasts.

To better understand the mechanisms by which the *mab-3* and *lin-32* pathways converge to direct V ray development, we are currently screening for mutations that suppress the *mab-3(e1240)* lack of V rays. We expect this screen to reveal targets of *mab-3* regulation as well as genes that compensate for loss of *mab-3* by upregulating activity of the *lin-32* pathway. We have identified two mutations, *ez1* and *ez2*, which confer a phenotype that we have provisionally called Smt (Suppressor of mab in tail). Both *ez1* and *ez2* act recessively to suppress *mab-3(e1240)*, partially restoring V ray formation. Suppression by *ez1* and *ez2* is sensory ray-specific; neither mutation

suppresses the ectopic yolk expression in the *mab-3(e1240)* male intestine. Progress toward characterizing *ez1* and *ez2* and isolating additional suppressors will be reported. Identifying the nature of mutations that suppress *mab-3* will provide insight into the genes that act with *mab-3* and *lin-32* to direct male peripheral nervous system development. Finding homologues of these *mab-3* suppressors may reveal whether conservation between *mab-3* and *dsx* extends to include additional downstream regulators of male sexual development.

[1] Yi and Zarkower (1999). *Development* 126: 873-877.

[2] Yi *et al.* (2000). *Development* 127: 4469-4480.

766. Secreted Signaling Ligand HER-1A Interacts with the TRA-2A Transmembrane Receptor to Regulate Sexual Cell Fates

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In the *C. elegans* sex determination pathway, *her-1* acts as a switch to specify male sexual fate by negatively regulating *tra-2*. Since sequence data suggests HER-1A is a small, secreted protein and TRA-2A is a large membrane-spanning protein, it has been hypothesized that HER-1A functions as a signaling ligand and TRA-2A as its receptor. In XX hermaphrodites, *her-1* is transcriptionally silenced and *tra-2* is active. However, HER-1A is expressed in XO animals, where it could interact with and inhibit TRA-2A, directing cells to adopt male fates.

We have used a highly specific antiserum to show that HER-1A is capable of entering the secretory pathway *in vivo* and *in vitro*. Since the HER-1 protein is very rare in worms, we used Sf9 cells to produce HER-1A in larger quantities. To assay binding we expressed the TRA-2A protein in COS-7 cells and exposed them to the HER-1A protein. We have shown that recombinant HER-1A binds to cultured cells expressing TRA-2A, providing the first evidence for a physical interaction between these two proteins.

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767. *C. elegans* SMC complexes: from dosage compensation to chromosome segregation

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The highly conserved structural maintenance of chromosome (SMC) proteins serve essential roles in chromosome segregation and condensation in mitosis and meiosis. The typical eukaryotic SMC proteins function within a multimeric complex, containing a pair of SMC heterodimer and other non-SMC subunits. In *C. elegans*, the SMC proteins have been adapted for a third essential process, dosage compensation. This process equalizes X-linked gene products between XX hermaphrodites and XO males. A dosage compensation specific SMC protein, DPY-27, along with mitotic and meiotic proteins form a complex that reduces gene expression from the hermaphrodite X chromosomes. The sex-determination and dosage compensation (SDC) proteins in hermaphrodites target this dosage compensation specific SMC complex (DCC) to the X chromosomes. We are interested in identifying and characterizing the components of the SMC complexes in *C. elegans* to elucidate their functions in dosage compensation and other chromosome dynamics.

The SMC protein, MIX-1, serves dual roles in dosage compensation and in mitotic chromosome condensation. Immunoprecipitation of MIX-1 from embryonic extract co-purified a 200-kDa protein, encoded by the ORF Y110A7A.1. Injections of dsRNA against this gene resulted in embryonic lethality. Analysis of the RNAi-treated embryos revealed severe chromosomal abnormalities. Subsequent biochemical experiments demonstrated that Y110A7A.1 associates exclusively with the mitotic SMC complex and not with the DCC. Reiterative BLAST searches found that Y110A7A.1 is distantly related to the XCAP-D2 subunit of the Xenopus mitotic 13*S* condensin. Hence we refer to this protein as CeCAP-D2. Interestingly, CeCAP-D2 is also homologous to the dosage compensation protein, DPY-28, which is found in the DCC and not in the mitotic SMC complex. The CeCAP-D2 and DPY-28 proteins may be paralogs, which raises an intriguing possibility that these two proteins may have similar functions within their respective SMC complex. Work is in progress to identify additional proteins that co-precipitate with MIX-1 and CeCAP-D2.

Aside from identifying protein co-factors by immunoprecipitation, we are also interested in defining the X sequences bound by the SDC proteins and the DCC. We have developed a chromatin precipitation (ChIP) protocol to show interaction between the SDC proteins and the *her-1* gene, an autosomal gene regulated by SDC proteins. We have examined the DNA precipitated by SDC-2 and SDC-3 antibodies, and found by PCR and southern blot analyses that genomic *her-1* DNA is specifically co-precipitated. We are applying this approach to identify the X sequences bound by the SDC proteins.

768. Molecular Characterization of the Dosage Compensation Gene, *dpy-21*

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Dosage compensation in *Caenorhabditis elegans* equalizes X chromosome expression between XO males and XX hermaphrodites. DPY-21 is one of several proteins required for proper dosage compensation. However, DPY-21 is unique among known dosage compensation proteins. *dpy-21* mutations cause only 20% lethality and affect both XX and XO animals while other dosage compensation mutations cause maternal-effect lethality and affect only XX animals. Furthermore, assays of different X-linked gene expression have shown that *dpy-21* mutations can cause both elevation and reduction in X-linked gene expression in XO animals. *dpy-21* was mapped and then cloned by injecting single-stranded RNA from candidate ESTs that map to a YAC close to *dpy-21*. Two positive ESTs were found to represent the same gene and were used to assemble the *dpy-21* mRNA sequence in conjunction with 5' and 3' RACE products. To verify that we had identified *dpy-21*, we sequenced six *dpy-21* alleles and found DNA lesions within the predicted coding sequence: three premature amber STOP codons, two missense mutations, and one in-frame deletion. The 5610 nucleotide *dpy-21* transcript, which is both SL1 and SL2 spliced, encodes a novel 1651 amino acid protein. While there is no sequence similarity to known proteins, putative homologues are present in *Drosophila melanogaster* and human genomes.

769. The *let381* gene

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The *let-381* gene encodes for a Forkhead transcription factor. Forkhead transcription factors have a highly conserved DNA-binding domain and have proved to be involved in development in many species.

The let-381 mutants die as embryos or L1 and few percent develops to sterile adults. This shows that the Let-381 protein is important for proper development at different times.

We will discuss potential target genes and why the Let-381 protein is important for development

770. Characterizing the *C. elegans* ortholog of STATs

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STAT proteins are latent transcription factors that are activated by tyrosine phosphorylation in response to extracellular signals and have been identified in a variety of organisms. To study the biological function of the STAT gene in *C. elegans* and its underlying signal transduction pathway, we cloned the nematode ortholog of mammalian STAT genes. It encodes a protein of 82KD that contains a DNA binding domain, SH2 domain, and site for tyrosine phosphorylation conserved with its mammalian counterparts. Promoter::GFP studies and immunohistochemistry using rabbit antibody against the endogenous protein show that it is highly expressed in pharynx, intestine, body muscle and most of the neuronal cells. RNAi experiment failed to show any detectable phenotype. We then screened a Tc1 transposon insertion and deletion library using *mut-2* strain MT3126, and obtained a mutant that has a 2.1kb genomic deletion in the gene, resulting in complete loss of protein expression. We have completed background cleaning and now are in the process of characterizing the mutant phenotype.

771. Characterization of CeTRAP240, a C. elegans Homolog of a Component in Multi-protein Transcriptional coactivator Complexes

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Recent studies have identified several mammalian multi-protein complexes, such as TRAP, DRIP and ARC, that function as coregulators for the transcriptional control of protein-encoding genes by RNA polymerase II. It is well established that these complexes can enhance the activator-stimulated gene transcription in vitro. However, the functions, including the physiological roles, of individual component in these complexes are mostly unknown. We are employing C. elegans as a model system to dissect the roles of CeTRAP240, a worm homolog of a component of TRAP/DRIP/ARC complexes, in the worm development. Elimination or reduction of CeTRAP240 expression in worms by RNA-mediated interference results in a high degree of embryonic arrest. Interestingly, although CeTRAP240 is ubiquitously expressed in embryos, RNAi of CeTRAP240 shows the specific reduction of HLH-1 (muscle cell differentiation marker) expression, but not PHA-4 (digestive tract marker), SCM (seam cell marker), UNC-47 (GABA neuron marker) or JAM-1 (epithelial cell marker). Thus, CeTRAP240 may play a specific role in muscle differentiation. In contrast to CeTRAP240, RNA-mediated interference of another worm homolog of a component of TRAP/DRIP/ARC complexes, CeTRAP230 (sop-1), only causes a small degree of embryonic arrest. However, many adult animals develop burst vulva and/or dumpy phenotypes, which are often associated with defects in hypodermal development. The mutation in sop-1 gene is able to suppress the pal-1(e2091) mutant male tail phenotype. The pal-1(e2091) mutants specifically lose pal-1 expression in the postembryonic neuroblast cell V6, which results in failure of the generation of V6 rays in adult male tail. We found that RNAi of CeTRAP240 also can suppress the pal-1(e2091) phenotype. Thus, CeTRAP240

may function in the same pathway with sop-1 to regulate pal-1 gene expression. Future experiments will take advantage of the C. elegans genetic system for developing screens designed to identify genes that specifically interact with these coregulators.

772. *skn-1* is required postembryonically for detoxification gene activation and resistance to oxidative stress

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The *C. elegans* transcription factor SKN-1 initiates formation of the feeding and digestive system by specifying the fate of the early embryonic cell EMS, and also has later postembryonic functions in the intestine that have not been elucidated. Our laboratory has determined that in portions of its DNA binding and transcriptional activation regions, SKN-1 is related to the vertebrate NRF (NF-E2-related factor) bZIP proteins, orthologs of which are not encoded in *C. elegans* (Walker, et al., JBC 275, 22166-22171). This similarity suggests that SKN-1 might represent an NRF protein ortholog, even though it binds to DNA through a highly divergent mechanism. In mice, *nrf* genes are required to resist oxidative stresses, and NRF proteins activate oxidative stress-induced enzyme genes directly, indicating that *skn-1* might have similar functions.

Supporting this model, we have determined that homozygous *skn-1*(*zu67*) L2 and L3 larvae are subject to significantly increased lethality when exposed to either heat or the oxidative stress inducer methyl viologen (paraquat). We also find that *skn-1* is expressed in the larval and adult intestine, and that in *C. elegans* multiple consensus SKN-1 sites are located within 1 kb of the 5' start site of multiple NRF target gene orthologs. These *C. elegans* genes include gamma-glutamylcysteine synthetase (*gcs-1*), glutathione synthetase, NAD(P)H:quinone oxoredutase and some glutathione S-transferase (GST) isoforms. We have investigated whether *skn-1* is required for expression of *gcs-1*, which is essential for synthesis of the crucial reducing agent glutathione. In wild type *C. elegans*, a *gcs-1::gfp* transgene reporter is expressed at high levels in the pharynx and low levels in the intestine, but its intestinal expression is induced robustly by either paraquat or heat. In contrast,

in *skn-1*(*zu67*) homozygotes *gcs-1::gfp* expression is detected only in the pharynx under either normal or inducing conditions. Transgenic deletion and mutation analyses indicate that intestinal *gcs-1::gfp* expression depends upon a promoter region that contains multiple SKN-1 sites, including a composite element like that to which SKN-1 appears to bind directly to activate its embryonic target genes *med-1* and *med-2*. We find that a SKN-1 binding site in this element is essential for intestinal *gcs-1::gfp* expression. Our findings indicate that SKN-1 and NRF proteins have preserved some parallel functions, and that SKN-1 acts through conserved transcriptional mechanisms that are involved not only in the initial specification of the feeding and digestive system, but are also important for one of its most important and ancestral functions.

773. Analysis of PHA-4 activity on the *myo-2* promoter and identification of a new PHA-4 DNA binding site

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PHA-4 is a forkhead/winged helix transcription factor that is essential for pharyngeal organogenesis. PHA-4 is expressed in all five classes of pharyngeal cells. Ectopic expression of PHA-4 leads to ectopic expression of pharyngeal markers. We wish to identify other factors that cooperate with PHA-4 to confer cell-type specificity. In pharyngeal muscles, PHA-4 binds to a site in the *myo-2* promoter to activate *myo-2* transcription. A potential transcriptional cofactor of PHA-4 is PEB-1, a novel DNA binding protein that is expressed in the pharynx and binds to the *myo-2* promoter at a site that overlaps with PHA-4 (**1**). To test for PHA-4 and PEB-1 interactions we have expressed both factors in yeast to investigate their ability to activate transcription of a reporter gene regulated by the binding sites found in the *myo-2* promoter. Both PHA-4 and PEB-1 (weakly) activate transcription when expressed alone. However, we detect neither synergy nor interference between PHA-4 and PEB-1 when they are coexpressed. Other experiments also suggest no evidence for cooperative binding but rather that PEB-1 may interfere with PHA-4 binding (**1**).

To identify PHA-4 target genes, we are determining the preferred PHA-4 binding site. The *pha-4* gene produces three PHA-4 protein isoforms called PHA-4A, B and C that differ only at their N-termini. After six rounds of DNA site selection from a pool of random oligonucleotides 20 bp long, baculovirus-produced PHA-4B selects the sequence TGTGG(C/T); this differs somewhat from the known TGTTTG PHA-4 binding site in the *myo-2* promoter. We are currently putting this selected sequence in the promoter of a GFP reporter gene to determine if it functions as an

enhancer *in vivo* and continuing site selection with PHA-4A and C.

(**1**) See the abstracts by Laura Beaster-Jones and Anthony Fernandez.

774. The LIM homeobox gene *ttx-3*: a paradigm for the complexity of transcriptional control in cell fate specification in the nervous system

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In organisms ranging from vertebrates, to flies and to *C. elegans*, the LIM homeobox class of transcriptional regulators has been shown to be important in the determination of aspects of terminal differentiation of neurons. While there has been much information detailing expression patterns and implicating these transcription factors in the terminal differentiation of neurons, little light has been shed on the identity of the downstream targets that are ultimately responsible for these characteristics that are imparted by this class of transcription factors.

Previously, our lab has identified a transcriptional cascade in the AIY interneuron involving *ttx-3*, one of the *C. elegans* LIM homeobox proteins, and two other homeobox genes, *ceh-10* and *ceh-23* (Altun-Gultekin, *et al.*, *Development* in press). In this cascade, *ceh-10* is required to initiate expression of *ttx-3*, which then positively regulates its own expression as well as the expression of *ceh-23*. In addition to *ceh-23*, the AIY terminal differentiation markers *sra-11*, *ser-2*, *kal-1*, and *unc-17*, which code for two G-protein coupled receptors, a cell surface protein, and an acetylcholine vesicular transport protein, respectively, were also described as being either direct or indirect targets of *ttx-3*, with *sra-11* also under the regulatory control of *ceh-23*. Intriguingly, by studying the expression patterns of these transcriptional regulators along with the expression pattern of their downstream targets, it became clear that while this regulatory cascade is critical for AIY cell fate specification, expression of *ceh-10* or *ttx-3* in other cellular contexts does not lead to an AIY-like differentiation pattern. For example, while *ceh-10* is also expressed in the CAN neuron, its expression is not sufficient to turn on the expression of *ttx-3*. Likewise, *ttx-3* expression in the ASI and AIA neurons is not sufficient to lead to expression of *ceh-23*. Additionally, as with AIY, CAN neuron fate specification, which includes the expression of

ceh-23 and *kal-1*, requires *ceh-10* expression. However, in CAN these cell fate markers are induced without utilizing *ttx-3* as an intermediary. This complexity of control in neuronal fate specification has led us to investigate the modulatory nature of the promoters of these downstream genes to elucidate why some promoter elements are used in specific cellular contexts.

In order to address this question, as well as to determine whether these downstream genes are direct or indirect targets of *ttx-3*, we have made deletions and point mutations in the transcriptional regulatory regions of these genes (*ttx-3*, *ceh-23*, *kal-1*, *sra-11*, *ser-2*, *unc-17*) fused to GFP as a reporter. By assaying for reporter expression in the AIY class of neurons, we have identified a 12-nucleotide presumptive TTX-3 binding site that is common to the regulatory regions of the *kal-1* and *ttx-3* genes and is necessary for *kal-1* and *ttx-3* GFP reporter expression in AIY. Through gel shift analysis, we intend to show whether this sequence is in fact a TTX-3 binding site and what other factors might be involved in cooperative binding to this proposed AIY enhancer element. In addition, through further promoter analyses with the other presumptive downstream targets of *ttx-3*, we hope to further define a consensus TTX-3 binding site. We then intend to use this consensus sequence to search the *C. elegans* genome for additional candidate downstream targets of *ttx-3*.

775. Functional redundancy between *pes-1* and *fkh-2*(T14G12.4).

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pes-1 is expressed in several lineages during early embryogenesis and encodes a fork head transcription factor. This suggests that *pes-1* has an important role in the regulation of gene expression during early embryonic development. Inactivation of *pes-1* however, either by RNAi or by a Tc1-mediated deletion, does not give rise to any obvious phenotype. We now know this is because of genetic redundancy and *pes-1* functions redundantly with another *C. elegans* fork head gene, *fkh-2* (T14G12.4).

fkh-2 and *pes-1* have strikingly similar expression patterns. *fkh-2*::*gfp* expression appears to start one round of cell divisions later than *pes-1*::*gfp*. The D lineage component for both of these expression patterns completely overlaps whereas partial overlap is seen in the AB lineage component of both of these expression patterns. Interestingly, double antibody staining suggests a reciprocal level of expression between *fkh-2* and *pes-1* within the AB lineage. This similar expression pattern suggested to us that *fkh-2* function could overlap with that of *pes-1* even though these fork head genes do not share particular sequence similarity. Inactivation of *fkh-2* alone by RNAi, results in a subtle phenotype of restricted L1 movement. Disruption of both *fkh-2* and *pes-1* function, however, results in a penetrant lethal phenotype. 12% of progeny arrest during variable late stages of embryogenesis and a further 80% of progeny arrest at the first larval stage. Preliminary examination of the arrested larvae reveals anterior pharyngeal defects.

A screen is being undertaken for a Tc3-mediated deletion mutant of *fkh-2*. The double mutant phenotype for *pes-1* and *fkh-2* will then be studied in more detail.

776. The role of TLF in RNA Polymerase II transcription

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Metazoans possess two TATA-binding protein homologs: the general transcription factor TBP and a related factor called TLF. In *C. elegans*, TLF is required to express a subset of RNA Polymerase II (Pol II) genes, including *pes-10*, in pregastrula embryos (1). Two models could explain the non-redundant role of TLF during embryogenesis. First, TLF could function as a component of the basal transcription machinery and replace TBP at certain promoters. This model predicts that i) individual promoters should depend on either TBP or TLF, but not both, and ii) TLF should bind its target promoters near the transcription start site. Alternatively, TLF could have co-opted the TBP DNA binding domain for a new purpose, such as functioning as an enhancer-binding activator. The second model predicts TLF binds farther upstream and recruits the TBP-containing basal machinery to activate transcription. Our current goal is to distinguish between these models by using a putative TLF target (the *pes-10* promoter) to identify TLF binding sites and to determine the role of TLF binding *in vivo*.

1. Kaltenbach et al., Molecular Cell, 6, 705-713 (2000).

777. Sins and saps: investigating components of the *Caenorhabditis elegans* Sin3-histone deacetylase complex.

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The significance of histone acetylation and deacetylation in transcriptional regulation has only been realised within the last five years. Hyperacetylation of lysine residues in histone tails correlates to up regulation of transcription and, conversely, hypoacetylation correlates to down regulation of transcription. Removal of some acetyl groups by deacetylase enzymes, is mediated by a Sin3-containing protein complex. Sin3 interacts with many transcriptional repressor proteins directly (such as MNF-β and MAD), or indirectly through N-CoR and SMRT.

The Sin3-deacetylase complex is evolutionarily conserved and has been found in organisms ranging from yeast to mouse to human. The following members of this complex: *sin3*, *sap18* and *sap30* have been identified in *C. elegans* by analysis of the complete genome sequence. We have investigated the function of these components by both the construction of reporter gene fusions, and through examination of phenotypes generated by double-stranded RNA interference (RNAi).

Ce-sin3::gfp begins expressing in embryos around the 8 E-cell stage, and through to adulthood in head and tail ganglia, the ventral nerve cord, intestine, hypodermis, pharynx and somatic gonad. RNAi for this gene affects the egg-laying rate of F1 progeny. *Ce-sap18*::gfp shows extensive expression in embryos, becoming restricted to the head in early larvae. Some expression is also observed in adult heads. No phenotype was observed from RNAi experiments targeted to this gene. The putative *Ce-sap30* when fused to GFP, is solely expressed in the excretory cell and canals, however when crossed with N2 males some expression has been seen in pre-elongated embryos. The RNAi phenotype for this gene is a highly penetrant embryonic lethal.

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778. What is TLF doing during the *C. elegans* cell cycle?

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Transcription is finely regulated during the cell cycle. In most organisms, genes are actively transcribed during interphase, but not during mitosis when DNA is condensed, nascent transcripts are released, and transcription factors are inactivated by phosphorylation and discharged from DNA. How then do cells re-establish the appropriate transcriptional repertoire in newly born daughter cells? One attractive idea is that cells mark genes to specify those that are actively being transcribed during interphase. The molecular nature of the mark is currently unknown.

We are using *C. elegans* embryos to investigate cell cycle regulation of RNA Polymerase II (Pol II) transcription. Like other organisms, *C. elegans* probably inactivates transcription at mitosis since i) Pol II in mitotic cells lacks a phosphorylated epitope normally associated with transcriptional elongation (1, 2) and ii) transcription factors are dispersed at this stage of the cell cycle (L. Kaltenbach and S.E.M., unpublished). Since embryonic blastomeres cycle very rapidly (~15-30 minutes), we envision that gene marking and rapid re-establishment of transcription after mitosis are critical for normal embryonic development.

One appealing candidate for a marking protein is the TATA-binding protein (TBP) paralog TLF. TLF is required for Pol II transcription during *C. elegans* embryogenesis, suggesting it performs a unique function distinct from that of TBP (2, 3). TLF is required to activate a subset of Pol II promoters and facilitates the re-establishment of transcription after mitosis (2). In addition, preliminary data indicate that TLF may associate with mitotic chromosomes during the cell cycle (L. Kaltenbach and S.E.M., unpublished). This association contrasts with TBP, which is released during mitosis (L. Kaltenbach and S.E.M., unpublished). These observations suggest that TLF has a critical role during the cell cycle, possibly to mark genes for their timely reactivation during the next interphase. Our current goal is to test this hypothesis using both *in viv*o and *in vitro* approaches.

1. Seydoux and Dunn, Development 124, 2191-2201 (1997)
2. Kaltenbach et al., Molecular Cell, 6, 705-713 (2000).
3. Dantonel et al., Molecular Cell 6, 714- (2000)

779. Evolution of homeotic gene function in nematodes

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We are studying the evolution of vulval cell fate specification in nematodes by comparing *Pristionchus pacificus* with *Caenorhabditis elegans*. Many features of the posterior body region of nematodes are determined by the homeotic gene *mab-5*. In *C. elegans*, the MAB-5 protein regulates the expression of different target genes in response to upstream signals like PAL-1 or the Wnt pathway. *Cel-mab-5* plays an important role during male ray formation, M lineage specification and also during hermaphrodite vulva formation. *P. pacificus mab-5* mutants have a similar ray phenotype, whereas the phenotype in the M lineage and the vulva differs strongly from *Cel-mab-5*. For instance, the "multivulva phenotype" caused by the ectopic differentiation of P8.p in *Ppa-mab-5* mutants depends on interactions between P8.p and the misspecified M lineage. Given the pattern of conservation and change of MAB-5 function, we want to study if regulatory and/or coding differences are responsible for these evolutionary alterations. In the present work, we expressed *Ppa-mab-5* in a *Cel-mab-5* mutant background under the control of the *Cel-mab-5* gene and studied the rescue of the posterior rays. We cloned the *Ppa-mab-5* ORF in a construct containing the complete *Cel-mab-5* gene including the 6 kb upstream region. This basic construct has a frameshift mutation in the hexapeptide (HD-) to eliminate the function of the *Cel-mab-5* gene but conserving its regulatory regions. Preliminary results show that *Ppa-mab-5* ORF can rescue *Cel-mab-5* during ray formation as efficient as *Cel-mab-5* (HD+) or *Cel-mab-5* ORF cloned in the same way. Further experiments are under way to study MAB-5 function in other tissues and to compare various Hox genes in the ray rescue assay.

780. THE MOLECULAR CHARACTERIZATION OF NOVEL bHLH PROTEINS IN CAENORHABDITIS ELEGANS.

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The helix-loop-helix (HLH) family of transcription factors is responsible for the expression of genes that regulate many aspects of development in eukaryotes including cellular differentiation and specification, morphogenesis, and growth. The nematode, Caenorhabditis elegans, possesses many different transcription factors, including HLH proteins, many of which have been identified and characterized. Through Blast searches, we have identified putative HLH-encoding genes in the C. elegans genome, some of which are homologous, at varying degrees of identity and similarity, to members of the Achaete-Scute (As), Hairy-related (Hes), and Atonal-related (Ato) subfamilies regulating neurogenesis. While As and Ato-related proteins form heterodimers with ubiquitous HLH proteins in order to facilitate transcription, Hes proteins dimerize with universal HLH proteins to repress transcription of a target gene. We have sought to determine which of the hypothetical genes are expressed in wild-type C. elegans by RT-PCR and by Northern blotting, and to determine the spatial and temporal expression patterns using in situ hybridization. We have also performed RNA interference assays to assign a preliminary function to the genes that are expressed.
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781. Characterization of tissue-specific targets for the Pax factor EGL-38

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The *C. elegans Pax* gene *egl-38* plays a role in the development of several structures including the hindgut, the hermaphrodite egg-laying system and male tail structures. Genetic analysis has identified hypomorphic alleles that preferentially disrupt a subset of *egl-38* functions. Since these tissue-preferential alleles all correspond to amino acid substitutions in the DNA binding domain of EGL-38, they indicate EGL-38 regulates different target genes in different tissues (Chamberlin et al., 1997). To better understand the mechanism of how a transcription factor might regulate different genes in different tissues, we have begun to characterize how EGL-38 regulates two genes expressed in hindgut cells: *lin-48* and *cdh-3*.

lin-48 encodes a zinc finger transcription factor similar to Drosophila OVO that is necessary for the development of a subset of hindgut cell types (Chamberlin et al., 1999). *cdh-3* encodes a cadherin-related cell adhesion molecule expressed in hindgut cells (Pettitt et al., 1996). Although each of these genes is expressed in cells in addition to the hindgut, we have shown the hindgut expression is specifically reduced in *egl-38* mutants. This result indicates that *lin-48* and *cdh-3* are downstream of *egl-38* in hindgut cells. To further investigate the relationship among these genes, we tested expression of *lin-48* in *cdh-3* mutants, and *cdh-3* in *lin-48* mutants, and found that expression of each gene was unaltered in the mutant background. This result indicates that the two genes may be independently regulated by EGL-38, rather than be part of a linear regulatory cascade. We are creating constructs for ectopic expression of *egl-38* and *lin-48* to further test the genetic relationship among these genes.

To better understand how EGL-38 may regulate *lin-48* and *cdh-3*, we have begun an analysis of the promoters of these two genes. Using *in vivo* analysis of deleted and mutant reporter constructs and *in vitro* EMSA, we have shown *lin-48* is a direct, tissue-specific target for EGL-38 in the hindgut. Experiments to test the DNA binding ability of proteins corresponding to the different tissue-specific mutant alleles are underway. Further analysis of *lin-48* and its regulation should provide evidence for how EGL-38 regulates this gene in hindgut cells but not in the other tissues where EGL-38 is present. In contrast to *lin-48*, experiments with *cdh-3* suggest it is indirectly regulated by EGL-38. We have identified a 65 bp region of the *cdh-3* promoter important for its expression in hindgut cells. Since this region contains no similarity to sequences which bind Pax factors, we are investigating what factors might act through this regulatory element. This analysis may identify genes that regulate *cdh-3* in response to EGL-38.

Chamberlin et al. (1997). *Development* 124, 3919-3928.

Chamberlin et al. (1999). *Genetics* 153, 731-742.

Pettitt et al. (1996). *Development* 122, 4149-4157.

782. *gon-2* and interacting genes.

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gon-2 encodes a predicted cation channel belonging to the TRP superfamily. Loss-of-function mutations in *gon-2* severely impair the mitotic divisions of the gonadal precursors. Our working hypothesis is that *gon-2* functions within Z1 and Z4 to allow the influx of cations (probably Ca^{2+}), which then stimulate G1 progression. In order to identify genes that act in conjunction with *gon-2*, we have screened for extragenic revertants of the temperature sensitive allele, *gon-2(q388)*. To date, we have identified four distinct *gem* loci (*gon-2* extragenic modifier) that can mutate to suppress *gon-2(q388)*. *gem-1* X and *gem-4* IV are represented by multiple alleles, whereas *gem-2* II and *gem-3* III are represented by a single allele each. We are currently performing SNP mapping and transformation rescue assays to determine the molecular identities of the *gem* loci.

As another means of identifying genes that interact with *gon-2*, we screened for mutations that cause similar gonadogenesis defects. *gon-11(dx5)* is a recessive, temperature sensitive allele that produces a phenotype similar to that of *gon-2(q388)*. *dx5* has relatively low penetrance, so it is only marginally useful. Therefore, we used non-complementation screening to search for more robust alleles of *gon-11*. This resulted in the identification of *gon-11(dx106)*, which causes fully penetrant late embryonic lethality. We are currently using *dx106* for SNP mapping and transformation rescue assays.

This work is supported by NIH RO1 GM49785

783. Ten-1, the *C. elegans* homologue of the *Drosophila* pair rule gene ten-m is involved in somatic gonad formation.

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The ten-m gene was identified by Baumgartner et al. [1994] in *Drosophila* . It is the first pair rule gene which is not a transcription factor but a cell surface protein. In addition to the early embryonic expression it is also expressed in the developing nervous system of the fly. Members of the Ten-m protein family were also identified in vertebrates and have been named "teneurins" [Minet et al., 1999]. Vertebrate teneurins are highly expressed in the nervous system. They are type II transmembrane proteins. The N-terminal intracellular part is followed by a single transmembrane domain. The extracellular domain is around 2500 aa and contains EGF-like repeats. The *C. elegans* teneurin is located on chromosome III and is mapped to the cosmid R13F6. By 5'-RACE analysis we identified an alternative transcription start located 8 kb upstream of the predicted one. Both teneurin forms are expressed early in *C. elegans* morphogenesis. In post-embryonic development the protein from the upstream promoter is expressed in the developing somatic gonad, the pharynx and a subset of neurons. The protein from the downstream promoter in adult worms has a broad neuronal expression. RNAi aimed against teneurin transcripts results in a low penetrance phenotype affecting worm morphogenesis in various stages of development. Embryos arrest at the two- or three-fold stage. Larvae burst during hatching and the survivors often burst at later stages of development. Adult hermaphrodites have very little or no sperm. The gonad is often displaced. In order to further analyse the function of teneurin in *C.elegans*, we screened a mutant library for deletions in the ten-1 gene. No mutant carrying a deletion of the downstream transcription start that would result in a complete null could be rescued. However, a mutant carrying a deletion of the upstream transcription start including the three first exons was recovered. We conclude that this results in a null mutation of the longer form of teneurin. This mutation affects various aspects of the

somatic gonad development. The distal tip cell migrates along the gonad or the gut and occasionally makes an extra turn. It may penetrate the gut or the uterus. The width of the gonad arms is uneven along their length. The vulva-uterine connection is often not established. The worms have less sperm and endomitotic oocytes are formed. In addition to the malformation of the gonad, ten-1 mutants show neuronal migration defects and are constipated.

We postulate that teneurin is important for cell-cell interactions and that it is a receptor for morphogenetic cues and regulates cell migration and cell shape.

784. A Structure/Function Analysis of the *sys-1* Gene

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We are interested in understanding how a complex organ of the correct size, shape, and function is generated. To address this, we are studying formation of the *C. elegans* gonad. We have identified the *sys-1* gene that is required for hermaphrodite gonad development, but is not essential for male gonad development. Hermaphrodites that are homozygous for *sys-1(q544)*, a putative null allele, exhibit two interesting defects. First, they lack distal tip cells, and the cells that normally make distal tip cells can generate extra anchor cells (Miskowski et al., 2001). This phenotype is similar to that described for mutants in the Frizzled homolog, *lin-17*. (Sternberg and Horvitz, 1988). Second, they fail to form the somatic gonadal primordium during late L2, which rearranges cells into a prepattern of the adult organ.

To gain insight into the mechanism by which *sys-1* acts, we have pursued cloning of the *sys-1* gene. The *sys-1* locus was mapped near *fog-3* on *LGI* by three-factor mapping and deficiency analysis. In that region, a gene was identified that rescues the *sys-1* mutant phenotype and mimics the *sys-1(q544)* defect by RNAi. This gene encodes a novel, cytoplasmic protein. A putative *C. briggsae* homolog of this gene has been identified; it is over 50% identical to its *C. elegans* counterpart at the amino acid level. We are currently investigating the function of the *C. briggsae* gene by RNAi. Using the sequence similarity between *C. briggsae* and *C. elegans*, we are deleting the most conserved regions from the *C. elegans* gene and testing the activity of these reconstructed genes by transformation rescue. We hope that this analysis will help elucidate the function of this novel protein.

Miskowski, J.A. et al. (2001) Dev Biol 230: 61-73.

Sternberg, P.W. and Horvitz, H.R. (1988) Dev Biol 130: 67-73.

785. Coaction of DNA topoisomerase III_{alpha} and RecQ homologue during the germline mitosis in *Caenorhabditis elegans*

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DNA topoisomerase III can interact with RecQ family DNA helicases functionally and physically in eukaryotic cells, contributing to genome stability. Among four RecQ homologues predicted from the *Caenorhabditis elegans* genomic DNA sequence, T04A11.6 is the most similar to Bloom syndrome¡¯s protein in humans. To investigate a possible interaction of the protein with topoisomerase IIIα (Top3α), as observed between Top3 and RecQ homologues in yeast and human, the *top3*α gene expression was suppressed by RNA interference in a *C. elegans* mutant, *him-6*(*e1104*) with an amino acid substitution in the RecQ homologue, T04A11.6(communicated by Drs. F. Mueller and C. Wicky). Germ cells in the gonads of the progeny showed severe chromosomal abnormalities and were arrested during mitosis with a subsequent failure of entering meiosis. These phenotypes were also observed in the progeny produced by double RNA interference of the *top3*α and *recQ* gene expression, though at a reduced level. The severe phenotypes resulting from the double deficiency of *top3*α and *recQ* contrasted with the results in yeast, where a *recQ* mutation suppressed some phenotypes of *top3* null mutation. Therefore, in *C. elegans*, Top3α and the RecQ homologue (T04A11.6) act either together in a complex or separately in two redundant pathways, which contribute to genome stability during germline mitosis.

786. Functional requirement for the histone deacetylase HDA-1 during gonadogenesis in *C. elegans*

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Histone hyperacetylation is correlated with transcriptional activation, while hypoacetylation is correlated with repression. Histone deacetylases (HDACs) catalyze the de-acetylation reaction and have recently been shown to function as transcriptional repressors. However, the biological functions of HDACs in multi-cellular organisms are only beginning to be identified.

Here we show that a previously isolated *C. elegans* mutant *gon-10* (for gonadogenesis defective-10) carries a point mutation in *hda-1*, a homolog of the human *hdac-1* gene. The *gon-10* animals lack HDA-1 protein and display three major developmental defects that impact on somatic gonad tissue formation, germ cell and vulval development. The latter two processes are regulated by the Notch and Ras signaling pathways, respectively. We find that wildtype HDA-1 but not a mutant lacking the catalytic activity can rescue *gon-10* fully. This suggests that 1) the point mutation in *hda-1* is responsible for the observed phenotypes in *gon-10* animals; 2) the catalytic activity of HDA-1 is important for its biological functions. Our findings thus reveal a novel and specific function for a ubiquitously expressed histone deacetylase in *C. elegans* gonadogenesis.

Further data will be reported on the mutant defects as well as potential target genes deregulated by HDA-1.

787. A cytochrome P450 involved in gonad development identified in RNAi screens

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We started a reverse genetic screen using RNAi that was originally designed to identify new genes in the dauer/aging pathways. As we are particularly interested in peptide and steroid hormone signalling pathways, we focused on homologs of genes involved in steroid biosynthesis including cytochrome P450's as well as on a number of homologs to mammalian peptide hormone receptors.

We found that RNA interference with one cytochrome P450 resulted in a severe sterile phenotype. Injected P0 animals showed disruption of gonad structure eventually leading to sterility by day 2 after injection. Among the F1 progeny, we found different phenotypes probably reflecting the degree to which the RNAi effect had spread in the P0 mothers at the time the eggs were formed. Most eggs contained arrested embryos at different stages of development. Hatched larvae showed a lack of coordination in different tissues and were arrested in L2/L3 for 2-4 days. They then recovered to sterile adult animals forming a well developed vulva, but only rudimentary gonad tissue. These results demonstrate that a cytochrome P450 is involved in development as well as maintenance of gonad structure and function, potentially indicating a new steroid hormone signalling pathway in *C. elegans*.

We are currently characterizing the effect of the cytochrome P450 on gonad development in more detail and investigating its expression pattern. We are also screening other candidate genes for phenotypes by RNAi.

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788. Role of IP_3 signaling pathways in regulating contractile activity of gonadal sheath cells

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We recently identified a ClC-type anion channel encoded by *clh*-3 that is functionally expressed in *C. elegans* oocytes. CLH-3 is activated during oocyte meiotic maturation suggesting that the channel plays a role in meiotic cell cycle progression, ovulation, fertilization, and/or early development. Disruption of channel expression by RNA interference has little effect on various reproductive events. However, in worms injected with *clh-3* dsRNA, we observed that ovulatory contractions of the gonadal sheath cells were initiated prematurely This suggests that CLH-3 functions in inhibitory signaling pathways that modulate sheath cell contractile activity (Rutledge et al., *Curr. Biol.* 11: 161-170, 2001).

Oocytes are coupled to sheath cells by gap junctions (Hall et al., *Dev. Biol.* 212:101-123, 1999) indicating that the two cell types may communicate via electrical and chemical signals. We postulated that activation of CLH-3 during meiotic maturation depolarizes the oocyte and electrically-coupled sheath cell plasma membranes. We also postulated that depolarization modulates sheath cell contractile activity by regulating calcium influx via receptor-activated calcium channels that are triggered by depletion of IP3-sensitive intracellular calcium stores.

To begin testing this model, we have developed an isolated gonad preparation. Sheath cells continue to contract for at least 1-2 h after gonad dissection. Removal of extracellular calcium completely inhibits sheath contractions. Increasing calcium influx with the ionomycin induces tonic sheath contraction. Sheath cells load readily with the fluorescent calcium probe fluo-3, and voltage-sensitive fluorescent dyes suggesting that it may be possible to directly quantify intracellular calcium signaling events and membrane voltage during the contractile cycle.

GFP reporter studies have demonstrated that the IP3 receptor gene *itr-1* is expressed in sheath cells (Baylis et al., *J. Mol. Biol.* 294:467-476, 1999). To determine whether IP3 signaling events play a role in regulating sheath cell contractions, we have begun analyzing sheath contractile activity by video microscopy in worm strains harboring mutations in *itr-1*. In wild-type worms, sheath cells exhibit a basal contraction rate of 8.6 +/- 0.7 contractions/min with a displacement of 2.3 +/- 0.01 μm. The force and frequency of sheath contractions increase dramatically 3-4 min after maturation is initiated. These ovulatory contractions reach a peak value of 21.3 +/- 1.7 contractions/min with a displacement of 4.6 +/- 0.03 μm.

The JT73 nematode strain harbors a loss-of-function mutation in *itr-1* (Dal Santo et al., *Cell* 98:757-767, 1999). JT73 worms exhibit virtually no basal sheath contractions. Mean basal contractile activity measured in four worms over >3 h of video recording was 0.1 +/- 0.02 contractions/min. Rare basal contractions were observed, but never exceeded a rate of 1 contraction/min. The displacement of these contractions was 2.1 +/- 0.01 μm.

Ovulation in JT73 worms is severely disrupted. All worms analyzed had oocytes present in the spermatheca at the beginning of video recording. The most proximal oocyte in these worms underwent meiotic maturation triggering what appeared to be ovulatory contractions. These contractions lasted 5.7 +/- 0.4 min and reached a peak value of 5.8 +/- 0.7 contractions/min with a displacement of 3.7 +/- 0.1 μm. Each worm exhibited 3-4 of these apparent ovulatory contraction cycles during the period of observation. However, no successful ovulations occurred.

Ovulation was unsuccessful in the worms we analyzed because the previously ovulated oocyte failed to exit the spermatheca. Our results demonstrate that IP3 and calcium signaling pathways play critical roles in regulating sheath cell contractile activity as well as events that control the release of fertilized oocytes into the uterus.

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789. IDENTIFICATION AND CHARACTERIZATION OF *lin-35* Rb PATHWAY GENES THAT ANTAGONIZE RAS SIGNALING DURING VULVAL INDUCTION

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The synthetic multivulva (synMuv) class A and class B genes define two functionally redundant pathways that antagonize Ras signaling during vulval induction. Cloned class B synMuv genes encode proteins similar to the mammalian tumor suppressor pRB and the pRB-binding heterodimeric transcription factor DP/E2F. We propose that the class B synMuv proteins act as transcriptional repressors to negatively regulate Ras signaling. Specifically, we propose that a DPL-1 DP/EFL-1 E2F heterodimer binds DNA and recruits LIN-35 Rb and other synMuv proteins involved in chromatin modification, including LET-418 Mi-2, LIN-53 RbAp48 and HDA-1 HDAC, to repress the transcription of genes required for vulval cell-fate specification. To further investigate this RB signaling pathway, we cloned additional known class B synMuv genes and performed a genetic screen to identify new class B synMuv genes.

The class B synMuv genes *lin-52* and *lin-54* were identified by Chip Ferguson and Jeff Thomas, respectively, two former graduate students in our laboratory. We cloned both genes and found that each encodes a protein of unknown function. We obtained candidate null alleles of each gene and are using these alleles to characterize the multivulva, sterile and other abnormalities caused by *lin-52* or *lin-54* loss of function. We are focusing additional functional studies on *lin-54*. We are currently assessing whether a human *lin-54*-like gene can functionally substitute for *lin-54* and are conducting a range of protein-protein interaction studies with LIN-54 and the human LIN-54-like protein.

Together with graduate students Frank Stegmeier and Melissa Harrison we screened for additional class B synMuv genes. Using a *lin-15A* background, we screened 6,500 mutagenized haploid genomes and obtained at least 51 class B synMuv mutations. Our analyses to date have identified at least five new candidate class B synMuv genes. We will describe our genetic and molecular characterizations of these genes.

790. sur-7 positively regulates ras-mediated signalling during vulva development

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We describe identification and characterization of sur-7 (suppressor of ras) that functions to regulate a receptor tyrosine kinase-Ras-MAP kinase-mediated signal transduction pathway during hermaphrodite vulva development. The single sur-7 allele (*ku119*) completely suppresses the multivulval phenotype of let-60ras(n1046) mutants from approximately 157% percent induction of vulval precursor cells in n1046 animals to 101% in n1046;ku119 double mutants. However, *ku119* animals have no phenotype on their own. sur-7 acts at the level of ras in the signalling cascade. *ku119* is capable of suppressing activating mutations in the RTK pathway upstream of Ras, but fails to suppress mutations in this pathway downstream of Ras.

Molecular cloning of the affected locus in *ku119* animals has been hampered by its position on the right arm of the X chromosome where the absence of useful genetic markers has prevented detailed genetic mapping. We are currently employing single nucleotide polymorphism (SNP) mapping to more accurately place *ku119* on the physical map, and hope to report cloning and further characterization of sur-7.

791. Subcellular Localization of MPK-1 Using GFP Fusion

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The Mitogen-Activated Protein Kinase (MAPK) cascade is an essential pathway conserved from yeast to human. In C.elegans, the function of the MAPK pathway in vulval development is well characterized. The MAPK pathway has also been suggested to be important in olfaction (Nature 404:289). To study where it is expressed and what its role is there we generated an integrated line in which mpk-1::GFP is expressed from its own promoter. Our result showed that MPK-1::GFP is expressed mostly in neurons in the nerve ring, ventral cord, tail and pharynx. The pharyngeal neurons NSM, M4, M5, MI, I2, I6 are stained. Consistent with the result from Takaaki Hirotsu et al, MPK-1::GFP is also expressed in olfactory neurons.

ERK1 and ERK2, the mammalian homologues of MPK-1, are translocated from cytosol to nucleus by certain stimuli, where they phosphorylate nuclear targets. MPK-1::GFP is more concentrated in the cytoplasm than in the nucleus in most cells, and some neurons show exclusively cytoplasmic staining. We are searching for conditions in which mpk-1 changes its location. In parallel, we are examining the regulation of mpk-1 by western blotting with an MPK-1 antibody against phosphorylated ERK1/2 antibody. Based on the fact that a muscarinic receptor can signal through MAPK pathway in mammalian neurons, we treated worms with acetylcholine agonists, arecoline, but we couldn’t see a big change of subcellular localization of MPK-1.

We are also examining whether the MPK-1::GFP construct can complement an mpk-1 null mutation.

792. Characterization of a New Gene Involved in Ras-mediated Vulval Induction

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The development of the *C. elegans* vulva requires the activity of the Ras signaling pathway. To identify components of this pathway, we screened for mutations that suppress the multivulva phenotype caused by *let-60 (n1046gf*), a mutation that constitutively activates the *ras* gene. Forty-three alleles comprising 20 complementation groups were identified. These genes include conserved members of the Ras signaling cascade, such as Raf (*lin-45),* KSR (*ksr-1*), MEK (*mek-2*), MAPK (*mpk-1*), and an ETS transcription factor (*lin-1*). We are now analyzing *n2528*, an allele that defines a new complementation group. The *n2528* mutation partially suppresses the *lin-1 (e1275)* multivulva phenotype, suggesting that the affected gene may act downstream of or in parallel to the transcription factor *lin-1*. *n2528* mutants also display a cold-sensitive larval lethal phenotype that can be partially rescued by *let-60 (n1046).* Using high resolution mapping relative to single nucleotide polymorphisms, we mapped *n2528* to a 17.5 kilobase interval on LG X. We are currently attempting transformation rescue to identify the gene.

793. Cloning of negative regulators of the RAS/MAPK pathway in *C. elegans*.

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The EGF/RTK/RAS/MAPK signal transduction pathway has been conserved during evolution. It controls cell differentiation in organisms as different as insects, worms and vertebrates. Its activity must be tightly controlled for the correct development of the organism and its incorrect activation can lead to tumour formation in vertebrates.

In C. elegans, an EGF/RTK/RAS/MAPK signalling pathway is involved in vulval development. The anchor cell expresses the lin-3 ligand, a TGF-a homolog that activates the RTK/RAS/MAPK pathway in the P6.p cell, one of the six equivalent vulva precursor cells (VPC). In turn, the P6.p cell recruits the two neighbouring VPCs P5.p and P7.p through a lateral signal. The three cells then differentiate and form a functional vulva. Constitutive activation of the RTK/RAS/MAPK causes all six VPCs to be activated resulting in a multivulva (Muv) phenotype. Mutations that inactivate the RTK/RAS/MAPK pathway cause a vulvaless (Vul) phenotype because the VPCs remain uninduced and fuse with the hypodermis.

We are cloning new factors involved in the negative regulation of the EGF/RTK/RAS/MAPK pathway in C. elegans. We carried out an EMS mutagen screen in a gap-1(0) background and selected for multivulva animals in the F2 generation. Gap-1 is a negative regulator of the RAS pathway that does not have a phenotype as single mutant. The new mutants look normal in a wild type background and show the multivulva phenotype only in a gap-1(0) background. For this reason we named them "multivulva enhancer of gap-1Ó(meg). We isolated six mutations that fall into four complementation groups, ie meg-1, meg-2, meg-3, meg-5 and meg-4 (see also presentation of Gopal Battu). We will report

about the cloning of meg-3 that maps on chromosome II between the unc-4 and unc-53 markers.

794. *sur-9*, a Suppressor of Activated let-60(n1046) in the Vulva.

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The *C. elegans* vulva is induced by an EGF like signal that activates the Ras/MAPK pathway. Constitutively active alleles of *ras* lead to hyperactivity of the signal transduction pathway and result in a multivulva (Muv) phenotype where numerous pseudovulvae are formed from vulval precursor cells (VPCs). By initiating suppressor screens of activated *let-60 ras*, many previously unknown components of this pathway have been identified. One gene, *sur-9*, has been defined by a semi-dominant allele isolated in a screen for temperature sensitive mutations that suppress the *let-60(n1046)* allele. While homozygous, *sur-9(ku258)* can suppress *let-60(n1046)* from 80% Muv to <1% Muv. *sur-9(ku258)*/ + also suppresses the *let-60(n1046)* phenotype to 10% Muv. Additional genetic analysis has suggested that *sur-9* acts at a late step in the Ras/MAPK signaling pathway. Animals carrying the *sur-9(ku258)* mutation are also unhealthy, semi-sterile and show defects in other developmental processes. *sur-9(ku258)* has been mapped to LG III and fine mapping is currently being carried out. We hope to report the cloning and elucidation of *sur-9*'s molecular identity which could contribute to our knowledge of a highly conserved and important biological pathway.

795. Analysis of *lin-1* Mutants and Screen for Suppressors of *lin-1(n383)*

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A highly conserved RTK/Ras/MAP kinase pathway promotes the primary vulval cell fate by negatively regulating the ETS transcription factor LIN-1. Bob Horvitz and colleagues isolated 41 alleles of *lin-1* in a variety of screens for vulval defects. These alleles include loss-of-function and gain-of-function mutations of different severity. To identify residues and domains that are important for LIN-1 structure and function, we used DNA sequencing to determine the molecular lesions of these alleles. The loss-of-function mutations include several missense mutations that affect the ETS domain and a series of nonsense mutations. The DNA-binding properties of the ETS domain mutants are being investigated.

To identify genes that function downstream or in parallel to *lin-1* to promote the primary cell fate, we are screening for mutations that suppress the multivulva (Muv) *lin-1(n383)* loss-of-function phenotype. We have screened about 5,500 haploid genomes and identified ten suppressors of *lin-1* Muv. These ten mutations cause sterility. These suppressor mutations will being mapped relative to polymorphisms between the N2 strain and the Hawaiian CB 4856 strain. *lin-1* is epistatic to the well characterized genes in the RTK/Ras/MAP kinase pathway, therefore these mutations may affect new genes that function downstream of *lin-1* to regulate the primary cell fate.

796. GENETIC SCREENS FOR POSITIVE REGULATORS OF RAS SIGNALING

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RTK/Ras/Raf-MEK-ERK signaling pathway is a highly conserved signal transduction pathway required for multiple aspects of *C. elegans* development. The genes *ksr-1* and *sur-8* positively regulate Ras signaling at a step between *let-60 ras* and *lin-45 raf*. To identify positive regulators of the Ras pathway that may function with either *ksr-1* or *sur-8*, or at another step in the pathway, we have been performing an Enhancer Of Raf (Eor) screen. Screening for enhancers of weak hypomorphic alleles of *lin-45 raf* has already successfully identified mutations in *ksr-1*, *sur-6*, and previously unidentified regulators *eor-1* and *eor-2* (see abstracts by Howard et al. and Kao et al.). In addition, this screen has identified interesting alleles of *sem-5* and *let-341*, as well as hypomorphic alleles of *sur-2* and *lin-25*. Although this screen has identified multiple alleles of *eor-1*, *eor-2*, *sur-2* and *lin-25*, it is far from saturated.

As an alternative approach, we have initiated an Enhancer Of Sur-8 (Eos) screen. Screening in the *sur-8* mutant background for mutations that synergize with *sur-8* is expected to identify mutations in positive regulators that may function with *ksr-1* and *sur-6*. We believe this screen will also identify mutations in *eor-1* and *eor-2*, as well as new loci, while reducing some background mutations identified in the Eor screen. The results of these screens as well as reverse genetic analysis of candidate genes that may function to positively regulate Ras signaling will be presented.

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797. Characterization of a lin-12(d) suppressor, sel-7

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LIN-12/Notch proteins function as transmembrane receptors for intercellular signals during development. They are activated by binding of their ligands, DSL (Delta/Serrate/LAG-2) proteins. Ligand binding triggers at least one proteolytic processing event in the extracellular domain and then SEL-12/presenilin-dependent proteolysis within the transmembrane domain. This latter event releases the intracellular domain, which translocates to the nucleus and activates transcription of target genes in a complex with LAG-1, SEL-8, and presumably other proteins.

The anchor cell (AC)/ventral uterine precursor cell (VU) fate decision has served as an important model for LIN-12/Notch cell signalling events. Constitutive activation of LIN-12, by lin-12(d) mutations, causes the presumptive AC to be transformed into a VU. As no AC is made, no vulva is induced, leading to an egg-laying defective (Egl) phenotype. Reversion of the Egl phenotype yields intragenic and extragenic suppressor mutations. Such reversion screens have been carried out on a large scale (Tax et al., 1997; L. Vallier, I. Katic, J. Chen, and I. Greenwald, unpublished observations). Many of the extragenic suppressors, sel genes (suppressor/enhancer of lin-12), have proven to be critical components of LIN-12/Notch signal transduction. An extragenic suppressor mutation, n1253, isolated by Tax et al. 1997 defined the sel-7 gene; our lab has identified an additional allele, sel-7(ar516). We have characterized the genetic interaction of these alleles with various alleles of lin-12 and glp-1. We have also cloned sel-7, and found that it encodes a novel protein. We are currently exploring its expression and subcellular localization pattern, and hope to have some insight as to its function in LIN-12/Notch signalling by the meeting.

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798. spr GENES: SUPPRESSORS OF THE sel-12 PRESENILIN EGG-LAYING DEFECTIVE PHENOTYPE

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The *C. elegans sel-12* gene encodes a functional homolog of mammalian presenilin. *sel-12* was initially isolated as a suppressor of a *lin-12* gain-of-function allele (1, 2). Presenilin was identified as a gene implicated in familial early-onset Alzheimer's disease. It is now believed that presenilin mediates transmembrane cleavage of LIN-12/Notch proteins, a critical step in signal transduction by these receptors, as well as transmembrane cleavage of beta-amyloid precursor protein, a critical step in the generation of a peptide that can cause Alzheimer's disease. Hermaphrodites carrying a *sel-12* loss-of-function mutation are unable to lay eggs. The Egl phenotype of *sel-12* null mutants resembles that of *lin-12* partial loss-of-function mutants, although it is not yet certain if the reduction in *lin-12* activity is the sole basis for the *sel-12(-)* Egl phenotype.

To identify factors that influence presenilin activity, synthesis and stability, and possibly factors that influence *lin-12* activity, synthesis and stability, a screen for suppressors of the Egl phenotype of *sel-12(-)* mutants has been conducted. Previous work identified 14 mutations that act as highly penetrant suppressors of the Egl defect of *sel-12(ar171)* (3). Molecular characterisation of one of these *spr* genes (for suppressor of presenilin) has revealed that it encodes the C. elegans ortholog of mammalian SET, a protein that has been implicated in chromatin remodelling (as well as other biochemical processes) (3).

We have undertaken the characterisation of other *spr* genes. For one of the *spr* loci, we have found that loss of *spr* function can also partially suppress the defects in the AC/VU decision caused by reduced *lin-12* activity and supresses the maternal effect lethality caused by partial loss of *glp-1* activity. These observations suggest that this *spr* gene may play a more general role in LIN-12/Notch signalling. We have mapped this *spr* gene between single nucleotide polymorphisms using the Hawaiian strain CB4856 (4) and have cloned it by sequencing the predicted ORFs in the region and finding mutation in one of the ORFs. This ORF appears to have homologs in humans and Drosophila and its predicted protein product suggests a role in the modulation of transcription. We are currently attempting to characterise this *spr* gene further genetically and molecularly, and to assess its function in the LIN-12/Notch pathway. We will report on our progress at the meeting.

1- Levitan, D. and Greenwald, I. (1995). *Nature 377:351-354*.

2- Levitan, D. and Greenwald, I. (1996). *PNAS 93:14940-14944*.

3- Wen et al. (2000). *PNAS 97:14524-14529*.

4- Genome Sequencing Consortium (http://genome.wustl.edu/gsc/CEpolymorph/snp.shtml) and additional identified SNPs.

799. Receptor tyrosine phosphatase CLR-1 negatively regulates LET-23 EGFR signaling

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We demonstrate that *clr-1*, a previously identified negative regulator for *egl-15* mediated FGFR signaling, also regulates *let-23* EGFR-dependent signaling. Specifically, a reduction-of-function mutation in *clr-1* suppresses the lethality and vulvaless phenotypes of non-null mutations in *lin-3* EGF, *let-23* EGFR, *sem-5* Grb2, and *let-341* Sos. Furthermore, a *clr-1(rf)* mutation synergized with an activated *let-60* Ras mutation and with mutations in other negative regulators of *let-23* signaling, including *sli-1*, *ark-1*, *unc-101*, and *gap-1*, in causing a multivulva phenotype. Animals harboring a gain-of-function allele of *let-23* display a highly penetrant multivulva phenotype, while *clr-1(rf)* animals do not. These data, as well as the epistasis analysis, indicate that a *clr-1(rf)* mutation results in hyperactivation of a signaling component downstream of LET-23. We are continuing our epistasis analysis, and are testing if *clr-1* acts on the *let-23* pathway directly, or through amplification of an *egl-15*-dependent Ras signal.

800. A second *ksr*-like gene in *C. elegans*: Is it *pex-1*?

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KSR (Kinase Suppressor of Ras) is a conserved, Raf-related protein that positively regulates Ras signaling. Drosophila KSR (DKSR) is essential for Ras signaling (*Dksr* and *Ras1* mutants have similar lethal phenotypes; Therrien et al. 1995), whereas *C. elegans* KSR-1 is a non-essential positive regulator of Ras signaling (*ksr-1* mutants have only a few weakly penetrant *let-60* Ras-like defects; Kornfeld et al. 1995; Sundaram and Han 1995). Recently, the *C. elegans* genome sequencing consortium identified a second *ksr*-like sequence corresponding to the predicted gene F58D5.4, which we refer to as *ksr-2*. RNA-mediated interference (RNAi) of *ksr-2* causes no obvious somatic defects in wild-type, but causes nearly complete rod-like larval lethality in a *ksr-1* mutant background. Thus, *ksr-1* and *ksr-2* are redundant for viability and may together be essential for Ras signaling.

Ras signaling also functions in *C. elegans* germline development, being necessary for pachytene progression (Church et al. 1995). Mutations in the gene *pex-1* display a pachytene arrest phenotype similar to that of *let-60* Ras mutants, but do not exhibit either the rod-like larval lethality or vulvaless phenotypes. *pex-1* genetically maps in the vicinity of *ksr-2* and *ksr-2(RNAi)* in wild-type causes a sterile phenotype resembling that seen in *let-60* Ras and *pex-1* mutants. Experiments are underway to determine if *ksr-2* and *pex-1* are the same gene. If so, then germline *ksr* activity may be provided exclusively by *ksr-2*.

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801. Genetics and genomics of heterochronic regulatory RNAs

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The heterochronic pathway of *C. elegans* controls the progression of temporal development. Two developmental transitions are controlled by genes encoding small (21-22nt) RNAs. *lin-4* regulates the progression through the L1 and L2 stages, and *let-7* regulates the transition from late larval stages to adulthood. The *let-7* RNA is conserved in other metazoans, suggesting that genes required for its production and function will also be conserved and other regulatory RNAs could play developmental roles in *C. elegans* or other organisms.

One tissue affected by the heterochronic pathway is the lateral hypodermis, or seam. A wild-type animal hatches with 10 seam cells. The number increases to 16 by adulthood because of duplications of a subset of seam cells during the second larval stage. Heterochronic mutants can result in a change in the number of seam cells observed at the late L4/young adult stage in two ways, either by altering the timing or expression of the L1 and L2 stage-specific fates or by inappropriately reiterating cell divisions as young adults. *lin-4(lf)* animals reiterate L1 divisions and fail to execute L2 patterns of division; therefore, the number of seam cells remains at 10. *let-7(lf)* animals reiterate seam cell divisions as young adults. We screened directly for mutants with an altered number of seam cells in the late L4/young adult stage in a background carrying a seam cell-specific GFP marker (J. Rothman, personal communication). Such a screen could isolate not only mutations in genes involved in the production or function of the small RNAs and their regulatory targets but also in heterochronic genes that may be tissue specific regulators of temporal development. From a pilot screen of 1500 haploid genomes, we recovered three independent alleles that alter the number of seam cell nuclei in late L4s/young adults. One has up to 22 seam cell nuclei as an adult, and the other two both have 10-11 seam cell nuclei as adults. We are currently analyzing the mutant phenotypes to determine which developmental stage is affected.

We are also using a computational screen to search for other small RNAs in the *C. elegans* genome. The *let-7* and *lin-4* genes produce >60nt precursors predicted to fold into stem-loop structures, and the smaller RNAs are processed from the 5’ end of the stem. Our search looks for ~100nt regions of the genome that could fold into stable secondary structures and then checks for conservation of sequence and structure in the Drosophila genome. A test run of the program on the X chromosome identifies hundreds of such regions, but the only region with good sequence conservation at the 5’ end of a long stem loop structure is *let-7*. We are currently screening the remaining chromosomes.

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802. Regulation of *let-7* RNA Expression by upstream Components of the Heterochronic gene Pathway.

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The heterochronic gene pathway controls developmental timing in *Caenorhabditis elegans* larvae. The temporal mechanisms that specify the appropriate larval or adult programs are mediated by two small regulatory RNAs, *lin-4* and *let-7* , which act as translational repressors by base-pairing with the 3'-UTRs of target gene mRNAs. *lin-4* RNA starts to be expressed by mid L1 and translationally downregulates *lin-14* and *lin-28* . The decrease of LIN-14 and LIN-28 levels is required for progression through L1, L2, and later developmental programs. Accordingly, *lin-4(0)* animals exhibit a retarded development, where L1 programs are reiterated at later larval stages and L2 to adult(A) developmental programs are not expressed. Conversely, *lin-14(lf)* and *lin-28(lf)* mutants exhibit precocious phenotypes, deleting the L1 and L2 programs, respectively. In these precocious mutants, subsequent stage-specific programs, including the adult-specific differentiation of the hypodermis, occur prematurely. *let-7* influences the timing of the transition from L4 to A stage. The accumulation of *let-7* RNA at the beginning of the L4 translationally downregulates *lin-41* , and permits the switch from larval to adult programs at the proper time during development. *let-7(0)* mutants developed through the L1 to L4 stages on schedule, but express larval fates instead of adult fates at the L4 molt.

To test whether the upstream components of the heterochronic pathway control the timing of the L4-to-A transition by regulating the timing of *let-7* expression, we performed Northern blotting analysis of total RNA from staged populations of the wild type and heterochronic mutants. We observed higher levels of *let-7* in precocious mutants compared to wild type animals at the L3 stage, consistent with the negative regulation of *let-7* expression at early stages. Although levels of *let-7* RNA are dramatically decreased in retarded mutants compared to wild type at both L4 and A stages, a basal level of *let-7* RNA was still detectable. These results suggest that several signals, perharps both positive and negative, temporally regulate *let-7* expression.

803. Control of *ins-33* transcription by *lin-14* in the *C. elegans* hypodermis

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The heterochronic regulator *lin-14* affects certain somatic cell-fate choices during post-embryonic development in *C. elegans*. In *lin-14* mutants, cells express stage-specific developmental programs, such as cell divisions or dauer entry, at inappropriate times in development.[1,2] Loss-of-function mutations in *lin-14* result in the precocious execution of L2-specific programs during the first larval stage and the omission of L1-specific programs, while gain-of-function mutations retard the execution of L2-specific programs during the second larval stage and instead cause the re-iteration of L1-specific programs.

We chose to study the molecular mechanisms of *lin-14* function using two complementary approaches. First, we characterized *lin-14* biochemically by analyzing its association with nuclei *in vivo* and by performing *in vitro* DNA binding assays. We also performed microarray analysis to identify genes misregulated under *lin-14* loss-of-function or gain-of-function conditions. We identified a number of genes whose transcript levels are regulated either positively or negatively by *lin-14*, and which could be among *lin-14*'s *in vivo* targets. Using Northern blot and GFP-reporter fusion analysis we have begun to investigate whether these putatuve targets are transcriptionally controlled by *lin-14*, and whether this control is direct or indirect. The expression of one of these targets, *ins-33*, a *C. elegans* insulin homolog, appears to be controlled by *lin-14* at the level of transcription *in vivo*. LIN-14 protein can bind the *ins-33* promoter *in vitro* at at least two sites. We are currently characterizing the *in vivo* significance of these sites to *lin-14* dependent *ins-33* transcription.

1. Ambros, V., and H.R. Horvitz, Genes and Development (1987) 1:398

2. Liu, Z., and V. Ambros, Genes and Development (1989) 3:2039

804. Regulation of the *C. elegans* heterochronic gene *lin-41* by the small RNA *let-7*

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Genetic studies have shown that heterochronic genes govern the timing of development in *C. elegans*. The 21 nucleotide RNA, *let-7*, is predicted to bind to complementary sites in the 3'UTR of its target gene, *lin-41*, causing the down-regulation of *lin-41* at the L/A (larva-to-adult) switch. Together these two heterochronic genes act to regulate the timing of proliferation vs. differentiation in seam cells at the L/A switch through an unknown mechanism. Gene regulation by 3'UTRs is used by all eukaryotic cells, but little is known about the mechanism. Similarly, while there is presently substantial interest in gene regulation by small RNA molecules (e.g. RNAi), very little is known about their mechanism of action.

A genetic screen for *let-7*-like mutants was performed in order to find genes that may function with *let-7*. Two recessive mutations recovered in the screen, *mg281* and *mg283*, define a new gene *lin-63* that most likely acts with *let-7* to regulate *lin-41*. Similar to *let-7* mutants, seam cells fail to terminally differentiate correctly in *lin-63* mutant adults. Moreover, like in *let-7* mutants, a reporter gene bearing the *lin-41* 3'UTR fails to be turned off in adult staged *lin-63* mutants. *lin-63* maps near *lin-29* but complements *lin-29*. Purified *let-7* RNA binds to and gel shifts RNA from *lin-41* 3'UTR, but not RNA deleted for the *let-7* complementary sites. To identify potential proteins that recognize the *let-7*/*lin-41* duplex we performed a yeast 3 hybrid screen with the *let-7*/*lin-41* RNA duplex as bait. One interactor maps close to where *lin-63* maps, making it a good candidate for *lin-63*. We are testing whether our *lin-63* alleles are in this gene. Molecular identification of *lin-63* is underway and should help determine the mechanism by which these small RNA molecules act to

down-regulate their target molecules.

805. Characterization of the effect of early expression of the small RNA *let-7* on developmental timing in *C. elegans*.

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Timing of development in *C. elegans*is controlled by the expression of the small RNAs *lin-4* and *let-7*. Expression of *lin-4* during the L1 stage downregulates the expression of *lin-14*and *lin-28* to allow the execution of L2 developmental programs. Similarly, expression of*let-7*during the L3 stage downregulates expression of *lin-41*, relieving repression of *lin-29*and allowing the execution of adult developmental programs. *lin-4* and *let-7*encode RNA precursors that are processed to 22- and 21-nucleotide mature transcripts, respectively, that function by base-pairing to the 3 UTR of their targets.

In order to answer the question of whether expression of *let- 7*is sufficient to drive the execution of adult developmental programs, we have designed a construct to drive the early expression of *let-7* during the L1 phase when *lin-4* is normally expressed. We have fused the putative *lin-4* promoter and *lin-4* precursor to the 5 end of the *let-7*precursor. This construct was injected into wild-type (N2) animals and the resulting transgenic lines examined for heterochronic phenotypes. We expected that early expression of *let-7* might result in a *lin-41* loss of function phenotype (precocious development of adult alae, and sterility), since early downregulation of *lin-41*expression should occur. Our transgenic animals develop a protruding vulva as young adults and exhibit egg-laying defects but do not exhibit the classic *lin-41*phenotype. Therefore, we will determine whether a construct including more of the genomic region upstream of the *lin-4* precursor provides stronger expression of *let-7*, resulting in the expected phenotype for early *let-7*expression. We would then screen for mutations that suppress the phenotype caused by early expression of *let-7*.

We are currently determining whether the injected arrays provide *lin-4* and *let-7*activity sufficient to rescue mutations in these genes. If the *let-7* transgene is expressed early, a putative negative regulator of *let-7* gene action could be responsible for the lack of a precocious phenotype. We would then perform a screen for mutations that allow early expression of *let-7* to result in a phenotype suggestive of early execution of adult developmental programs.

806. Temporal Regulation of the *let-7* stRNA

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The heterochronic gene pathway in *C. elegans* controls the timing of developmental events. Within this pathway of at least ten genes, there are two genes, *lin-4* and *let-7*, which instead of coding for proteins encode short RNA products. *lin-4* and *let-7* RNAs accumulate during the first and fourth larval stages respectively and act as timing switches that initiate a cascade of developmental changes in the somatic cells between the first and second larval stages and the fourth larval and adult stages respectively. These are therefore referred to as small temporal RNAs (stRNA). The *let-7* stRNA is also temporally regulated during development of other animals including Drosophila and zebrafish. Since in *C. elegans*, the appearance of the stRNAs correlates with temporal transitions in cell fates after molting, we can distill the timing of temporal transitions down to the timing of appearance of the stRNAs. We are therefore interested in understanding how *let-7* is regulated.

Since both *lin-4* and *let-7* are first transcribed as ~70 nt pre-RNAs that are processed to the mature 21-22 nt transcripts, there are multiple potential points where regulation could take place, i.e., processing, transcription, RNA stability. To test the simple hypothesis that the *let-7* RNA is temporally regulated at the level of transcription, we fused the *let-7* promoter to GFP and saw that the temporal expression of LET-7 recapitulates that observed in developmental Northern blots. Therefore *let-7* appears to be temporally regulated at the transcriptional level. Preliminary studies with functional knockouts of *ama-1* support the hypothesis that RNA polymerase II transcribes LET-7. Additionally, Northern blot analysis indicates that many of the heterochronic genes upstream of *let-7* seem to play a role in the regulation of the *let-7* stRNA product. Further studies are underway to confirm these initial findings and also to elucidate other *cis*- and *trans*-acting elements involved in this temporal

and spatial regulation. This study may provide information pertinent to regulation of stRNAs in higher animals.

807. *lin-64*, A NEW GENE INVOLVED IN DEVELOPMENTAL TIMING

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Genes of the heterochronic pathway regulate developmental timing in *C. elegans*. *let-7*, a late-acting heterochronic gene, regulates the switch from proliferation to terminal differentiation in seam cells during the L4 to adult transition. *let-7*, a 21-nucleotide RNA molecule negatively regulates *lin-41*, and possibly other heterochronic genes as well through interaction with *let-7* complementary sites in the 3UTR of these genes. However, the molecular mechanism of *let-7*-mediated regulation remains unclear. To clarify the mechanism, we sought to identify other genes involved in *let-7* regulation. We performed a screen for mutants showing a Let-7-like phenotype (e.g. retarded development, egg-holding, protruding-vulva).

One gene isolated from this screen is *lin-64*, for which we have at least one mutant allele, *mg285*. Genetic mapping has placed *lin-64* near *lon-2* on chromosome X. Further mapping utilizing polymorphisms and deficiencies is being done. Complementation analysis is being carried out between *mg285* and other mutant alleles isolated in this screen to determine whether we have additional alleles of *lin-64*. *lin-64* appears to complement *daf-12* and *lin-64* mutants are not dauer defective. We have taken advantage of *gfp* constructs that mark either seam cell nuclei (*ssm::gfp*) or cell junctions *(jam-1::gfp*) to further characterize *lin-64*s mutant phenotype. Preliminary phenotypic analysis indicates that *mg285* animals are unable to appropriately make the seam cell differentiation vs. proliferation decision during the transition to adulthood. These mutants have extra seam cells and abnormal alae. We are also investigating possible genetic interactions between *lin-64* and other heterochronic genes.

We are studying the ability of *lin-64* mutants to suppress the precocious heterochronic phenotypes of *lin-41* mutants and *lin-28* mutants. We are also examining *lin-41* expression in the *lin-64* mutant using a *col-10-lacZ-lin-41* 3UTR reporter construct to determine whether *lin-64* participates in the regulation of *lin-41* by *let-7*.

808. *dre-1*, a new heterochronic gene affecting gonadal and extragonadal developmental age

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The *daf-12* nuclear hormone receptor specifies L3 options of dauer formation and reproductive growth, and couples dauer and heterochronic pathways. Genetic analysis suggests that *daf-12* is necessary for dauer diapause, but only partly required for the promotion of reproductive growth. As evidence, candidate null mutants of *daf-12* have completely penetrant dauer defective phenotypes, but impenetrant delayed heterochronic phenotypes starting in the third larval stage. In contrast, recessive gain-of-function alleles are dauer defective and display penetrant heterochrony in gonadal and extragonadal tissues. In a simple model, receptors of the gain-of-function alleles interfere with a redundant activity from another locus. If so, then screens for mutants that enhance the heterochronic phenotypes of *daf-12* null alleles should reveal this activity. We identified one locus, *dre-1* (***d****af-12* **re**dundant function **1**) in genome-wide genetic screens. Another locus, *lin-46*, we found taking a candidate gene approach. Both loci interact with *daf-12* in a stage and tissue-specific manner.

dre-1 on its own displays an impenetrant heterochronic delay of adult programs in the seam and gonad. In *dre-1daf-12* double mutants, the gonadal phenotype is dramatically enhanced. Notably, distal tip cells fail their L3 dorsal movements and instead migrate into head and tail, similar to *daf-12* gain-of-function alleles. *dre-1* maps to the center of chromosome V. We are currently positionally cloning *dre-1*.

lin-46 was first identified by Moss and Ambros, and has impenetrant delayed L3 and Adult seam phenotypes. *daf-12* null mutants also show impenetrant L3 seam phenotypes. Double mutants display synergistic and penetrant heterochronic defects in the seam, but not the somatic gonad.

In summary, *dre-1* and *lin-46* act in parallel to *daf-12* to regulate third and later stage programs in gonadal and extragonadal tissues. Conceivably, the DAF-12 nuclear receptor may serve to coordinate heterochronic circuits in different tissues by a hormonal mechanism.

809. The role of RNAi related genes in developmental timing in C. elegans

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In C. elegans two small RNA genes, lin-4 and let-7, regulate temporal development. The lin-4 gene encodes a ~22nt RNA that functions in early larval development and the let-7 gene encodes a ~21nt RNA that regulates later larval to adult transitions. let-7 RNA is widely expressed across animal phylogeny. Thus, the factors that regulate the expression and function of this small temporal RNA (stRNA) are also likely to be conserved. To uncover such genes we performed a screen looking for mutants that phenocopied the loss of let-7 RNA - defective vulval maturation and inability to generate the adult specific cuticle structure termed alae. One particularly interesting mutant, mg286, inappropriately reiterates larval cell fates and exhibits additional developmental phenotypes such as slow growth and scrawniness. These combined phenotypes closely resemble the defects displayed by worms undergoing RNAi of a homolog of the RNAi defective gene rde-1 (see also abstract by Grishok, et. al.). Although rde-1 mutants show no apparent developmental abnormalities, elimination of this closely related C. elegans protein, which we call argonaute like gene (alg-1) based on its similarity to the argonaute gene that has been described in Arabidopsis and Drosophila, causes misregulation of the developmental timing pathway controlled by stRNAs. The observation that a protein homologous to an RNAi factor is required for normal temporal development suggests that there may be connections between RNAi related genes and genes that control the timing of development in C. elegans. An obvious molecular parallel between the mechanisms that direct these processes is the ~22nt size of the RNAs that direct RNAi and temporal development. Thus, we expect that

mutants isolated in our let-7 phenocopy screen may reveal factors key to both of these pathways for regulating the generation and function of such small RNAs. Specifically, we are focusing on mutants that affect the expression and function of let-7 RNA as well as the ability to direct RNAi.

810. The heterochronic gene *lin-57* encodes a hunchback-like protein and is temporally regulated via its 3' UTR

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The *lin-57(ve18)* mutation causes the precocious terminal differentiation of the lateral hypodermal seam cells during the L3 molt. In *lin-57; lin-29* double mutants, the hypodermal seam cells never terminally differentiate, indicating that *lin-57* acts through *lin-29* to control this event. The *ve18* mutation affects a splice site and appears to represent a reduction-of-function allele. Progeny of *lin-57* RNAi treated animals exhibit two main phenotypes: precocious seam cell terminal differentiation or embryonic lethality. Because only one *lin-57* splice form has been detected and there are no genes sharing high sequence similarity to *lin-57*, this result suggests that the *lin-57* null phenotype is embryonic lethality. One possibility is that *lin-57* may play two roles in development, one during embryogenesis and one during the L3 stage. Non-complementation screens have yielded a candidate *lin-57* allele (*ve54*) which is embryonic lethal when homozygous. *lin-57* encodes a HUNCHBACK-like protein (a.k.a. HBL-1; Fay et al., 1999) which contains nine zinc finger motifs, six of which share similarity to the six *D. melanogaster* HUNCHBACK zinc fingers. Thus, *lin-57* is likely to act as a transcriptional regulator within the heterochronic gene pathway to control the time of seam cell terminal differentiation. Non-rescuing *lin-57:gfp* constructs are expressed during embryogenesis through the L3 stage. Expression decreases during the L4 stage and is not detected in adult animals. This regulation appears to be achieved post-transcriptionally via the *lin-57* 3 UTR since similar constructs containing the *unc-54* 3' UTR are expressed during all stages of development. The *lin-57* 3' UTR contains eight putative binding sites for the 21nt *let-7* regulatory RNA (Reinhart et al., 2000). A subset of these sites are conserved in *C. briggsae* and *C. remanei*. The temporal expression pattern of *let-7* (late

L3-Adult) suggests that the downregulation of *lin-57* could be mediated by *let-7*. Constructs bearing point mutations in the putative *let-7* binding sites are now being tested for their ability to cause *lin-57* mis-regulation. We also noted a single putative *lin-4* binding site in the *lin-57* 3 UTR. However, the functional significance of this site is unclear; *lin-4* is epistatic to *lin-57* which suggests that *lin-57* is not a downstream target of the *lin-4* gene.

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811. A MOLECULAR LINK BETWEEN DEVELOPMENTAL TIMING AND CIRCADIAN RHYTHMS

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The heterochronic genes of *C. elegans* control the timing and sequence of many post-embryonic developmental events, including the terminal differentiation of the lateral hypodermis during the L4-to-adult molt. Mutations in the heterochronic genes alter the timing of this differentiation event, resulting in adults with larval-type hypodermis or larvae with adult-type hypodermis. For example, hypodermal seam cells in *lin-42* mutants terminally differentiate and secrete a morphologically adult cuticle during the L3 molt, one stage earlier than in the wild type.

We cloned *lin-42* and showed that the predicted *lin-42* protein is most similar to the PERIOD family of proteins from *Drosophila* and other organisms. The region of similarity between LIN-42 and *Drosophila* PER is mostly restricted to a protein interaction domain known as the PAS domain. This similarity is especially interesting since PERIOD is involved in a second type of biological timing mechanism, the control of circadian rhythms. The connection between these two timing mechanisms is further highlighted by experiments that demonstrate that *per* mutants exhibit defects in growth rate.

We are investigating the extent to which the sequence similarity between LIN-42 to *Drosophila* PER reflects a similarity in gene regulation. A hallmark of *per* in flies is that its mRNA levels oscillate with a 24-hour periodicity. We found that, like *per, lin-42* message levels also oscillate, but with a faster rhythm that is synchronized to the ~6-hour molting cycles of post-embryonic development. Unlike PER, which participates in a negative autoregulatory mechanism, LIN-42 is not required for the oscillation of its message levels. In flies, the PAS domain of PER mediates an interaction with a second circadian rhythm protein called TIMELESS. A *timeless*

homologue in *C. elegans*, *tim-1*, was revealed by the genome sequencing project. To date we have not detected an interaction between LIN-42 and TIM-1.

Yeast two-hybrid screens have indentified the gene products of F34H10.4 and R08E3.4 as putative LIN-42-interacting proteins. However, RNAi experiments have yet to reveal a role in developmental timing for either candidate gene. Further analysis of potential LIN-42-interacting proteins will be discussed.

812. Identification of genes regulating synaptic remodeling of DD motor neurons

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The ability of neurons to change synaptic patterns to adapt environmental variations or to satisfy the needs during development is remarkable. Such neural rewiring phenomena have been observed in diverse organisms from invertebrates to vertebrates. In *C. elegans*, the development of DD motor neuron circuitry is a striking example of neural remodeling (1). DD motor neurons are born embryonically. In L1 stage, they receive cholinergic synaptic inputs from other neurons on the dorsal side, and form synaptic outputs onto ventral muscle. At later developmental stages, the polarity of DD neurons is completely reversed; they form synapses onto the dorsal muscle, and receive synaptic inputs from the ventral side.

Using a SNB-1::GFP that is expressed in DD and VD neurons, we previously reported that DD remodeling takes place 3~5 hours at the end of L1 stage (2). We are interested in the temporal and spatial mechanisms controlling DD neuron connectivity. To observe the event of DD remodeling more precisely, we have generated animals that carry two transgenes, one expresses the synaptobrevin-GFP fusion protein under the control of a DD specific promoter (a generous gift of Chris Li), and the other expresses *hlh-8:*::CFP (3). The SNB-1::GFP puncta represent the pre-synaptic termini of DD neuron (4). The *hlh-8*::CFP marks the M-cell lineage and provides a fine timing control for L1 stage.

We used these transgenic animals in a pilot clonal screen to isolate mutants disrupting either the timing of DD remodeling or the localization of synapses in the L1 stage. From 500 haploid genomes of L1 larvae, we isolated twelve mutants with abnormal DD synaptic pattern. Majority of these mutants is also associated with adult sterility and larval arrest. Several new alleles of known genes were obtained in this screen including *cnd-1*, *unc-129,* and *mua-3.* We are characterizing the phenotypes of these mutants as well as mapping these genes. The

details of our screening strategy and progress will be presented.

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813. Role of *lin-41* in Developmental Timing of Vertebrates

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The heterochronic pathway in *C. elegans* is involved in the temporal regulation of developmental events. *lin-41* is regulated by a small RNA, *let-7*, a late acting gene in this pathway, and together these genes regulate the timing of a proliferation versus differentiation decision by skin cells in *C. elegans*. Both *lin-41* and *let-7* are conserved across phylogeny in such organisms as Drosophila, Zebrafish, Humans, and Mice. The protein encoded by *lin-41* is an oncogenic-like Ring finger B-box Coiled Coil (RBCC) protein. This family of proteins plays many significant roles in vertebrate development and health. We are interested in determining the significance of the *lin-41* and *let-7* genes in mammals as well as further characterizing their interactions. Using *in situ* hybridization experiments on mouse embryos we have determined the stage and tissues that *lin-41* and *let-7* are expressed in the developing mouse. *Mlin-41* is expressed temporally in somites and limb buds, two tissues known to be under control of a developmental clock. We hope that we will gain insight into the timing of human development in our use of the mouse as a model organism for these experiments.

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814. Characterization of the *mua* gene *vab-10* in C. elegans

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In *Caenorhabditis elegans*, locomotion is driven by the transmission of force generated by body wall muscles across basal lamina and hypodermis to the cuticle. The mechanical pathway, whereby skeletal muscles transmit contractile force, is composed of various cytoskeletal proteins and matrix components. Mutations that affect normal assembly or integrity of this linkage system exhibit a paralyzed phenotype. Mutated genes of the *mua* (*m*uscle *a*ttachment) class result in a progressive paralysis due to post-embryonic failure of the structural linkages during normal use. To date, all *mua*-class genes appear to affect hypodermal links within this pathway. Specific mutations result in detachment initiated at characteristic locationsadhesion of muscle to hypodermis, adhesion of hypodermis to cuticle, or integrity of the hypodermis.

One *mua* gene of interest is the *vab-10* gene. Mutations in this gene result in viable worms that are only partially paralyzed, and are characterized by a "bent head" phentotype. In *vab-10* mutants, muscle assembly and differentiation are normal, but are followed by spontaneous muscle detachment from the body during growth.

vab-10 maps to an interval of chromosome I that contains the *C. elegans* plectin and kakapo homologs. Both of these genes are contained within the cosmid ZK1151. We injected this cosmid into animals and found that ZK1151 rescues *vab-10* mutants. The ZK1151 cosmid houses three predicted genesZK1151.1 encodes an unknown protein with spectrin-like repeats, while ZK1151.2 and ZK1151.3 encode for proteins with kakapo and plectinlike homology. Both the plectin and kakapo-like genes prove to be promising candidates for *vab-10* . Using restriction digestion followed by religation, specific genes are being knocked out of the ZK1151 cosmid and the resulting subclones microinjected into mutant *vab-10* animals to assess rescue and the identity of the *vab-10* gene.

Further characterization of *vab-10* will also include immunological techniques. The primary site of muscle detachment in *vab-10* mutants is believed to be between hypodermis and muscle. Recent work with GFP-tagged intermediate filaments has shown that *vab-10* mutants exhibit a pulling away of the muscle from the body wall with the hypodermis remaining attached to the cuticle (Wura Omotosho, personal communication). Whether peeling occurs from muscle and basal lamina or from hypodermis and basal lamina, and if other structures are affected will be determined with further immunostaining. In addition, other *mua* genes will be examined using immunofluorescent probes for specific muscle, basal lamina, and hypodermal proteins including *mua-1* (encoding a transcription factor) and *mua-3* (encoding a protein associated with fibrous organelles).

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815. The Myosin Tailpiece is Essential for Thick Filament Function

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Myosin heavy chain (MHC) is a major component of muscle thick filaments, important for both cell structure and contractility. MHC is a large molecule, containing distinct functional domains. The N-terminal myosin head contains the motor activity. The long coiled-coil rod is the site of dimerization, and the filament-forming domain. The small tailpiece region lies at the extreme C-terminus of the body-wall isoforms, MHC A and MHC B, and contains predicted phosphorylation sites. In other myosins that contain tailpieces, such as smooth muscle and non-muscle myosins, phosphorylation of the tailpiece is thought to regulate assembly of myosin into filaments. In these experiments, we have tested the role of the MHC A tailpiece in the striated body-wall muscle cells through site-directed mutagenesis. Our results suggest that the tailpiece plays a minor role in assembly, but is essential for thick filament function.

To test the role of the tailpiece region in myosin assembly and function, we generated MHC A constructs lacking the tailpiece domain. MHC A performs an essential function at the filament center, and can also substitute for the major isoform, MHC B, to form the filament arms. The T2 construct contains no predicted phosphorylation sites, producing a truncated protein lacking 9 residues of predicted coiled-coil as well as 25 C-terminal non-helical residues. We tested T2 function in vivo, and found that the truncated protein could rescue the lethality of mutants lacking MHC A, indicating that the tailpiece is not required for filament initiation activity. Antibody staining of embryos showed a small delay in the assembly of T2 into ordered structures, but staining was close to that of wild type. In addition, T2 transgenes could restore motility in mutants lacking MHC B, demonstrating competence to form the filament arms. Polarized light microscopy of rescued adults showed a pattern of birefringence that was near-normal, but not as crisp as wild type. Using TEM, we determined that the thick filaments containing the truncated protein were morphologically normal, suggesting that the tailpiece is not required to specify filament structure.

To determine whether a functional thick filament containing only truncated MHC could be formed, we tested our T2 trangenes in a double mutant background lacking any endogenous MHC in the body wall muscle. These experiments revealed that the truncated T2 myosin could not rescue the double mutant to viability, nor improve the terminal phenotype of the transgenic double mutant. Antibody staining revealed that the truncated MHC showed delayed assembly into ordered structures, but ordered staining was evident at the earliest appropriate stages. In contrast to the apparently minor assembly defect, time-lapse video microscopy revealed that transgenic double mutant animals show no twitching or coordinated movement, a phenotype indistinguishable from that of the MHC A null mutant, which lacks normal thick filaments. Because the protein appears to assemble, our results suggest that the tailpiece is required for filament function rather than filament formation.

We are currently testing the function of construct T1, which lacks only nonhelical sequences and contains a single potential phosphorylation site. T1 shows wild-type localization in embryos and adults, but has not yet been tested in the double mutant background. To determine the importance of this potential phosphorylation site for wild-type assembly, we are currently testing a T1 construct in which the serines within the coiled coil have been replaced with alanines.

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816. sem-3 encodes a cis-prenyltransferase homolog, an enzyme required for production of an essential lipid.

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In *C. elegans*, the egg-laying muscles are descended from a pair of M-derived cells known as the sex myoblasts (SMs). The SMs are born in the posterior of the animal during the L2 stage and migrate anteriorly, in response to multiple cues, to flank the center of the developing gonad.1 The development of functional egg-laying muscles requires their proper differentiation, attachment, and innervation in addition to the proper migration of the SMs.

sem-3(n1655) was identified as a spontaneous Egl mutation with defects in SM migration and vulval muscle attachment. The final positions of the SMs in *sem-3(n1655)* hermaphrodites lack the precision found in wild type, but are very close to normal. The sex muscles derived from these SMs are normal both in number and in their expression of appropriate reporter constructs, but often fail to attach properly. The subtle nature of the SM migration defect makes it likely that the vulval muscle attachment defects are the cause of the Egl phenotype of *sem-3(n1655)* mutants.

sem-3 was mapped to linkage group IV and found to be allelic to *let-654.* Standard germline transformation rescue of the Let and Egl phenotypes was used to identify the *sem-3* open reading frame from among cosmids in the region; the presence of lesions in multiple *sem-3* alleles confirmed the identification of the *sem-3* gene. By sequence analysis, *sem-3* encodes a member of the cis-prenyltransferase class of enzymes. This class of enzymes is responsible for the production of dolichol, a lipid molecule with essential functions in N-linked glycosylation, formation of GPI anchors, and regulation of membrane fluidity.2 BLAST analysis shows that SEM-3 is the only member of this class of enzymes in *C. elegans* and suggests that the lethality associated with *sem-3* mutations may be due to a lack of dolichol. Consistent with an essential role for SEM-3 in a ubiquitous process such as N-linked glycosylation a *sem-3*::GFP reporter construct is expressed in a wide variety of tissues throughout development. Models for SEM-3 function in vulval muscle attachment will be discussed.

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817. C. elegans Plakin Protein: Another Molecular Similarity Between Fibrous Organelles and Vertebrate Hemidesmosomes

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Cell-adhesion complexes containing intermediate filaments are essential for tissue integrity in many organisms. In *C. elegans,* the best characterized of these structures are hypodermal fibrous organelles which help to attach muscle cells to the bodywall. These strucutres are morphologically and functionally similar to vertebrate intermediate filament-containing structures called hemidesmosomes. Like vertebrate hemidesmosomes, fibrous organelles consist of membrane-associated plaques that appear to anchor intermediate filaments to the membrane. A number of components have been identified whose distributions correlate with the presence of fibrous organelles, two of which (MUA-6/IFA-2 and IFA-3) share homology with vertebrate hemidesmosomal proteins (intermediate filament proteins). To identify other molecular similarities between fibrous organelles and vertebrate hemidesmosomes we searched the *C. elegans* database for predicted peptides homologous to known hemidesmosome proteins. Three peptides, encoded by ZK1151.1, .2 and .3, share homology with the plakin family of proteins, some of which have been shown to link intermediate filaments to the membrane at hemidesmosomes.

Our analysis of RT-PCR products suggests the ZK1151.1, .2 and ,3 sequences all constitute a single gene, which we refer to as the ZK1151 gene. In addition, the data suggests two sets of transcripts are encoded by this gene, each containing a different 3-prime end. One set of transcripts contains ZK1151.1 sequences spliced to ZK1151.3 sequences (short form) and the other contains ZK1151.1 sequences spliced to ZK1151.2 sequences (long form). We were unable to obtain any evidence for transcripts containing both ZK1151.2 and .3 sequences. The ZK1151 protein shares sequence homology with *D. melanogastar* kakapoo and human macrophorin, two plakin family members thought to interact with microtubules and actin. In contrast to ZK1151, the *D. melanogastar* and the human genes express messages containing sequences homologous to ZK1151.1, .2 and .3 in a single transcript.

Analysis of RNAi-generated ZK1151.2 and .3 mutants suggests members of both the long and short classes of ZK1151 transcripts are essential. The ZK1151.2 mutants are larval lethals and the ZK1151.3 mutants are embryonic lethals. Immunofluorescence analysis of the distribution of intermediate filaments in the ZK1151.3 mutants suggests at least some short ZK1151 transcripts are involved in linking intermediate filaments to the membrane at fibrous organelles. The ZK1151.2 mutants show defects in both the hypodermis and the pharynx suggesting the long ZK1151 transcripts may be expressed in both these tissue.

Interestingly, RNAi-generated ZK1151.1, .2 and .3 mutants all show defects in the tissue distribution of proteins recognized by monoclonal antibody MH5. Furthermore, the data suggest ZK1151 may encode the MH5 protein, a protein whose distribution correlates with the presence of fibrous organelles and is present in the pharynx. In both these tissues, MH5 appears closely associated with regions of the cell membranes where intermediate filaments are anchored. This distribution is consistent with a role in linking intermediate filaments to the membrane as suggested by the ZK1151.3 mutants. In conclusion, we believe the open-reading frames ZK1151.1, .2 and .3 represent a single gene that encodes members of the plakin family of proteins. The gene encodes two sets of proteins with different carboxy-termini, different tissue distributions and possibly different functions.

818. PARAMYOSIN-GFPs RESCUE MUTATIONS AND ARE USEFUL TO STUDY EMBRYONIC LETHALITY OF *CAENORHABDITIS ELEGANS*

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Paramyosin is a core structural component of thick filament in invertebrate muscle. In *C. elegans*, many paramyosin mutations are in the C-terminal region of the molecule; *e1215* (Q809R), *su228* (R837C), *su2000* (EH702D). Thus this region may play an important role in assembly by the interaction of charged residues. The paramyosin mutant *e1402* has hydrophobic substitution (L799F) in the C-terminal region. This mutation causes temperature sensitive Unc and embryonic lethal phenotypes, but the underlying molecular mechanisms are not well known. To study the relationship between paramyosin disassembly and embryonic lethality, we made transgenic animals with paramyosin GFP fusion proteins (PM-GFPs, GFP tagged at the N-terminal: GFP-PM or C-terminal: PM-GFP of paramyosin). Null mutation *e1214* animal was recovered its motility with GFP-PM, but not with PM-GFP. Recovered motility was proportion to fluorescent filament assembly of the transgenic animals. Quantities of paramyosin and PM-GFPs were also detected on Western analysis by using paramyosin and GFP antibodies. In transgenic animals of *unc-15* semidominant alleles (*e73, e1215, su228, su2000*), GFP-PM was a good marker for monitoring filament assembly. In temperature sensitive (*e1402*) mutant, GFP-PM was also powerful to visualize the thick filament during development. Although over-produced PM-GFPs in the transgenic worm from N2 disrupted filament assembly and impaired locomotion of the worms, GFP-PM assembled same as that of native paramyosin and was a good marker to insight into the cause of the embryonic lethality. Our analyses, using PM-GFPs will allow to indicate how the embryonic lethality occurs due to disassembling of paramyosin.

819. The effects of the mutant troponin C on embryogenesis and muscle contraction in *Caenorhabditis elegans* .

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Troponin C (TnC) is a component of the troponin complex and regulate the contraction of striated muscle as a Ca^{2+}-dependent trigger. TnC contains four Ca^{2+} binding motifs namely site I-IV. In *Caenorhabditis elegans*, *pat-10* encodes body wall muscle TnC which has two Ca^{2+}-binding motifs at the site II and IV. We detected two point mutations in *C.elegans* TnC at positions of D64N and W153* of site II and IV respectively which together causes a Pat (paralyzed arrest at embryonic two-fold stage) phenotype. Using bacteria mutated proteins PAT-10-m1 and PAT-10-m2 for D64N and W153* respectively, we found that the D64 site is necessary for Ca^{2+}-dependent conformation change and the W153 site at C-terminal H-helix is essential for troponin I binding. This was the first troponin C mutant analysis in any animal (*Terami, et al*. 1999 J. Cell Biol.146: 193-202).

Our interest now is to know the kind of behavior in mutant animals having each of the two different *pat-10* mutations. We constructed the injection vectors (pPAT-M1 or pPAT-M2) using site-directed mutagenesis. Injection of pPAT-M1 together with a marker into the mutant animal (RW3613: *pat-10* (*st575*)/*unc-11* (*e47*) *dpy-5* (*e61*)) rescued the Pat phenotype, suggesting that Ca^{2+}-binding on site II is not necessary for the muscle contraction. We are currently getting transformed animals haboring pPAT-M2. Characterization of these mutant phenotypes will help us to understand the function and mechanism of troponin C in embryonic developent and muscle contraction. Finaly, we are also studying interactions between troponin C isoforms from pharynx and body wall and four troponin I isoforms (*Razia et al*, this meeting poster).

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820. Tissue localization and epitope-based functional analysis of the ryanodine receptor of *Caenorhabditis elegans*

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Muscle contraction is triggered by the release of Ca^{2+} from the sarcoplasmic reticulum (SR) via a Ca^{2+} release channel which is known as the ryanodine receptor. The ryanodine receptor of *C. elegans* (CeRyR) is encoded by a single *unc-68* gene, which is composed of 5,071 amino acids. Although the knockout mouse of skeletal or cardiac RyR is lethal, the *unc-68* null mutants show weak Unc and are viable. The *unc-68(kh30)* animals show intermittent convulsion before paralysis in anesthetic ketamine solution. The *kh30* mutation causes substitution of Ser1,444 to Asn, at a predictive phosphorylation site of protein kinase C. To know the detail localization and functional domains of CeRyR, we made the fusion proteins of the putative functional regions in CeRyR and raised antiserum against them.

We have obtained the region-specific antibodies of CeRyR by affinity purification of antiserum. Only one antibody against the region corresponding to the *kh30* mutation site was useful for immunostaining. CeRyR was found to be located in body wall, pharyngeal, vulval, enteric and sex muscles, but not in neurons and intestine. We confirmed that CeRyR was present in muscles of the *kh30* animal but not in those of *unc-68* null mutants. CeRyR was expressed from comma stage as well as myofilament components. The expression at comma stage was weak but strongly from 1-1/2 fold. Ca^{2+} bound to the regions of two EF-hand motifs and the C-terminal. Calsequestrin (CSQ-1) which is Ca^{2+} binding protein in SR could not bind CeRyR directly. On the basis of these results, we propose a model based on the functional domains of CeRyR. Our model agrees well with a model based on the results of proteolysis and cross-linking analyses of mammalian skeletal RyR. It suggests that CeRyR may be a primitive RyR and is useful for analysis of the function of the ryanodine receptor not only *C. elegans* but also in other animals.

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821. Effect of the alpha 2 Ca^{2+} channel subunit UNC-36 on calcium transients in pharyngeal muscles.

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Voltage-gated calcium channels are protein complexes that mediate calcium transients essential for muscle contraction and synaptic transmission. Although the properties of the pore-forming alpha-1 subunit have been studied extensively in vertebrate systems, less is known about the role of the associated subunits. We are using recently developed calcium imaging techniques to investigate the effects of loss of function in one of these subunits, the putative α2 subunit encoded by *unc-36*. *unc-36* has several loss of function alleles and encodes an 85kD protein with 28% similarity to the human α2/δ1 subunit CACNA2D1. Loss of function alleles of *unc-36* produce a moderately severe uncoordinated phenotype and reduced rates of pharyngeal pumping, indicating that the α2 subunit is functionally important in *C. elegans*.

Although other studies have implicated a role for *unc-36* in neurons, we are initially characterizing the role of *unc-36* in the pharyngeal muscles. Preliminary GFP expression data indicates that *unc-36* is expressed in the pharyngeal muscles. We have chosen the pharynx as our model system because it is well-characterized electrically and pharmacologically. We are using the genetically encoded cameleon calcium sensor driven by the pharyngeal-specific *myo-2* promoter to record calcium transients in intact worms. We quantify the pumping behavior by measuring the pumping frequency, the duration of each transient, and the rate of increase in the calcium. In the two alleles we have tested, *unc-36(e251)* and *unc-36(ad698)*, we found that the duration of transients is similar to that of wild-type but that the rate of calcium increase is 30-40% greater. This suggests that the role of *unc-36* on the pharynx is to reduce the calcium flux. The simplest explanation is that the pharyngeal channels are decreased in number or conductivity in the presence of UNC-36 and suggests a possible negative regulatory role for the α2 subunit in muscles. Alternatively, UNC-36 could be acting directly in neurons and the observed effect in muscle could be an acute or developmental response to the altered neuronal input. The reduced rate of pumping in *unc-36* mutants is consistent with this hypothesis: the pharyngeal nervous system is required for rapid pharyngeal pumping. This suggests that UNC-36 may facilitate the activity of calcium channels in neurons.

To distinguish between these two hypotheses, we are eliminating synaptic input by conducting experiments in a *snt-1* background. *snt-1* encodes the only known synaptotagmin in *C. elegans* and is thus thought to be essential for evoked neurotransmitter release. Experiments on *unc-36* mutants in this background should allow us to distinguish between the effect of UNC-36 in muscle and in the nervous system. Results from these experiments can be verified by laser ablation of the MC neuron, the only neuron in the pharyngeal nervous system responsible for inducing rapid pharyngeal pumping. We also intend to rescue the *unc-36* mutants with the pharynx specific *myo-2::unc-36* construct to further characterize the effect UNC-36 in the nervous system.

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822. Depletion of PKL (KIN-4) in Muscle Results in L1 Paralysis

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In *C.elegans kin-4* encodes two isoforms of a PDZ containing S/T protein kinase (PKL). PKL1 and PKL2 are identical over 1333 amino acids but have different C termini that are generated by omission or inclusion of the penultimate exon of the gene. MAST205, a mammalian protein kinase, has catalytic and C terminal PDZ domains that are homologous with corresponding domains in PKL. However their N and C terminal sequences have limited but potentially significant homology with those of PKL.

I have used *C.elegans* and mammalian systems to probe PKL functions. Immunostaining revealed that PKL1 is aligned along filaments of body-wall muscle (adjacent to actin) in *C.elegans*. PKL2 is enriched in pharyngeal muscle (adjacent to actin), the lateral surface of intestinal cells and the gonad. Both PKLs are associated with large, punctate structures in numerous cell types. MAST205 is expressed principally in testis, skeletal muscle and heart. In a skeletal muscle cell line MAST205 is enriched in focal adhesions and actin cytoskeleton. The kinase accumulates at "zipper like" cell-cell junctions in a spermatocyte-derived cell line. These junctions and MAST205 are dispersed in cells treated with cytochalasin B. Large punctate structures that contain MAST205 are also evident in these cells. ^{32}P-labeled PDZ domains from MAST205 and PKL avidly bind mouse skeletal muscle beta-tropomyosin (TM). Binding is dramatically reduced when C-terminal amino acids of beta-TM, (TSL, a potential PDZ ligand) are replaced with NNL, NSL or NSA. *C.elegans* TMs, CeTMI and CeTMIII bind both full length PKL1 and a 40kDa PKL1 fragment that includes the PDZ domain. CeTMI and CeTMIII are also phosphorylated by PKL.

CeTMI and CeTMIII lack PDZ ligand sequences at their C-termini. Deletion analysis revealed that 16 C-terminal amino acids of CeTMI are not required for binding with the PDZ-region of PKL. However, deletion of 63 or 113 C-terminal amino acids from CeTMI decreased binding activity by 60 and 80 %, respectively. Immunofluorescence analysis showed that PKL1 and CeTMs are co-clustered in filaments of body wall muscle. Thus, PKLs may be targeted to actin filaments in body wall and pharyngeal muscle via interaction with tropomyosin. Anchored PKL/MAST205 may catalyze phosphorylation of tropomyosin and/or troponin subunits, thereby regulating contraction.

Transgenic nematodes expressing anti-sense PKL RNA in muscle are paralyzed at L1 and move slowly at subsequent larval stages. This correlates with a >70% reduction in PKL1 protein. Wild-type PKL1 and "kinase-dead" PKL1 are being selectively expressed in body wall muscle of transgenic nematodes. Effects on *C. elegans* movement and muscle structure will be assessed in order to obtain additional clues regarding functions of PKL. PKL may be a novel regulator of muscle physiology.

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823. Calcium Regulatory Sites in UNC-68 Ryanodine Receptor Channels

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Ryanodine receptors (RyRs) are intracellular, homotetrameric ion channels in muscle cells that gate the release of calcium (Ca^{2+}) from the sarcoplasmic reticulum (SR). RyR channels flood the myoplasm with Ca^{2+} following depolarization of the muscle surface membrane, causing contraction. RyRs are regulated by myoplasmic Ca^{2+}. Depolarization is accompanied by a modest influx of Ca^{2+} through voltage-gated Ca^{2+} channels, which activates RyR channels. Subsequently, RyRs are inactivated by the higher Ca^{2+} levels present after SR Ca^{2+}-release, thus creating a self-terminating burst of Ca^{2+} during muscle contraction. *C. elegans* has a single RyR gene (*unc-68)* that encodes a 5074 amino acid RyR protein. In body-wall muscle cells, UNC68 channels are localized in SR vesicles between the surface membrane and the myofilament lattice. *unc68* null mutant animals are viable, but have defective locomotion. We are examining the biological role of Ca^{2+} binding sites in UNC-68 RyRs that may be involved in activation or termination of Ca^{2+} release in body-wall muscle cells. The predicted UNC-68 protein has two EF hand-type Ca^{2+}-binding motifs at amino acids 4198-4246 (Sakube, et. al. 1997 JMB **267**:849). We have eliminated one or both EF hands using alanine substitution, and analyzed $^{45}Ca^{2+}$-binding to wild type and mutant fusion proteins containing the EF-hand region. Both the EF hand motifs bind $^{45}Ca^{2+}$, as shown by $^{45}Ca^{2+}$-overlays and equilibrium binding. We have expressed GFP-tagged *unc-68* genes containing wild-type EF-Hands or the EF-Hand mutations in *unc68* null mutant animals. Proper expression and localization of the GFP-tagged RyRs have been confirmed using in vivo localization of the GFP tag, and western blot analysis of purified, detergent-solubilized RyR proteins. Both intact EF hands are required for rescue of motility (i.e. only the WT gene rescues normal locomotion). The two genes with a single EF hand eliminated ($EF1^{+}$ $EF2^{-}$ or $EF1^{-}EF2^{+}$), give partial rescue of motility, and restore sensitivity to paralysis by ryanodine. The gene lacking both EF-Hand calcium-binding sites does not rescue motility or restore sensitivity to ryanodine. Interestingly, when expressed in *unc-68(0)/+* or wild-type animals, arrays containing all three EF-Hand mutant RyR genes exert semi-dominant effects on motility. One interpretation of this semi-dominance is that the mutant RyR subunits form heterotetramers with Wild Type subunits. Further characterization of the purified mutant RyR channels should reveal whether the EF-Hand calcium-binding sites are involved in activation or inactivation of calcium release during body-wall muscle contraction.

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824. Structural and Functional Disruption of Muscle Caused by Troponin I Mutation

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Muscular performance varies enormously among animals, as well as even within a single organism, and some of the variation is speculated to arise from adaptations of troponin, which regulates tension development in striated muscle. Like vertebrates, *C. elegans* has multiple genes coding for troponin I (TnI), the inhibitory subunit within the ternary troponin complex. Phylogenetic analysis of the four *C. elegans* TnIs suggested that diversification of TnI isoforms occurred independently in vertebrates and invertebrates. Nonetheless, the domain architecture of TnI is preserved in all isoforms. RNA interference (RNAi) of TnI-1 (F42E11.4), expressed in embryonic body wall muscle and hermaphroditic gonad, led to no discernible defect. Similar results were obtained following RNAi of TnI-4 (W03F8.1), expressed in the pharyngeal muscle. RNAi of the larval and adult body wall muscle TnI, Unc-27/TnI-2 (ZK721.2), produced rigid paralysis mimicking that of *unc-27* mutants. Elimination of TnI-3 (T20B3.2) yielded constipated and egg-laying defective worms, consistent with detection of TnI-3 mRNA in vulval muscles and intestine.

Three mutant alleles of *unc-27* exist: *e155* (Gln10stop), a presumed null; *su142*sd (Gln122stop), which lacks the domain thought necessary for inhibition at sub-micromolar Ca^{2+}; and *su195*sd (Glu207stop), which eliminates only the *C*-terminus. Each of the mutant alleles severely compromised swimming behavior of adult homozygous hermaphrodites ($p<0.0001$), and among the mutants, *su142*sd worms showed the lowest tail-beat frequency ($p<0.001$). Even at the L1 stage, *su142*sd worms differed from wild type in swimming ($p<0.05$). Both polarized light microscopy and anti-vinculin labeling of mutant body-wall muscle revealed a loss of sarcomeric organization. The wild-type pattern of obliquely arranged, alternating isotropic and anisotropic bands was absent. Dense bodies, marking the ends of the sarcomeres, were evenly spaced along parallel rows in wild-type worms, but their disposition was greatly distorted in the mutants. Electron microscopy of body wall muscle confirmed a prevalent disorder of dense body positioning and a less well defined sarcomere structure. The reason for this appearance is identified from transverse sections. In wild-type worms this orientation showed a regular alternation of the sarcomere bands. Dense bodies were followed by I bands, composed of thin filaments, then by overlap zones of A bands, with regularly packed thick filaments surrounded by rings of thin filaments, and next by the H zone of A bands, containing only thick filaments. In the mutants, the same bands were seen, but they were not in a regular pattern. Small islands of thin filaments were interspersed within the overlap region of A bands and even within the H zone. In the overlap region the content of thin filaments was quite variable, and the H zone was fragmented into several small areas of the cross section. The defects were strongest in the *su142*sd mutants. This structural pattern could arise from unregulated contraction of the sarcomere, in which small portions of each myofibril shorten irregularly and independently from one another, thereby distorting the filament disposition.

Collectively, the functional and structural analyses are consistent with a primary role of TnI in inhibiting force development and highlight the dependence of sarcomeric organization on proper contractile activity. The exacerbated deficits exhibited by *su142*sd worms strengthen the view that the *N*-terminal portion of TnI participates in enhancing force development in an active muscle and thus suggest that design variations within this portion of TnI isoforms could contribute to the diversity of tension-producing capability of muscle.

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825. CAM-1/KIN-8 Receptor Tyrosine Kinase is Important for Coordinated Movement

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The neuromuscular junction (NMJ) is critical for neuronal control of muscle movement and coordination. Work from our lab indicates a Ror-like receptor tyrosine kinase, CAM-1/KIN-8, is expressed at the NMJ, and may play an important role in the development and/or function of the NMJ.

CAM-1/KIN-8 as an important player in V-cell polarity and CAN cell migration (Forrester *et al*., 1999, *Nature*, 400(6747):881-5). CAM-1/KIN-8 can also act in the dauer formation pathway as a regulator of DAF-7/TGF-β expression (Koga *et al*., 1999, *Development* (23):5387-98). Our lab independently generated a *cam-1/kin-8* deletion mutant, *ak37*, and found that CAM-1/KIN-8 may be necessary for proper function of the NMJ.

Using cell-specific GFP markers, we have examined muscle arm morphology and synaptic density at the neuromuscular junction. *cam-1/kin-8(ak37)* mutants have significantly smaller muscle arms and decreased acetylcholine receptor (AChR) density at the neuromuscular junction. *cam-1/kin-8(ak37)* mutants also show a mild resistance to Aldicarb. We are planning to use electrophysiology and the AChR-specific drug levamisole to further characterize NMJ function in *cam-1/kin-8(ak37)*animals.

Transgenic worms that overexpress the full-length CAM-1/KIN-8 protein under its native promoter appear wild type, but worms overexpressing the CAM-1/KIN-8 receptor under the *myo-3* promoter have severe morphological and behavioral defects including spiny muscle outgrowths, deformed vulval muscles, and paralysis. Paralysis caused by this muscle-specific overexpression is unchanged in the *cam-1/kin-8(ak37)*background. Worms overexpressing a truncated form of CAM-1/KIN-8 (lacking the kinase domain and putative intracellular protein-binding domains) are also paralyzed. These data suggest the CAM-1/KIN-8 extracellular domain may act as a ligand or signaling molecule independent of CAM-1/KIN-8 kinase activity.

826. PRODUCTION OF ANTIBODIES AGAINST *C elegans* PROTEINS

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To construct antibody libraries covering *C elegans* whole proteins, we have been in an effort to produce monoclonal antibodies by the methods of cell fusion and phage display. As a source of these antibodies supply, we chose the spleen cells of Balb/c mouse immunized with homogenates of *C elegans* whole body or with proteins derived from *C elegans* cDNA expression library supplied by A.Fire.

In a preliminary work, we tried to produce monoclonal antibodies by the fusion of P3U1 myeloma cells with spleen cells from the mice immunized with *C elegans* whole proteins mixed with Freunds adjuvant. We obtained the antisera showing strong reaction to many kinds of *C elegans* proteins, and could produce 16 kinds of monoclonal anti-*C elegans* antibodies named 4A7, 4A3, 4A1, 1E1, 2G5, 4A9, 1B1, 4C10, 2B8, 2B6, 3B12, 2H3, 3A6, 1F6, 1D12 and 1C10. When we immunized the mice with *C elegans* whole proteins alone instead of mixture with Freunds adjuvant, we obtained antisera showing weak reaction to *C elegans* proteins and could produce only one kind of monoclonal anti-*C elegans* antibody named 3D7. We applied these antisera and monoclonal antibodies on western blotting analysis to identify their antigens. The antisera reacted with many kinds of *C elegans* proteins. On the other hand, monoclonal antibody 4A7, 4A1, 1E1, 2G5, 2B6, 3B12 and 2H3 reacted with 40kDa *C elegans* protein alone suggesting these antibodies detecting a single molecule. In the same way, 55kDa protein was suggsted as a sole antigen of 3A6, 1F6 and 1D12. A number of antigens were suggested for 4A3, 4A9, 1B1, 4C10, 2B8 and 3D7. The immunohistochemical localization of these antigens in *C elegans* is under investigation.

827. Biochemical Characterization of *C. elegans* Malate Dehydrogenase

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In my laboratory, we are overexpressing and biochemically characterizing *C. elegans* malate dehydrogenase (MDH), the last enzyme in the citric acid cycle. This enzyme, encoded by putative gene F20H11.3, appears to be the mitochondrial form of MDH, since it contains a mitochondrial import presequence. We have overexpressed and purified a version of this enzyme with the mitochondrial import presequence removed at the predicted cleavage site of the mitochondrial processing protease. The purified MDH enzyme has malate dehydrogenase activity that follows Michaelis-Menten kinetics when plotted versus oxaloacetate concentration, and the resulting Km is similar to the Km values for other MDH enzymes. Interestingly, the temperature dependence of the enzyme is a bell-shaped curve with a peak at approximately 30°C, suggesting that the enzyme is adapted to the growing temperature of *C. elegans*. We are grateful to Yuji Kohara for providing the cDNA clone of MDH.

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828. Loss of a dynamin related protein MGM-1 causes excessive mitochondrial fragmentation

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Our lab studies the functions of dynamin and dynamin related proteins. These proteins form a small family of large GTP-binding proteins. We have recently shown that the C. elegans dynamin related protein DRP-1, is involved in the scission of the mitochondrial outer membrane (1). Since little is known about mitochondrial division and mitochondrial morphology in animal cells, we are investigating the mechanisms that regulate these processes. MGM-1 is another member of the dynamin family present in animal cells. As of now we know that this protein is present in yeast, C. elegans, and humans. Unlike other members of the dynamin family, MGM-1 has an N-terminal mitochondrial leader sequence. MGM-1 is also more closely related to bacterial dynamin-like proteins than it is to dynamin or DRP-1, suggesting that this protein has followed a different evolutionary path. We speculate that MGM-1 was introduced into eukaryotic cells by the progenitors of mitochondria. This suggests that MGM-1 has a function that is different than DRP-1. Here we investigate the function of MGM-1 using C. elegans muscle cells.

Expression studies using ß-galactosidase under the control of the mgm-1 promoter show high levels of expression in intestines, body wall muscles, and neurons. These high levels might reflect high metabolic rates in those tissues, because the MGM-1 expression pattern is similar to that of another dynamin-related protein, DRP-1, which is also important for mitochondrial maintenance. Mgm-1 RNAi causes worms to be lethargic, slows their growth rate and doubles their life span. This RNAi phenotype led us to investigate eat-3, a previously identified mutant that maps to the mgm-1 locus and has a similar phenotype. We sequenced the mgm-1 gene in eat-3 and found a point mutation in the GTPase domain. We have also partially rescued the eat-3 phenotype by injecting a wildtype mgm-1 construct. We show that RNAi, mgm-1 antisense cDNA, and dominant negative MGM-1 induce excessive fragmentation of mitochondria. We investigated the ultrastructure of mitochondria in wildtype and mutant worms by electron microscopy. Wildtype mitochondria have cristae that look tubular and are usually perpendicular to the length of the mitochondria. The mitochondria of eat-3 mutants are small and round and have few or no detectable cristae. Many mitochondria look like they are further subdivided by septae of inner membrane. We find that MGM-1::GFP is localized to the mitochondrial matrix, suggesting that MGM-1 regulates the morphology of the mitochondrial inner membrane.

1. Labrousse, A.M., Zappaterra, M.D., Rube, D.A., and van der Bliek, A.M. Molecular Cell 4: 815-826. 1999.

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829. Search for novel proteins affecting mitochondrial inner and outer membrane division.

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Mitochondria are dynamic structures that often divide and fuse throughout the life of a cell. Mitochondrial division is necessary to keep up with cell growth and division and it is often also necessary in response to changes in the cellular environment. Mitochondrial division is a complex process requiring coordination between inner and outer membrane division. We have previously shown that a dynamin-related protein, called DRP-1, is required for division of the mitochondrial outer membrane, but not for division of the inner membrane (1). Although we suspect that DRP-1 acts as part of a larger protein complex, other factors controlling outer membrane division in C. elegans are unknown, nor are inner membrane factors known. In a companion abstract by Rube et al. the possible role of other endocytic components in mitochondrial outer membrane division is explored further. Here, we look for novel factors that control outer and inner membrane division.

We are testing candidate proteins by injecting antisense constructs under control of the myo-3 promoter, which is expressed at high levels in C. elegans bodywall muscles, along with GFP targeted to mitochondria. For the outer membrane, we have started with some of the known outer membrane proteins such as components of the voltage dependent anion channel (VDAC). One component of VDAC, the peripheral benzodiazepine receptor (PBR), is of special interest, because benzodiazepine has been shown to induce mitochondrial division in mammalian cells. We are currently testing the possible role of C. elegans PBR in division, using GFP-tags and antisense PBR under control of the myo-3 promoter. We are also exploring the possible roles of mitochondrial matrix proteins in division. A large number of novel matrix proteins were identified using a bioinformatics approach (2). Our preliminary results suggest that some of these affect division of the mitochondrial inner membrane. These results will be presented at the meeting.

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2. Marcotte, E.M., I. Xenarios, A.M. van Der Bliek, and D. Eisenberg (2000) Localizing proteins in the cell from their phylogenetic profiles, Proc Natl Acad Sci U S A 97, 12115-12120.

830. Nematodes with Mitochondrial Diseases

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Caenorhabditis elegans is a simple animal with a mammalian-like mitochondrial respiratory chain. We have isolated mutations in two nuclear genes, *nuo-1* and *atp-2*. The *nuo-1* gene encodes the 51 kDa subunit of complex I (NADH:ubiquinone oxidoreductase), which contains the NADH and the FMN binding sites. The *atp-2* gene encodes the beta subunit of complex V (ATP synthase). Both mutations are lethal and produce developmental arrest in the third larval stage with gonad development being even more severely impaired. Swimming, pharyngeal pumping, and defecation are also impaired. The *atp-2* mutant is dauer defective, while the *nuo-1* mutant is unable to exit dauer once entered. We believe that it is the impairment of energy production that is the common event in both mutants and the root cause of the developmental arrest. We predicted that any interference with mitochondrial biogenesis should also produce larval arrest. Ethidium bromide, chloramphenicol, and doxycycline all produce a third larval stage arrest that is phenotypically similar to the *atp-2* and *nuo-1* mutants. We conclude that mitochondrial respiratory chain dysfunction in *C. elegans* is lethal and produces a distinctive larval arrest phenotype. The phenotype can be elicited by interfering with nuclear or mitochondrial genes encoding subunits of the respiratory chain. We estimate there are approximately 200 genes necessary for the production of a functional respiratory chain. The *atp-2* and *nuo-1* mutants define the first two members of this large family of essential genes.

831. RNAi-Mediated Embryonic Lethality by Three Genes Encoding Complex II Subunits of the Electron Transport Chain.

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The electron transport system consists of four complexes that alternatively accept and donate electrons, generating ATP in the process. We have demonstrated previously that the *mev-1* gene encodes one (Cyt-1) of the four subunits (Ip, Cyt-1, Cyt-S and Fp) of complex II. mev-1 mutants are hypersenstive to oxidative stress and age precociously, particularly at elevated oxygen concentrations. To more systematically explore the consequences of complex II inactivation, we performed RNAi, specifically the soaking method, directed against three of the four subunits of complex II. Embryonic lethality was the predominant result in each case, with hatching reduced by 87%, 75% and 98.3% after functional inactivation of Ip, Cyt-1, and Cyt-S, respectively . The survivors were healthy and not hypersensitive to hyperoxia. The inviable zygotes arrested at various times throughout embryogenesis in proportions specific to the subunit inactivated. For example, Cyt-S inactivation yielded almost 70% inviable zygotes that arrested immediately after fertilization (either prior to nuclear fusion or at the one-cell stage) whereas as almost 60% of Cyt-1-treated embryos reached at least the late morula stage. This embryonic lethality is not surprising given the energy demands throughout embryogenesis.

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832. Mitochondrial Complex I and II Defects Result in Excessive Superoxide Anion and Apoptosis.

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Electron transport is facilitated by four complexes that are bound to the mitochondrial inner membrane. In *C. elegans*, the mutation *mev-1(kn1)* causes a defect in the cytochrome b large subunit (Cyt-1) of complex II, and mutations in *gas-1* result in a defective 49 kDa iron-sulfur protein subunit of complex I. Both mutations render animals susceptible to oxidative stress. We now present a series of interrelated observations on *mev-1* and *gas-1*. First, the levels of the Reactive Oxygen Species (ROS) superoxide anion are substantially elevated in isolated sub-mitochondrial particles (SMP) from both *mev-1* and *gas-1* mutants, particularly when reared under hyperoxic conditions. Interestingly, superoxide anion levels in intact *gas-1* mitochondria were actually lower than in wild type. Second, the concentration of reduced glutathione (GSH), which is an important antioxidant, is substantially reduced in both *mev-1* and *gas-1* animals. These two phenotypes undoubtedly translate into higher levels of oxidative stress in *mev-1* and *gas-1* mutants. Two manifestations of this burden are as follows: i) as evidenced by microscopic examination of animals exposed to the reagent JC1, inner mitochondrial membrane potentials were significantly lower *mev-1* and *gas-1* animals; and ii) as measured using Western blots, the concentrations of mitochondrially localized *ced-9* gene product were different in wild type versus the two mutants; specifically, CED-9 levels increased as a function of oxygen concentration in N2 but decreased in *mev-1* and *gas-1*. These latter two observations help explain the fact that both *mev-1* and *gas-1* embryos contain significantly more apoptotic cells than wild type. In the case of mev-1, the abnormal cell death is completely suppressed by inclusion of either loss-of-function *ced-3* or *ced-4* mutations into the genetic background, indicating that supernumerary apoptosis operates via the normal *ced-9/ced-4/ced-3* apoptotic pathway. This epistasis experiment has not yet been conducted with the *gas-1* because the double mutant is very difficult to maintain. Collectively, these data demonstrate that mutations (*mev-1* and *gas-1*) in different complexes of the electron transport chain have very similar consequences, namely heightened susceptibility to oxidative stress and apoptosis. This underscores the importance of mitochondrially induced oxidative stress in aging.

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833. The Effects of Electron Transport Inhibitors on Survival of Wild-type *C. elegans*

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Electron transport is mediated by four complexes that are anchored to the mitochondrial inner membrane. Rotenone, antimycin, TTFA and azide selectively inhibit complex I, II, III and IV, respectively. We have measured the lethal effects of these four inhibitors at two oxygen concentrations. Given that free radical generation is directly proportional to oxygen concentration, this should allow us to access the relative contribution of each complex to oxidative stress. As is our custom, survival was determined as the ability of L1 larvae to attain adulthood under a particular experimental condition.

The concentrations of the complex I inhibitor rotenone required to reduce survival to 50% (i.e., LD50) were 0.75 mM under 21% oxygen and 0.26 mM under 90% oxygen. Similarly, the LD50's for the complex II inhibitor TTFA were 5.2 mM under 21% oxygen and 2.9 mM under 90% oxygen. Thus, hyperoxia potentiated the lethal effects of the complex I and complex II inhibitors by factors of 3 and 1.8, respectively. Conversely, oxygen had little or no effect on the resistance of *C. elegans* to either the complex III inhibitor antimycin (LD50 = 15-16 uM under both oxygen concentrations) or the complex IV inhibitor azide (LD50 = 2.5 and 2.2 mM under 21% and 90% oxygen, respectively). These data indicate complexes I and II play important roles in mediating oxidative stress, which squares nicely with the fact that genetic disruption of either complex I (*gas-1*) or complex II (*mev-1*) render mutant animals hypersensitive to hyperoxia. On the other hand, since the literature indicates that most superoxide anion is generated at complex III, we expected the sensitivity to antimycin to be modulated by oxygen concentration. It is possible that we saw no such effect because this compound was several orders of magnitude more potent than the other three inhibitors. We were also surprised to find that the complex II defect did not render *mev-1* animals more susceptible to TTFA. Collectively, these data further our understanding of the roles played by electron transport constituents in oxidative stress.

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834. Cool dauers: video capture + thermal stage microscopy = sequence + morphology

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Although it is commonly stated that *C. elegans* is a soil nematode, hardly anyone has isolated it directly from soil extract, as opposed to from decomposing organic matter. Presumably, the dauer is the stage most likely to be encountered in soil, but it is nearly impossible to morphologically distinguish the dauer of *C. elegans* from that of other rhabditid species, especially when preserved in fixative and mounted in permanent slides. As a result, the ecology of *C. elegans* remains unknown: there are no primary data on its natural population dynamics, prevalence, dispersal or associations with phoretic vectors.

PCR-based identification has now become quite easy, but is expensive to apply routinely in a survey situation, and limited in quantitative resolution. In order to study the species ecology of *C. elegans* and relatives, we have therefore set out to develop a protocol that allows us to combine morphological and molecular data from individual nematodes, and especially from cryptic stages such as the dauer.

Our basic equipment consists of a microscope equipped with Differential Interference Contrast optics, with a thermal stage and with a video camera. The camera is connected to a personal computer equipped for Video Capture and Editing (VCE) with a generic capture card and non-linear editing software. Individual nematodes are immobilised by cooling on the microscope stage, and the morphology of different body regions is captured as videofiles on hard disk, while manually focusing through the specimen at highest magnification. The nematodes are then taken off the microscope, lysed in extraction buffer, used for PCR of ribosomal loci, and sequenced. Next, the obtained sequences are matched with known sequences, to determine the identity of each individual. Finally, the obtained identifications are used to compare the multifocal VCE files and search for morphological characters allowing consistent distinction between dauers from different species. We present the first ecological data obtained in this manner, from samples collected in central and southern California.

The equipment used is similar to a stripped-down, low cost 3-D version of a 4-D microscope, consisting mostly of generic components that are widely available from various suppliers and easily configured with minimal expertise. The system is highly versatile, and can e.g. also be used for non-destructively capturing the detailed morphology of mutants, recording ephemeral staining patterns, exchanging data across the internet, teaching, and assembling a VCE reference archive. Each series of videofiles from one nematode basically represents a "virtual microscope slide", bypassing the need for permanent slides. The size and contents of the captured files can be optimized with VCE, and the resulting compressed files combine the ease of storage and distribution of still images, with the depth of information and representation of multifocal videofiles.

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835. Cryofixation of *C. elegans* for ultrastructure, immunogold labeling and GFP fluorescence of the nervous system.

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Ultrastructural and antigenic preservation are often mutually exclusive when conventional fixations are applied to biological samples for transmission electron microscopy (TEM). This has been especially true for the nervous system of *C. elegans*, which is rich in phospholipids that are readily extracted if osmium tetroxide (OsO_4) is not included in the fixation. Since OsO_4 often masks and/or destroys antigenicity, it is usually excluded in experiments requiring immunolabeling, resulting in low-contrast, extracted samples. Cryofixation offers advantages to both structural and immunoEM studies because molecules are immobilized within milliseconds, which reduces conformational changes in antigens and preserves membrane dynamics in a life-like state. In this paper, we have tried to improve freezing quality using the low-cost Metal Mirror Freezing (MMF) method, and we have developed post-freezing sample treatments designed specifically for ultrastructural, immunolabeling or GFP visualization purposes.

Freezing quality is of utmost concern, especially when using MMF, since the depth of good freezing is limited by this method. We use a low vacuum to pull dilute suspensions of live worms down onto 0.45 µm millipore filters and then wick excess water from the filter just before freezing. Transferring frozen worms from liquid nitrogen temperatures (-196º C) directly to freeze substitution media at -85º results in a higher percentage of freeze damaged worms, presumably from recrystallization of vitreous ice. We have increased the ratio of well-frozen worms by warming from -196º to -85º over a 24-hour period. Cellular water must then be completely removed by the substitution media at -85º or recrystallization artifacts can occur. To ensure complete dehydration, we use an extended substitution time at -85º for at least one week with agitation during the last two days.

The fixative composition of the freeze substitution media is dependant upon the goal of the study, but we have found that the optimal base solvent for all purposes is a 3:1 ratio of acetone to methanol. For ultrastructural characterization, we include 1% OsO_4, and 0.5% uranyl acetate, slowly warm to 4ºC, rinse with acetone/methanol and embed in Embed 812 epoxy resin which gives higher membrane contrast than Spur's resin. When immunogold labeling is the goal we omit the OsO_4 and include 1% tannic acid and 0.5 % uranyl acetate, neither of which seems to interfere with antigenicity of the proteins we have tried to label thus far. Embedding in the acrylic resin LR Gold gives better preservation of synaptic vesicles than LR White, and there is less tissue shrinkage. GFP fluorescence and antigenicity is preserved during freeze substitution in straight acetone without fixatives. Bright GFP fluorescence with little background can be observed after gradual rehydration with 0.1 M PIPES buffer (pH 7.4) at 4ºC. Fluorescence lasts up to one week in these worms when stored at 4º C in the dark. Aqueous aldehyde fixatives can also be applied following rehydration but GFP fluorescence is reduced and background autofluorescence is increased.

Ultrastructural preservation of well-frozen material is superior in most cases to conventionally fixed worms, particularly in the eggs and larvae that are too small to cut open during chemical fixation. In the nervous system, plasma membranes and microtubules show good preservation and contrast, and synaptic vesicles seem to be associated with a fuzzy, cytoplasmic material. In worms processed for immunogold localization, we have been successful in labeling several neuronal proteins, including UNC-41, UNC-13 and GFP-fusion proteins (using commercially available anti-GFP antibodies). Cytoplasmic components of the nervous system in these worms are well preserved, although not as contrasted as in osmicated material.

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836. CAMBIO Initiative: Computational Algorithms for Multidimensional Biological Image Organization

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The microscope has been a ubiquitous tool of biological research for over two centuries; indeed it has almost become a symbolic icon for biomedical research. At the present time, more than ever before, there is a demand for microscopy, to study the structure and dynamics of cellular machinery. Much of this demand is being fueled by the extraordinary advances in genomics during the past few years, which is providing the complete parts lists of several key model organisms. The new challenge is to see how all these parts fit together and function as a living ensemble. Four Dimensional (4D) Microscopy, which uses a combination of optical sectioning microscopy together with computational techniques to study the structural dynamics of developing organisms, has become a powerful way to meet this challenge. By using 4D microscopy it is now possible to study three-dimensional dynamics of living tissue. We have established a collaboration between the Molecular Biology Laboratory, the Space Science and Engineering Center and the Computer Science Departments at the University of Wisconsin to develop an integrated software suite that will be used to capture, archive, visualize, analyze and distribute multi-focal plane, time-lapse (4D) recordings of embryonic development. Advanced visualization aids including roaming in space and time, 3D rendering and arbitrary plane slicing will facilitate perception of complex structural dynamics. A comprehensive annotation system will enable extensively labeled canonical developmental sequences to be established in a database thereby providing a powerful educational resource for students of embryology. This initiative will not only establish a means for 4D image manipulation, storage and dissemination of embryo data, but will also establish a framework for sharing and visualizing 4D data that are generated in other biological studies such as cytoskeletal dynamics and organogenesis.

837. Electron Tomography at an Organismal Level, a Unique Application of a Powerful Tool

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It can be difficult to obtain three dimensional(3D) information with traditional transmission electron microscopy(TEM). Electron microscope tomography using thick sections represents an alternative to reconstructing 3D images from serial thin sections. In some circumstances this technique can provide better depth resolution, require less time and be less subject to certain types of artifacts. As a result, some types of questions that were possible but difficult to address with thin section are now more practical. These include taxonomic comparisons and the production of developmental time series at an ultrastructural level. The method has been utilized here to examine cuticular structures in Zeldia punctata (Rhabditida: Cephalobidae) and to compare the tomographic reconstruction with information obtained via thin section TEM. Limitations and possible methodological improvements are discussed. Electron tomography has been used primarily at a macromolecular to sub-cellular spatial scale. Due to their small size, nematodes provide a unique opportunity to apply this tool as a means of asking questions at an organismal level. Possible applications to Caenorhabditis elegansinclude the comparative characterization of cuticle mutations, elucidation of the connections between neurons and the incorporation of higher quality 3D information into ultrastructural descriptions of morphogenetic processes.

838. CAENORHABDITIS ELEGANS (NEMATODA) AS TEST SUBJECT FOR CRYOIMMOBILISATION

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Compared to chemical fixation, considerable morphological improvements have been achieved with hyperbaric cryofixation followed by cryosubstitution. The duration for cryofixation takes circa 20 milliseconds, so that fast processes (vesicleformation,) in the cell are instantly halted.

Along the entire section of the nematode, no shrinkage was observed. Specimen can be fixed as a whole, no permeabilisation steps are required. Organelles are highly contrasted, and the contents of the vesicles is not leached out. Especially the eggs, with fully formed eggshell, were excellent preserved in the uterus with no visible artefacts at all.

Due to the crosslinking features of chemical fixatives, visualisation of proteins and protein reactions was optimalized. Cryofixation immobilises the specimen without crosslinking and with subsequent preservation of antigenicity. Comparable results were obtained with immunocytochemical treated, chemical fixed and cryofixed specimen, with the difference that the concentration of antibodies to be used is much lower in cryofixed specimen.

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839. The Low-Cost Worm Lab

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The equipment for Molecular Biology and Genetics research traditionally has been expensive, due to a variety of factors, including: low production volumes, high precision requirements, and complex sales/distribution systems. We have tried to improve this situation through the use of innovative or alternative technologies, and the adaptation of consumer and industrial mass-produced equipment for use in the biology laboratory.

While a variety of approaches have been pursued toward the efficient DNA-mediated transformation of *C. elegans* (Papp et al., 1997; Wilm et al. 1999), so far, no method has rivaled the effectiveness of direct microinjection (Mello et al., 1995). A major "difficulty" with microinjection is the cost of an inverted microscope with Differential Interference Contrast (DIC) optics and a Microinjection Controller. In response to this, we developed a low-cost electronic foot pedal controlled Microinjection Controller (Papp et al., 1991), and more recently, coupled it to a low-cost inverted microscope that utilizes custom-made Hoffman Modulation Contrast (HMC) optics, along with an inexpensive glide stage and micromanipulator. These high numerical aperture HMC optics yield a DIC-like image of oocytes and syncytial nuclei. While not ideal for demanding cell lineage studies, this system is more than adequate for microinjection. We can retail our complete system for $10,000, about one-third the cost of traditional systems, making it a nice dedicated microinjection station.

Proper maintenance of *C. elegans* cultures, particularly temperature-sensitive mutants, requires a precisely temperature-controlled incubator. Incubators with the ability to both heat and cool are traditionally expensive devices employing both Freon compressors and heating coils that fight against each other to control the temperature. In response to this, we developed a low-cost microprocessor-controlled incubator, based on the Peltier thermoelectric effect, which is now commonly employed in consumer-grade coolers. This unit quickly learns just the right amount of power to apply to the heat pump, in order to maintain the set temperature with about 0.1 degree Celsius precision, and it has sophisticated alarms to inform the user if anything has gone wrong. These incubators are available for less than $800, about one-third the cost of traditional heating and cooling incubators.

C. elegans genetics experiments require many media-filled Petri dishes. Traditional Sterile Media Dispensers utilize unreliable peristaltic pumps, or unreliable syringe based pumps, both of which are expensive and often cumbersome. In response to this, we developed a low-cost, all-digital microprocessor-controlled Sterile Media Dispenser based on a miniature autoclavable electromagnetic pump. This unit has a variety of advantages included memory storage for common sizes of Petri dishes, and retails for $650, about one-third the cost of other Sterile Media Dispensers.

Samples of this equipment, as well as a discussion of ideas in the pipeline will be at the Poster Session.

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840. A High Resolution Digital 4-D-Microscopy

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A High Resolution Digital 4-D-Microscope

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The small size and transparency of the C. elegans embryo in combination with the known lineage offers the unique chance to analyse the consequences of manipulations, mutations or RNAi experiments and expression patterns of genes on the single cell level. 4D-microscopy is an ideal tool to follow and analyse the fate of many cells. The disadvantage of 4D-microscopes so far was that the high resolution required to "completely" analyse embryos was coupled to very expensive and special equipment as well as high running costs for the storage media. We recently designed a new system which can be built from commercially available parts and which stores high quality pictures. A 4D-microscope requires an excellent Nomarski optics, a matching analog camera (e.g. Hamamatsu Newvicon, PCO VX44), a frame grabber (Inspecta-2), a very precise control of the stage for the z-scans and a shutter for the light beam. The microscope is controlled by a PC (Windows NT) and the pictures are stored on a large hard disc as a series of bitmap files (BMP format). To save space on the hard disk an algorithm compresses the pictures by a factor of eight to ten without any visible quality loss (Wavelet compression, Luratech, Berlin); this works much better than for example JPEG compression. A full recording of 20.000 pictures only has 800 MB with this compression algorithm. When the Zeiss Axioplan Imaging with its new stage control is used the precision is high enough to acquire very precise z-series. Former models can be equipped with a piezo mover and an external shutter for the light beam. The new program allows to control the motorized functions of the Zeiss microscope. A special setup menu helps to find the optimal parameters for the recordings. Parameters like time, increment and position of the upper level can be corrected any time during the recording. The video window contains functions for an online enhancement of the pictures. The program also offers the necessary functions to document fluorescent pictures. We also designed a dual channel microscope which permits the alternate recording of Nomarski and fluorescence pictures which is suitable for the precise analysis of GFP expression patterns. The lineage analysis program SIMIBiocell was adapted to read the digital pictures.

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841. A Computer-Assisted System For Reconstructing And Behaviorally Analyzing In 4D Every Cell And Nucleus During *C. elegans* Embryogenesis

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During embryogenesis the body plan of an organism is determined, tissues are demarcated, and organ systems formed. Because of its limited cell number, transparency, and invariant cell lineage, *C. elegans* provides an excellent model system for the detailed analysis of embryogenesis. Until now, however, there have been no computer-assisted systems available for the reconstruction and motion analysis of an entire embryo (i.e., including every cell, nucleus, and cytoplasmic domain) from fertilization to the end of cleavage. The methods that have been available have been for the most part two dimensional and devoid of motion analysis software. In recent years, we have developed computer assisted systems (3D-DIAS) for reconstructing and motion analyzing in 3D the surface, nucleus, and pseudopodia of individual crawling cells (1,2). Using DIC optics and stepper motors, cells are optically sectioned in a two-second period. Optical sections are digitized and the edges of the cell, nucleus, and pseudopodia are identified by automatic and manual methods. The outlines are converted to β-spline models that are stacked and used to construct faceted 3D images of a crawling cell every two seconds. The series of 3D reconstructions is converted into a 3D computer movie that can be viewed at any angle through a stereo workstation. Every component of the dynamic 3D image (cell surface, pseudopod, nucleus) can then be individually reconstructed in 3D over time, and motion analyzed in 3D, allowing the assessment of high-resolution behavioral phenotypes.

3D-DIAS has been used to elucidate the mechanism of chemotaxis and the roles of a number of regulatory and cytoskeletal components in *Dictyostelium discoideum* (e.g., 3).

We will present here for the first time a new computer-assisted system, $3D\text{-}DIAS_{emb}$, that we have developed to reconstruct and motion analyze developing embryos in three-dimensions. This system reconstructs the surface of every cell and nucleus in 3D. *C. elegans* embryogenesis was used as the initial model to develop this system. 60 optical sections are collected through the embryo in two seconds using differential interference contrast microscopy, and the process repeated every 5 seconds. The optical sections are stacked and converted to 3D faceted reconstructions of every cell and nucleus through embryogenesis. The audience will be provided with red and blue glasses in order to view 3D reconstructions of wild-type embryos from 1-50 cells, in which each cell and each nucleus is converted into a 3D faceted image. This system allows the user to view a developing embryo from any angle through time, to remove any cell or nucleus at any time and analyze its behavior and lineage (i.e., every morphometric change, every nuclear division, every association with other cells over time), and color code cells and nuclei in any desired manner. Cell surfaces can be subtracted and only nuclear divisions reconstructed and motion analyzed over time. The system also provides vector flow plots that reflect cytoplasmic flow at any depth and in any desired cell over time. The power of the technology for analyzing mutant phenotypes will be demonstrated by comparing wild-type and *par-1* embryogenesis.

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842. New! Improved! Fixation protocols for TEM

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We continue to test alternate methods for preparing worms for transmission electron microscopy. We will describe new protocols, and will demonstrate what makes them better [or different] in comparison to previous methods (Hall, 1995). We still like simple immersion fixation and chopping open the animals by knife blade, and have made minor changes in the starting solutions to get optimum results. For early larval stages, which have never fixed well by immersion, and which are too little to chop open easily, we have adapted a new microwave protocol which gives very good results on intact worms. The resulting fixation looks equivalent to our immersion preparations of adults. Microwave fixation is proving very useful in the analysis of arrested animals from RNAi preparations, and should be excellent for looking at late embryos or dauers.

Fast freezing methods offer a quite different approach, and the quality of tissue preservation can be superb. Both metal mirror freezing and high pressure freezing can produce excellent results, and they are achieving wider use over the past few years (Mohler et al., 1998; Rappleye et al., 1999). The inherent contrast after freeze substitution is often much greater, in part because the primary fixation contains only osmium, or a combination of osmium and aldehyde together. These methods allow much more rapid fixation. We can capture more "life-like" views of biological events in action, particularly for events such as vesicle fusions at the plasma membrane. Delicate cytoskeletal elements such as microtubules are also well preserved. We continue to try new combinations of fixatives and solvents to improve the appearance of nerve processes and synapses by fast freezing. Kent McDonald has been very helpful in suggesting improvements to these protocols.

Laserhole fixations of embryos are technically rather difficult to accomplish, but can facilitate the passage of fixatives and embedding resins through the eggshell. We are continuing to use the protocol worked out by Carolyn Norris. See our website for details.

843. Cell lineage acquiring system for early embryogenesis

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We have developed a computerized system that automatically acquires a cell lineage of early C. elegans embryo. At first, the system prepared a 4D Nomarski DIC microscope image of a target embryo that consists of 3D images taken every minute for 2 hours. A 3D image consists of more than 50 focal plane images at 0.5-micrometer intervals. The system then detected the regions of cell nucleus in each focal plane image. We found that the image entropy efficiently distinguishes nucleus regions from cytoplasm regions because it measures the roughness of images. All the focal plane images, whose size was about 600x600 pixels, were scanned by a small square window of 10x10 pixels measuring the image entropy of the inside regions, in order to convert the original images into entropy-value images. The regions of lower entropy are the nucleus regions. Finally, the system created a cell lineage based on the nucleus regions. Nucleus regions that represent the same nucleus at the same time point were grouped into a 3D nucleus region, then each pair of them were connected if they represent the same nucleus at two consecutive time points. The connections generated the lineage of 3D nucleus regions, which is the cell lineage. The system output three-dimensional positions of nuclei at each time point and their lineage. Currently, our system can generate the cell lineage from the 1-cell stage to about the 20-cell stage.

844. NEXTDB: The nematode expression pattern database.

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We are updating "NEXTDB" that integrates all the information of ESTs, gene expression patterns and gene functions of *C.elegans* which are being produced and analyzed in this laboratory, and, are preparing to make it open by the worm meeting.

Images of whole mount in situ hybridization for mRNA were taken by CCD cameras equipped on Zeiss Axioplans, loaded to the Sun workstation to process and arrange them properly in the database. Then, they were annotated with respect to developmental stages and expression patterns on the database. Images of immunostaining taken on Zeiss LSM510 confocal microscope, and images and descriptions of RNAi phenotypes (See Hirono et al), were also stored in the database.

New version of NEXTDB contains about 12,000 unique cDNA groups, in situ images of about 7,600 cDNA groups, RNAi phenotype images of 160 groups, and immunostaining images of 60 groups. The new version will be demonstrated and will be available over the Internet.

http://helix.genes.nig.ac.jp/db/index.html

845. Correlation between transcriptome and interactome data in *S. cerevisiae*

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The genome sequences of *Saccharomyces cerevisiae*, *Caenorhabditis elegans* and *Drosophila melanogaster* have led to the prediction of 6,000, 19,000 and 13,600 genes, respectively. For each organism, various functional genomic approaches are being carried out to provide annotations for the large number of uncharacterized genes. Ideally the information emerging from these different approaches should be integrated to generate biologically meaningful hypotheses. Here, we investigated the potential relationship between data sets obtained from expression profiling of the transcriptome and physical protein-protein interaction mapping of the interactome of *S. cerevisiae*. Transcriptome/interactome correlation maps were generated to compare the interaction pattern of proteins encoded by genes that belong to common expression clusters versus that of proteins encoded by genes that belong to different clusters. Our results suggest that genes with similar expression patterns are more likely to encode proteins that interact with each other. This might have implications on how transcriptome and interactome data sets can be used for global functional annotations of uncharacterized genes. The approach described here should be applicable to integrate functional genomic data in higher organisms such as *C. elegans*.

846. Identifying promoter motifs and predicting gene expression patterns in *C. elegans* using data mining tools.

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With the completion of several genome sequencing projects, one of the major challenges in computational biology lies in the discovery of significant features or motifs in the sequences. We are particularly interested in sequences controlling the expression of genes, which are much less understood than sequences coding for proteins. Using data on expression patterns gathered by Shawn Lockery and Oliver Hobert, and found in ACeDB and Wormbase, as well as the published literature, we are attempting to discover DNA motifs important in the control of gene expression in *C. elegans*. For our initial work, we are searching for motifs only in the non-coding promoter region 5' to the translational start site of genes of interest; we are not yet considering introns or regions 3' to the coding region. Our assumption is that important regulatory motifs presumably correspond to binding sites for transcription factors, and should occur repeatedly among genes with similar expression patterns. We hope both to identify important motifs, and to use knowledge of these motifs to predict expression patterns of genes.

It seems likely that combinations of motifs may be involved in control of gene expression. Thus, our analysis involves two major steps: identifying potential DNA motifs of interest, and trying to determine whether particular combinations of motifs may be required to produce a specific expression pattern. We divided our data into a training and test set in order to evaluate the usefulness of our analysis. In order to identify putative motifs, we made extensive use of a motif discovery tool called MEME (1). This open source package uses the

expectation maximization (EM) algorithm to identify the 'best' motifs that are found in common among the input sequences. In preliminary experiments, MEME identified known transcription factor binding sites best when smaller numbers of fairly short non-coding regions were used as input sequences. Therefore, we divided our database into groups of 4-7 genes with similar expression patterns. The promoter regions were further divided into the 1500 bases most proximal to the translation start site, and the remainder of the promoter region. Using these groupings of genes, we are currently using MEME to generate putative motifs. We then plan to use a data mining technique in which rules will be generated that allow classification of genes into expression pattern classes based on the presence of particular combinations and spacing of motifs. We will report on preliminary results of our analysis at the meeting.

(1) Bailey, Elkan, and Grundy. MEME, Multiple EM for Motif Elicitation. http://meme.sdsc.edu/meme/website/meme-input.html

847. Design of a Whole-Genome *C. elegans* Microarray for Investigating Protein-Genome Interactions

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DNA is brought to life and transformed into a genome by processes that occur in the cell's nucleus, such as transcription, replication, recombination, and DNA repair. These hallmarks of life are controlled by regulatory networks that ultimately modulate, as a function of time and environment, just two aspects of DNA-dependent enzymes: the level of their activity, and where they act. Therefore, a critical part of understanding the mechanism and logic of cellular regulatory networks is understanding where enzymes and their regulatory proteins interact with the genome *in vivo*. From this, we can determine the genomic features that specify protein binding, and simultaneously identify genes or other chromosomal elements whose function is affected by the binding. High resolution, genome-wide maps of protein-DNA interactions can be created by hybridizing DNA enriched in chromatin immunoprecipitations to DNA microarrays containing elements that represent not only all ORFs, but all intergenic regions in the genome as well. This procedure has the advantage of not requiring any prior knowledge of a protein's binding targets, and by the same token is not prejudiced by existing notions of where a given protein should bind. Furthermore, the determination of functional targets is direct, and avoids the pitfalls of analyzing what could be secondary effects on gene expression in mutant strains.

Experiments in yeast that produced a detailed map of the sites at which four key regulatory proteins, Rap1p, Sir2p, Sir3p and Sir4p, bind to the genome will be presented. While yeast will continue to be an important model for addressing DNA-target specificity in fundamental cellular processes, tackling more complex developmental issues requires a new multicellular model for the

chromatin-IP-microarray strategy. *C. elegans* is sufficiently complex to address metazoan issues such as cell-cell signaling, organogenesis, and the development of neuronal networks, muscle, germline, and sexual dimorphisms, but has a genome of just 97 Mb, more than 30 times smaller than human. The entire genome, excluding introns, could be represented at 1.5 kb resolution on a 50,000-element, single-slide array that can be printed routinely.

The design of a pilot array that would initially consist of 13,000 genomic DNA fragments will be presented. All X-chromosome intergenic regions, broken into 1.2-kb segments, would be represented by 9,702 unique arrayed spots. In addition, the pilot will contain spots representing all 2,631 predicted X-linked ORFs, and the ORFs and promoters of key developmental loci, such as the 83 homeobox and 235 nuclear hormone receptor genes. To establish the utility of our approach in *C. elegans*, the first DNA-binding proteins to be mapped will represent diverse mechanisms of DNA-association, be functionally well-defined, abundantly expressed in many tissues, and have several predicted binding targets. Although the initial focus will be on transcription factors, coverage of an entire chromosome on the pilot array also allows one to study long-range regulatory mechanisms and whole-chromosome phenomena, such as mitotic chromosome condensation, dosage compensation, or homolog pairing in meiosis.

848. Integration of functional genomics approaches: protein interaction mapping using genes enriched in the C. elegans germline

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Since the complete genome sequence of C. elegans became available at the end of 1998, several functional genomics projects have been initiated to functionally annotate the many predicted genes that had remained previously uncharacterized. The data generated by these projects can be viewed as hypotheses until validated further. We have proposed that the likelihood of such hypotheses to be biologically meaningful might increase as the data from different projects are integrated. For example, genes that cluster in expression profiling, protein interaction maps and phenotypic analysis might have a relatively high likelihood to functionally interact in the same biological process.

Recently, microarray analysis has led to the identification of a set of genes that show increased expression in the germline (Reinke et al, Mol. Cell. 6, 605-616, 2000). We have cloned these ~750 open reading frames and transferred them into yeast two-hybrid vectors using Gateway cloning. Subsequently, we have generated a 750 X 750 protein interaction map. The data obtained and its integration with the RNAi data obtained by Fabio Piano and colleagues will be discussed.

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849. Mapping the genome(s) of the human nematode parasite *Brugia malayi*

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The human-infective filarial nematode parasite *Brugia malayi* contains three genomes : the mitochondrial genome, the genome of the rickettsia-like *Wolbachia* endosymbiont and the nuclear genome.

We have sequenced the mitochondrial genome of *Brugia malayi* and compared it to the other sequenced nematode mitochondria. The genome is, as expected, very similar to that of *Onchocerca volvulus*, and is remarkably different from *C. elegans* in gene order and sequence. Phylogenetic anlysis of nematode mitochondrial DNAs conflict with phylogenies derived from nuclear genes.

Most filarial species harbour an bacterium that is believed to be in mutualistic symbiosis with the nematode. The bacteria are closely related to the *Wolbachia* endosymbionts of arthropods. The genome of the *Wolbachia* endosymbiont is being mapped and sequenced by a consortium headed by Barton Slatko at New England Biolabs.

The nuclear genome of *B. malayi* is estimated to be 100 Mbp with an expected gene number comparable to *C. elegans*. To date, the Filarial Genome Project has produced 22,441 ESTs from 11 different cDNA libraries from various stages of the life cycle. These are estimated to represent ~8000 different genes.

As a prelude to whole genome sequencing we are now in the process of constructing a physical map for the nuclear genome. A BAC library is being end sequenced, and BAC-derived end-probes hybridised to the gridded library to create contigs in a sampling-without replacement strategy. A number of EST clones have also been hybridised. In the course of this mapping program we have defined the distribution of sattelite repeats in the library, and have identified at least two families of retrotransposon-like elements.

850. NEMATODE.NET, a Tool for Navigating Sequences from Parasitic and Free-living Nematodes

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Two web sites have been established to allow easier access to nematode sequences from species other than *C. elegans* and *C. briggsae*; WWW.NEMATODE.NET is maintained by the Genome Sequencing Center (GSC) at Washington University in collaboration with North Carolina State University, and WWW.NEMATODES.ORG is maintained by Mark Blaxter's lab at the University of Edinburgh.

Useful features being built for NEMATODE.NET include the following - 1) Searches: All nematode expressed sequence tags (ESTs) generated at the GSC, currently 32,000 from 10 species, and NemaGene clusters built from these ESTs, are available for BLAST and text searching. Searches can be directed by species, library, or nematode clade in a way that is not possible using the NCBI EST database dbEST. 2) FTP: All EST project data can be downloaded for local analysis including FASTA files and sequence trace image files. 3) Trace Viewer: Fluorescent trace representations for each EST can be viewed. Traces can sometimes provide additional sequence information not included in the EST due to quality value cut-offs. 4) Project Updates: Information is available about libraries in construction and sequencing in progress as the project expands toward 235,000 ESTs. 5) Clone Requests: Details on clone availability and ordering procedure are provided. 6) Links: The site includes an up-to-date set of 300 links to information on human, animal, and plant parasitic nematodes.

Further plans for NEMATODE.NET include linking of ESTs to their closest *C. elegans* homologues by DAS third-party curation of Wormbase. This work is funded by NIH-AI-46593, NSF-0077503, and a Merck / Helen Hay Whitney Foundation fellowship.

851. The worm is not enough - comparative genomics of *Ancylostoma ceylanicum*

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Ancylostoma ceylanicum is a parasitic nematode which infects humans and domesticated animals. We have taken a comparative genomic approach to understanding *A. ceylanicum* parasitism by constructing cDNA and genomic DNA libraries from this parasitic nematode. We build cDNA libraries using directional, SL1-oligo dT, RT-PCR amplification, followed by topoisomerase I- based A/T cloning into a pCR-XL-TOPO (Invitrogen). PCR products were size selected by horizontal gel slicing and fractions were subsequenty separately cloned to build sub- libraries. In our view this strategy allows rapidly identification of highly complex sub- libraries and therefore eliminate fractions containing highly redundant inserts. Using similar strategy we build a small insert genomic library. Briefly, genomic DNA was sheared, gel fractionated and cloned into pCR4Blunt-TOPO (Invitrogen). Both cDNA and gDNA libraries were therefore build in high-copy plasmid based vectors useful for direct sequencing. To characterize our libraries we sequenced over one hundred clones. We find that about half of SL-cDNA clones show apparent homology to genes predicted by *C. elegans* genome project. Results of trial sequencing and characteristics of sub- libraries will be summarized.

852. Isolation of Long-lived C elegans Mutants by Transposon Mutagenesis

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The soil nematode *Caenorhabditis elegans* has proven to be an excellent model for studying ageing. Several genes, Age genes, have been identified which when mutated, extend the lifespan of the nematode. Identification of novel Age genes may improve our understanding of the ageing process and we have therefore performed mutant screens to identify such genes. Stress resistance has been shown to be an associated phenotype of most Age mutants. We have isolated stress resistant mutants both in screens for resistance to hydrogen peroxide and resistance to heat. The high transposition frequency of transposable elements in the mutator strains MT3126 [*mut-2*(*r459*)] and NL917 [*mut-7*(*pk720*)] were utilised to generate mutants. The isolated mutants show increases in mean lifespan ranging from approximately 20 to 100% compared to the wild-type N2 strain. We are currently assessing if the isolated alleles define novel Age genes.

853. Genetic Screen for Delayed Reproductive Tract Senescence

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Declining fertility is a characteristic of the aging process. In many animals the reproductive system senescences before most other organ systems, especially in females. The mechanisms that control this process are poorly understood. The *C.elegans* hermaphrodite may be a useful model for studying aging of the reproductive system since hermaphrodites cease egg-laying before death and mutations have been identified that control other aspects of the aging process. We characterized the average time for N2 hermaphrodites to cease egg-laying when allowed to self fertilize or when outcrossed to N2 males up to 15 days past the L4 stage. It is notable that many hermaphrodites retain the ability to generate a small number of cross progeny for several days after they cease to produce fertilized self progeny. We are conducting a F2 clonal screen for mutations that delay senescence of the hermaphrodite reproductive system using two strategies. F2 hermaphrodites are mated to males early in adulthood and screened for extended egg-laying capabilities late in life. F2 hermaphrodites are mated late in adulthood and screened for the ability to lay an increased number of viable progeny late in life.

854. Characterization of a Short-Lived *C. elegans* Mutant

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Our lab screened 20,000 haploid genomes for long- and short-lived mutants and found at least ten short-lived mutants. We would like to know whether these animals are aging more rapidly than normal, or whether they are simply sick and unhealthy due to their mutations. Preliminary characterization of short-lived mutants reveals one candidate that appears healthy in early life but then dies early. We are using high-power microscopy and DNA microarray analysis to determine if this mutant has an accelerated progression of age-related tissue degeneration and biomarkers.

855. Screening for Mutations Involved in Stess Resistance and Aging in *Caenorhabditis elegans*

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Aging is an intriguing and fundamental process that remains poorly understood. The analysis of a number of *C. elegans* mutations has revealed a positive correlation between stress resistance and life span extension. In a sense, the increasing probability of death over time that is evident from standard mortality curves reveals an increasing susceptibility to stress. We are attempting to exploit the age-related susceptibility to stress to identify genes that are involved in the aging process. We characterized the ability of aged N2 worms to withstand a heat stress, and compared this to the ability of aged *daf-2* and *age-1* mutants. Conditions were identified that caused N2 worms to have a significantly greater decrease in body movement and pharynx pumping than *daf-2* and *age-1* worms. We are conducting an F2 clonal screen for aged mutants with an increased ability to withstand a heat stress. Candidate mutants will be characterized to determine if they display additional aging phenotypes.

856. Identification of genes involved in oxidative stress response and aging in *Caenorhabditis elegans*

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Aging is a multifactorial phenomenon; yet, the accumulation of oxidative damage to macromolecules appears to play a crucial role. There is an intimate relationship between longevity and the ability to prevent/repair chronic damage to macromolecules. The efficiency of anti-damages mechanisms can be revealed by the ability to endure environmentally imposed stress (Such "external" stress can be considered as an exacerbation of the chronic endogenous accumulation of damages). All environmental or genetic interventions that increased longevity also increased resistance to environmental stress. It appears that the capacity to control the accumulation of damages to macromolecules modulates both longevity and environmental stress resistance.

In order to get insight into the mechanisms of resistance to oxidative stress and its relation with aging, we have isolated *C. elegans* mutants showing an increased resistance to the oxidative stress inducing compound Juglone (5-hydroxy-1,4- naphthoquinone). Juglone, is a redox active quinone causing reduction of O_2 to $\cdot O_2^-$, and thus exerting an oxygen-dependent toxicity. We analyzed six independent mutant lines. Four of these lines show a concomitant life-span extension (up to 36% increase in mean life-span), substantiating the strong relation between oxidative stress and aging. None of these mutants are Daf-c at 27°c, suggesting that their life-span extension is not due to mutations in the *daf*-2 - *daf*-16 pathway. We are currently using Transposon Insertion Display to identify the mutated genes (transposon tagging).

857. Longevity and gerontogenes in model organisms

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Mutations leading to life-extension have revealed molecular mechanisms that regulate longevity and aging in model organisms. Approximately sixty-five gerontogenes have been identified in model organisms, ranging from nematodes to mammals. The vast majority have been identified in the nematode, Caenorhabditis elegans. Thus, C. elegans represent a paradigm for understanding the specification of lifespan in other organisms. Most C. elegans gerontogenes act as negative regulators of longevity and also affect resistance to environmental stressors. Such gerontogenes may regulate a group of genes needed for increased longevity and other biological processes including environmental perception, metabolism, reproduction, stress resistance and others. This poster describes gerontogenes in organisms ranging from C. elegans to the mouse and discusses molecular mechanisms specifying longevity.

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858. A genetic screen to identify novel players in *C. elegans* lifespan regulation

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Multiple genetic pathways play a role in worm lifespan control. Most notably, mutations leading to reduced *daf-2* insulin-like signaling extend the lifespan of *C. elegans* up to 4-fold. The longevity phenotype associated with *daf-2(lf)* is completely suppressed by loss-of-function mutations in the forkhead transcription factor *daf-16*, suggesting *daf-16* likely regulates genes that control *C. elegans* lifespan. To identify *daf-16* targets, as well as genes in other parallel pathways, which regulate lifespan, we performed a genetic screen to identify mutants that exhibit an extended lifespan phenotype in a *daf-16* null background. From a pilot screen of 1600 haploid genomes, we recovered three independent mutants with lifespans significantly longer than the starting *daf-16(mgDf47)* strain. Among the three mutants, *age-3(mg312)* displayed the greatest lifespan extension, up to 3-fold that of control strain. *age-3(mg312)* is pleiotropic, including slight uncoordinated movement, arrested germ line development and sterility. These pleiotropies allowed rapid mapping of this mutation to a small region around +2 on chromosome I. Cosmid rescue and sequencing of genes in the region suggested that *age-3(mg312)* resulted from a nonsense mutation in the only *C. elegans* leucyl-tRNA synthetase gene. Single gene rescue experiments and further characterization of *age-3* are underway. While it is not clear how such a fundamental player of gene expression specifically regulates lifespan, other basic cell machinery components have been previously implicated in longevity, including the *WRN* DNA helicase in human, the *Indy* Krebs cycle transporter in *Drosophila*, and the *SIR2* histone deacetylase in worm and yeast. On the other hand, given the lifespan effects of germ line ablations, the sterility associated with *age-3* could indirectly contribute to its lifespan phenotype.

Phenotypic analysis and mapping of the other two longevity mutations is also in progress. In addition, a large-scale genetic screen to isolate more mutations with an extended lifespan phenotype is ongoing. Taking into account that mutations which affect global metabolism may modulate *C. elegans* lifespan, as well as other developmental processes, mutants with significant extension in lifespan, but no other pleiotropies, will be of the highest priority for mapping and cloning. Furthermore, we are also using the recently generated chromosome I RNAi library (A. Fraser, *et. al*., Nature, 2000.) to survey for genes which, when inactivated, result in long lifespan.

859. A Screen for Mutations that Affect *Caenorhabditis elegans* Aging

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How aging is controlled is a fundamental biological question that remains largely unanswered. *C. elegans* promises to be a powerful model system for studying the genetic basis of aging, as it ages rapidly and mutations in genes such as *age-1* and *daf-2* have been shown to extend the lifespan significantly. We have initiated a screen for mutations that delay the aging process. We characterized the average time for N2 worms to cease egg-laying, display significantly reduced body movement and pharyngeal pumping, and die. Using EMS mutagenesis, we are conducting an F2 clonal screen for mutants with a persistence of the youthful condition for one or more of these processes. We have screened about 1700 (haploid) genomes and identified several mutations that significantly delay one or more of these senescence traits.

860. The knock-out of the peptide transporter gene *pep-2* results in delayed development and extended life-span in *Caenorhabditis elegans*

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Peptide transporters of the PTR-family are integral plasma membrane proteins, that mediate the electrogenic proton-coupled transport of di- and tripeptides and peptide-like drugs across cell membranes. The physiological role of PEPT1, one member of this family in vertebrates, is the uptake of small peptides into epithelial cells of the intestinal tract and the kidney proximal tubule. In *Caenorhabditis elegans* a homologue to mammalian PEPT1 is encoded by the *pep-2* gene.

By injecting a *pep-2*::GFP expression construct in wild-type animals, we localised *pep-2* expression in the intestinal cells and a subset of sensory neurons in the head of the animal. To study the physiological role of the PEP-2 transporter in vivo, a *C.elegans pep-2* mutant was constructed. The animals deficient in PEP-2 show a remarkable phenotype with pronounced signs of malnutrition. The larval development is severely retarded with a 2-fold extended generation time compared to wild-type animals. Moreover, they carry less eggs in the uterus, have a severely reduced brood size and possess a prolonged mean life-span compared to wild-type animals. It was possible to rescue the phenotype by the expression of the wt *pep-2* gene in the mutant. The *pep-2* phenotype is similar to that of an *eat-2* mutant that is feeding-defective resulting in caloric restriction. The observed starved phenotype in *pep-2* mutants might therefore be best explained by the reduced intestinal absorption of peptide bound amino acids that are required for protein synthesis and energy metabolism and provides the first direct evidence for the predominant role of the intestinal peptide transporter in amino acid absorption. *pep-2* is additionally expressed in neuronal cells and experiments are in

progress to identify these neurons. However, the physiological role of a proton-coupled peptide transporter in neurons is currently unknown but *C.elegans* provides a useful model system to study this function *in vivo.*

861. Phosphorylation profiles of a quantitative trait loci (QTLs) on chromosome III, IV, V and X controlling longevity of *Caenorhabditis elegans*

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We have identified several highly significant quantitative trait loci (QTLs) that affect adult life span of *C. elegans. Life span QTLs (lsq's) present on chromosomes III, IV, V and X were back-crossed for 20 generations into one of the parental strains, to construct congenic (near-isogenic) lines. These backcrossed lines have only a short span of one chromosome that has been introduced into a uniform genetic background, and thus differ from the recurrent-parent genome only by this short region. Each of these congenic lines has now been demonstrated to differ significantly and reproducibly from the recurrent parent with respect to longevity. We have now performed in vitro phosphorylation studies on these lines, comparing congenic lines with recurrent parental strains. The patterns of protein phosphorylation produced by whole animal lysates in vitro show several labeled bands distinguishing two of the congenic lines (on chromosomes III and IV) from their recurrent-parent. This assay thus may provide a powerful tool for use in gene mapping, facilitating the identification of proteins involved in longevity determination.*

862. Enzymatic and *in vivo* functional characterization of a *C.elegans* RecQ DNA helcase

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The *Caenorharbditis elegans* E03A3.2 gene product is a member of the RecQ family DNA helicase, which includes Sgs1 in *S. cerevisiae* and RecQL, Bloom's Syndrome protein (BLM), Werner's Syndrome protein (WRN), Rothmund-Thomson Syndrome protein (RecQ4), and RecQ5 in human. Deficiency in either of Sgs1 and three human homologs (BLM, WRN, RecQ4) results in phenotypes suggesting their common roles in the maintenance of genomic stability. To examine enzymatic characteristics of the E03A3.2 protein which is most similar to human RecQ5 among 4 RecQ homologs in *C. elegans*, the recombinant protein was overexpressed in *E. coli* cells as a fusion protein with glutathione-S-transferase. The purified protein exhibited DNA-dependent ATPase and helicase activities. As a helicase, the RecQ5 protein preferentially displaced a primer with a 5'-overhang than a primer with a 3'-overhang, both of which had been annealed to M13 single-stranded DNA, indicating a 3' to 5' directionality in the helicase movement. To study the *in vivo* function, *in situ* mRNA hybridization and reporter gene expression, and double-stranded RNA interference were carried out. The mRNA was expressed in the gonad, oocytes, and early embryos and was localized in gut cells of the late embryos. This intestinal localization was also observed for a reporter protein (GFP) expression induced by the promoter from the late embryonic to adult stage. dsRNA*i* of the recQ5 gene expression resulted in a reduction of life-span by 2.3 days, suggesting that the RecQ5 protein may be involved in a cellular reaction affecting the aging of *C. elegans*.

863. Genetic analysis of the DAF-2 insulin-like signaling pathway of *C. elegans*

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The evolutionarily conserved DAF-2 insulin-like signaling pathway regulates development, metabolism, and longevity in *C. elegans*. Genetic analysis has identified several components of the pathway, including the DAF-2 insulin receptor, AGE-1 PI 3-kinase, DAF-18/PTEN, AKT-1/2 and PDK-1 serine/threonine kinases, and the DAF-16 Forkhead transcription factor. Consistent with experiments in mammalian systems, epistasis analysis in *C. elegans* suggests the existence of AGE-1 PI 3-kinase-dependent and -independent outputs from the DAF-2 insulin receptor. To date, no AGE-1 PI 3-kinase-independent components of this pathway have been identified.

In an effort to identify these and other components of the DAF-2 insulin receptor signaling pathway, we have performed *Daf-c* screens in three different genetic backgrounds that attenuate DAF-2 insulin receptor signaling. A 25^0C F_2 *Daf-c* screen in a *daf-2(e1370);daf-18(mg198)* background yielded 11 independent mutants from approximately 17,000 mutagenized haploid genomes. 7 mutants have been characterized in detail. 3 are alleles of *pdk-1,* 2 are alleles of *akt-1*, 1 is an allele of *age-1*, and 1 is likely an allele of *daf-11*. We are currently in the process of analyzing the remaining 4 mutants. The identification of multiple new alleles of genes in the DAF-2 insulin-like signaling pathway indicates that the use of attenuated strains as substrates for mutagenesis is effective in sensitizing genetic screens for components of this pathway.

We have also performed genetic screens in two other attenuated backgrounds. A 25^0C F_2 *Daf-c* screen in an *age-1(mg44);akt-1(mg144)* background yielded 1 mutant from approximately 6,700 mutagenized haploid

genomes, and a 25^0C F_2 *Daf-c* screen in an *akt-1* null background yielded 32 independent mutants from approximately 21,000 mutagenized haploid genomes. These mutants are being mapped for molecular analysis.

864. A screen for mutations affecting the *daf-16*/FH-independent *daf-2*/IR signaling pathway

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TGFb -like and insulinlike signaling pathways regulate both dauer entry and recovery. Since the Daf-c (dauer formation constitutive) phenotype caused by *daf-2(lf)* (insulin receptor-like) or *age-1(lf)* (PI3 kinase) can be fully suppressed by *daf-16(lf)* (fork-head-like transcription factor) at the non-permissive temperature of 25° C, *daf-16* has been considered to be the terminal dauer-promoting gene of the insulin-like pathway and the sole output of the *daf-2*/IR signal. Likewise, *daf-3* (smad transcription factor) is considered to be the terminal dauer-promoting gene of the *daf-7*/TGFb -like pathway. The two signaling pathways may converge to regulate dauer formation by affecting the *daf-12* (nuclear hormone receptor) activity, based on the genetic epistatic analysis. In addition, a third signaling pathway for dauer arrest has been implicated by the finding that *daf-16(0); daf-3(0)* animals can still form partial dauers in response to pheromone. The above working model is based on the hypothesis that the *daf-11* (guanyl cyclase) signaling cascade functions upstream of, instead of in parallel to TGFb -like and insulinlike signaling pathways.

In order to identify the components in the putative third signaling pathway, we screened for Daf-c mutations in a *daf-16(0); daf-3(0)* background. No Daf-c mutations in either the TGFb or insulin-like pathways were expected to be isolated from this screen. *mg293*, one of the two isolates from this screen, displayed a transient Daf-c phenotype in the original *daf-16(0); daf-3(0)* background and a non-recoverable Daf-c phenotype in the wild-type background. Unexpectedly, *mg293* turned out to be an allele of *daf-2*, based on map position and a complementation test. We confirmed this result by constructing a *daf-16(0); daf-2(0); daf-3(0)* strain, using a known *daf-2(0)* allele. This triple mutant forms transient partial dauers at all temperatures tested. These results suggested that *daf-16(0)* does not suppress the *daf-2* phenotype in the

absence of the *daf-3* activity. This further suggests that there is a *daf-16*-independent *daf-2* output, which is normally masked by the *daf-3(+)* function. It is noteworthy that this *daf-3(+)* function is to repress dauer arrest. Different from the known *daf-16*-dependent *daf-2* signal, which regulates both dauer entry and recovery, the *daf-16*-independent *daf-2* signal specifically regulates dauer entry. Moreover, a *daf-16(0); age-1(0); daf-3(0)* strain also showed the transient Daf-c phenotype, with lower penentrance compared to *daf-16(0); daf-2(0); daf-3(0)*, suggesting that *age-1* is involved in the *daf-16*-independent *daf-2* signal. Finally, introduction of a *daf-12(0)* mutation into *daf-16(0); daf-2(0); daf-3(0)* prevented the transient Daf-c phenotype, indicating that *daf-12* is a target of the *daf-16*-independent *daf-2* signal.

Since the *daf-16*-independent *daf-2* signal regulates dauer entry but not recovery, some of its downstream effectors should also specifically regulate dauer entry. To identify such effectors, we performed a screen in the N2 background, aiming for mutants that mimic the transient Daf-c phenotype associated with *daf-16(0); daf-2(0); daf-3(0).* We have obtained four candidate mutants from 12,000 mutagenized haploid genomes. Characterization of these mutants is underway.

865. GENETIC ANALYSIS OF THE NICTATION BEHAVIOR OF DAUER LARVAE

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In unfavorable environmental conditions *C. elegans* larvae can enter a facultative larval stage, the dauer stage. Dauer larvae (dauers) are specialized for long-term survival and dispersal through changes in physiology, morphology and behavior specific to the dauer stage. Dauers display a unique behavioral repertoire. They are lethargic, often motionless and suppress pharyngeal pumping. However, dauers are capable of active movement, respond to touch stimuli and have the tendency to climb up objects, stand on their tails and thrash vigorously. The latter behavior has been called *nictation* or *winking* (Croll, N.A. and Matthews, B.E. (1977) *The Biology of Nematodes*, Halsted Press, New York) and is thought to facilitate dispersal through attachment to passing insects.

To identify changes in the *C. elegans* nervous system that underlie the altered behavior of dauer larvae we have begun a genetic anaylsis of the nictation behavior. We are analyzing mutations known to affect neuronal function for effects on dauer behavior, and we are performing screens to identify new genes that affect dauer behavior.

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866. The role of *egl-32<i/> in egg laying and its interaction with the TGFbeta pathway*

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A TGFbeta-related signaling pathway regulates dauer larval development and egg laying in *C. elegans*. Mutations in *daf-7* ligand, *daf-1* type I receptor, *daf-4* type II receptor, and *daf-8* and *daf-14* Smads result in Dauer-constitutive and Egg-laying defective animals. These mutations are suppressed for both defects by mutations in *daf-3* Smad. We are interested in the role of this pathway in egg laying. Two other genes that affect egg laying, *egl-4* and *egl-32*, are also implicated in this pathway by their suppression by *daf-3*. We then undertook a molecular analysis of *egl-32*. We narrowed down the *egl-32* interval using chromosomal deficiencies to the region between *unc-29* and *mec-8* on LGI. Cosmids mapping to this interval were tested for the ability to rescue *egl-32(n155)*. One cosmid from this interval, C26G6, which overlaps the sequenced cosmid T08G11, rescued the *egl-32* phenotype. Among several subclones of this cosmid, one containing two predicted ORFs T08G11.2 and T08G11.3, but not one containing only T08G11.3, also rescued *egl-32*. Therefore, *egl-32* likely corresponds to predicted ORF T08G11.2. We further tested this hypothesis by RNAi, and found that injection of L4 animals by dsRNA from this gene results in the Egl defect. Injection of dsRNA into adults gave no phenotypes in the offspring.

Finally, we cloned T08G11.2 from the *egl-32* mutant and determined its sequence. A single base substitution was found that causes a missense mutation in the predicted protein sequence (D209G). Taken together, these results support the identification of T08G11.2 as the *egl-32* open reading frame. The predicted ORF encodes a small (282 aa) protein with a putative SH2 domain.

The existing allele of *egl-32* is temperature sensitive, so we used temperature shift experiments to determine the critical period of development in which *egl-32* activity is needed. These experiments indicated that the critical period is during the L4 stage, and that *egl-32* activity is dispensable during adulthood when egg-laying behavior is actually seen. This temperature-sensitive period also coincides with the period during which animals are sensitive to dsRNA injection.

EGL-32 is a member of an uncharacterized protein family in *C. elegans* that includes at least one closely related paralog and two more distantly related homologs. To characterize the function of *egl-32* and its homologs, we have submitted these genes for targeted disruption by The *C. elegans* Gene Knockout Consortium. The first two knockout strains deleting *egl-32* and its putative paralog have recently become available. The phenotype of the *egl-32* deletion will be analyzed to determine the null phenotype of *egl-32*. Previously, only a single allele of *egl-32* was available. A knockout of the gene may be more or less severe than the existing allele. One possibility that would cause a knockout to be less severe is if *egl-32* is redundant with its homolog. We will test this possibility by creating the double mutant.

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867. Candidate downstream targets of TGF-beta signaling in dauer formation

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Depending on environmental conditions, *C. elegans* enters a developmentally arrested dauer larva stage or proceeds through larval development to adulthood. Several genes have been identified that are required for normal regulation of dauer formation, and molecular analysis of some of these genes revealed that a TGF-beta-related pathway influences the dauer versus non-dauer decision. Components of this pathway are encoded by *daf-7* (a TGF-beta ligand), *daf-1* (a type I receptor), *daf-4* (a type II receptor), *daf-8* and *daf-14* (two Smad proteins). Mutations in any of these genes lead to a dauer constitutive (Daf-c) phenotype. This Daf-c phenotype can be suppressed by mutations in *daf-3* (another Smad protein) or *daf-5* (a novel protein that interacts with DAF-3 and has weak homology to chromatin remodeling proteins [see abstracts by Hu, Tewari, Ruvkun, and Vidal and by Sun and Patterson]). This epistasis analysis suggests that TGF-beta signaling promotes reproductive development by negatively regulating *daf-3* and *daf-5*, which are both required for dauer formation.

Although much insight has been obtained into how and when this TGF-beta-related pathway functions, little is known about its downstream targets. In order to identify some of these downstream factors, a screen was performed to isolate suppressors of the dauer constitutive phenotype of a *daf-7* mutation. These suppressors are necessary for dauer formation, and indeed, several alleles of known *daf-7* suppressors, such as *daf-3*, *daf-5*, and *daf-12*, were isolated. In addition, this mapping and complementation testing suggests that at least four new loci have been identified. We have been mapping and analyzing these potential new components of the TGF-beta pathway as well as beginning our investigation into many of the uncharacterized suppressors. Our observations indicate that the strength of the individual suppressors varies widely. The strongest suppressors allow 80-85% of animals to bypass the dauer constitutive *daf-7* phenotype and become reproductive adults while the weaker suppressors only produce 30-35% bypassers. To determine if the weaker suppressors are more effective in suppressing weaker Daf-c mutations, we are currently creating strains carrying these suppressors in *daf-8* and *daf-14* mutant backgrounds.

In another approach to better understand downstream signaling events, we are currently searching for *C. elegans* homologues of mammalian proteins that interact with Smad1. The *C. elegans* homologues of these interactors are candidate cofactors of *daf-3* and *daf-5*, or they may be involved in another pathway that is also under TGF-beta regulation. Several potential Smad1 interactors were isolated in a two-hybrid screen performed by Tongwen Wang and collaborators using Smad1 as bait. One protein isolated, SNIP1, suppresses TGF-beta signaling by binding to the CBP/p300 cofactor (Kim, et al. Genes and Dev. 14: 1605-1616). We will be testing the effects of knocking out the *C. elegans* proteins with homology to SNIP1, as well as the homologues of the other candidate proteins, on various developmental pathways influenced by TGF-beta signaling (dauer formation, body size, and axon pathfinding).

868. Defining new components of TGF-beta signaling pathways in *C. elegans*

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Ligands of the transforming growth factor-beta superfamily are multi-functional growth and differentiation factors responsible for regulating many diverse biological processes in both vertebrate and invertebrate species. Among the most dramatic of TGF-beta's effects are those associated with specification of cell fates during development and inhibition of cell cycle progression. The core TGF-beta signaling pathway has now been described using a synergistic combination of genetic and biochemical approaches. Heteromeric complexes of specific transmembrane receptors with intrinsic protein serine kinase activity bind ligand in the extracellular milieu and then phosphorylate intracellular proteins known as Smads. Phosphorylated Smads form heterooligomers and translocate into the nucleus where they can modulate transcriptional responses. To identify additional components of this pathway, including potential regulators and target genes, we have carried out genetic screens in the nematode, *Caenorhabditis elegans*. Disruptions in the TGF-beta signaling pathway in *C. elegans* result in worms with altered body size or dauer phenotypes. Our screens have focused on identifying body size mutants (Sma and/or Lon). We have also carried out a screen to identify suppressors of *lon-2*, an upstream component in the Sma/Mab pathway (see Gumienny abstract). The success of these screens has been confirmed by the identification of each of the known components of the TGF-beta signaling pathway including ligands, receptors, Smads, and schnurri. In addition, we are now cloning and molecularly characterizing other genes identified in this screen that either mediate TGF-beta signaling or modify pathway activity. We will present the most recent data regarding these mutants, particularly *sma-10*, *sma-11*, *sma-12*, and *sma-13*.

869. TGF-beta Signaling and Gene Expression

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TGF-beta like signaling in C. elegans occurs in two pathways, the dauer and the SMA/MAB pathways. While they are distinct in function, both pathways share a common Type II receptor, Daf-4. Although the major components of both pathways are fairly well studied, downstream effectors and target genes have yet to be characterized. To address this question we have chosen to do global expression studies using cDNA microarrays.

The first experiment involves comparing mRNA generated in *daf-4* mutant animals to N2 mRNA from a comparable developmental stage. We are in the process of analyzing this data. We are particularly interested in expression changes that affect cell cycle regulation and in identifying novel targets of TGF-beta signaling. In a complimentary experiment, screens are underway to characterize the link between TGF-beta and the cell cycle using genetic screens built around an *rnr::GFP* reporter. Mutants identified by this screen should represent downstream effectors of TGF-beta that influence cell cycle control. Together these experiments should provide a wealth of information for our continued study of TGF-b signaling and the cell cycle. In addition, because our lab works mainly with the SMA/MAB pathway, we are interested in downstream effector molecules that are influenced solely by this pathway. Previous and ongoing experiments in the lab include a screen for small body size, as well as *lon-2* and *lon-1* suppressor screens, which have already uncovered many of the essential components of the pathway (see abstracts from Gumienny and Zimmerman). Experiments are underwayto analyze mRNA from animals expressing a *dbl-1* overexpression construct (Long body phenotype) and compare that to mRNA isolated from *sma-6* mutant animals. Because *dbl-1* is the SMA/MAB specific ligand, and *sma-6* is the SMA/MAB specific Type I receptor, this should give us great insight into the downstream targets of the pathway. When coupled with the

information already available from our genetic screens, these experiments will shed much needed light on the targets of TGF-beta signaling in C. elegans.

870. The new progress about sma-3 in TGF-beta signaling in C. elegans

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In the nematode *C. elegans*, a TGF-beta signaling pathway regulates body size and male tail morphogenesis. Mutations in *dbl-1* ligand, *sma-6* type I receptor, *daf-4* type II receptor, and *sma-2*, *sma-3*, and *sma-4* smads result in similar defects. Mutants hatch at about same size as wild type, but grow more slowly and are about half the normal size at adulthood. To look at SMA-3 localization, we constructed two full length SMA-3::GFP fusion proteins, with GFP inserted either at N- or C- terminus. Only N-terminal insertion was able to rescue a *sma-3* (*wk30*) mutant. The fusion protein expressed in intestine, pharynx, hypodermis and etc., but not in the gonad. The expression begins at late embryonic stage and lasts almost whole life long. After translated, the fused protein accumulates into cell nuclei in all expressing cells. Next, we tested the role of putative C-terminal sequence SMT. In other smads, the last two serines in the consensus sequence SSXS are phosphorylated during activation. In SMA-3 sequence SMT, the serine and threonine could be the phosphorylation sites. We constructed several kinds of C-terminal mutations of sma-3 in which SMT is deleted or changed into AMA, YMY or DME. The constructions were transformed into wild type (*N2*) worm and *sma-3* (*wk30*) mutant. The results show the *sma-3* mutations can not rescue *wk30*, even they can inhibit wild type *sma-3* function (dominant negative). The inhibition is dose dependent. Although the wild type transformed worms have small body sizes, the male tails are more like wild type ones. It is consistent with other results suggesting body size is more sensitive to reduction in pathway activity then is the male tail.

871. *sma-9* and TGFbeta Signaling

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A TGF-beta-related signaling pathway regulates body size and male tail morphogenesis in *C. elegans*. Mutations in *dbl-1* ligand, *sma-6* type I receptor, *daf-4* type II receptor, and *sma-2*, *sma-3*, and *sma-4* Smads result in similar defects. Mutants hatch at about the same size as wild type, but grow more slowly and are half the normal size at adulthood. In the male tail, defects in morphogenesis and patterning result in crumpled spicules and sensory ray fusions. In a genetic screen for additional Small mutants (C.S.D. and R.W.Padgett, unpublished), four alleles of a novel gene *sma-9* were isolated. *sma-9* mutants have defects in all of the tissues described above, but in each case, the *sma-9* phenotype differs slightly from that of the other mutants. In body size, L1 and L2 *sma-9* animals have the same size and growth rate as TGF-beta Sma mutants, but after this time, their growth rate increases to a wild-type rate. In the male tail, *sma-9* animals have crumpled spicules, but at a lower penetrance. Sensory ray fusions are seen between rays 8 and 9, but never between rays 4 and 5 or rays 6 and 7. *sma-9* alleles from a noncomplementation screen show the same ray and spicule phenotypes. Based on the phenotypic analysis, we suggest that *sma-9* is a co-factor or modulator of the TGF-beta pathway. To understand better how *sma-9* interacts with the signaling pathway, we are analyzing double mutants between *sma-9* and TGF-beta Sma mutants. Surprisingly, the phenotype of *sma-3;sma-9* doubles is less severe than that of *sma-3* alone. *lon-1, lon-2* and*lon-3* are genes whose mutants grow one third longer than wild type. We analyzed double mutants between *sma-9* and *lon* mutants. Interestingly, *lon-1; sma-9* display similar body size as wild type, and so does *lon-3; sma-9*, while *lon2 sma-9* shows small size. The *lon-1; sma-9* phenotype was unexpected because *lon-1; sma-2* and other doubles are long. So, *sma*-9 may act in parallel with *lon-1* in body size. To further identify *sma-9* function in TGF-beta signal pathway, gene cloning is proceeding.

872. Regulation of endocytosis of albumin in the intestinal epithelial cells of *C.elegans* by TGFbeta-like signaling

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In *C. elegans*, TGFβ-like signaling pathways regulate a range of developmental processes such as dauer formation (Estevez et al., 1993), body size growth and male tail formation (Morita et al., 1999; Suzuki et al., 1999), and axon guidance (Colavita et al., 1998). Recently, Siddiqui et al. have shown that *daf-4* (TGFβ-like-RII) and *daf-1* (TGFβ-like-RI) (at 25^{o}C) mutants show poor uptake of FITC-BSA (fluorescein-5-isothiocyanate isomer- bovine serum albumin) in the intestinal cells when compared to wild-type animals. Our aim is to identify the genes in the TGFβ-like pathways that affect endocytosis of albumin in the intestinal epithelial cells. We will examine the uptake of FITC-BSA in the mutants of the TGFβ-like pathway in comparison to its uptake in wild type. *daf-4::gfp* (Patterson et al., 1997) and *daf-1::gfp* (Gunther et al., 2000) are expressed in the intestinal cells. Expression and regulation of other TGFβ-like receptors, Smad proteins and the downstream trancription factors in the TGFβ-like signaling pathway will be examined in the intestinal cells of wild type and various *daf* mutants, in the presence and absence of albumin, by immunofluorescence and immunoprecipitation assays. This approach will determine the role of TGFβ-like signaling in endocytosis of albumin, and also uncover any possible cross talk between such pathways in the process.

873. Characterization of Sma Mutants from a *lon-2* Suppressor Screen

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In higher organisms, transforming growth factor beta (TGF-beta) plays critical roles in regulating proliferation, cell cycle, and fate determination. Disruption of the TGF-beta pathway is evidenced in many different cancers. In *C. elegans*, two TGF-beta pathways have been fleshed out. One controls entry into the dauer stage (the Daf pathway), and the other regulates body size and male tail ray development (the Sma/Mab pathway).

Our lab focuses on the Sma/Mab TGF-beta pathway. The extracellular TGF-beta ligand, DBL-1, is recognized by the complex of SMA-6 (Type I receptor) and DAF-4 (Type II receptor). Signaling is transduced from the cell surface to the nucleus through the Smads SMA-2, SMA-3, and SMA-4, which hetero-oligomerize and translocate to the nucleus to regulate transcription of target genes. *lon-2* has been shown by epistasis tests to act upstream of *dbl-1* in this pathway.

We carried out a screen to isolate suppressors of *lon-2* to identify novel members of the Sma/Mab TGF-beta pathway. We isolated mutants of several components of the Sma/Mab pathway, including *dbl-1*, *sma-6*, *sma-2*, and *sma-4*, demonstrating the efficacy of the screen. In addition, we isolated a worm homolog of Schnurri, a TGF-beta pathway regulator in *Drosophila*. Furthermore, we acquired several mutants that complement known Sma/Mab pathway genes.

To better understand these novel suppressors, we are characterizing their defects: body size, male tail ray abnormalities, crumpled spicules, and whether they affect the dauer pathway. We are also mapping them as a prelude to cloning some to gain a deeper insight on issues of body size and TGF-beta pathway signaling.

874. Genetic analyses of the adenylyl cyclase family in *C. elegans*

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Adenylyl cyclases convert intracellular ATP into cyclic AMP, a major second messenger molecule in the cell. Mammalian membrane-bound adenylyl cyclases consist of a short cytoplasmic N-terminal sequence, a six-transmembrane spanning region and a cytoplasmic catalytic domain, followed by a second six-transmembrane spanning region and a second cytoplasmic catalytic domain. In *C. elegans*, at least four genes show significant sequence similarity to mammalian adenylyl cyclases: *sgs-1*, *acy-2*, *acy-3* and *acy-4*.

sgs-1 was previously reported to encode an adenylyl cyclase that shows a general neuronal expression pattern (Korswagen *et al.*, 1998). We have isolated a null allele of *sgs-1*, and animals homozygous for this allele show retarded development and arrest in variable larval stages. Furthermore, these animals exhibit lethargic movement and pharyngeal pumping, and, while sterile, have a life span that is nearly twice as long as that of Bristol N2 animals. We isolated an extensive set of reduction-of-function mutations in *sgs-1* in a screen for suppressors of a neuronal degeneration phenotype induced by expression of a constitutively activated version of the heterotrimeric G-alpha-s subunit of *C. elegans*. These reduction-of-function mutants do not have altered lifespan or pharyngeal pumping compared to Bristol N2 animals, but preliminary results suggest that they have reduced locomotion rates. The reduction-of-function alleles show differences in their degree of suppression of the neuronal degeneration, in their locomotion rate and in their growth in trans to the null allele. Based on that, we have placed the reduction-of-function alleles in an allelic series.

acy-2 was previously reported to have a more restricted expression pattern than *sgs-1*, and loss of *acy-2* results in early larval lethality (Korswagen *et al.*, 1998). Probably, ACY-2 performs an essential function in the CAN cells together with G-alpha-s.

acy-3 and *acy-4* are most similar to mammalian adenylyl cyclase type V. *acy-3* is expressed in support cells of ciliated neurons in head and tail ganglia and in 2 pairs of neurons in the retrovesicular ganglia. In addition, it is highly expressed in the spermatheca. We are currently studying the expression pattern of *acy-4*, and we are screening a chemical deletion library to isolate *acy-3* and *acy-4* null alleles.

References:

H.C. Korswagen, A.M. van der Linden, and R.H.A. Plasterk. (1998) G protein hyperactivation of the *Caenorhabditis elegans* adenylyl cyclase SGS-1 induces neuronal degeneration. Embo J. 17, 5059-5065

875. *mig-14* functions in multiple developmental processes regulated by Wnt signaling

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P3.p P8.p express the Hox gene *lin-39* and become the vulval precursor cells (VPCs). Activation of the Ras and Notch pathways in the VPCs causes them to adopt induced cell fates and divide to form the vulva. Evidence from our lab and others suggests that in addition to the Ras and Notch signaling pathways, a Wnt signaling pathway is also required for proper vulval cell fate specification. *bar-1* encodes a beta-catenin/armadillo-related protein that is required for the expression of the Hox gene *lin-39* in the VPCs. In *bar-1* mutants, loss of LIN-39 can cause VPCs to adopt the F fate, which is to fuse with the hypodermis without dividing. Loss of the APC homologue *apr-1* can also cause VPCs to adopt F fates, suggesting that a Wnt pathway regulates fate specification in the VPCs. In addition to the defects in vulval development, *bar-1* mutants also have defects in the migration of the progeny of the neuroblast Q_L and in cell fate specification by P12. Both of these processes are known to be regulated by Wnt signaling.

Like *bar-1*, a single mutation in a locus previously called of *pvl-2(ga62)* was identified in the screen for mutants with a Pvl phenotype. *pvl-2(ga62)* mutants display defects in VPC fate specification, migration of the progeny of Q_L and in fate specification by P12, like those of *bar-1* mutants, suggesting that *pvl-2* may function as a component of a Wnt signaling pathway. This is further supported by the fact that *ga62* fails to complement *mom-3*, which was identified by the Bowerman lab in a screen for endoderm/mesoderm specification mutants. *mom-3*mutants display maternal effect lethality resulting from an E to MS cell fate transformation, and *pvl-2/mom-3* animals die as embryos showing that the two loci are allelic. *ga62* also fails to complement *mig-14(mu71)*for both the Egl and P12 phenotypes. *mig-14(mu71)*was identified by the Kenyon lab in a screen for Q_L neuroblast migration mutants. This locus, previously known as

mig-14 (Kenyon, Nishiwaki), *mom-3* (Bowerman), *let-553* (Hodgkin), and *pvl-2* (Eisenmann) will now be called *mig-14*. As *mig-14* functions in multiple processes regulated by Wnt signaling, we are attempting to map and clone this locus. In collaboration with the Bowerman lab, we have mapped *mig-14* to the interval on LGIIR between the *jsp304*and *jc101* polymorphisms. We have obtained rescue with a YAC which covers this interval. Currently, we are in the process of generating YAC subclones, as much of this interval is not covered by cosmids. RNAi of the ORFs in this region is also being employed. Details of progress will be reported. As there are no known Wnt pathway components or regulators in the genetic interval where this locus lies, *mig-14* may encode a novel Wnt pathway component.

876. Identification of EGL-27 molecular interactions

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We are interested in the control of cell polarity and cell migration during *C. elegans* development. Our approach has been to identify mutations that disrupt the polarity of the T cell in the hermaphrodite tail. Mutations in the Wnt gene *lin-44* and in the *frizzled*-related gene *lin-17* have been shown to cause a reversal and a loss of T cell polarity, respectively. Mutations in the *egl-27* locus also cause a loss of T cell polarity as well as defective QL daughter cell migrations, which is another Wnt-controlled (*egl-20*) process. Additionally, certain *egl-27* mutations and *egl-27* RNAi animals cause embryonic patterning defects. Three different transcripts are produced from the *egl-27* locus. Two transcripts, roughly corresponding to the C-terminal half of EGL-27, encode glutamine-rich proteins. A third, larger transcript, corresponding to all of EGL-27, encodes a product that contains an N-terminal region having similarity to mammalian MTA (metastasis-associated) proteins. MTA proteins have been biochemically linked to nuclear protein complexes having a putative functional role in chromatin remodeling and histone deacetylation. We hypothesize that *egl-27* controls T cell polarity and QL daughter cell migrations by regulating transcription at the chromatin level, in response to Wnt signaling. We have used the two-hybrid system to identify proteins that might interact with EGL-27 to mediate transcriptional regulation. Clones recovered from screening a *C. elegans* two-hybrid library (a gift from R. Barstead) correspond to four genes having predicted nuclear functions. Notably, two of these genes encode members of an evolutionarily conserved transcriptional regulatory complex. Like *egl-27*, the RNAi phenotypes of three of the two-hybrid interactors reveal essential functions. The fourth interactor did not display a detectable RNAi phenotype. Analysis of post-embryonic gene function using a zygotic RNAi technique is underway. *In vitro* binding assays are being performed to confirm the two-hybrid interactions.

877. Identification of *lin-44(sa490ts)* suppressors

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We are interested in genes involved in the control of cell polarity. We have focused on the postembryonic T blast cells in the tail whose polarities are controlled by *lin-44*/Wnt.

The goal of this research is to identify additional genes that function with *lin-44* to control cell polarity. A proven genetic technique for identifying interacting genes in a genetic pathway is to isolate mutations that suppress or enhance a weak mutation affecting a gene in the pathway. This can often be achieved by using a conditional allele, such as a temperature sensitive (ts) allele, and adjusting the conditions such that only a weak defect is observed; for example, by placing a ts mutation at a temperature intermediate to the permissive and restrictive conditions. We have determined that *lin-44(sa490)* is temperature sensitive and have used this allele in genetic screens for *lin-44(sa490ts)* suppressors. To date, we have identified three suppressors. Preliminary genetic analysis indicates that all three suppressor mutations are extragenic. Currently, we are continuing our genetic analysis by mapping the suppressor mutations and pursuing efforts to identify new *lin-44(sa490)* suppressors.

878. Neurodegeneration in *C. elegans*: analysis and cloning of a new *deg* gene

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Cell type specific neurodegeneration has been identified in invertebrate and vertebrate animals across Phyla. Yet the molecular mechanisms underlying postdevelopmental neuron cell death are poorly understood. Clues from genetic model organisms are rapidly narrowing the gap in our understanding of neurodegeneration. We have identified and are characterizing a mutation that causes degeneration of the *C. elegans* sensory ASH neurons.

Several characteristics of the ASH neuron make it particularly amenable to the study of neurodegeneration. Survival and morphology of the ASH neuron are easily observed *in vivo*. The sensory ending of the ASH neuron directly contacts the environment. When the ciliated sensory ending of the ASH neuron is morphologically intact, the neuron can take up fluorescent lipophilic dyes including DiD, allowing direct visualization of the neuron. Survival of the ASH neuron is assessed by detection of green fluorescent protein (GFP) driven by an ASH neuron specific promoter. A neuron that fails to take up DiD, but expresses GFP has degenerated, but has not died. This system provides a visible phenotype for identification of changes involved in neuronal integrity and survival.

A recessive, cold sensitive mutation was isolated in a genetic screen for ASH neuron specific degeneration. 90% of the ASH neurons are lost by adulthood in *rt70* mutant animals that are raised at 15^{o}C. However, if *rt70* mutant animals are raised at 27^{o}C, ASH neurons survive and are morphologically normal. In addition to ASH neurons, at least one class of neurons in the anterior ganglion and one class of ventral nerve cord neurons are affected in rt70 mutant animals. In contrast to phenotypes associated with apoptotic death, neurons in *rt70* mutant animals swell and vacuolate prior to death. This degeneration phenotype suggests

misregulation of osmotic balance. Four gain of function alleles that cause similar neurodegeneration in *C. elegans* encode ion channels (CGC# 2167, 1299, 2711, 1871). Interestingly, *rt70* maps to a 700 kb region on the X chromosome that contains seven putative ion channels. Currently, single nucleotide polymorphisms are being used to map *rt70* more precisely. Cosmids within the region will be injected to identify the rt70 mutation. A molecular, genetic, and functional analysis of *rt70* will provide important information about degeneration of specific sets of neurons.

879. A transgenic *C. elegans* model for Parkinson´s Disease

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Parkinson’s Disease (PD) is the second most common neurodegenerative disorder with resting tremor, muscle rigidity, bradykinesia and postural instability as primary diagnostic signs. The movement dysfunction results from specific degeneration of dopaminergic neurons in the substantia nigra. The neuropathological hallmark of PD is the Lewy body, a cytoplasmic inclusion largely composed of fibrils formed by the polymerized, presynaptic protein alpha-synuclein. The point mutations A53T and A30P in the alpha-synuclein gene are linked to an autosomal dominant form of familial PD suggesting that a toxic gain of function may participate in the pathophysiology of PD. As *C. elegans* has shown to be a powerful model organism for the study of human neurological diseases like Alzheimer’s Disease and Huntington’s Disease, we established a nematode model for PD based on the expression of human alpha-synuclein. We generated worms carrying extrachromosomal and integrated arrays of human wildtype and mutant alpha-synuclein controlled by the *C. elegans* promoters *unc-119* and *sel-12*. Transgenic worms containing constructs expressing alpha-synuclein in neurons produce axonal deposits with strong anti alpha-synuclein immunoreactivity implying transport to the presynapse and aggregation in the axon. Furthermore, we detected a high molecular alpha-synuclein aggregate that migrated in the stacking gel in immunoblotted worm protein extracts. Transgenic *C. elegans* expressing human alpha-synuclein display noticeable phenotypes including locomotor dysfunction, egg laying defect and morphological abnormalities suggesting alpha-synuclein toxicity. As it has been reported for the Drosophila model of PD we did not detect major phenotypic differences between worms containing wild type or mutant alpha-synuclein. Experiments to determine the fate of the dopaminergic neurons and the structure of

alpha-synuclein deposits in the transgenic animals are in progress. In summary, we have created a transgenic model of PD that replicates key features of PD pathology.

880. Dissecting mechanisms of X-adrenoleukodystrophy neurotoxicity using *C. elegans*

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X-linked adrenoleukodystrophy (X-ALD) is a progressive degenerative disorder that affects the nervous system and adrenal glands in affected males. X-ALD is a peroxisomal disease in which peroxisome structures are present in the cell, but very long chain fatty acids (VLCFA) are not transported into the peroxisome to undergo degradation. Consequently, excess VLCFAs accumulate especially in the adrenal gland and the brain. The protein that is missing or defective is the ALD protein (ALDP), which is a member of the ABC transporter protein family. In a related finding, a gene that works directly upstream of the ALDP in the degredation of VLCFAs, the VLCFA CoenzymeA synthetase, is implicated in neurodegeneration in the *Drosophila*. This enzyme normally activates the VLCFAs for subsequent degradation in the peroxisome. In the fly, absence of this gene (called *bubblegum*), causes late-onset neurodegeneration. These two findings imply that excess very long chain fatty acids in the cell may have a role in triggering neurodegeneration. The mechanism through which this occurs is unknown but is of clear clinical interest.

We have identified homologs for both the ALDP and the VLCFA synthetase genes in the *C. elegans* genome. While there are numerous fatty acid synthtases in the worm, there is just one protein (Y65B4Bl.5) that contains high homology to the two signature motifs that are specific for the growing "bubblegum" family of VLCFA synthetases. Members of this "bubblegum" family of synthetases are expressed in the nervous system in human, mouse, and fly. We are examining the expression pattern of the *C. elegans* homolog Y65B4BL.5 with GFP analysis. Preliminary experiments using RNAi suggest that knockdown of this gene may result in embryonic lethality. Due to the possibility of the neural expression of this gene, and the

associated difficulty of targeting RNAi to the nervous system, we have constructed a neuron-specific foldback RNA construct under control of the *mec-7* promoter. Identification of neurodegeneration as a result of knockdown of this gene in the worm would enable us to genetically dissect the cellular and molecular pathogenesis of X-linked adrenoleukodystrophy. We will report our results on developing this model for VLCFA toxicity.

881. FUNCTION OF *C. ELEGANS apl-1*, A GENE RELATED TO HUMAN AMYLOID PRECURSOR PROTEIN.

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Alzheimer's disease is a neurodegenerative disorder characterized by the accumulation of dense extracellular plaques and intracellular tangles in the brain. The major component of the plaques is the β-amyloid peptide, which is a cleavage product of the Amyloid Precursor Protein (APP). The early onset form of Alzheimer's Disease has been linked to mutations in several genes, including *APP*. The function of APP remains unclear. A large family of APP-related proteins has been identified, suggesting that a function is conserved through evolution. We are studying the function(s) of the APP-related gene, *apl-1*, in *Caenorhabditis elegans*. *apl-1* encodes a putative single pass transmembrane protein that is expressed in over 50 cells in the animal. *apl-1* shares many similarities with the APP family of proteins, but does not contain the β-amyloid peptide.

To analyze the function of APL-1, we isolated eight *apl-1* alleles. Loss-of-function (lof) alleles have a larval lethal phenotype. Animals are unable to molt and have several other morphological defects. Western blot analysis indicates that animals carrying the *yn10* and y*n23* alleles do not produce an APL-1 protein, suggesting that these alleles represent complete lof alleles. Expression of the extracellular domain alone or expression of full-length *apl-1* in neurons rescues the lethality. These results suggest that APL-1 plays an important role in development and that the extracellular domain of APL-1 is essential for viability. We are determining which regions within the extracellular domain are necessary for *apl-1* function.

By Western blot analysis, animals carrying the *yn5* allele produce a truncated APL-1 protein that presumably corresponds to the extracellular domain of APL-1. *yn5* animals show several phenotypes that can be phenocopied by microinjection of wild-type animals with

constructs encoding the extracellular domain of APL-1. These results suggest that the extracellular domain of APL-1 is involved in multiple behaviors.

As with mammalian APP, APL-1 may be cleaved to release its extracellular domain. We are in the process of determining whether APL-1 is cleaved. In addition, we will determine whether secretion of the extracellular domain of APL-1 is required for rescue of the *apl-1* lethality.

882. CHARACTERIZATION OF THE DEG-3 nAChR, A RECEPTOR INVOLVED IN NEURONAL DEGENRATION

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DEG-3 is a subunit of a nicotinic acetylcholine receptor (nAChR) that can mutate to cause neuronal degeneration. The degeneration causing mutation *deg-3(u662)* affects a residues in the pore lining domain of the receptor and is thought to cause degeneration by its effect on channel gating. To further understand how a single missense mutation leads to such a dramatic effect we are using electrophysiological analysis of the normal and mutant DEG-3 channel in an accessible heterologus expression system, Xenopus oocytes. DEG-3 channel activity is seen when co-expressed with *des-2*, also a nAChR subunit. This gene was originally identified as a supressor of *deg-3(u662)*. It is also part of the *deg-3* operon, and is therfore likely to be a subunit of the DEG-3 receptor in-vivo. The DEG-3/DES-2 reconstruction of DEG-3 channel activity shows that indeed the *u662* mutation affects channel gating, including a defect in channel desensitization. However, such a defect does not provide an explanation to DEG-3(*u662*) induced degenerations as the limiting factor for ACh gated channel activity is the elimination of ACh by acetylcholinesterases, and not receptor desensitization. Electrophysiological analysis also revealed that choline, a metabolite that is constitutively present in most organisms, is an agonist of the DEG-3 receptor. This finding suggested that low constitutive levels of choline, when combined with the *u662* mutation, may lead to *deg-3* dependent degenerations. Indeed oocytes expressing *deg-3(u662)* and not *deg-3* die when incubated with physiological concentrations of choline. Thus the heterolgous expression of DEG-3(*u662*) and DES-2 allows reconstruction of the cytotoxic processes induced by *deg-3(u662)*. Additional details of the analysis of the DEG-3 channel will be presented.

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883. Expression analysis of C. elegans homologues of the human CLN3 gene involved in neuronal ceroid lipofuscinosis

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The neuronal ceroid lipofuscinoses (NCL) are the most common hereditary neurodegenerative disorders of childhood with an incidence as high as 1 in 12,500 in Northern Europe and the United States, and 1 in 100,000 elsewhere. Symptoms of this heterogenous group of devastating lysosomal storage diseases are progressive visual failure, seizures, and psychomotor deterioration. The different NCL types can be distinguished by age of onset, clinical signs and structural characteristics of the storage material. We focus on the juvenile form (Batten disease, gene symbol: CLN3), which is the most frequent form of the disease. Genetic studies have indicated that CLN3 encodes a novel integral membrane protein. The CLN3 protein has been localized to the lysosome, confirming that the primary defect in NCL is in lysosomal function, but the function of the CLN3 protein is still unknown.

Homologues to the human CLN3 gene have been identified in several organisms, including the nematode *Caenorhabditis elegans*. In *C. elegans* three genes, *cln-3.1*, *cln-3.2*, and *cln-3.3*, homologous to the human CLN3 gene have been identified by sequence comparison. We have decided to exploit the genetic power of *C. elegans* as a model for Batten disease in order to dissect the processes, in which the CLN3 proteins play a role, and to provide further insight into the function of the *cln3* genes and in the mechanism of neurodegeneration in NCL. We have cloned the putative promotor regions of the CLN3 homologues in front of the GFP gene in *C. elegans* expression vectors and analyzed the spatial and temporal expression pattern of the *cln3* genes in transgenic worms. Preliminary evidence suggests that the *cln-3.1*-GFP fusion protein is expressed in the distal part of the gut. No fluorescent signals were observed with GFP fusion constructs containing the intergenic regions in front of the *cln-3.2* and *cln-3*.3 genes, suggesting that these genes are part of operons. We expect that the determination of the spatial and temporal expression pattern of the *cln-3* genes will be helpful in the characterization of *cln-3* deletion mutants.

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884. Exploring necrotic cell death in *C. elegans*

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Necrotic cell death can be induced by cytotoxic insults and plays fundamental roles in neuronal degeneration in stroke and ischemia. Unlike apoptotic cell death, little is understood about molecular mechanisms of necrosis. At the level of light microscopy, necrotic-like cells appear as swollen vacuoles several times larger than normal cells. Under the electron microscope the first visible step in necrotic-like death is the involution of the plasma membrane, followed by the appearance of membrane whorls. Later, the electron density of the cytoplasm is reduced, the cell swells and cytoplasmic vacuoles arise. The chromatin forms aggregates and becomes fragmented. Organelles swell and finally lyse. The dead cell is removed by phagocytosis. The key characterized initiators and regulators of apoptotic cell death do not influence the initiation or progression of necrotic cell death.

A variety of different insults can initiate necrotic-like neurodegeneration in *C. elegans* and lead to similar morphological changes in the affected cells. Necrosis-inducing genes include dominant alleles specifying hyperactive variants of the *mec-4* and *deg-1* degenerins which encode ion-channel subunits with similarity to mammalian epithelial Na^+-ion channels (ENaC's), a dominant allele of the *deg-3* acetylcholine receptor α-subunit channel, and a transgenically expressed constitutively activated mutant of the G-protein subunit Gαs.

In our lab we are focusing on a gain-of-function mutation in the touch mediating degenerin ion-channel subunit *mec-4(d)* to study molecular requirements for necrotic-like cell death. When mutated, the MEC-4(d) ion channel is locked in the open conformation, leading to increased cation influx, which is the trigger of the degeneration of the touch cells. The morphological features observed in this model share similarities with those induced by other cytotoxic insults mentioned above. Interestingly, the membranous inclusions occurring at the early stage of necrosis resemble ultrastructural features seen during some excitotoxic cell death in mammalian models.

In order to gain more information about the molecular nature of necrotic-like cell death, we ectopically expressed *mec-4(d)* in the ventral nerve cord and screened for mutations that prevented consequent paralysis. This approach allowed us to isolate 14 suppressor alleles in previously unknown loci influencing cell death. Further analysis defined 8 genes required for *mec-4(d)*-induced necrosis. One of the loci identified by the screen proved to code Ca-binding calreticulin (see abstract by Keli Xu).

Here we report on the detailed genetic and phenotypic analysis of the other suppressor loci, including mapping, genetic interactions, strength of suppression of vacuolation, influence on *mec-4* expression, and effects on other death-inducing stimuli.

Our long term goal is to clone these loci to learn about molecular details of necrotic death mechanisms.

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885. *unc-79*: A Gene Involved in Anesthetic Responsiveness in *C. elegans*

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Despite their continuous usage for 150 years and their pivotal role in surgery, the method of action of volatile anesthetics is unknown. We are studying the effects of genetics on the response of C. elegans to volatile anesthetics and have characterized three genes that cause hypersensitivity to volatile anesthetics. One of these genes, *unc-79*, has been the focus of this abstract.

The *unc-79* phenotype can be explained by single transcript. Mutant rescue experiments have shown that the cosmid E03A3 is capable of reversing the *unc-79* phenotype. Additional mutant rescue experiments have shown that the 19 kb DRAII cut of E03A3, is the smallest rescuing fragment. RT-PCR experiments have shown that a single 6.3 kb transcript is produced from the 19 kb genomic fragment. In 4/4 cases this transcript has either a frameshift or a nonsense mutation in the *unc-79* alleles.

The transcript exists in several alternatively spliced forms. There is also a form with an alternative poly adenylation site. However all forms of the transcript which we had been able to find have stop codons in the middle of the transcript. We have *unc-79* alleles that have molecular lesions both before and after the stop codons. A non-complementation test between an upstream mutant and a downstream mutant indicates that the *unc-79* transcript produces a single protein. It is also noteworthy that an EST from the *Drosophila* homologue of *unc-79* has stop codons similarly placed.

Northern analysis indicates another unusual feature of the *unc-79* transcript. Although the *unc-79* cDNA is 6.3 kb in length, the only band which appears after Northern analysis is greater than 10 kb in length. Additional Northerns have been performed using introns and also display a band greater than 10 kb in length. We therefore conclude that band present on the Northern is due to an unspliced/partially spliced message. Turnover of any spliced message must be very rapid since only unspliced message is seen on the Northern.

Expression analysis using eGFP indicates that *unc-79* is expressed primarily in the nervous system. This data is consistent across species---of the greater than 50 human ESTs homologous to *unc-79* all expression in non-cancerous tissue is confined to the central nervous system. Our future work on this project is directed relating the *unc-79* gene products to the other genes we have identified as hypersensitive to volatile anesthetics. Additional work is also being done to resolve the stop codons present in the message.

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886. SNAP-25, a Rat General Anesthetic Target Implicated by *C. elegans* Genetics

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The SNARE proteins syntaxin and SNAP-25, both anchored in the presynaptic membrane, and VAMP (also called synaptobrevin), which is anchored in the synaptic vesicle membrane, form a tight ternary complex that drives membrane fusion and hence neurotransmitter release. We previously showed that a mutation in *C. elegans* syntaxin, *unc-64(md130)*, dominantly blocks the effect of the volatile anesthetics (VA) isoflurane and halothane at clinical concentrations. We believe this and other results are most consistent with the *md130* product blocking the binding or transduction of binding of VAs to a syntaxin interacting protein. To ask directly whether one of the SNARE proteins might be a VA target, we produced recombinant *C. elegans* and rat SNARE proteins and measured their ability to bind isoflurane by ^{19}F- NMR spectroscopy. *C. elegans* and rat syntaxin, SNAP-25 and VAMP, each His_6-tagged and lacking its C-terminal transmembrane domain (syntaxin and VAMP) or its palmitoylation (SNAP-25), were cloned into vectors (pHO2c or pHO4d), expressed in BL21(DE3) bacteria, and purified by Ni-NTA agarose chromatography. Isoflurane binding to the recombinant proteins was measured by ^{19}F-NMR spin-spin-relaxation (T2). Resulting T2 times are indicative of binding when significantly lower than buffer. The recombinant SNARE proteins had distinctively different isoflurane binding characteristics. Rat SNAP-25 clearly lowered the isoflurane T2 in what appeared to be a dose-dependent manner at clinical concentrations of isoflurane (0.1 - 0.6 mM). In contrast, rat syntaxin binds isoflurane in a non-dose-dependent manner with a very low T2 time, not saturating over a 100-fold range of isoflurane concentrations (0.06 - 6.0 mM). This is suggestive of many low-affinity non-specific binding sites. Rat VAMP and the *C. elegans* orthologs of SNAP-25, syntaxin, and VAMP produced T2 values comparable to buffer alone therefore not binding to isoflurane. However, unlike r-SNAP-25, our recombinant ce-SNAP-25 does not appear to be capable of forming a ternary complex with syntaxin/VAMP and may not have a tertiary structure resembling that *in vivo.* In conclusion, SNAP-25 is the first neuronal protein shown to bind VAs and may be a relevant VA target mediating general anesthesia *in vivo.*

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887. Defining the structural requirements for *unc-64(md130)*'s general anesthetic resistance

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unc-64 encodes a homolog of vertebrate syntaxin 1A, which is expressed ubiquitously in the *C. elegans* nervous system. Syntaxin is a t-SNARE composed of 5 functional domains (Ha, Hb, Hc, H3, and TM) and is involved in membrane fusion of synaptic vesicles. Its H3 helical domain is known to interact with other SNARE proteins during neurotransmission. We have shown previously that mutations in *unc-64* profoundly alter the volatile anesthetic (VA) sensitivity of *C. elegans*. Animals with the *unc-64(md130)* mutation are semidominantly resistant to isoflurane and halothane while worms carrying other hypomorphic *unc-64* mutations are 30 times more sensitive to these anesthetics. The *md130* lesion is a G to A mutation at the splice donor site of intron 6 of *unc-64*. By RT-PCR, *md130* produces a small amount of wild type-mRNA along with truncated forms lacking half of the H3 domain and the entire TM domain, rendering them unable to properly interact with other SNARE proteins. We want to define the structural requirements for *md130*'s resistance to VAs. First, we transformed N2 worms with a plasmid containing genomic *unc-64* carrying the *md130* lesion and found that these animals exhibit the same behavioral and VA phenotypes as *unc-64(md130)* homozygous animals. *unc-64(null/+)* animals transformed with the same plasmid are also VA resistant, and transformed *unc-64(null)* homozygotes are viable. Another plasmid with an added stop codon that produces only the truncated *md130* product and no wild type product conferred VA resistance similar to *unc-64(md130)* and predictably failed to rescue the lethality of *unc-64(null)*. N2 transformed with syntaxin containing only the H3 and TM domains (delta-Habc H3 TM) are very Unc (too Unc to test their anesthetic sensitivity), slow growing and have small brood sizes. N2 animals transformed with *unc-64* lacking only the H3 domain (Habc delta-H3 TM) are uncoordinated and slower growing compared to N2 and are not VA resistant. This plasmid also does not rescue the lethality of *unc-64(null)*. Other *unc-64* deletion constructs and amino acid substitution mutations are currently being made.

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888. Gain-of-function mutations in *egl-30* produce resistance to volatile anesthetics

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Because mutations in the neuronal syntaxin gene *unc-64* alter volatile anesthetic (VA) sensitivity, we have screened for *unc-64(rf)* suppressors in hopes of identifying regulators of VA action. For different reasons, Owais Saifee in Mike Nonet's lab performed a similar screen. A total of 38,000 genomes were screened by the two labs, and 21 suppressor mutations were isolated. The suppressors were outcrossed from *unc-64(rf)* and fell into two phenotypic classes loopy and jerky that cosegregate with *unc-64(rf)* suppression. Two semidominant loopy suppressors mapped by their behavioral and VA resistant phenotypes to chromosome IL near *egl-30*. *egl-30* was sequenced in the mutants, and one of the alleles *js126* was found to have a missense mutation in the coding sequence. We did not find an *egl-30* mutation in the other strain. *egl-30(tg26gf)*, kindly given to us by K. Iwasaki, was also loopy and resistant to VAs. Testing of strains transformed with a constitutively active *egl-30* array (thanks to Carol Bastiani - Sternberg lab) were also resistant to VAs confirming that *egl-30(gf)* could indeed produce VA resistance. Furthermore, we treated N2 with phorbol-ester, which activates diacyl glycerol-binding proteins and effectively enhances EGL-30 signaling; treated worms were resistant to VAs. Finally, we found that *egl-30(js126)*, *egl-30(tg26)*, the *egl-30(gf)* array-containing strains, and phorbol ester treated strains were all aldicarb hypersensitive. Aldicarb hypersensitivity suggests that presynaptic release of acetylcholine is increased. Thus, consistent with our previous results for *goa-1(rf)* and *unc-64(rf)* that suggested a presynaptic mechanism of VA action, *egl-30* regulates VA sensitivity, presumably by controlling transmitter release. Currently, we are constructing double mutants between various VA resistant and hypersensitive strains to place *egl-30* in the VA sensitivity pathway.

889. TOWARDS THE CLONING OF THE SUPPRESSORS OF *UNC-1*

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Volatile anesthetics perform their function by acting on unknown neuronal mechanisms. To date, the functions of *C. elegans* genes linked to sensitivity to volatile anesthetic do not create a clear picture of the targeted processes. UNC-1 is a stomatin, a large transmembrane protein, that has been shown to have an affect on volatile anesthetic sensitivity when mutated. *unc-1* is understood to be involved in membrane trafficking. *unc-24*, another stomatin gene, is epistatic to *unc-1* and affects the distribution of UNC-1 to the membrane. To better understand the role of *unc-1*, we are pursuing suppressors of *unc-1*. One of the genes identified by this screen, *unc-8*, is a sodium channel gene that had already been shown to have an affect on volatile anesthetic sensitivity.

Two other suppressors remain to be identified. Both were selected for suppression of the uncoordinated phenotype of *unc-1 (e580)*, a phenotypically null variant of *unc-1*. Both have since been shown to also affect *unc-1* sensitivity to volatile anesthetics.

Single nucleotide polymorphism (SNP) mapping with the CB4856 strain has linked both suppressors to roughly two centimorgans. One on chromosome IV near Y57G11B, and the other on chromosome V near F38A6. Interestingly, one of the suppressors, *fc71*, converts from recessive to semi-dominant in the CB4856 background. The identification of the suppressors of *unc-1* will extend our understanding of the neuronal machinery targeted by volatile anesthetics.

890. *egl-2(lf)* Mutants are Volatile Anesthetic Resistant

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In screening through new and existing mutants for resistance to volatile anesthetics (VAs), strains carrying *egl-2(rf)* mutations were found to be VA resistant. *egl-2* codes for a homolog of the *Drosophila*ether-a-go-go potassium channel (*eag*). Drosophila *eag(lf)* mutants were originally isolated based on their convulsion-like phenotype in the presence of the VA diethyl ether. Of the various mutants that we have found to be abnormally sensitive to VAs, *egl-2(rf)* mutants are unique in that they do not appear to have alterations in synaptic transmitter release. Thus, we hypothesized that *egl-2* may regulate VA sensitivity through a mechanism distinct from the *goa-1, egl-30, unc-64* mechanism(s), all of which regulate both VA sensitivity and synaptic transmitter release. We constructed double mutants containing *egl-2(null)* and either *goa-1(null)*(VA resistant) or *unc-64(js21)*(VA hypersensitive) or *unc-64(md130)*(VA resistant). In the case of the *unc-64* doubles, the VA phenotypes of double mutants were not significantly different from the *unc-64* single mutants. However, the resistance of the *goa-1(null);egl-2(null)* strain was significantly greater than *goa-1(null)* and similar to that of *egl-2(null)*. These results suggest that *egl-2* functions upstream of *unc-64* and downstream or parallel to *goa-1* to regulate VA sensitivity. We are currently measuring the VA sensitivity of non-null *egl-2* alleles to understand better the sequence requirements for the VA resistance of *egl-2(rf)*. We are also attempting transformation rescue of the VA resistance of *egl-2(lf)* with cosmids spanning the *egl-2* gene.

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891. *mab-21* expression is regulated by genes controlling anesthetic response.

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mab-21 gene encodes a novel protein that determines cell fate and cell identity in male tail development. Our previous study in *Xenopus* indicated that inhibition of frog *mab-21* homolog expression resulted in posterior body patterning defect similar to that in embryos with FGF signal depleted. This observation promoted us to examine if *mab-21* is acting in the FGF pathway. Using a functional *mab-21::gfp* fusion transgene as a reporter, we examine if the expression and subcellular localization of the protein is regulated by worm FGF components, *egl-17* and *egl-15*. It was noted that mutations in these genes had no effect on *mab-21* expression. Instead, we observed as enhanced expression of *mab*-21*::gfp* particularly in the hypodermal tissue in *unc-1* mutant, a linked mutation used in this FGF pathway analysis.

The results suggest that strong expression of *mab-21::gfp* transgene in hypodermis may be related to the functional pathway that controls sensitivity of *C. elegans* to volatile anaesthetic chemicals. To ascertain such possibility, the same reporter transgene was crossed into a number of mutants known to be part of this functional complex. Our result showed that the *mab-21::gfp* expression was up-regulated in *unc-8 and mec-2* mutations, while it was repressed by *unc-24* and *unc-79* mutations. *unc-1* encodes stomatin like protein and *unc-8* belongs to the degenerin family. Both of these genes code for integral membrane proteins, arguing that MAB-21 expression level and its function may be regulated by the signaling process downstream of these membrane proteins.

Since the reporter was a fusion protein, the strong expression may be due to either enhanced transcriptional activity or protein stability. Experiments are underway to differentiate the two possibilities and additional experiments addressing the functional role between these molecules as well as the biological significance of such relationship will be presented.

892. Ethanol Sensitivity Genes in the nematode *Caenorhabditis elegans*

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Alcohol has many effects in higher organisms, especially in the nervous system. The mechanisms and sites of action of ethanol are not fully understood. In the hope of understanding the mechanisms of ethanol, we identified genes that control sensitivity to ethanol and anesthetics in the invertebrate system Caenorhabditis elegans. 15 mutations that confer resistance to the anesthetic effect of ethanol were identified, either by chemical mutagenesis or transposon insertion mutagenesis. We have cloned two of the genes responsible for the resistant phenotypes by mapping and rescue, and by transposon tagging. One of these, jud-1, encodes a protein similar to contactin. The cDNA analysis of the jud-1 gene shows that it is alternatively spliced. The shorter transcript has a single Ig domain, while the longer transcript has three Ig domains and one fibronectin type III domain. The functions of the two alternatively spliced transcripts are not known at this moment. In the mammalian system, contactin is known to be a cell adhesion/recognition molecule in the nervous system and has roles in the formation of axon projections in the developing nervous system. In C. elegans, jud-1 is also expressed in some neurons as shown by GFP expression analysis. jud-5, an ethanol sensitivity gene cloned by transposon tagging, encodes a novel protein. From the expression pattern of its GFP fusion protein, it could be seen that jud-5 is expressed in most muscles including pharyngeal, body wall, tail and vulva muscles. Further studies of these mutants are under way.

893. A Genetic Analysis of the Effects of Ethanol on Egg Laying

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Ethanol causes dose-dependent suppression of egg laying in *C. elegans*. Quantification of tissue ethanol concentrations from *C. elegans* after exposure to different exogenous doses suggests that suppression of egg laying occur at doses relevant to human intoxication. To elucidate the molecular targets of ethanol, we screened for mutants showing ethanol-resistant or -inducible egg-laying behavior. We completed a screen of 7,500 haploid genomes and identified 20 candidate ethanol resistant mutants. In this screen we isolated seven *slo-1* alleles (see also abstract by Davies, Thiele, and McIntire). *slo-1* encodes a calcium activated potassium channel (personal communication, Nonet and Salkoff). There is in vitro evidence for an activating effect of ethanol on vertebrate slo channels (Dopico et al. 1999 Neurochem. Int. 35:103-106). We are presently characterizing additional ethanol resistant mutants.

Several of the resistant mutants show ethanol-inducible egg laying behavior at relatively low exogenous ethanol concentrations that have a weak inhibitory effect on egg laying in N2 animals. We have therefore screened for mutants laying eggs without food in the presence of low concentrations of ethanol. Thus far we have completed a screen of 4,000 haploid genomes and identified 11 candidates. This screen has generated a class of ethanol-inducible egg laying mutants as well as a class of mutants exhibiting egg-laying independent of food and ethanol. Further mapping and molecular characterization of these mutants will be presented.

894. Reproductive and Acute Toxicity of Platinum-based Chemotherapy Drugs in C. elegans

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We are using C. elegans as a model system to determine the mechanism by which platinum-based chemotherapy drugs kill germ cells and non-dividing somatic cells. We have treated Bristol N2 worms with cisplatin, carboplatin and transplatin. Continuous exposure to cisplatin killed worms in a time and dose-dependent manner. Treatment of age-synchronized populations showed the L1s to be more sensitive than older worms to both the acute toxicity and reproductive toxicity of cisplatin. Worms were unable to reproduce in concentrations of cisplatin above 250uM. Carboplatin also inhibited reproduction, but ten-fold higher concentrations were required for similar levels of inhibition. Transplatin had no effect on reproduction or viability even when tested at the limit of solubility of the drug. The relative potency of these drugs in C. elegans is similar to that observed in humans. We are selecting for drug resistant mutants and testing the drug sensitivity of a series of knockout strains to identify the molecular mechanisms responsible for the selective toxicity of these drugs to specific cell types.

895. Towards the Cloning of Gene That Can Mutate to Confer Lithium Resistance In *C. elegans*

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Little is known about how ions permeate the *C. elegans* gut and cuticle. How various ions influence development and behavior is also not understood. One ion with considerable impact in human biology is Li^+, which modulates bipolar disorder by an unknown mechanism. In particular, the targets of lithium that cause side effects remain to be identified. We are using *C. elegans* to genetically identify targets of lithium and to elaborate on mechanisms of transport, which may have an impact on human health. Li^+ has dosage-dependent effects on *C. elegans* embryos. When L4 animals mature on NGM plates with Li^+ added to the final concentrations of 10mM-20mM, they produce embryos that are unable to hatch. We demonstrated that this failure to hatch is due to defects in cytokinesis that result in multi-nucleated embryos and symmetrically partitioned cells. Li^+ also has a dosage-dependent effect on larval development. When adult hermaphrodites are permitted to lay embryos on 10mM-20mM Li^+ NGM plates, the resulting offspring experience a developmental delay proportional to the concentration of Li^+. The offspring become progressively paralyzed as they reach adulthood. We demonstrated earlier that there is a delay in the entry into the S phase of the cell cycle larval stages. To learn more about the biology of Li^+ sensitivity, we screened for Li^+ resistant mutants. We developed three screens that took advantage of the embryonic arrest and the larval delay caused by Li^+. We isolated one mutant *bz71*, in a screen of 44,000 haploid genomes, that is resistant to both the larval and embryonic blocks

of 16mM Li^+. *bz71* is dominant. Using classical mapping techniques, we positioned it on LGIII between *unc-32* and *dpy-18*. We are currently using SNP strategy to obtain a higher resolution map and we hope to report on the identification and cloning of this locus.

896. A genomic approach to understanding the actions of fluoxetine

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Re-uptake blockers are a class of antidepressants proposed to achieve their clinical effects in people by inhibiting presynaptic norepinephrine and/or serotonin re-uptake transporters, which normally function to terminate transmitter signaling at synapses. It has never adequately been explained, however, why therapeutic relief of depression takes weeks when this particular action of re-uptake blockers occurs within hours of drug administration.[1]

A requirement for some form of adaptation at synapses is a frequently cited possibility, but recent work has also highlighted that there are a myriad of non-classical targets for re-uptake blockers, such as EAG-like K^+-channels (blocked by imipramine)[2] and novel membrane-spanning proteins typified by NRF-6 and NDG-4, identified as fluoxetine targets in a recent screen in worms[3].

With the recent discovery of mutants in *mod-5*, a gene encoding the *C. elegans* serotonin re-uptake transporter,[4] a new approach to tackling this long-standing question has emerged.

Utilizing the DNA microarray facility at Stuart Kim's laboratory in Stanford, we have begun work on a gene chip project to perform a four-way comparison between N2 and *mod-5(n3314)* worms (*n3314* is a 1.6 kb deletion in the *mod-5* genomic locus), each on and off fluoxetine. We hope to understand what baseline transcriptional differences exist between the two genotypes, how similar the

effects of chronic fluoxetine in N2 are to the effects of a complete absence of MOD-5 in *mod-5(n3314)* worms, and to identify which gene expression changes induced by fluoxetine are MOD-5-dependent and which are MOD-5-independent.

A preliminary experiment is expected to be completed by the end of April, 2001.

[1] Schafer WR, *Cell* **98**:551-554

[2] Weinshenker D *et al. J Neurosci* **19**:9831-9840

[3] Choy RK *et al. Mol Cell* **4**:143-152

[4] Ranganathan R and Horvitz HR, 1999 International Worm Meeting, abstract 70

897. Steps Toward Solving the Drug Problem in Worms

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Amphetamine (AMPH) and its methylated derivatives are among the most widely abused illegal drugs. These compounds are known to inhibit the reuptake of monoamines by various plasma membrane transporters; and the rewarding and addictive properties are thought to be mediated by an increase in synaptic monoamines. We are using a genetic screen in *C. elegans* to further characterize the monoaminergic signalling pathways in worms and possibly to identify additional sites of action of psychostimulants that may retain a conserved function in vertebrates. Toward this end, we have begun to characterize the behavioral modifications seen with chronic and acute treatments of AMPH.

Based upon the inhibition of reuptake of multiple monoamine transmitters, it is expected that AMPH would have myriad effects upon *C. elegans* behavior; and indeed AMPH does have inhibitory effects upon locomotion, pharyngeal pumping, egg laying and the overall rate of development. Within a single behavior, AMPH exhibits distinct concentration dependent effects. For instance, egg-laying of drug naïve wild-type worms on AMPH containing plates is stimulated at lower concentrations during the initial 15-30 minutes but inhibited immediately at higher concentrations. Locomotion is also suppressed with increasing dosage to the point of paralysis, while pre-treatment with a lower dose results in uncoordinated movement. Pharyngeal pumping is also dramatically suppressed by acute AMPH, and perhaps consequently, chronic treatment with AMPH from embryonic or L1 stages slows the rate of development to adulthood. The inhibitory effects seen in locomotion, egg-laying and pumping upon acute exposure to amphetamine are reversible, and the length of time to recovery is dose dependent. Importantly, *cat-1* (vesicular monoamine transporter) and *cat-2* (tyrosine hydroxylase) mutants are partially resistant to some of these AMPH effects.

We have initiated genetic selections for resistance to AMPH, focusing on the selection of mutants that can escape the AMPH induced slowing of the rate of development. Screening of 4500 EMS mutagenized haploid genomes yielded 12 mutants that exhibit varying degrees of resistance to AMPH. Classes of mutants include those that exhibit hyperforaging behavior as well as mutants that exhibit increased egg-laying at normally inhibitory concentrations of AMPH. Genetic mapping and molecular characterization of these loci may further our understanding of the mechanism of action of psychostimulants.

898. Functional roles of flavin-containing monooxygenase (FMO) genes in *C. elegans*: EMS-induced gene lesions screened using denaturing high-performance liquid chromatography (DHPLC)

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Mammalian flavin-containing monooxygenases (FMOs) have been traditionally characterised as "drug-metabolizing enzymes" as they catalyze the oxidative metabolism of numerous xenobiotics, including drugs and pesticides, as well as dietary-derived compounds, eg. trimethylamine (TMA). Of the five mammalian FMOs (FMOs 1-5), the most relevant with respect to human foreign compound metabolism is considered to be FMO3. Loss-of-function mutations in the corresponding gene, *FMO3,* underlie "fish-odor syndrome" - an inherited defect in TMA *N*-oxidation. However, FMOs may play a physiological role in biogenic amine homeostasis as suggested by their expression in the central nervous system and their ability to metabolize biogenic amines *in vitro*. Biogenic amines have known potent neuromodulatory activities and we hypothesize that their inactivation may, in part, be mediated *via* FMO action. To further explore this potential role, we are investigating the function of homologous FMOs in *C. elegans*. The *C. elegans* genome contains seven predicted genes encoding products exhibiting significant (26-45% amino acid identities) with mammalian FMOs. We have isolated and sequenced cDNAs encoding six of these seven putative worm FMOs and, apart from some minor mistakes, the predicted exon-intron organization of the corresponding genes were found to be correct. The deduced amino acid sequences exhibit predicted hydrophilicity profiles and secondary structure features very similar to those predicted for mammalian FMOs. All mammalian FMOs also contain two copies of a consensus "fingerprint" predicting a Rossman fold characteristic of a binding domain for the ADP moiety of dinucleotides. One such ADP domain is located close to the *N*-terminal and binds FAD while the other is located further towards the *C*-terminal

and binds NADP. Two such domains are also present in each *C. elegans* FMO at the same relative locations as they are found in mammalian FMOs. To investigate potential physiological role(s) for the putative *C. elegans* FMOs we are attempting to create mutant lines for each FMO gene using an adaptation of the TILLING (Targeting Induced Local Lesions IN Genomes) procedure developed originally in *Arabidopsis*. Single F1 L4 larvae, originating from a synchronised EMS-mutagenised P0 population, are transferred to individual wells of flat-bottomed 96-well plates and cultured for 1 week. Half of the resulting F2 progeny of each F1 worm are then frozen and the remainder used for DNA isolation. PCR, designed to amplify a targeted region of the gene of interest, is performed in 96-well plates and the resulting amplicons are subjected to DHPLC to identify heteroduplexes indicative of a potential mutation within the amplified region. Direct sequencing of the original PCR product identifies the location and nature of the sequence change and, if appropriate, the corresponding frozen F2 worms can then be recovered and mutant lines generated.

899. Neural integration of thermosensory and chemosensory information

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C. elegans relies on thermosensory and chemosensory cues to search its environment in behaviors called thermotaxis and chemotaxis respectively. The neural mechanisms underlying thermotaxis and chemotaxis have been studied extensively as separate behaviors[1], however, less is known about neural mechanisms that may integrate thermotaxis and chemotaxis. As a first step to characterize the neural integration of thermosensory and chemosensory information in *C. elegans*, we measured the chemotaxis performance of wild-type worms (N2) as a function of different background temperatures. Populations of wild-type worms were cultivated at 20 º C on bacteria until the adult stage and then tested for chemotaxis to ammonium chloride at different temperatures (15, 22, 28, 32, and 35 º C) for one hour[2]. We found that chemotaxis performance was high for assays carried out at 15, 22 and 28 º C. At 32 and 35 º C, however, chemotaxis performance was significantly reduced relative to performances at lower temperatures; a result consistent with previous observations for sodium chloride chemotaxis[3]. Subjective observation of locomotion revealed that most animals moved well at 32 º C, but many moved poorly at 35 º C. To determine whether the reduction in chemotaxis performance at 32 º C was due to a non-specific reaction to elevated temperature, or due to the specific activity of neurons in the thermotaxis network[4] we tested the chemotaxis performance of mutants having defects in thermotaxis neurons. We tested *ttx-1* mutants, which have abnormal thermosensory neurons (AFD)[5], and *ttx-3* mutants, which have abnormal interneurons (AIY)[6] that may relay information between AFD and the ASE chemosensory neurons since AFD and ASE have reciprocal chemical synapses[7]. Both *ttx-1*

and *ttx-3* animals performed chemotaxis at 32 º C significantly better than wild-type animals (avg ctx indices[2] ± 95% confidence interval: WT = .26 ± .10, *ttx-1* = .60 ± .08, *ttx-3* = .74 ± .11, greater than 20 assays per animal type, F = 23, $p < 0.001$). These results suggest that normal function of neurons within the thermotaxis neural network is required to integrate temperature information with chemosensory information to reduce the chemotaxis response at high temperature. We plan to screen for other mutants that can perform chemotaxis at high temperature to identify additional genes involved in thermosensation, thermotaxis and the integration of thermosensory and chemosensory information.

1. Bargmann & Mori (1998) in C. elegans II; 2. Bargmann & Horvitz (1991) Neuron 7, 729-42; 3. Dusenbery et al. (1978) J Exp Zool 206, 191-98; 4. Mori & Ohshima (1995) Nature 376, 344-8; 5. Perkins et al. (1986) Dev Biol 117, 456-87; 6. Hobert et al. (1997) Neuron 19, 345-57; 7. White et al. (1986) Philos Trans R Soc Lond B Biol Sci 314, 1-340.

900. Reverse genetic approach to reveal sensory signal transduction pathway involving TTX-4 nPKC-epsilon

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We found that TTX-4, a homolog of nPKC ("novel" PKC)-epsilon, is essential for sensation of environmental signals such as temperature, volatile and soluble compounds, and osmolarity (Okochi et al., this meeting). Biochemical analyses mostly based on mammalian cell cultures have revealed that nPKC species are activated only by diacylglycerol (DAG) or other lipids such as phosphatidylinositol-3,4,5-trisphosphate (PIP_3) or phosphatidylserine without requirement of calcium ion which is necessary for cPKC ("conventional" PKC) activation. Little evidence has been reported, though, to prove *in vivo* activation of nPKC by any of those lipids. Moreover, *in vivo* substrates of nPKC, whose activities are regulated by nPKC-dependent phosphorylation and required for transducing specific signal from nPKC, are poorly understood. To address these questions, we are undertaking reverse and molecular genetic approaches to identify upstream and downstream molecules of the TTX-4 nPKC-epsilon. We identified *C. elegans* homologs of candidate genes for nPKC pathway from the genome database, and have been making GFP reporter constructs of those genes. If the candidates are expressed in the same cells as those expressing *ttx-4*, we will screen TMP/UV library for knockout mutants (Inada et al., this meeting).

(I) Upstream candidates: DAG is a strong candidate molecule as an activator of TTX-4. DAG can be generated through hydrolysis of phosphatidylinositol-4,5-bisphosphate (PIP_2) by phospholipase C (PLC) or through dephosphorylation of phosphatidic acid (PA) by

PA phosphatase (PAP). Thus, those enzymes are candidates for upstream gene products of TTX-4. We are currently analyzing expression pattern of these genes.

(II) Downstream candidates and binding proteins: Several proteins have been reported to be phosphorylated by nPKCs in mammals. Biological significance of those phosphorylations however remain questionable. We are currently analyzing a couple of homologs for these proteins, beta'-COP (F38E11.5) and adducin (F57F5.4). Interestingly, the adducin (F57F5.4) is located adjacent to *ttx-4* (F57F5.5) on the physical map. We are also screening yeast two hybrid library using wild type or kinase negative TTX-4 catalytic domain as a bait.

901. Warm avoidance is a major response of *C. elegans* to a thermal gradient

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It has been proposed that *C. elegans* remembers a temperature in association with food conditions, and on a thermal gradient, migrates toward a growth temperature while avoids a starvation temperature. However, this idea seems to be derived from only a part of the behavior, or from behaviors under the condition where normal movement was suffered, and therefore it remains disputable. In this report, we established an improved assay with a linear temperature gradient, and examined a distribution of a worm population and movement of a single worm. The results showed that fed wild type worms dispersed in a region which spanned more than several degrees. Within the area worms migrated in both directions of the gradient at similar frequencies. Worms who came close to the warm limit of the distribution changed their directions to stay inside the area, and if artificially put in a warmer place they showed taxis down the gradient until they reached the distribution area. These results indicated that *C. elegans* avoids a warm temperature but is indifferent to a gradient in moderate temperatures. The avoiding temperature depended on a growth temperature, whereas starvation for several hours made worms tolerant to a warm temperature that was avoided by fed worms. However specific responses to neither the growth temperature nor the starvation temperature were detected. For the warm avoidance the *tax-2* and the *tax-4* genes were both essential, and the *eat-4* gene also seemed to be involved. Mutations in the *ttx-3* gene, on the other hand, made worms sensitive to low temperatures which wild type worms did not avoid. Therefore sensory and inter-neurons expressing these genes likely receive or transmit the warm signal, or modulate the response. Mutant studies also suggested that the mechanisms of the warm avoidance is at least some part different from those involved in the escape from a very high, noxious temperature.

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902. The trampoline assay: A new method for measuring the step response of the thermotaxis mechanism in *C. elegans*.

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Worms in a thermal gradient migrate to the temperature at which they were cultivated (thermotaxis). Qualitative analysis of the effects of mutations and neuronal ablations on thermosensory behavior[1,2] suggests that thermotaxis may involve a mixture of two strategies: isothermal tracking, in which the head is aligned orthogonal to the thermal gradient, and pirouetting, in which course correction is achieved by a cluster of sharp turns, as in chemotaxis[3].

As a direct test of whether pirouettes play a role in thermotaxis, we devised a new procedure--the "trampoline assay"--in which worms were placed on a thin (~100 μm) agarose film suspended over a chamber filled with buffer at the cultivation temperature (21°C). After a 5 min. adaptation period, we quickly replaced the first chamber with a second containing buffer at a lower temperature (18°C), and observed the worm for an additional 5 min. Temperature measurements on the surface of the film indicated that the time constant of the temperature shift was ~4 sec.

When we shifted wild type worms (n = 14) to the lower temperature (down-shifts), we observed a transient increase in the probability of initiating sharp turns (time to peak = ~30 sec; decay time constant = ~50 sec). This result is consistent with a role for pirouettes in thermotaxis because, in a spatial temperature gradient, a pirouette induced by a temperature drop tends to return the worm to its cultivation temperature. The increase in sharp turn probability is almost certainly not a mechanical artifact because we saw no increase in turning when the second chamber was at the cultivation temperature (n = 8). Conversely, when we down-shifted the cryophilic mutant *ttx-3*(ks5) (n = 15), we observed a sustained decrease in sharp-turn probability. In a spatial temperature gradient, a decrease in turning induced by a temperature drop tends to move the worm toward lower temperatures, consistent with the cryophilic phenotype of *ttx-3*. We conclude that pirouettes are likely to be important for thermotaxis, at least for excursions below the cultivation temperature. Experiments are in progress to determine if the same is true for excursions above cultivation temperature.

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2. Mori, I. & Ohshima, Y. *Nature* 376, 344-348 (1995).

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903. Characterization of the thermotactic response in C. elegans

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We are analyzing the thermotactic response in C. elegans, both the behavior of the organism as it navigates thermal gradients and the activity of the neurons in response to thermal stimuli. Using custom-built equipment, we track worm motility on spatial and temporal gradients, and are establishing the rules of the thermotactic strategy. Using phluorins, we are attempting to measure vesicle recycling in the neurons underlying the thermotactic response. We are also attempting to detect calcium transients in these neurons using aequorin, a bioluminescent indicator of Ca2+.

904. New mutants defective in thermotaxis behavior

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Thermosensation is one of the major sensory systems for animals in order to ensure their survival in the environment. The molecular components involved in thermosensation however have not been revealed very much. To identify new molecular components involved in thermosensation, we conducted a screen for thermotaxis-defective mutants(1). Through screening about 6,000 genomes using the interaction between dauer formation and thermosensation, over ten mutants were isolated. Some mutants displayed defects in thermotaxis only, the other displayed defects in thermotaxis and chemotaxis. By complementation test, we found that , of these, *ttx-4(nj1, nj3)*, *ttx-5(nj2, nj6)*, *nj7*, *ttx-6(nj8)*, *nj20* and *nj21* are novel thermotaxis-defective mutants, and *nj9ts*, *nj17* and *nj18* are allelic to the *tax-4* mutations. Based on the thermotactic defects, these mutations are categorized into the following three groups. For *ttx-4*, see the abstract by Okochi et al..

cryophilic: *nj7*, *ttx-6(nj8)*, *nj20*, *nj21* and *nj19*

thermophilic: *ttx-4(nj1, nj3)*

athermotactic: *ttx-5(nj2, nj6)* and *tax-4(nj9ts, nj17, nj18)*

nj7 mutants showed cryophilic behavior, grown at 15 and 20-degrees, although they showed normal thermotaxis, grown at 25-degrees. *nj7* mutants showed normal chemotactic behavior to NaCl. *nj19* mutants showed cryophilic behavior and normal chemotaxis behavior to NaCl. *nj20* and *nj21* mutants showed cryophilic behavior, independently of cultivation temperature. Although *nj20* mutants showed normal chemotaxis to volatile odorants, *nj21* mutants showed mild defective chemotaxis to some volatile odorants. *tax-4(nj9ts)* mutants showed normal thermotaxis, cryophilic and athermotactic, and athermotactic behavior, when grown at 15, 20 and 25-degrees, respectively.

ttx-6(nj8) mutants always move toward lower temperatures, independently of cultivation temperature. The *ttx-6* mutant animals thus manifest cryophilic behavior. *ttx-6* mutants displayed mild defects in the osmotic avoidance response, but showed normal chemotaxis toward the water-soluble attractant NaCl and volatile odorants. These results suggest that *ttx-6(nj8)* mutation, which was found to be a reduction of function allele, affects thermotactic behavior specifically. We mapped *ttx-6(nj8)* to the *unc-29*, *lin-11* interval on the chromosome I. We are attempting to rescue the *ttx-6(nj8)* mutant phenotype with DNA fragments.

1. Okochi et al., 1999, International *C. elegans* Meeting

905. Systematic gene knockout to identify genes required for thermotaxis in *C. elegans*

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C. elegans can sense and memorize temperature, and change its temperature response, depending on the past experience. For example, after cultivation with sufficient food at 20 degree, *C. elegans* migrates to its cultivation temperature (i.e. 20 degree) and then moves isothermally on a thermal gradient without food. This behavior is called thermotaxis, and several mutants defective in thermotaxis have been isolated. Of these, *tax-4* and *tax-2* show athermotactic phenotype, and both *tax-4* and *tax-2* gene were found to encode two different subunits of cyclic nucleotide-gated cation channel (CNG channel), which functions in several sensory neurons including AFD thermosensory neurons. The TAX-4/TAX-2 CNG channel shows higher affinity to cGMP than cAMP, suggesting that cGMP acts as a second messenger in thermosensory signal transduction. Thermotaxis must also require execution of some form of associative learning between temperature and food. Recently, our laboratory has been isolating mutants designated *aho* (abnormal hunger orientation), which are likely to be defective in mechanism of associative learning between temperature and food (see abstract by Mohri et al).

The completion of *C. elegans* genome project makes us possible to systematically analyze the function and development of the nervous system by reverse genetic approach. Among 19,000 genes that exist in the *C. elegans* genome, about 1,700 genes are thought to function in the nervous system. In order to dissect the molecular mechanism of thermotaxis, we have started to construct TMP/UV-induced deletion mutants for the genes that likely function in neurons required for thermotaxis. Since (1) cGMP could be a second messenger in thermosensation and (2) associative learning in thermotaxis can be established only in a few

hours (0.5 ~ 4 hr), which implicate the importance of change in neuronal activity, we focus on genes for (1) molecules related to cGMP signal transduction and (2) ion channels required for modulation of neuronal activity. For the first aim, we plan to knockout genes for guanylyl cyclase (GC), cyclic nucleotide phosphodiesterase (PDE), and cGMP dependent protein kinase (PKG). In *C. elegans*, there are at least 33 genes for GC, and at least two of these genes, gcy-8 and gcy-12, are expressed in AFD thermosensory neurons. Also, there are several genes for PDE and a single gene for PKG. For the second aim, we are paying attention to the following genes; 6 genes for voltage-gated chloride channel, and 11 genes for transient receptor potential (TRP) ion channel. To date, we screened about 2,400,000 genomes and obtained deletion mutants for GCY-12, a chloride channel, and a TRP ion channel. We are currently analyzing behavioral phenotype of these deletion mutants.

906. Photoresponse of *Caenorhabditis elegans*

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Chemotaxis and thermotaxis, the two sensory mechanisms that *C. elegans* uses to interact with its environment have been reported in detail. However little is known of phototaxis, another operative sensory mechanism. To investigate photoresponse in the worm, we used two approaches. In (1), phototaxis of wild type worms were observed on NGA plates using two different light sources and (2) photoresponse of a single worm was determined under limited light conditions. Phototaxis is shown as phototaxis rate (PR) = Number of worms in area after irradiation/ worms before irradiation. In 30 independent assays using white light, PR ranged between 3.0 and 0.8. Using a thermostat filter to maintain constant temperature, PR decreased to between 1.35 and 0.75. This result indicates that the worm is sensitive to temperature increase during irradiation. We are yet to obtain clear results with a light source of 533 nm filter and we are trying other approaches for precise determination. Photoresponse was observed after irradiation shift from weak to strong light and percentage of responsive animals were classified as avoidance, weak avoidance and no response. In 15 independent tests, 25% of the worms responded to irradiation. Using mutant worms of chemotaxis and thermotaxis, the percentage irradiation response was decreased proportionally to the morphology of the amphid cilia. This suggests that some molecules in the amphid cilia could respond to light. The relationship between phototaxis and photoresponse and its link to chemotaxis and thermotaxis are still underway.

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907. Light-increased reversal frequency and spontaneous reversal frequency are affected by starvation.

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C elegans was shown previously to reverse direction in response to light (Burr 1985). In that study, 40-80% increases in reversal frequency over the background level varied with light intensity and wavelength in a normal way and were shown not to be due to radiant heating. Because large sample sizes are needed in order to detect small increases on top of the fluctuating frequency of spontaneous reversals, we have developed a computer automated tracking system that makes experimentation possible in a reasonable length of time. Under continuous near-infrared illumination, video images are digitized once each second and processed to find the new coordinates of up to 23 worms. Another program identifies reversals from the coordinates along the track. These are counted during a 10 s light period (1.6×10^4 uW cm^{-2} blue-white broadband light) and a preceding 10 s dark period in each 40 s measurement cycle. There are 5 cycles per 200 s run. The number of reversals per 10 s measurement period, averaged over all worms and cycles during a run, counts as one independent measurement. Data are analyzed as a generalized randomized complete block design using the Proc Mixed procedure of SAS version 8. For detecting the effect of light, the difference in reversal frequency between the paired light and dark periods is analyzed in the same way.

A new sample of worms was used for each run. About 30 synchronous six day-old, well-fed worms were washed off an OP50 culture plate and sedimented twice through behavior buffer (5.5 mM Tris + 34 mM NaCl). The worms were transferred in a drop of buffer to the edge of a 50-mm behavior plate and the plate was rotated to distribute the worms around the edge. The behavior plates were prepared by making 1.8% agar in 90% behavior buffer then drying to 90% of poured weight. Just before using, about 100 uL distilled water was distributed around the edge and allowed to soak in. The resulting lower osmolarity at the agar perimeter discouraged the worms from crawling near the edge. Worms were allowed to crawl into the 22x28 mm field of view and were tracked within 30-50 min of removal from the food. All procedures were carried out at 19 C.

Sensitivity of the system under actual conditions was estimated by simulation, starting with data from an experiment with fed worms during which the light beam was blocked. Reversals were added to the data during the 'light' periods at frequencies of 0.01 to 0.10 per 10 s period (perP), and the data were reanalyzed. The increase in reversal frequency became significant at 0.050 perP ($P = 0.037$, $n = 21$ runs), a 45% increase over the background (dark period) frequency. A greater sensitivity would be expected at higher sample sizes.

In all our experiments with well-fed worms, light had no significant effect on reversal frequency. To investigate the influence of starvation, we pooled worms from several food plates and put half on a food-free plate and half on a fresh food plate. After a starvation period, the fed and starved worms were tested in alternate runs. Light increased reversal frequency significantly in worms starved 20 h (+0.040 perP, +62%, P=0.049, n=51), but not significantly in worms starved 6 h (+0.013 perP, +12%, $P = 0.4$, $n = 39$). In both experiments, light had no significant effect on the fed controls (-0.023 perP, -15%, $P = 0.2$, $n = 58$ and -0.015 perP, -9%, $P = 0.4$, $n = 42$). In addition to being more responsive to light, starved worms had a significantly lower background (dark period) frequency. For the 0 h, 6 h and 20 h starved worms, average dark frequency (+/- SE) was 0.15 (0.013) perP, 0.11 (0.013) perP and 0.064 (0.012) perP, respectively. Thus, both background reversal frequency and responsiveness to light appear to be affected by starvation. In the previous study in which light responses were observed (Burr 1985), the worms may inadvertently have been starved.

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908. Factors that affect the subcellular localization of $GABA_A$ receptors

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$GABA_A$ receptors are the major inhibitory neurotransmitter receptors in the mammalian brain. The postsynaptic response to GABA depends heavily on the density of $GABA_A$ receptors at the synapse. Receptor density is determined by the rates of receptor synthesis and trafficking to synapses, receptor internalization, and receptor degradation. These processes are not well understood. We are developing *C. elegans* as a model system to study $GABA_A$ receptor synthesis, trafficking, internalization, and degradation.

The *C. elegans unc-49* gene encodes a homolog of the $GABA_A$ receptor. *unc-49* encodes three $GABA_A$ receptor subunits by complex alternative splicing. Two of these subunits, UNC-49B and UNC-49C, are produced at high levels, and co-assemble to form a heteromultimeric $GABA_A$ receptor at the inhibitory neuromuscular junction. We are using a functional GFP fusion to the UNC-49B subunit to study receptor synthesis and trafficking to synapses, internalization, and degradation. Defects in any of these processes are expected to cause the UNC-49B::GFP fusion protein to become mislocalized within the cell. We have discovered four factors which affect the subcellular localization of UNC-49B::GFP: 1. The sequence of a large intracellular loop in the UNC-49B subunit. Sequences required for localization to synapses are contained within a stretch of 53 amino acids, and we are presently defining the minimal sequence requirements. 2. The structure of the *unc-49* mRNA. UNC-49B::GFP is localized nearly completely to synapses when it is encoded by a full-length genomic construct. By contrast, it appears to accumulate in the endoplasmic reticulum when it is encoded by a minigene, suggesting a defect in the assembly or trafficking process that may be linked to mRNA processing. We are determining which specific difference between the genomic and minigene UNC-49B::GFP constructs is responsible for this defect. 3. Wild type function of other genes. We have isolated at least three mutants that accumulate UNC-49B::GFP in an intracellular compartment resembling a late endosome. We expect these mutants will be defective in genes required for $GABA_A$ receptor assembly, or trafficking in the biosynthetic or degradation pathways. 4. Exposure to agonist. Chronic exposure to the $GABA_A$ receptor agonist muscimol causes a compensatory down-regulation of the UNC-49 receptor at the synapse. This downregulation is accompanied by the appearance of fluorescent puncta in the membrane, suggesting increased $GABA_A$ receptor internalization by clathrin-mediated endocytosis. We are determining which steps in the biosynthetic, trafficking, internalization, and degradation pathways are the important control points that govern the agonist-dependent downregulation of $GABA_A$ receptors. These studies should provide important insights into the function of the human brain because $GABA_A$ receptor biosynthesis, trafficking, internalization and degradation, and agonist-dependent downregulation are likely to take place by conserved mechanisms in the *C. elegans* and mammalian nervous systems.

909. DEG-3 LOCALIZATION REVEALS NEURONS WITH A COMPLEX MORPHOLOGY

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DEG-3 is a subunit of a nicotinic acetylcholine receptor (nAChR) that can mutate to cause neuronal degeneration. In order to gain more insight into the normal function of the DEG-3 receptor we generated antibodies against the C-term of this protein. Immunohistochemical analysis using these antibodies shows diffuse staining of neuronal processes and of cell bodies. This pattern is not the typical synaptic localization that is expected for a nAChR. Interestingly, in adults this analysis also revealed web like structures that appear to be highly branched neuronal processes. Such a complex morphology is unexpected in view of the simple, unbranched morphology of C. elegans neurons as described by White et al. To confirm this observation and further characterize these processes we generated a full length DES-2::GFP fusion. In this construct GFP is fused to the intracellular loop of DES-2, a subunit of the DEG-3 receptor that is encoded by the *deg-3* operon. Animals expressing this fusion show GFP fluorescence in the same web like structures as seen in the antibody staining, thus confirming the original staining results. Observation of these processes in wild-type and *mec-3(e1338)* animals suggests that the highly branched processes emanate from the PVD and FLP sensory neurons. Detailed characterization of these processes in adults and during development will be presented.

910. MUTATIONS THAT AFFECT SYNAPTIC LOCALIZATION OF GLR-1.

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The orderly flow of information in the nervous system requires that proteins are specifically targeted to pre- and post-synaptic specializations. We are interested in identifying the molecules that target and maintain proteins to synapses. We have used the GLR-1 AMPA type glutamate receptor as a marker for post-synaptic localization. A wild type animal expressing a GLR-1::GFP fusion exhibits punctate spots along the ventral nerve chord representing clustering of the glutamate receptor at specific synaptic termini. To identify genes involved in synaptogenesis, we are screening mutagenized GLR-1::GFP worms for defects in GLR-1 localization.

We predict classes of mutants to represent trafficking, targeting, localization and stabilization molecules necessary to initiate and maintain synaptic architecture. Approximately 7000 genomes have been clonally screened so that sterile or inviable mutants may be recovered as heterozygotes. Nine complementation groups have been isolated which define loci important for the proper localization of GLR-1 in the nervous system. Several of the loci are in genes whose identities are already known. These include mutations in unc-11, an AP180 homologue, and unc-101, a medium chain subunit of the AP-1 clathrin adaptin complex. The implication of these mutants on receptor trafficking and maintenance at synapses will be discussed.

911. A conserved mechanism of synaptogyrin localization

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Synaptogyrin is an integral membrane protein of synaptic vesicles that is conserved from worms to man. We have studied the localization of synaptogyrin family members. Both native and GFP-tagged *C. elegans* synaptogyrin (SNG-1) are expressed in neurons and synaptically localized. Deletion and mutational analysis using GFP-tagged SNG-1 has defined a 38 amino acid sequence within the C-terminus of SNG-1 and a single arginine in the cytoplasmic loop between transmembrane domain two and three that are required for SNG-1 localization. These domains may represent components of signals that target synaptogyrin for endocystosis from the plasma membrane and direct synaptogyrin to synaptic vesicles, respectively. In chimeric studies, these regions were sufficient to relocalize cellugyrin, a non-neuronal form of synaptogyrin, from non-synaptic regions such as the sensory dendrites and the cell body to synaptic sites. Furthermore, GFP-tagged rat synaptogyrin is synaptically localized in neurons of *C. elegans* and in cultured hippocampal neurons. Similarly, the C-terminal domain of rat synaptogyrin is necessary for localization in hippocampal neurons. To identify trans-acting factors that regulate targeting, we examined the localization of SNG-1 and/or GFP-tagged SNG-1 in a variety of *C. elegans* mutants, but found no disruption of localization in these mutants. Our study suggests that the mechanisms for synaptogyrin localization are likely to be conserved from *C. elegans* to vertebrates.

912. Identification of domains of the UNC-43 CaMKII that are essential for its localization within neurons.

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Cell components need to be localized not only at the right place but also at the proper concentration for their function. To examine how neurons control the localization of proteins, we are studying glutamatergic synapses in *C. elegans*. Reiner, Newton, Tian, and Thomas (1999) have shown that UNC-43 encodes a type II calcium and calmodulin-dependent protein kinase (CaMKII). In previous studies, Rongo and Kaplan (1999, 2000) showed that UNC-43/CaMKII regulates the transport of the AMPA-type glutamate receptor GLR-1 from neuron cell bodies to synapses. Nematode neurons that lack UNC-43 fail to transport GLR-1 to axons and accumulate GLR-1 in their cell bodies. UNC-43/CaMKII is also required in these neurons to maintain a high density GLR-1-containing synapses. These results suggest that UNC-43/CaMKII has at least two functional roles for regulating GLR-1 localization in neurons. To understand the mechanisms of UNC-43/CaMKII and GLR-1 localization, it is important to determine the trans-acting factors that interact with UNC-43/CaMKII.

In this study, we are trying to identify the domains of CaMKII responsible for proper localization of CaMKII and GLR-1 by mutational analysis. Several mutations that inhibit interaction with substrates of CaMKII have been reported in *in vitro* biochemical assays. Most of these mutations still maintain the kinase activity of CaMKII, except for mutations in the catalytic site. We will generate transgenes that express mutated versions of UNC-43 in interneurons under the control of the *glr-1* promoter. We will express both GFP::UNC-43 translational fusions and untagged UNC-43 cDNA in an *unc-43* null strain, and examine transgenic nematodes for the localization of UNC-43 and GLR-1. In the former case, we can check which domains are required for the accumulation of UNC-43/CaMKII in interneurons. In the latter case, we can check which domains of

UNC-43/CaMKII are important for GLR-1 localization. In the future, we plan to perform yeast two-hybrid screens using the domains identified above to identify novel trans-acting factors that interact with UNC-43/CaMKII.

913. Regulation of *unc-13* and Subcellular Localization of its Protein Products

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We are examining regulation of synaptic neurotransmission. We are studying the *unc-13* gene, which is part of a signal transduction pathway affecting neurotransmitter release. At least one product of this gene is involved in the step between synaptic vesicle docking and fusion. While most studies of UNC-13 have examined the 200 kDa form of the protein, multiple forms of UNC-13 proteins are expressed. At least three types of proteins are made from the gene, and these proteins may have different functions in cells. We are interested in understanding the roles of the different forms of UNC-13 in *C. elegans*. In particular, we are asking where the protein products localize and how protein expression is regulated. To approach these questions, we are developing antibodies to identify the subcellular localization patterns of the different UNC-13 proteins. Antibodies were made previously to recognize the 200 kDa form of the protein, but these antibodies cannot label all forms of UNC-13. We are interested in determining whether all forms of the proteins are found in neurons and whether they localize in synapses. We are also examining the possibility that an internal promoter is involved in gene regulation. We are constructing GFP fusions to determine whether an internal region of the *unc-13* gene is capable of driving transcription of a subset of *unc-13* mRNA.

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914. A screen for dominant enhancers of *rpm-1*, a gene that regulates presynaptic terminal organization

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Presynaptic terminals contain highly specialized subcellular structures to facilitate neurotransmitter release. In a presynaptic terminal, synaptic vesicles are localized in the vicinity of the active zone, an electron dense structure at the presynaptic plasma membrane, where synaptic vesicles dock and fuse. The active zone is surrounded by the periactive zone. In *Drosophila*, several synaptic proteins including Fas II and SIF are localized at the periactive zone and involved in synaptic development and growth (1).

rpm-1 mutants have abnormal presynaptic terminals at the GABAergic neuromuscular junctions (NMJs) (2). In *rpm-1* mutants, many NMJs have multiple active zones within a single presynaptic terminal. The RPM-1 protein has an RCC1-like guanine nucleotide exchange factor (GEF) domain and RING-H2 finger. RING-H2 fingers have been shown to be involved in ubiquitin mediated protein degradation. RPM-1 is most similar to the *Drosophila* presynaptic protein Highwire (HIW) and the mammalian Myc binding protein Pam. RPM-1 appears to be localized to the periactive zone of the presynaptic terminal. We hypothesize that *rpm-1* may regulate the spatial arrangement, or restrict the formation, of presynaptic structures by linking an unknown G protein signaling with protein degradation.

To identify the signaling components of *rpm-1*, we are performing a screen for dominant enhancers of a temperature sensitive *rpm-1* mutation. At 15°C, only 10% of the synaptic SNB-1::GFP marker expression in the dorsal cord of *rpm-1 (ju44)* animals are abnormal, whereas those in the ventral cord are essentially wild-type; at 25°C, 80-90% of the synaptic SNB-1::GFP marker expression in both the dorsal and ventral cords are abnormal. From screening 6,500 EMS mutagenized haploid genomes, we have isolated 11 mutants that enhance the *rpm-1 (ju44)* phenotype at 15°C. Preliminary characterization of these mutants will be presented.

(1) Sone et al. Development 2000.

(2) Zhen et al. Neuron 2000.

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915. Molecular genetic analysis of synaptogenesis in mechanosensory neurons

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In an attempt to identify proteins that regulating synapse formation, we previously described a screen for mutations that alter GFP-tagged synaptic vesicle localization in mechanosensory neurons. Mutants from this screen, designated sam (synaptic vesicle tag abnormal in mechanosensory neurons), were classified based on several phenotypic criteria. These criteria suggest that the genes (rpm-1 V, sam-3 II, sam-6 II, and sam-8 X) likely regulate aspects of synapse development in multiple distinct cell types.

rpm-1(aka sam-1) encodes an extremely large protein expressed throughout the nervous system. The primary sequence contains motifs suggesting protein-protein interactions and exchange factor activity. In C. elegans, RPM-1 is required during synapse formation, and acts cell-autonomously in presynaptic mechanosensory neurons. RPM-1 is conserved, with homologs in Drosophila and human. Biochemical functions of these homologous proteins have not yet been described, though mutations in the Drosophila homolog also expressed a synaptogenesis defect.

sam-6 and sam-8 exhibit very similar phenotypes as rpm-1. Each exhibits a variably penetrant defect in formation of GFP-tagged synaptic varicosities. Double mutant combinations between each of the three loci resemble rpm-1 mutants in severity. Progress towards molecular cloning of sam-6 and sam-8 will be described. sam-3 shows a similar defect in accumulation of SNB-GFP in the ventral nerve cord, but in addition, exhibits an accumulation of SNB-1-GFP and altered morphology of soma. In rpm-1 sam-3 double mutants the sam-3 cell body phenotype is suppressed sugggesting that rpm-1 is required for expression of sam-3 defects. Furthermore, sam-3 exhibits several other phenotypes seen in rpm-1 mutants. SAB neurons exhibit altered morphology, and the lateral process of PLM axons often over grow and desend into the ventral nerve cord. Our current working hypothesis is that sam-3 regulates rpm-1 activity or localization. sam-3 has been mapped to a 0.2 map unit interval of II.

916

916. Are input and output defects linked in *unc-4* mutants?

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The UNC-4 homeoprotein interacts with its Groucho like co-factor UNC-37 to repress transcription. *unc-4* and *unc-37* mutants are defective in backward locomotion. This defect has been attributed to miswiring of synaptic inputs from command interneurons to VA class motor neurons. UNC-4 and UNC-37 are also required for maintaining normal levels of synaptic vesicles (SVs) in VA motor neurons (Lickteig et al., 2001, *J. Neurosci.* 21, 2001-2014). Thus, *unc-4* and *unc-37* mutations affect both the specificity of inputs to VA motor neurons as well as the strength of their outputs onto muscle cells. Our research is examining whether there is a causal relationship between the miswiring event and the decreased levels of SVs in *unc-4* and *unc-37* mutants. For example, tetanus-toxin induced cleavage of the SV protein, Synaptobrevin, in *Drosophila* neurons alters presynaptic inputs to the affected cells (Baines et al., 1999, *Current Biology* 9, 1267-1270). Our preliminary data indicate, however, that neither decreased activity of Choline acetyltransferase (ChAT), in the temperature sensitive allele *cha-1*(*y226)*, nor reduced function of Synaptobrevin (SNB-1) lead to permanent movement defects similar to that of *unc-4* and *unc-37* mutants. This finding suggests that a decrease in the activity of at least these two SV-associated proteins is not sufficient to alter presynaptic inputs to VA motor neurons. We are currently using transgenic animals that express human caspase (Y. Zheng et al.,1999, *Neuron* 24:347-361) to ablate the command interneurons in order to determine if the failure to receive proper synaptic inputs leads to decreased numbers of SVs in VA motor neurons. Finally, we have exploited the negative effect of *unc-4* mutations on SV protein levels to devise a genetic screen for mutations in UNC-4 target genes. We are using the *unc-4* promoter to drive expression of GFP-tagged SNB-1. In an *unc-4* or *unc-37* mutant background, SNB-1::GFP is diminished especially in the VC motor neurons that synapse with the vulva. We hypothesize that UNC-4 and UNC-37 are acting to repress a negative regulator of synaptic vesicle stability or assembly. Thus, mutations in such a gene should restore normal levels of SNB-1::GFP to VC synapses in *unc-4* and *unc-37* mutants. The goal of our screen is to identify UNC-4 target genes in order to understand the mechanism by which UNC-4 affects SV number and possibly synaptic choice.

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917. Examination of DD remodeling in Wnt signaling mutants

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Neuronal dendritic-axonal asymmetry, once established, is normally stabilized in order to maintain information flow. However, polarity changes can occur in response to developmental changes or in culture. The six *C. elegans* GABAergic DD motoneurons remodel their synaptic connectivity patterns as the worm matures from the L1 to the L2 stage. (1) In L1 worms these DD motoneurons form neuromuscular junctions (NMJs) to ventral muscles. In the L2 to adult stage the NMJs are reformed onto dorsal muscles without an alteration in the morphology of these motoneurons.

A recent study in the mouse CNS has shown that cerebellar granule cells secrete factors that induce axon and growth cone remodeling in mossy fibers. (2) This effect is blocked by a Wnt antagonist and mimicked by Wnt7a which is expressed by granule cells. To investigate whether DD remodeling may employ Wnt signaling, we are examining the effect of various existing Wnt signaling mutants. We have used the *Punc-25* driven SNB-1::GFP marker to visualize the presynaptic pattern of DD neurons. (3) Preliminary results reveal that four maternal effect Wnt signaling mutants, *mom-2, mom-4, lit-1 and pop-1* do not have a dominant effect on DD remodeling. We are currently examining the zygotic effect of these mutants. The zygotic Wnt signaling mutants *egl-20* and *lin-17* do not have any effect on DD remodeling. In *egl-27* mutants however, DD motoneurons appear to form synapses to dorsal muscles in the L1 stage. We are further investigating whether this effect may be due to a cell fate alteration.

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918. Visualizing synapses in the motor neuron circuit

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A major emphasis of this laboratory is to unravel the mechanism whereby the UNC-4 homeoprotein regulates synaptic specificity in the motor neuron circuit. In unc-4(e120) mutants, the usual inputs to VA motor neurons from AVA, AVD, and AVE command interneurons are replaced with gap junctions from AVB, which are normally reserved for VB motor neurons. The unc-4 wiring defect was initially detected by John White and colleagues by EM reconstruction of serial sections. We are now developing an alternative approach to detect motor neuron-specific synapses in living animals in the confocal microscope. The idea is to label presynaptic and postsynaptic proteins with different colored GFPs and then to use specific promoters to drive expression of these GFP-tagged markers in the command interneurons and in their postsynaptic motor neuron partners. The Zeiss LSM 510 confocal microscope provides argon laser lines that are ideal for discrete excitation of CFP (458 nm) and YFP (514 nm). An authentic synapse in the C. elegans motor neuron circuit labeled in this manner should appear as superimposed CFP and YFP "spots" in the ventral nerve cord.

We have constructed YFP-tagged synaptobrevin (SNB-1), a membrane component of neurotransmitter vesicles, and used the nmr-1 promoter to drive expression in AVA and AVD command interneurons. Animals expressing the nmr-1-SNB-1-YFP transgene show YFP puncta in the ventral nerve cord. We are now testing candidate proteins for marking the postsynaptic membranes in motor neurons. The vertebrate protein spinophilin (aka, neurabin II) interacts with actin microfilaments and is concentrated at the postsynaptic membrane where it is believed to tether Protein Phosphatase I (PP1) for modulation of glutamate receptor activity. Neurabin II is also more widely expressed in vertebrate tissues and may interact with additional classes of cytoplasmic proteins and receptors at the cell surface. A related vertebrate homolog, neurabin I, is largely expressed in the brain. C. elegans contains a single neurabin-like gene, nab-1 [C43E11.6, P = 5 x e-49]. We fused GFP to the NAB-1 C-terminus at a site that does not perturb localization of spinophilin in mammalian neurons. Transgenic animals expressing GFP-tagged NAB-1 under the control of nab-1 gene regulatory sequences show punctate staining in the nerve ring and in axial nerve cords, a pattern that is strikingly similar to that of other GFP-tagged synaptic proteins. We also see transient GFP-localization to hypodermal cells midway through embryogenesis. The goal now is to determine if NAB-1 localizes to the motor neuron postsynaptic membrane and then to confirm that a GFP-tagged version of NAB-1 can be used to score specific synapses between command interneurons and motor neurons in the ventral nerve cord.

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919. In search of UNC-4 target genes, mediators of synaptic specificity.

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Mutations in the UNC-4 homeodomain transcription factor disrupt backward locomotion. In *unc-4* mutants, VA motor neurons fail to receive synapses from their usual interneuron partners and instead accept inputs normally reserved for their lineal sisters, the VB motor neurons. Work done in this laboratory has shown that UNC-4 and the Groucho homolog UNC-37 function together in VA motor neurons to repress VB-specific genes. GFP reporters constructed from two genes, *del-1* (DEG/ENaC sodium channel subunit) and *acr-5* (α-like nicotinic acetylcholine receptor subunit), are ectopically expressed in the VA motor neurons in *unc-4* and *unc-37* mutants. As cell surface proteins and ion channel components, ACR-5 and DEL-1 are attractive candidates for mediators of neuron-specific synapses.

We have now performed genetic experiments, however, that rule out a **necessary** role for either ACR-5 or DEL-1 in the specification of VB-type inputs. Deletion mutants of *del-1* and *acr-5*, as well as the double mutant (*acr-5;del-1*), do not perturb forward locomotion as would be expected for a mutation which disrupts normal inputs to the VBs. Assays designed to quantitate locomotion have detected no differences between wildtype worms and these mutants. In addition, placement of *acr-5* and *del-1* mutations in an *unc-4* background does not suppress the Unc-4 phenotype, suggesting that ACR-5 and DEL-1 are also not required to promote VB-type inputs in VA motor neurons.

Although *acr-5* and *del-1* are not determinants of synaptic specificity, they are regulated by UNC-4 and are therefore likely to contain UNC-4 Response Elements (U4REs) in their promoter regions that are also located in other UNC-4 target genes. A 1.8 kb upstream region of the *C. elegans* (Ce) *del-1* gene contains four subregions that are conserved in the promoter of the *C. briggsae* (Cb) *del-1* gene. These motifs will be targeted for deletion and their effects on *del-1* expression will be determined; removal of an U4RE should result in ectopic expression in VA motor neurons in wildtype animals. A comparison of the Ce and Cb *acr-5* upstream regions has not identified conserved sequences. However, deletion analysis of the Ce *acr-5* promoter has revealed a modular structure that segregates the U4RE region from sites that are likely to respond to other classes of transcription factors. The 4.2 kb *acr-5* promoter is expressed in head and tail neurons as well as in DB/VB motor neurons in the ventral nerve cord (VNC). We have found that a 1 kb segment of this region is sufficient to drive expression in only the VNC and is regulated by *unc-4*. Further deletion analysis of these regions should reveal specific sequence subdomains that can then be tested for direct UNC-4 binding. We plan to use the *C. elegans* microarray to detect genes that are de-repressed in *unc-4* and *unc-37* mutants. These candidate UNC-4 target genes will be scanned for U4RE sequences and then tested for expression and *unc-4* regulation *in vivo*.

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920. The Role of Rapsyn in *C elegans*

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Nicotinic acetylcholine receptors(nAChRs) are clustered at high density in the postsynaptic membrane of neuromuscular junction. Rapsyn(achR Associated protein at SYNapse) is a peripheral membrane protein that is required for AChR clustering at the neuromuscular junction. Rapsyn is conserved in many species including C. elegans. To understand rapsyn-mediated AChR clustering at the neuromuscular junction, we have examined various constructs of rapsyn fused to green fluorescent protein in transgenic animals and mutants. Rapsyn mutants(MN1581rap[ok148]) showed smaller brood size and slower growth rate compared to wild type worms. In order to identify the function of rapsyn in worms, we have co-injected rapsyn-GFP constructs with acetylcholine receptor-GFP into N2 and, rapsyn mutant animals. When AChR-GFP alone was microinjected into rapsyn mutants, GFP expression was detected in the embryo but only lasted until the L1 stage. When AChR-GFP was injected together with wild type rapsyn, AChR-GFP expression lasted until adulthood. In rapsyn mutants bearing AChR-GFP and dominant negative rapsyn in which histidine residues of zinc finger motif were substituted by glutamine, GFP expression of AChR was not detected. These results suggest that rapsyn plays a critical role in acetylcholine receptor(AChR) clustering and that the zinc finger motif is essential for this mechanism. To further analyze the motifs of rapsyn, we have co-injected various rapsyn-LacZ constructs with AChR-GFP into wild type worms and double-stained the transgenic animals. The results of double-staining showed abnormal of acetylcholine receptor clustering at the neuromuscular junction in transgenic animals containing mutant rapsyn forms. Examination of the transgenic animals bearing either wild type rapsyn or dominant negative mutant rapsyn in the genetic background of unc-29(AChR mutant), and ric-4(md1088) showed that both the functional presynaptic activity and functional receptors are required for normal localization and function of rapsyn. We will present results from examination of various deletion derivatives of rapsyn. We are currently dissecting the cis-acting regulatory element required for expressing rapsyn specifically in the muscles and neurons. We will also present data from this experiment.

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921. Yet another proteins that contribute to a periodicity of posterior body wall muscle contractions (pBoc) in *C. elegans*.

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Several groups of muscles in *C. elegans* exhibit continuous rhythmic activity throughout the life span of the animal. This motor activity supports a constant search for food and its processing, which is vital for survival. This rhythmic behavior exemplifies an ultradian cycle also observed in other species. In humans, an ultradian clock controls heartbeat and breathing, among other processes. The most studied, but yet incompletelyunderstood rhythmic behavior in *C. elegans* is the defecation motor program. It consists of two sequential longitudinal contractions of the body, followed by expulsion of the gut content. It takes 50 seconds on average to repeat each cycle. It was shown that an inositol trisphosphate (IP3) receptor expressed in intestinal cells regulates the motor program, through triggering calcium oscillation (1).

We report three proteins that function in intestinal cells and may contribute to the periodicity of posterior body wall muscle contraction (pBoc), the first step in the defecation cycle. F11E6.5 was identified as a predicted protein structurally similar to fatty acid (FA) elongases of the GNS1/SUR4 gene family. We showed that elimination of F11E6.5 activity significantly alters the FA composition of lipids in worms. These changes correlate with shorter (37.1 +/- 6.3 sec) pBoc intervals as compared to wild type N2. We hypothesized that F11E6.5 may contribute to pBoc cycle through regulation of IP3 production, dependent on FA composition of phosphoinositides (2). We also showed that F11E6.5 RNAi may compensate for *unc-43* and *dec-11* pBoc mutant phenotypes. In addition, we showed that a RNAi- mediated loss of F11E6.5 function is associated with a reduced body size and a pale intestine, suggestive of additional defects in nutrient storage.

In contrast to the F11E6.5 RNAi phenotype, interference with voltage-gated potassium channel genes, *kqt-2* and *kqt-3*, led to long and irregular pBoc cycles (see also 3). This suggested that suppression of these potassium channels, compromised a signal transduction event either up- or downstream of the time-keeper. This may be through altering the membrane potential or structural properties of the membrane. We showed that *kqt-2* and *kqt-3* RNAi phenotypes are dominant in a *flr-1* (*lf*) background, resulting in worms that displayed long irregular pBoc intervals. Based on our observation that *kqt-2* and *kqt-3* RNAi phenotypes in L3 animals are wild type, we hypothesized that the mechanism controlling pBoc in *C. elegans* is flexible and stage-dependent.

1. Dal Santo et al, (1999). Cell, vol.98, 757-767.

2. Carricaburu and Fournier, (2001). Eur. J.Biochem. 268, 1238-1249.

3. Wei et al., (2001). Abstract. Thirteenth International *C. elegans* Meeting.

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922. Genetic and Phenotypic Analysis of Defecation in *clk-1* Mutants.

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Mutations in the maternal-effect gene *clk-1* affect developmental, cellular, and behavioral timing. They result in a mean lengthening of embryonic and post-embryonic development, the cell cycle period, and lifespan as well as the defecation, swimming, and pharyngeal pumping cycle periods (1, 2). We have been carrying out a detailed genetic and phenotypic analysis of the slow defecation behavior of *clk-1* mutants. We have found that when wild-type worms are grown at 20°C and are then shifted to a new temperature, the defecation cycle length is strongly affected by that new temperature. In contrast, when *clk-1* mutants are shifted, the defecation cycle length is the same at the new temperature as it is at 20°C. The absence of *clk-1* activity, thus reveals an underlying physiological mechanism that maintains a constant defecation cycle length in the face of changes in temperature. *clk-1*, on the other hand, appears to be necessary for re-adjusting the defecation period in response to changes in temperature.

We reasoned that if *clk-1* is actively involved in regulating the timing of the defecation cycle it might work through other gene products. In order to identify genes that are required for defecation to be slow in *clk-1* mutants, we carried out a screen for suppressor mutations. We identified 8 mutations that correspond to 5 different complementation groups, which we call *dsc* genes (for defecation suppressor of *clk-1*). These suppressors fall into 2 distinct classes. One class of mutants suppresses the slow defection as well as the inability of *clk-1* mutants to react to changes in temperature, such that they suppress *clk-1* at 20°C and after a switch to a new temperature. In contrast, the other class of mutants cannot overcome the temperature insensitivity, such that although they can suppress at 20°C, they cannot, or can only poorly, suppress after a switch to a new temperature.

We will present phenotypic analysis of the *dsc* mutants, and genetic interactions among the *dsc* genes, and will discuss possible models for the action of the *dsc* genes and their interactions with *clk-1*.

1. Wong et al, 1995. Genetics 139:1247-1259.

2. Branicky et al, 2000. BioEssays 22:48-56.

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923. Genetic Analysis of the Ultradian Defecation Rhythm of *C. elegans*

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Biological rhythms are widespread in nature and regulate processes such as vertebrate and invertebrate heartbeats, and invertebrate swimming and gut peristalsis. Such rhythms are called ultradian since they have a period of less than 24 hours. The defecation cycle of *C. elegans* is an ultradian rhythm which is amenable to genetic analysis. Every 45 to 50 seconds, wild type *C. elegans* initiates a series of muscle contractions known as the defecation motor program. The first muscle contraction is the pBoc, or posterior body-wall muscle contraction. About three seconds later, the anterior body-wall muscles contract (aBoc), immediately followed by the enteric muscle contraction, which expels gut contents. Mutants have been identified that are lacking one or more of these steps; however, none of these have a large effect on cycle period. This indicates that the mechanism controlling the timing of the contractions is distinct from the motor program itself. The defecation rhythm shares several characteristics with other defined molecular clocks. First, it exhibits temperature compensation over a range of temperatures between 19-30 degrees Celsius. Second, it can be reset by an external stimulus. Specifically, light touch with an eyelash to the head region resets the phase of the clock. Finally, the clock can keep time in the absence of the defecation motor program. When wild type worms spontaneously leave a lawn of food, the contractions of the motor program cease, and on return to food the next defecation tends to be in phase with the original cycle (Liu and Thomas, 1994). Thirteen genes have been identified that can mutate to alter the length of the cycle. One of these Dec genes, *itr-1*, has been shown to encode the only inositol trisphosphate receptor in *C. elegans*. Loss-of-function mutations in *itr-1* cause the cycle period to be lengthened or eliminated, while overexpression of *itr-1* produces a short period. In addition, mosaic analysis has shown that ITR-1 function in intestinal cells is necessary and sufficient for the defecation rhythm (DalSanto *et al*, 1999). Four other Dec genes have been cloned: *unc-43* encodes the only CaMKII in *C. elegans* (Reiner *et al*, 1999), flr-1 encodes a DEG/ENaC sodium channel, and *flr-3* and *flr-4* share homology with protein kinases (Take-Uchi *et al*, 1998). The molecular identification of other Dec genes may provide more information regarding the signaling pathways that control the timing of the clock. In addition, better characterization of the phenotypes and gene interactions among the Dec genes may allow inference of formal genetic pathways that regulate the clock. I have begun work towards identifying *dec-2*. *dec-2*(sa89) causes a defecation cycle period of ~100 seconds. I am also analyzing Dec mutant responses to light touch and reduced concentrations of food and constructing *dec-2* double mutants for gene interaction studies. Current progress on the cloning of *dec-2* and the phenotypic analysis will be presented.

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Liu DW, Thomas JH. (1994) Regulation of a periodic motor program in *C. elegans*. J Neurosci 14: 1953-62.

Reiner DJ, Newton EM, Tian H, Thomas JH. (1999) Diverse behavioural defects caused by mutations in *C. elegans unc-43* CaM kinase II. Nature 402: 199-203.

Take-Uchi M, Kawakami M, Ishihara T, Amano T, Kondo K, Katsura I. (1998) An ion channel of the degenerin/epithelial sodium channel superfamily controls the defecation rhythm in *C. elegans*. Proc Natl Acad Sci USA 95: 11775-80.

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924. Probing the Go-Gq Signaling Network

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Synaptic transmission is the process by which neurons communicate with each other, or with other cells, at specialized structures known as synapses. During synaptic transmission, synaptic vesicles fuse with the presynaptic plasma membrane and release neurotransmitter, which then diffuses across a narrow extracellular space and activates postsynaptic receptors on another cell. The amount of synaptic transmission that occurs at synapses is likely to be highly regulated, and understanding this regulation may ultimately provide insights into how the nervous system establishes, maintains, and modifies behavior. With this goal in mind, we are investigating the signal transduction pathways that regulate neurotransmitter secretion.

Genetic studies in *C. elegans* have revealed a network of signaling proteins, centered around GOA-1 (Goα) and EGL-30 (Gqα), that appears to regulate synaptic transmission by controlling the production and consumption of diacylglycerol. Mutations that decrease or inhibit EGL-30 signaling cause phenotypes consistent with reduced synaptic transmission (aldicarb resistance, decreased rates of locomotion and egg laying), while mutations that increase or potentiate EGL-30 signaling cause phenotypes consistent with too much synaptic transmission (aldicarb hypersensitivity, increased rates of locomotion and egg laying).

Current models of the Go-Gq signaling network suggest that its complexity may extend beyond the known components. We are performing two kinds of genetic screens designed to identify additional proteins that may be important for proper function or regulation of the network. In the first screen we are looking for mutations that suppress the nearly paralyzed locomotion phenotype of *ric-8(md303)* mutants. RIC-8 (Synembryn) is a novel, conserved protein that appears to function upstream of EGL-30 in the Go-Gq network. The upstream role of RIC-8 provides the opportunity to identify bypass suppressors, which would appear as loss of function mutations in downstream negative regulators, such as *dgk-1* mutants, which we have previously identified as *ric-8* suppressors. In addition, since *ric-8(md303)* is a missense mutation, the suppressor screen could also reveal a protein that directly interacts with RIC-8. Using new screening methods, not used in a previous pilot screen, we have thus far identified 20 strong and 9 weak suppressor mutants among 15,000 EMS mutagenized genomes. One of the strong suppressor genes is defined by a single rare, dominant, extragenic mutation. This gene is a good candidate for encoding a protein that directly interacts with RIC-8.

The second way we are probing the Go-Gq signaling network is by screening for additional mutants that exhibit the hyperactive locomotion phenotype caused by excessive EGL-30 signaling. This screen is important because it puts no restrictions on where within the network the mutation must occur. From 9000 EMS mutagenized genomes screened so far, we have identified 46 hyperactive mutants. Some of the new genes identified in this screen may encode other negative regulators of EGL-30 signaling, positive regulators of GOA-1, or components of interacting pathways that regulate neurotransmitter secretion.

925. CaM-K signal cascade of *C. elegans* functions*in vivo*

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Ca^{2+}/CaM protein kinases (CaM-K) have significant roles for Ca^{2+}-dependent signal transduction. CaM-K-mediated signal cascade consists of the upstream CaM-kinase kinase (CaM-KK) and downstream CaM kinase I (CaM-KI) and CaM-kinase IV (CaM-KIV). A transcription factor cAMP-responsive element binding protein (CREB) is known to be activated by phosphorylation with downstream CaM-KIV.

In this study, we identified *C. elegans* orthologues of mammalian CaM-KK *(ckk-1*), CaM-KI (*cmk-1*) and CREB, and found that CaM-KK-CaM-KI-CREB cascade is conserved and operated both *in vitro* and in transfected cells. Furthermore, we investigated the physiological functions through this signal cascade by genetical approaches. The distribution of activated CREB was analyzed by using the transgenic worms that carries *4XCRE::gfp* fusion gene. GFP fluorescence cannot be seen in normal development, however, transgenic worms carrying constitutive-active form of *cmk-1* induces GFP-expression in small number of sensory neurons in head and tail. On the other hand, wild type and mutant *cmk-1* at T179A did not show any CREB activation, suggesting the requirement of Ca^{2+} mobilization and the upstream kinase which was identified as CaM-KK . In addition, CREB-deficeient worms show no GFP fluorescence reasonably. Interestingly, when the worms were maintained in starved condition, the number of GFP-positive neurons increased and the intensity of GFP fluorescence was dramatically enhanced. These results suggest that CaM-KK-CaM-KI-CREB cascade of *C. elegans* is conserved *in vivo* in the some sensory neurons and activated by the circumstantial stimuli.

926. Guanylate Cyclase Beta 2 Homologues in *C. elegans*

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Soluble guanylate cyclases (sGCs) catalyze the conversion of GTP to cGMP. In mammals, the most well-studied sGC is a heme-containing, heterodimer, composed of α1 and β1 subunits. Nitric oxide (NO) binds to the heme, which is ligated to the β1 subunit, and stimulates the activity of the enzyme 400-fold. A β1 homologue, β2, has been cloned from rat and human kidney cDNA libraries. This subunit appears to be expressed in the kidney collecting duct cells, but its heme binding status, sensitivity to NO, and intracellular localization are unknown. We want to elucidate the structure and function of this protein.

Analysis of the *C. elegans* genome predicted seven putative sGC β subunits, including five β2 homologues, (*gcy-32, gcy-34*, *gcy-35, gcy-36,* and *gcy-37*). We cloned these five homologues, by RT-PCR and RACE, and found that each of them contains the residues necessary for GTP binding, catalysis and heme binding. These findings suggest that the β2 homologues in *C. elegans* are receptors in a cGMP-dependent signaling system.

Our data are consistent with this hypothesis. Preliminary characterization of the bacterially-expressed N-terminus from *gcy-35* suggests that it can bind heme. Promoter::green fluorescent protein (GFP) fusion studies localize all of the β2 homologues to four candidate sensory neurons (URXL, URXR, AQR and

PQR). These neurons are all connected to the pseudocoelom, and might play a role in fluid homeostasis. This possibility is consistent with data demonstrating that their mammalian homologue, β2, is expressed in the kidney. We are now using transgenes to express Egl-1 in these four candidate neurons, so that we can eliminate them and elucidate their functions.

Does NO activate β2 and its homologues? Analysis of the *C. elegans* genome did not reveal an open reading frame for nitric oxide synthase (NOS). Furthermore, we have identified GC activity in *C. elegans* lysates and shown that this activity is NO-insensitive. These results suggest that β2 and its homologues define a novel class of NO-insensitive cyclases. We are using biochemical techniques to examine heme-binding and ligand specificity in the β2 homologues.

927. *pdl-1*: A gene involved in nervous system development?

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One major focus in our lab is to elucidate the role of *unc-119* in nervous system development. Through the course of this study we became interested in the predicted gene *pdl-1* (phosphodiesterase 6 delta like), which shows weak similarity to *unc-119*. A pdl-1 promoter::GFP fusion shows pan-neuronal expression beginning early in embryogenesis and maintained throughout the life of the worm. This suggests that *pdl-1*, like *unc-119*, is involved in nervous system development or function so we have decided to characterize this gene further. Additionally, *pdl-1* is highly conserved exhibiting 85% similarity to the mammalian protein PDE6D (rod cGMP phosphodiesterase delta). PDE6D is widely expressed in mammalian tissue with enrichment in the retina and appears to play a regulatory role through its interactions with other proteins. Specifically, PDE6D binding affects the solubility and/or nucleotide binding properties of the proteins with which it interacts. These include rod cGMP phosphodiesterase (PDE6), Arl3, and Rab13. Interestingly, *C. elegans* PDL-1 is able to bind and solubilize mammalian PDE indistinguishably from PDE6D[1], suggesting that the function of these proteins has been conserved.

To determine the function of *pdl-1* we are attempting to determine a knockout phenotype as well as studying its expression and protein interactions.

i. Knockout Phenotype
Deletion screening is ongoing through the *C. elegans* Knockout Consortium. We have made several attempts at RNAi, but so far we have been unable to knock out expression of pdl-1 in the nervous system. We have also looked for uncloned mutations in the region of pdl-1 which seem like possible candidates. Neither *dyf-13* nor *egl-42* is rescued by *pdl-1*.

ii. Expression
Northern analysis has confirmed that *pdl-1* is expressed as a single transcript of predicted size. Transcriptional and translational GFP fusions show expression throughout the nervous system with additional expression in pharyngeal and vulval muscle. As well, we are in the process of purifying antibodies which will allow us to determine the subcellular localization of PDL-1.

iii. Protein Interactions
We have performed a yeast 2-hybrid screen using PDL-1 as bait. We have identified six putative positives, two of which show interaction with mammalian PDE6D. Further characterization of these interactions, including domain analysis and CoIp, is underway.

1. Li and Baehr (1998). FEBS Letters. 440: 454-457.

928. A genetic analysis of *unc-119*

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Our understanding of what UNC-119 is doing in the worm is limited because this founding member of a novel family (with homologs in flies, fish and mammals) has no recognizable domains and only one conserved putative phosporylation motif. While a molecular analysis has proven tantalizing, genetic screens for conditional mutants or suppressors have met with mixed success.

Recently, in an unrelated screen using *unc-119* as a genetic marker, the Chamberlain lab identified several mutations that are synthetic lethal in an *unc-119* homozygous background. An update on our efforts to identify the genes involved will be presented as will an examination of the nervous system structure in affected animals.

Unc-119 mutants have a variety of neurite outgrowth defects including both ventral and dorsal elongation defects within the nerve ring. Examination of young animals suggests that fully-elongated but misguided axons are initially extended but are then retracted when their normal synaptic partners are not contacted. Retraction is followed by supernumerary branch formation. A comparison of the nerve ring defects in several other mutants places the *unc-119* phenotype in a unique category.

Defects in the anteroposterior position of commissures suggests a polarity defect in sprouting of the initial axon. However, there are no circumferential pathfinding defects in motor neurons, similar to those seen in *unc-6* , *unc-5* or *unc-40* mutants. Indeed, although some cell bodies are misplaced in *unc-119* mutants, their axons are often correctly targeted, implying that turning at choice points is somewhat independent of other pathfinding mechanisms.

Localized expression of an UNC-119::GFP fusion protein in neural subsets rescues the neural defects only in the cells in which it is expressed. Ectopic expression in muscles does not rescue any structural or behavioral defects. Thus UNC-119 acts cell-autonomously. The rat homologue, RRG4, has been shown to be associated with synaptic vesicles of photoreceptor ribbon synapses. We have raised antibodies to amino, middle and carboxyl portions of UNC-119 and will describe localization of the protein the worm.

929. Neuronal Defects Elicited by Ectopic Expression of *C. elegans* Kallmann Protein

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The Kallmann Syndrome gene, *KAL-1*, codes for a cell surface protein containing a protease inhibitor domain and three FNIII repeats. Mutations in this gene cause defects in axon targeting in the olfactory system of humans. The *C. elegans* ortholog, *Cekal-1*, shows high structural homology to human *KAL-1* and is expressed in a subset of *C. elegans* neurons as well as in the excretory canal and uterine lumen (1). To investigate potential effects of *Cekal-1* on the nervous system of *C. elegans*, we ectopically expressed the gene under a variety of neuron specific promoters.

Ectopic expression of CeKAL-1 in either the AFD thermosensory neuron or the AIY interneuron causes a cell-autonomous, highly penetrant, protein-specific neurite sprouting defect (1). A modifier screen of the sprouting defect in AIY yielded seven modifier mutants in five complementation groups; these mutants either suppressed or qualitatively enhanced the sprouting phenotype and only modified neurites generated by CeKAL-1 (1). We are currently testing whether the modifier mutants of the AIY sprouting defect are also capable of modifying the AFD sprouting defect.

To determine the extent of cellular responsiveness to ectopic CeKAL-1, we expressed CeKAL-1 pan-neurally using the promoter of *unc-119*, a novel pan-neural gene. We obtained four integrated lines and investigated these for neuronal and non-neuronal defects. The most striking non-neuronal defect exhibited is the vab (variably abnormal) phenotype, suggesting defective ventral enclosure of the hypodermis during embryogenesis. Consistent with this, we see embryonic expression of CeKAL-1 at the time of ventral enclosure in cells that appear to be neuroblasts. To investigate for neuronal defects in the lines in which CeKAL-1 is pan-neurally expressed, we systematically constructed doubles with a variety of

neuron-specific GFP reporters.

In the pan-neural CeKal-1 lines, we observe a highly penetrant (50%) severe short stop phenotype in the AIY interneuron, a phenotype notably distinct from the sprouting phenotype that results from cell-autonomous expression of CeKAL-1 in AIY. A double mutant expressing CeKAL-1 both in AIY and pan-neurally shows both the short stop and sprouting phenotypes, indicating that pan-neural expression of CeKAL-1 does not interfere with its cell-autonomous effect in this case.

In the pan-neural CeKAL-1 lines we also observe, in addition to several low penetrance (less than 10%) axon pathfinding defects, a striking and moderately penetrant (15-25%) axon pathfinding defect in the amphid sensory neurons. Whereas the axons of these neurons normally enter the nerve ring through the amphid commissure, in the pan-neural CeKAL-1 lines, DiI filling reveals altered pathfinding: These axons, as a fascicle, sometimes wander in any direction or more frequently enter the nerve ring directly via a lateral route. We note that this phenotype is unlikely to result from a general defect in dorsal to ventral pathfinding since dorsally to ventrally directed processes of the touch neurons show wild type routing. Visualization of this lateral entry phenotype using a GFP reporter specific for AFD reveals that the axons of AFDL and R appear to successfully meet in the nerve ring even when one or both partner takes a lateral entry route. We also see that this axon pathfinding defect is frequently associated with ectopic neurites originating at the neuronal cell bodies.

We note that in *sax-3(ky200)* and *unc-6(ev400)* mutants the amphid commisure axons also take a lateral route into the nerve ring (2), and we are investigating whether the pan-neural CeKAL-1 lateral entry phenotype may be dependent on ROBO or netrin.

1. Bülow, H., Berry, K., Zhu, J., and Hobert, O. Worm Breeders Gazette16(4): 28 (Oct. 1, 2000)
2. Zallen, J., Kirch, S., and Bargmann, C. (1999) *Development*, 126:3679-3692.

930. *C. elegans rac* GENES CONTROL AXON GUIDANCE, CELL MIGRATION, AND CELL-CORPSE ENGULFMENT

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Rac GTPases control cell polarity via regulation of the actin cytoskeleton. The pathways and developmental events controlled by Rac genes remain largely unresolved. There are three *C. elegans rac* genes, *ced-10*, *mig-2*, and *rac-2*. *ced-10* is necessary for the engulfment of cell corpses generated by programmed cell death, *mig-2* has a role in Q-cell migration, and *rac-2* has no previously ascribed function. *C. elegans* Rac regulatory proteins have been identified: CED-10 is regulated by CED-2 CrkII and CED-5 DOCK180 for phagocytosis, and UNC-73 Trio is a guanine nucleotide exchange factor that can act on Rac GTPases. To determine the developmental roles of and the pathways that control the three *C. elegans rac* genes, we examined axon guidance, cell migration, and engulfment in animals deficient for the function of one or more of the *rac* genes and/or Rac regulatory genes.

We found that *ced-10*, *mig-2*, and *rac-2* function redundantly to control axon guidance, as determined using ultrastructural studies and cell type-specific GFP reporters. Perturbing the function of one *rac* gene led to no defects in axon guidance, whereas perturbing the functions of any two *rac* genes did. All three *rac* genes appear to interact with *unc-73* for axon guidance. *ced-10; mig-2* double mutants showed severe defects in axon guidance, but *ced-2; mig-2* and *ced-5; mig-2* double mutants did not. Thus, *ced-10* acts independently from *ced-2* and *ced-5* for axon guidance, indicating that Racs can be regulated by different proteins for the control of different developmental events. *ced-10*, *mig-2*, and *rac-2* act redundantly, and together with *unc-73*, to control CAN cell migration; by contrast, *ced-10* and *mig-2* but not

rac-2 are each necessary for distal tip cell migration, indicating that the *rac* genes are redundant in some cell types and individually required in others. *ced-10* is the primary *rac* gene controlling the engulfment of cell deaths, with *mig-2* and *rac-2* having subtle roles only detected in genetically-sensitized backgrounds. *unc-73* had no detectable role in engulfment.

Our findings indicate that Rac signaling pathways are key integrators of cell polarity cues for multiple developmental processes and that distinct regulatory proteins modulate Rac activation and function in different developmental processes.

931. *In Vivo* Imaging of HSN Growth Cones

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A growth cone at the leading edge of a developing neuron responds to environmental cues to spatially direct growth by regulating the underlying cytoskeleton. We are studying the development of the HSN motorneuron in *C. elegans* using time-lapse confocal microscopy, as pioneered by Knobel *et al.* for VD motorneurons. This approach allows us to combine *in vivo* imaging with genetics so that we can begin to understand the extracellular and intracellular factors that control axon guidance in worms. Using an *unc-86* transgene to drive expression of soluble GFP, we have developed a detailed description of growth cone dynamics in HSN during its development.

The cell body of HSN is located in a lateral position slightly posterior to the vulva and has a single ventral process that fasciculates onto the ventral nerve cord and grows anteriorly to the nerve ring. In wild-type worms, HSN begins to extend filopodia and lamellipodia ventrally in the late L2 stage. Ventral growth of the lamellopod continues until it is impeded by the muscle attachment sites between the ventral body wall muscles and the hypodermis. After stalling for approximately 2-4 hours, HSN sends multiple processes through these attachment sites toward the ventral nerve cord. These processes also extend filopodia in all directions. Eventually only one of these processes is stabilized and becomes the axon, while the others retract. The stalling/outgrowth/retraction sequence is similar to what Knobel *et al.* have described for the VD motorneurons. By early L4, ventral growth is complete and the growth cone has begun its anterior trajectory.

HSN ventral guidance is controlled by attraction to a ventral source of UNC-6/netrin and by repulsion from a dorsal source of SLIT. In *unc-6* and *unc-40* null mutants, HSN lacks the ventral component of axon outgrowth and instead grows anteriorly along the lateral hypodermis. We are currently asking when this defect arises. We are also generating other markers to visualize the cytoskeleton and the leading edge of growth cones.

Knobel KM, Jorgensen EM, Bastiani MJ. (1999) *Development*. 126:4489-98.

932. A GENETIC SCREEN FOR DA/DB AXON GUIDANCE MUTANTS IN C. ELEGANS

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In order to identify novel factors involved in axonal guidance, we have taken a genetic approach and used C. elegans to identify mutant animals that have defects in axonal projections in a subset of motoneurons which normally project their axons dorsally, the DA and DB neurons. These axons are already known to be repelled by the netrin UNC-6 (Ishii et al., Neuron 9:873-881, 1992), and also require the TGF-beta family member UNC-129 derived from dorsal muscle for proper guidance (Colavita et al., Science 281:706-709, 1998). These neurons actually also express unc-129, so that their axons can be visualized by driving GFP expression from the unc-129 promoter. We have mutagenized unc-129::gfp worms with EMS and screened for mutants that have defects in projections of DA and DB axons. Reasoning that most of severely uncoordinated mutants with global axon guidance defects might have been isolated in previous screens, we focused our screen on mutants with minimal uncoordinated phenotypes and less severe axon guidance defects. We have isolated mutants in this screen, and named these mutants "max" for motor axon guidance defect. We have characterized and mapped one of the mutants, max-1.

933. Molecular characterization of the *seu-2* gene involved in axon guidance

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Our laboratory has identified *seu-2* as a suppressor of axon guidance defects induced by ectopic UNC-5 expression (Colavita & Culotti (1998) Dev. Biol. 194: 72-85). The *seu-2(ev523)* mutation on its own does not cause axon guidance defects, but was probably picked up in this suppressor screen because the screen is sensitized to the dose of components of the UNC-5 axon guidance signaling pathway.

Since the *seu-2(ev523)* mutant does not show any obvious phenotype, we had to use the rescue of the dorsalward axon guidance phenotype induced by ectopically expressed UNC-5 to identify a rescuing region. Using overlapping cosmids, we identified a rescuing fragment containing two predicted genes that encode a putative seven-pass transmembrane protein and a serine-threonine phosphatase PP2A. The two coding regions are very close and are likely to constitute an operon. To determine which one of these genes is actually *seu-2*, we are attempting to rescue the mutant phenotype with constructs carrying a premature termination mutations in each gene. The sequence analysis of the transcripts from both genes has not revealed any amino-acid change suggesting that the *seu-2(ev623)* mutation may affect untranslated regions of the gene. We are investigating the expression pattern of the gene using a translational fusion to GFP. Preliminary results reveal expression in dorsal and ventral muscles, in motorneurons and in amphid and phasmid neurons throughout development. We will check expression in touch neurons by using a longer promoter region and observing the reporter transgene expression during embryonic development. To further characterize the *seu-2* locus genetically and molecularly, we are currently doing a non-complementation screen to isolate new mutant alleles of the *seu-2* gene. The molecular analysis of the new mutations should provide new insights into the function of SEU-2 and possibly identify different functional domains of the protein.

934. Identification of Genes Specifically Required for Ventralward Axon Guidance in *C. elegans*

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Several conserved classes of guidance molecules direct axon guidance in *C. elegans* and other organisms. The UNC-6/netrin diffusible guidance cue is required for axon guidance along the dorsal-ventral axis in *C. elegans*, Drosophila and vertebrates. The UNC-5 guidance receptor in combination with UNC-40 mediates repulsive response to the UNC-6 which expresses in ventral nerve cord and ventral epidermis, to orient growing axons in a dorsal direction. Attractive responses to UNC-6 require UNC-40/DCC but not UNC-5. Does UNC-40 require a co-receptor for attractive responses to UNC-6, the way it requires a co-receptor (namely UNC-5) to mediate repulsive responses to UNC-6? To look for mutations in such a hypothetical co-receptor for attraction or in mechanisms that act in parallel with UNC-6 and UNC-40, we searched for mutants defective in ventralward guidance of the touch receptor axons AVM and PVM. The screen was carried out in a genetic background partially compromised for UNC-40 function, using *mec-7::gfp* to visualize sensory neurons. This screen yielded 12 mutations representing 6 genes. Two are unc genes (unc-44 and unc-51) previously identified as affecting axon guidance of a number of neurons, while mutations in the other 4 genes are not Unc and only appear to affect certain ventrally oriented axons. *avm-1* (allele *ev755*) is on LGIII, *avm-2* (allele *ev756*) is on LGI, *avm-3* (alleles *ev740*, *ev741*), is on LGX, *avm-4* (alleles *ev750*, *ev751*, *ev752*, *ev753*, *ev754*) is on LGI, and *avm-5* (allele *ev742*) is on LGX. We have rescued *avm-3* with a fragment of cosmid C26G2 corresponding to the Slit gene (F40E10.4), which encodes an axon guidance ligand for the SAX-3 receptor. The laboratory of Dr. C. Bargmann (UCSF) is currently characterizing these mutations. We have also rescued *avm-4* mutations with overlapping cosmids F14B11 and F32A7, and further localized the rescuing region to a single open reading frame. We have found that *avm-4* mutations enhance AVM and PVM guidance defects of an *unc-6* null allele, suggesting that

AVM-4 functions in an axon guidance pathway that acts in parallel with UNC-5, UNC-6, and UNC-40. We are currently determining in which cells this gene must function by expression and mosaic analyses. We are also cloning and characterizing *avm-1* and *avm-2*.

935. The *C. elegans* homologue of mammalian Hrs/Hrs-2 (C07G1.5) interacts with the *C. elegans* homologue of vertebrate EAST (C34G6.7), and the clathrin adaptor protein UNC-11A. Is there a link between clathrin mediated retrieval and synaptobrevin trafficking through the endocytic pathway? I. Characterization of Ce Hrs expression.

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Our general goal is to understand the mechanism(s) of synaptic vesicle (SV) recycling at the active zone. Specifically we want to address the contribution of clathrin-mediated endocytosis to this process. Our approach has been to characterize the synaptic transmission defects of mutants lacking the function of the monomeric clathrin assembly protein UNC-11. In the absence of *unc-11* function there is a generalized defect in neurotransmitter release probably as a result of a decrease in SV fusion. Ultrastructural analysis revealed an accumulation of vesicular structures that are larger than mature synaptic vesicles and the mislocalization of synaptobrevin to all neuronal membranes including those in pre- and post-synaptic compartments (Nonet et al., 1999, Krump et al., 2001).

Thus, we are testing the hypothesis that UNC-11 has a role in the trafficking of synaptobrevin from the pre-synaptic plasma membrane through the endocytic pathway. Interestingly, a yeast two-hybrid screen designed to identify proteins that interact with UNC-11A (one of six possible isoforms derived from the gene *unc-11*), identified two candidate-binding proteins which have been implicated in trafficking between endosomes and lysosomes, C07G1.5 and C34G6.7. The protein encoded by C07G1.5 is homologous to mammalian

Hrs/Hrs-2, whereas the open reading frame C34G6.7 is homologous to the vertebrate proteins EAST/Hbp. Both Hrs (**H**epatocyte growth factor **r**egulated tyrosine kinase **s**ubstrate) and EAST/Hbp (**E**GFR **A**ssociated protein with **S**H3 and **T**AM domains/**H**RS **b**inding **p**rotein) have been implicated in receptor-mediated endocytosis and promote internalization of proteins targeted to the recycling or degradation pathways (reviewed in Komara and Kitamura, 2001).

To characterize the expression pattern of Ce Hrs in the nematode we have generated antibodies against a recombinant, full-length, GST-fusion protein. A single protein of approximately 105 kDa is detectable in protein extracts using affinity-purified antibodies. Immunohistochemical analysis of whole worms has revealed that Ce Hrs is expressed in all cells in an intracellular punctate pattern that is reminiscent of endosomes. The expression pattern is dynamic and detectable throughout development. In the adult, Ce Hrs is enriched in the spermatheca, coelomocytes and the pharyngeal intestinal valve. At the ventral and dorsal nerve cords, Ce Hrs immunostaining only partially colocalizes with the synaptic vesicle marker UNC-17.

To determine the function of Ce Hrs *in vivo* we have used double stranded RNA interference (RNAi) to knock out the function of this protein in the progeny of the injected animals. The RNAi analysis indicates that Ce Hrs plays an important role in nematode development. About 82% of the RNAi treated progeny die at embryonic and larval stages. In addition, the surviving animals take three times longer to reach adulthood and in most cases develop phenotypes similar to those displayed by well-characterized mutants in signal transduction pathways. To analyze the function of Ce Hrs in the nervous system we are in the process of isolating deletion mutants and generating transgenic lines expressing dominant negative forms of the protein in the nervous system.

This work has been supported by UIC departmental funds to AA and an NIH grant (NS32449) to AA.

936. Molecular and functional analysis of the vacuolar protein sorting homolog, *Ce-vps45*, a member of the Sec1/Munc-18 family in *C elegans*

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The Sec1/Munc-18 family is an essential factor in both constitutive and regulated secretion, but the physiological role is still unclear. In yeast, 4 Sec1/Munc-18 proteins (Sec1p, Sly1p, Slp1p and Vps45p) are known to function in specific membrane trafficking steps. We have found that *C elegans unc-18*, homologous to Sec1p, is expressed in nervous system and loss of function mutations in this gene result in defects of neurotransmitter release. There are at least 5 members of this family in addition to *unc-18* on the *C elegans* genome. In this work, we determined the full length cDNA sequence of the *C elegans vps45* C44C1.4 whose orthologous protein in yeast plays an important role in membrane trafficking from Golgi's apparatus to vacuole. A comparison of genomic and cDNA sequences of *Ce-vps45* revealed that 11 exons encoded 547 amino acids having 40 % identity to Vps45, as predicted by Genefinder. The Ce-vps45::EGFP fusion protein was expressed in all tissues, predominantly in intestinal cells.

To get insight into the function of *Ce-vps45*, we isolated a putative null mutant, *tm246*, which had a 1.2 kb deletion covering the first to fourth exons of *Ce-vps45* gene, using TMP/UV mutagenesis. We found that the *tm246* mutation showed a maternal effect ts lethal phenotype, exhibiting developmental arrest in the early larva at 25¼C, while reaching to adulthood and were fertile at 15C. A genomic fragment og 7.5 kb containing the entire ORF complemented *tm246* mutation, resulting in normal growth of transgenic animals at 25C. *tm246* mutation causes amorphous autofluorescense in the

intestinal cells instead of intestinal lipofuscin granules, which are thought to be located in secondary lysosomes. When *Ce-vps45* cDNA was specifically expressed in the intestine of *tm246* animals under the *ges-1* (encoding gut -specific esterase) promoter, ts lethal phenotype of the mutant was rescued. These results suggest that *Ce-vps45* gene products may function in membrane trafficking from Golgi's apparatus to lysosome in mainly intestinal cells where lysosome is highly developed, and dysfunction of the lysosome resulting from *Ce-vps45* mutation causes growth arrest and larval lethality in *C elegans*. *tm246* mutant fed with Rhodamine-Dextran showed granules loaded with bright red fluorescence in the intestinal cells, even though blue fluorescence was amorphous, suggesting that the endocytosis was almost normal in the mutant. We speculate that the lysosomal function may be necessary for processing of neutrition uptaken into intestinal cells. To identify the functional domain for membrane trafficking into the lysosome in intestine, or the plasma membrane in neuron, chimeric experiments between *Ce-vps45* and *unc-18* are in progress.

937. Redundant and distinct interactions of mu1 adaptins with various cellular proteins in the Nematode *Caenorhabditis elegans*

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Clathrin-coated vesicles transport many cellular proteins, and clathrin-associated protein complexes(AP complexes) play an important role in this transport. Out of the 4 subunits of the AP complex, the medium chains(mu adaptins) are associated with the sorting of the cargo proteins. There are two mu1 adaptins in C. elegans similar to mammals. The expression pattern and functions of the two mu1 chains, unc-101 and apm-1 mostly overlap, but show some differences during various developmental stages of C. elegans.

We thought that unc-101 and apm-1, being mu adaptins, would have redundant and distinct functions in selecting cargo proteins in the cell. Therefore, yeast two-hybrid screenig was carried out to find proteins interacting with unc-101 and apm-1. From one million transformants of each screening using either unc-101 or apm-1 as bait, we found 37 fish proteins using unc-101 as a bait and 35 fish proteins using apm-1 as bait. Most fish proteins interact with unc-101 and apm-1 simultaneously and several proteins interact specifically. We are now characterizing the fish proteins and studying the relationships of the mu1 adaptins and their interacting proteins.

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938. The *C. elegans* homologue of vertebrate EAST (C34G6.7) interacts with the *C. elegans* homologue of mammalian Hrs/Hrs-2 (C07G1.5), and the clathrin adaptor protein UNC-11A. Is there a link between clathrin mediated retrieval and synaptobrevin trafficking through the endocytic pathway? II. Characterization of Ce EAST expression.

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Hrs and EAST are endosomal-associated proteins that are tyrosine phosphorylated in cells stimulated by growth factors such as EGF, HGF and PDGF. Both Hrs and EAST/Hbp have proposed roles in exocytosis as well as endocytosis (Bean et al., 2000; Murai and Kitamura, 2000). EAST is an avian protein that was initially identified as an EGF Receptor substrate and later was found to bind Eps15, a protein implicated in EGFR endocytosis. The rat homologue of EAST, Hbp, was found to bind Hrs via coiled-coil domains (reviewed in Komada and Kitamura, 2001). Since Hrs and UNC-11 also bind Eps15 (Ce Ehs-1 is homologous to mammalian Eps15), UNC-11, Ce Hrs and Ce EAST are likely to have a role in the trafficking of proteins from the plasma membrane through the endocytic pathway.

In a yeast two-hybrid assay the nematode proteins C07G1.7 (Ce Hrs) and C34G6.7 (Ce EAST) bind to each of three distinct UNC-11 isoforms with varying strengths. Furthermore, the interaction is direct, as we are able to co-immunoprecipitate either protein with UNC-11 using *in vitro* synthesized proteins. Although we have been unable to co-immunoprecipitate from nematode extracts endogenous UNC-11 with either of these proteins, we have documented an *in vivo* interaction between endogenous Ce Hrs and Ce EAST.

To characterize the role of Ce EAST in the endocytic trafficking we have characterized the pattern of expression of the protein encoded by the C34G6.7 open reading frame. We have generated antibodies against a recombinant, full-length, GST-fusion protein. A single protein of approximately 57 kDa is detectable in protein extracts using affinity-purified antibodies. Immunohistochemical analysis of whole worms has revealed that Ce EAST, like Ce Hrs, is ubiquitously expressed and is detected intracellularly in a punctate pattern that is reminiscent of endosomes. The pattern of expression is also dynamic and detectable throughout development. In the adult, Ce EAST, like Ce Hrs, is enriched in the spermatheca, coelomocytes and the pharyngeal intestinal valve. Furthermore, Ce EAST immunostaining, like that of Ce Hrs, only partially colocalizes with the synaptic vesicle marker UNC-17 at the ventral and dorsal nerve cords. However, Ce EAST is more abundant than Ce Hrs in the cell bodies of the ventral cord motor neurons.

To determine the function of Ce EAST *in vivo* we have used double stranded RNA interference (RNAi) to knock out the function of this protein in the progeny of the injected animals. In contrast to Ce Hrs, Ce EAST is not essential for viability and development. The progeny of injected animals look and develop like wild type animals. Since RNAi is known to be ineffective in inactivating the function of proteins in the nervous system we are in the process of isolating a loss of function mutant in Ce EAST to determine whether it would affect trafficking of the synaptic vesicle protein synaptobrevin.

This work has been supported by UIC departmental funds to AA and an NIH grant (NS32449) to AA.

939. Looking for the intracellular function of the sterol-sensing domain protein CHE-14 in ectodermal epithelial cells

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At least two processes contribute to the polarisation of epithelial cells: specialised junctions which separate apical from baso-lateral regions of the plasma membrane, and differential vesicle trafficking which specifically targets proteins to different domains.

We have previously identified the *che-14* gene which is characterised by a defect in secretion towards the apical surface in the hypodermis and support cells (Michaux *et al*, *Curr Biol* 2000, **10**:1098-1107). This hypothesis is based on i) analysis of the phenotype by electron microscopy studies, ii) the subcellular localisation of a CHE-14::GFP fusion protein, and iii) the similarity of CHE-14 with Patched, Dispatched and NPC1, three proteins with a sterol-sensing domain. NPC1 is implicated in lipid trafficking between late endosomes and lysosomes. Dispatched is responsible for the release of cholesterol-modified Hedgehog, of which Patched is the receptor, and thus these two proteins could also be implicated in vesicle trafficking.

The route from endoplasmic reticulum to the apical plasma membrane is not well known. Studies in mammalian cell lines have shown that specific adducts, like glycosylphosphatidylinositol (GPI), N- and O-glycans, and cholesterol via microdomains (or lipid rafts), can act as apical sorting determinants, rather than signals encoded by the amino-acid sequence. One possible function of CHE-14 could be to target such modified proteins to the apical domain. We are testing whether GPI- or cholesterol-modified protein targeting depends on CHE-14 activity by using proteins tagged with GFP and designed to be modified either by GPI or by cholesterol. These constructs will be injected into a *che-14* mutant background and should give us new insights about the role of CHE-14 in apical targeting.

940. A novel role of the TGF-beta signaling cascade in endocytosis in *C. elegans*

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TGF-beta and related members of the cytokines play a central role in growth and development in higher organisms. In *C. elegans* three important aspects of development have been described that are mediated through TGF-beta signaling pathway. These include the dauer formation (Estevez et al., 1993), control of body size, and male tail morphogensis (Morita et al., 1999; Suzuki et al, 1999), and the axonal guidance (Clovita et al., 1998). We have found that the TGF-beta signaling also mediates endocytosis in the intestinal epithelium of *C. elegans* . Mutants in TGF-beta receptor family of Ser/Thr kinases, *daf-1 and daf-4* (Georgi et al., 1990; Estevez et al., 1993), show abnormal endocytosis. Activation of TGF-beta receptor induced endocytosis of albumin in intestine is dependent on the endocytic machinery that includes, dynamin, clathrin heavy chain, *rab-5, rab-11,* and alpha and beta adaptin. We have extended these findings to show the role of TGF-beta receptors in albumin endocytosis in monolayers of endothelial cells by inhibiting endocytosis with receptor antibodies and its augmentation in cells transfected with cDNA encoding the receptor (Attisano et al., 1993). TGF-beta receptor mediated albumin endocytosis specifically activates Smad2 phosphorylation, Smad4 translocation, MAPK p38 phosphorylation, and promotes cell growth and is anti-apoptotic. We propose a model that suggests that activation of endocytosis promotes cell growth and is anti-apoptotic. Thus, interaction of albumin with TGF-beta receptors is critical in regulating its endocytosis and the downstream activation of the TGF-beta signaling pathway. We thank A. Malik for enthusiastic support, and lively discussions, D. Riddle, S. Uddin, L. Jacobson, J. Massague, P. ten-Dijke, K. Miyazono, A. Shajahan, P. Okemma, T. Orenic, T. Stiernagle, and the CGC for strains, reagents and invaluable suggestions.

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941. *unc-20* MAPS TO A REGION THAT INCLUDES TWO PREDICTED GOLGI COMPLEX PROTEINS

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The canonical weak *unc-20* mutation, *e112*, causes a characteristic coiling uncoordinated defect, as well as defects in axon outgrowth and pathfinding in several neurons, and increased sensitivity to serotonin. Strong *unc-20* mutations, recovered as part of a *smg*-dependent screen performed by Phil Anderson's lab, cause developmental arrest in late embryogenesis or at hatching. Given the pleiotropies of *unc-20* mutations, the gene may be involved in a central cellular process, either one specific to the nervous system or a general process.

Genetic mapping of *unc-20* places it between *imo-1* and *fax-1* on XL. This region is covered by genomic cosmid clone C15C7, however we have been unable to obtain stable transformants with this clone, preventing us from determining the location of *unc-20* by transformation rescue experiments. Cosmids to the left and right of C15C7 do not rescue *unc-20* and a large deletion just to the right of C15C7 complements *unc-20*. Therefore, we have focused our attention on the predicted genes on cosmid C15C7. We amplified genomic DNA from *unc-20(e112) and unc-20(r977)* homozygous animals from regions of C15C7 that correspond to predicted genes C15C7.3, C15C7.4 and C15C7.5; all sequences were the same as wild-type. RNAi experiments with a cDNA clone corresponding to C15C7.5 (yk64b6; obtained from Y. Kohara) did not generate any obvious visible phenotype. Therefore, we conclude that these three genes are unlikely to encode *unc-20.*

The two remaining candidates, C15C7.1 and C15C7.2, appear to be transcribed together in an operon. Both predicted genes encode products that are predicted to be localized to trans-Golgi. C15C7.1 encodes a protein that is similar to syntaxin-6, a SNARE that is thought to function in trans-Golgi vesicle trafficking. C15C7.2 encodes a protein that includes a coiled-coil region and a C-terminal GRIP domain. The GRIP domain is associated with vertebrate proteins that are localized to trans-Golgi. One can imagine mechanisms by which defects in vesicle trafficking and/or secretion may result in all known *unc-20* phenotypes. For example, axon pathfinding and extension defects may be the result of difficulties in supplying new membrane to growth cones or the result of compromised transport of receptor molecules. RNAi experiments with a cDNA clone corresponding to C15C7.2 (yk121h6) causes weak embryonic lethality, but does not cause the Unc phenotype characteristic of *unc-20* putative hypomorphs. We are currently amplifying DNA corresponding to both of these predicted genes from *unc-20* mutants; come to our poster for the outcome.

We are also embarking on an electron microscopic study of *unc-20* mutants. We will evaluate global nervous system phenotypes, such as integrity of axon bundles, as well as subcellular Golgi and vesicle anatomy.

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942. Analyzing a putative acetyl-CoA transporter gene involved in modification of carbohydrates in development and morphogenesis

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To understand the roles of glycoconjugates (glycoproteins and glycolipids) in development and morphogenesis, it is very important to know what kinds of proteins are indispensable for the synthesis, degradation and modification of glycoconjugates. It is also essential to know what kinds of molecules are recognizing and interacting with these glycoconjugates. We are currently performing systematic studies on molecules involved in glycoconjugate metabolism and modification as well as molecules interacting or recognizing glycoconjugates in the nematode *C. elegans*. In this paper, we would like to focus on molecules involved in glycoconjugate modification and recognition. In mammalian cells, several glycoconjugates are modified with O-acetylation. For instance, O-acetylated gangliosides are expressed in neuronal cells in specific timing and location in development, and O-acetylation of glycoconjugates is also shown to be important in virus binding, cell adhesion and in modulating immunogenicity of glycoconjugates. O-Acetylation of glycoconjugates is supposed to occur in Golgi compartment with colocalizing acetyl-acceptor glycoconjugates (e.g. gangliosides), acetyltransferases and acetyl-CoA transporters. One of the authors (Y. Hirabayashi) previously reported a cDNA cloning of mammalian acetyl-CoA transporter gene. The gene product is involved in O-acetylation of glycoconjugates in mammalian cells and its DNA sequence is well preserved from bacteria to human. To study the roles of this glycomodification gene in development and morphogenesis, we searched for homologous genes in *C. elegans* and tried to isolate deletion mutants of the orthologous gene (T26C5.3A,B) by TMP/UV method. In a deletion mutant, the deletion was mapped to the intron of the alternatively spliceable gene, and the deletion allele showed abnormal phenotypes and some of the mutant worms were viable and fertile. The allele showed slow growth rate, high embryonic death-rate, small dumpy morphology with decreased egg number. RNAi study of the gene showed that the 100% of the dsRNA injected worms died at a larval stage. The roles of putative acetyl-CoA transporter gene in development and morphogenesis will be discussed with the data obtained with 4D microscopy.

943. Mutations in *let-767*, a steroid processing gene, result in cholesterol hypersensitivity in *C. elegans*

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Caenorhabditis elegans is unable to survive without the supplemental addition of cholesterol to its media and is therefore sensitive to a cholesterol-limited environment. We have shown that a mutation in a steroid metabolism gene, *let-767,* results in hypersensitivity to reduced levels of cholesterol. The *let-767* gene encodes a protein that is similar to a family of mammalian steroid enzymes that are responsible for the reduction of 17-beta hydroxysteroid molecules. There are three alleles of *let-767* resulting in early larval, mid-larval, or maternal-effect lethality respectively. *let-767* is maternally contributed to the embryo and *let-767* transcripts are present during embryogenesis, however, LET-767::GFP is detectable only after hatching where it is localized specifically to the gut. We believe that *let-767* is required for the metabolism of a sterol compound in *C. elegans.* Four additional genes have been identified in *C. elegans* which have high sequence similarity to LET-767 (C06B3.4, C06B3.5, F11A5.12, and F25G6.5). All are located on chromosome V. We have evidence of expression for three of the four genes - C06B3.4, C06B3.5, and F11A5.12. F25G6.5 may be a psuedogene. We are currently investigating the role these genes play in development.

944. Isolation of Genes involved in the Regulation of Sterol Metabolism

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Steroids are required for normal growth and reproduction in C. elegans. In order to investigate the mechanisms that underly the steroid function in worm physiology, we tested the effects of three mammalian steroid biosynthesis inhibitors on worm growth and reproduction. Aminoglutethimide (AMG), an inhibitor for cytochrome p450 side-chain cleavage enzyme (cyp-11) and aromatase (cyp-19), used to treat breast cancer, has no significant effect on worm growth and reproduction. Nor did metyrapone, a cyp11b-1 inhibitor used to treat Cushing's disease. In contrast, adding ketoconazole, an anti-prostate cancer and anti-fungal drug that inhibits several steps of steroid biosynthesis in mammals and cytochrome P450-dependent 14 alpha-demethylase in fungii, to culture plates significantly inhibits growth and reproduction. Worms treated with low conc. (100mM) of ketoconazole grow slightly slower than worms that were grown in normal plates. The average brood size of ketconazole-treated worms is significantly smaller than worms grown in normal condition. These phenotypes are similar to phenotypes found when worms grown in cholesterol-deprived media. When worms were treated with high conc. (250 mM) of ketoconazole, a severe growth defect was found. Some worms arrested at early larval stage, and worms that survived remained at L1 or L2 stage for at least 7 days. Based on this phenotype, we have set up a screen to isolate the suppressors for the ketoconazole effects. From a pilot screen, we have isolated several homozygous mutants that can suppress the ketoconazole effect. We are currently conducting a large scale screen and will report our results in the meeting.

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945. *C. elegans* genes coding for protein involved in lipid metabolism

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We are interested in studying how organisms control and regulate lipid metabolism as it represents a complex process whose alterations affect the functioning of various tissues and organs, and the physiology and behavior of the entire organism. *C. elegans* is an attractive animal model for this study as it allows for the systematic identification of genes coding for the components of fatty acid metabolism.

b-oxidation of fatty acids, which occurrs in mitochondria, plays a central role in lipid metabolism and carnitine is a key molecule required for the transfer of fatty acids across the mitochondrial membrane. Two mammalian genes involved in carnitine biosynthesis have been identified, one, BBOX1, maps in the 11p14 region and one, BBOX2, maps in the Xq28 region. Both genes seemingly code for **gamma-butyrobetaine hydroxylases**. Systematic search of the *C. elegans* DNA sequence databases has revealed two predicted homologs of the human BBOX genes. We have named D2089.5, *gbh-1* and M05D6.7, *gbh-2* for **gamma butyrobetaine hydroxilases** 1 and 2. They appear to be orthologues of the human genes in that *gbh-1* aligns best with hBBOX1 while *gbh-2* aligns best with hBBOX2. We analyzed the expression pattern of *gbh-2*, using 2.7 kb upstream of the ATG to drive expression of GFP with and without a nuclear localization signal. The most prominent GFP expression was in intestinal cells while a weaker signal could be detected in head and body muscles. Expression begins very early in embryogenesis, is restricted to 8 intestinal cells and does not appear to change substantially during development. A similar construct for *gbh-1* showed an expression pattern identical to *gbh-2* in larval stages and in adults but different in the embryo where expression is not restricted to the gut, but appears more generalized. We have used *RNA interference* to reduce expression of *gbh-1* and *gbh-2* and to study the resulting phentoype(s). The main defects we have observed are: i.The presence of bubble-shaped fatty acid accumulation in the pseudocaelomic cavity of *RNAi* treated worms. The fat droplets are more easily visible after sexual maturity has been reached. Fat appears to accumulate not only in the extracellular space but also inside the cells of the intestine. We have used the dye Nile Red to confirm the presence of fat droplets in the intestinal and hypodermal cells of living *C. elegans*. ii. Many *RNAi* hermaphrodite worms have twisted gonads. In some worms the distal arm of the gonad is longer and misplaced to the ventral side. iii. Interfered worms have reduced fertility and often the germ line cells appear degenerated or abnormal. Interference with *gbh-1* and *gbh-2* probably results in reduced synthesis of carnitine. Since carnitine is necessary for fatty acid utilization it is conceivable that RNA interfered worms under utilize lipids as energy sources and accumulate fatty acids. The abnormal gonad morphogenesis and the reduced fertility of *gbh-1* and *gbh-2* interfered worms are probably secondary to the alteration in fatty acid metabolism. It is interesting that also in mammals, carnitine deficiency, due either to inefficient transport or to reduced biosynthesis, results in extensive fat accumulation not only in the cytoplasm of cells but also in the extracellular space and that it is associated with a variety of pathological conditions.

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946. Role of sphingolipids in *C. elegans*

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Sphingolipids are membrane components of all eukaryotic cells. Excess or lack of these complex lipids in mammals leads to improper development or lethal diseases. Ceramide and glucosylceramide are sphingolipid precursors proposed to have specific functions in cell signalling. For example, increased ceramide concentration following Tumor Necrosis Factor alpha treatment has been implicated in apoptosis, cell-cycle arrest and cell differentiation. These effects seem to be mediated via different effectors. In contrast, glucosylceramide has different and in some cases opposite effects. Increased levels correlate with keratinocyte proliferation and are required for axonal growth after induction by basic Fibroblast Growth Factor or laminin. Although significant progress has been made recently, the precise mechanisms underlying the multiple functions exerted by ceramide and glucosylceramide as well as the role of sphingolipids and their biochemical targets remain unclear.

We aim to clarify the role of sphingolipids during development and/or signalling employing the nematode *C. elegans*. Our approach is to generate mutant animals unable to synthesise certain sphingolipids, characterize their phenotype, and carry out genetic screens to identify genes that alter such a phenotype. We decided to initially concentrate on Ceramide Glucosyl Transferase (CGT). CGT catalyses the addition of glucose to ceramide thus forming glucosylceramide. This is an essential step for the synthesis of many complex glycosylated sphingolipids. Disruption of CGT results in the absence of glucosylceramide and in the accumulation of ceramide.

Homology searches revealed that *C. elegans* has three CGTs. Using a GFP vector (a gift from Dr. Andy Fire, Baltimore, USA) we looked at the expression of one of these CGTs and found that it is expressed in a very limited number of cells in the head and in the tail. We generated transient knockout animals using RNA interference and found that worms in which 2 of the 3 CGTs are inactivated stop development at an early larval stage. We then screened an EMS mutagenised library of approximately 1,400,000 genomes and identified a deletion, which reduces CGT function. Worms homozygous for this deletion arrest at an early larval stage when another CGT is inactivated by RNA interference. We are now in the process of carrying out a screen for genes that suppress the phenotype conferred by CGT inactivation.

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947. Deletion of glucosamine 6-phosphate acetyltransferase causes sterility in homozygotes

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De novo synthesis of the nucleotide sugar UDP-N-acetylglucosamine (UDP-GlcNAc) requires the enzyme glucosamine 6-phosphate acetyl transferase (GNA1/ PAT1 (yeast), EMeg32 (mouse)) of the hexosamine synthesis pathway. UDP-GlcNAc is an essential substrate in chitin synthesis, in N- and O-linked glycosylation of membrane and secreted proteins (by Golgi-resident transferases), in O-linked cytosolic protein glycosylation and in GPI protein anchoring. In yeast, deletion of GNA1 is lethal, causing cells to have defects at multiple steps in the cell cycle, resulting in some cells with multiple or no nuclei. Mouse embryos homozygous for a null mutation in EMeg32 (GNA1) die at E7.5 and *in vitro* differentiated null ES cells show multiple changes, including defects in proliferation and adhesiveness, resistance to apoptotic stimuli, decreased actin depolymerization increased PIP_2 levels and increased PKB activation.

To try to elucidate the role played by UDP-GlcNAc in development and the cell cycle, we have begun studies of GNA1 in *C. elegans*. RNA interference has been done using the two predicted worm homologues of GNA1 (B0024.12, T23G11.2). Interference with B0024.12 had no discernible effect on phenotype. However, interference with T23G11.2 resulted in F1 progeny that were sterile. We have isolated a mutant of T23G11.2 in which all four predicted exons have been deleted. Worms that are heterozygous for this mutation are viable and produce viable progeny. However, mirroring the RNAi results, homozygous mutants are viable but sterile, laying masses of eggs that do not hatch. We have rescued the aberrant phenotype using a line carrying the cosmid T23G11 in an extrachromosomal array. The mutant does not appear to be Spe, since it was not rescued by mating with wild-type males. Mating with *fem-1* is being done to determine if the mutant produces fertile sperm and UDP-GlcNAc in the mutant is being quantified by HPLC. In addition, we are characterising the phenotype of the embryos in the uterus by staining with DAPI, anti-tubulin and calcofluor (for chitin).

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948. Characterisation of mammalian syndecan homologue in *C.elegans*

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The syndecans are transmembrane heparan sulphate proteoglycans (HSPGs) expressed on all adherent mammalian cells. There are four distinct mammalian syndecans, which are expressed in cell-, tissue type and developmental specific manner. Based on the conserved transmembrane and cytoplasmic domains of the mammalian syndecans, *Drosophila*, *Xenopus* and ascidian syndecans have been cloned. The *C. elegans* genome also contains a single syndecan-like gene that shows homology to mammalian syndecans.

The syndecans have diverse functions ranging from regulation of growth factor signalling and enzyme activity to mediating cell-cell and cell-matrix interactions. The syndecans play important role in cell differentiation, morphological transitions, changes of tissue organisation and in pathological conditions such as wound healing. Interactions of syndecans with growth factors and matrix molecules lead to clustering of cytosolic signalling molecules and reorganisation of the cytoskeleton.

The syndecans mediate their functions through binding of matrix ligands and growth factors to their heparan sulphate (HS) chains. Various ligands show selectivity in their binding affinities to distinct HS structures. Studies using mice and *Drosophila* have shown that HS is essential in specific signalling pathways involved in cell differentiation and tissue morphogenesis during development.

We have recently begun studies on syndecan and HS biosynthetic enzymes in *C.elegans* with the view to understand their function in development and tissue maintenance. With the complete *C.elegans* genome available, we have cloned the syndecan-like (F57C7.3) cDNA and genomic DNA. Northern blotting analysis reveals the presence of a single mRNA. Current studies include characterisation of the expression pattern by whole mount immunohistochemistry, using a polyclonal antibody against the conserved cytosolic domain of syndecans, and by using GFP-fusion constructs. It is hoped that these studies will also provide information on functional roles of *C.elegans* syndecan.

We have also begun studies on the role of the enzymes involved in biosynthesis of complex HS structures, sulphotransferases in particular, in *C.elegans* development. We have cloned the putative 2-O-sulphotransferase (2-OST; C34F6.4) and 6-O-sulphotransferase (6-OST; Y34B4a.e) cDNAs. Northern blotting analysis reveals the presence of a single mRNA for both of these genes. Preliminary RNAi studies with the 2-OST indicate that loss of its expression results in multiple phenotypic effects including sterility or defects in egg laying, suggesting that this enzyme is essential for normal development.

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949. Molecular and Genetic Characterisation of Cyclophilin Encoding Genes from *C. elegans*: a Focus on *cyp-1* and *cyp-5*.

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The cyclophilins (CYP) are a class of peptidyl-prolyl *cis/trans* isomerase (PPI) enzymes, additionally they are the ligand for the immunosuppressing drug cyclosporin A. PPIs are involved in the isomerisation of Xaa-proline peptide bonds, a rate limiting step in the folding of many proteins. In *C. elegans* they are a large multi-gene family with 18 members. These genes and their encoded enzymes are being characterised to establish the role they play in protein folding.

We are focussing on the highly conserved cyclophilins, *cyp-1* and *cyp-5*, both of which contain an amino-terminus targeting sequence domain. Their spatial expression pattern was examined via *lac*Z promoter/reporter transgene analysis. *cyp-1* was expressed in a large variety of tissues, whereas *cyp-5* was expressed solely in the gut. Both *cyp-1* and *cyp-5* were constitutively expressed. However the timing of individual transcript expression differed throughout the life cycle. *cyp-1* expression increased steadily throughout the life cycle from the L1 larval stage to the adult stage. However *cyp-5* expression remained more or less constant during the life cycle. RNA mediated interference of *cyp-1*, *cyp-5* and the combination *cyp-1* and *cyp-5* in wild type *C. elegans* did not produce obvious phenotype.

Both *cyp-1* and *cyp-5* genes minus their targeting sequence domain were cloned into the bacterial pET-15b expression vector. N-terminus his-tag fusion proteins were expressed and purified. The peptidyl-prolyl *cis/trans* isomerase enzymatic activities of recombinant CYP-1 and CYP-5 were determined, as well as their inhibition by cyclosporin A.

Ongoing work is currently focussing on crystallizing and resolving the structure of CYP-1 and CYP-5. This work is a continuation of previous structural work on another highly conserved *C. elegans* cyclophilin with homology to human cyclophilin A: CYP-3 (Dornan et al. (1999). JBC. 274(49): 34877). This work may help elucidate a functional role for these conserved proteins in *C. elegans*.

950. ISOLATION AND CHARACTERIZATION OF A NOVEL NADPH-DEPENDENT FLAVIN-REDUCTASE IN C. elegans AND HUMANS

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Cytochrome P-450 reductases (CPR) are flavin-containing enzymes, associated with the endoplasmic reticulum membrane, found in bacteria, plants, yeast, and animals. They function as an internal electron transport chain, transferring electrons from NADPH to FAD to FMN and finally to cytochrome P-450 as well as to other heme-containing enzymes such as heme oxygenase and cytochrome b5. The nitric oxide synthases (NOS) also belong to the flavoprotein family of electron transferases. In contrast to CPR, which acts by passing reducing equivalents to other target proteins, the NOS proteins contain an N-terminal extension that includes an oxidase domain containing a heme. We have identified a novel C. elegans sequence, which encodes a cytosolic protein, with high similarity to the CPR and NOS. This novel member of the flavoprotein family contains binding domains for FMN, FAD and NADPH.

Analysis of the upstream region from the C. elegans reductase gene revealed the presence of another novel gene. We showed by RT-PCR analysis that the reductase and the upstream gene comprise a two-gene operon. We also cloned orthologs of both these genes from human. Based on the analysis of the promoter region in C. elegans and the results from transfected HEK cells we hypothesize a cooperative regulation of the reductase and upstream gene and the possible functional interaction of these proteins in both C. elegans and humans. Similarity with cytochrome P450 reductase enzymes and NOS indicates that the novel reductase is likely to transfer electrons from NADPH to heme- or transition metal-containing center of an appropriate redox partner. We show that the novel reductase is capable of reducing hemoproteins such as cytochrome c and that the upstream protein is required for its function in the cell

951. Examining the Role of Proprotein Convertases

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Many physiologically important proteins are synthesized as large inactive polypeptides. Activation commonly occurs by limited proteolytic cleavage at mono-, di or oligobasic residues, a reaction catalyzed by members of the kex2/subtilisin-like proprotein convertase family (PCs) of serine endoproteinases. *C. elegans* encodes four PC family members including *kpc-1, egl-3/kpc-2, aex-5*/*kpc-3* and *bli-4*/*kpc-4*. Mutations in all family members have been isolated and are currently being characterized in order to further our understanding of their developmental and physiological roles and substrate specificity. Candidate substrates are being identified based on several criteria. These include the analysis of genes which when mutated exhibit a phenotype similar to those displayed by PC mutants; identification of genes which genetically interact with PC mutants by enhancing or suppressing mutant phenotypes; and examination of conserved gene products which have been identified as substrates for processing by the PC family in other organisms. Using this approach it has been determined that the *kpc-1* gene products are likely responsible for processing transforming growth factor (TGF) beta precursors including those encoded by *daf-7*, *dbl-1* and *unc-129*. The *bli-4*/*kpc-4* gene products are required for processing cuticular procollagens. As yet, substrates recognized by AEX-5/KPC-3, the defecation-defective mutant, have yet to be determined although putative target precursors are being investigated using an in vitro peptide-cleavage assay. To support evidence generated by genetic analysis, cleavage of precursor proteins has been followed in vivo by immunofluorescent detection of epitope-tagged substrates as well as identification of cleavage products by western analysis. Together, these results suggest that substrate specificity is governed not only by the primary sequence of the precursor cleavage site but also by the temporal and spatial localization of both PC and precursor protein.

Many thanks to Ann Rose in whose laboratory this work was initiated and to Terry Snutch.

952. *CAENORHABDITIS ELEGANS* AS A MODEL ORGANISM IN THE STUDY OF NUCLEOSIDE TRANSPORTERS.

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Nucleoside transporters (NTs) are essential for nucleotide synthesis by salvage pathways in cells, such as protozoan parasites, that lack *de novo* biosynthetic pathways. In addition, they have important roles in many adenosine-mediated physiological processes in mammals, including neurotransmission, platelet aggregation and coronary vasodilation. Most eukaryotes possess multiple NTs, which can be grouped into two unrelated transporter families; the equilibrative nucleoside transporters (ENTs) and the concentrative transporters (CNTs). In order to understand the physiological functions of nucleoside transporters and the reasons underlying their biological diversity, we are using *C. elegans* as a model system. *C. elegans* possesses two genes encoding CNTs and six encoding putative ENTs. As the first step towards understanding the rationale underlying this diversity of transporters, we have expressed one of these, ZK809.4, in *Xenopus* oocytes and shown it to be a genuine ENT which we have designated CeENT1. It exhibits a broad substrate specificity for natural purine and pyrimidine nucleosides and also transports antiviral nucleoside analogues such as 3'-azido-3'-deoxythymidine (AZT). cDNAs for two other ENT genes K09A9.3 and K02E11.1 (which we have designated CeENT2 and CeENT3 respectively) have been obtained from Yuji Kohara and have also been expressed in

Xenopus oocytes. We have demonstrated that these are able to transport nucleosides, with adenosine being a good substrate for CeENT2 and adenosine and uridine being substrates for CeENT3. In an attempt to elucidate the biological roles of these and other NTs, we have used Green Fluorescent Protein reporter constructs to investigate the temporal-spatial expression patterns of these genes. Additional clues to the biological roles of NTs have been obtained by double-stranded RNA interference (dsRNAi). RNAi using dsRNA corresponding to CeENT1 failed to yield a discernible phenotype. Similarly, injection of animals with dsRNA corresponding to CeENT2 yielded no obvious phenotype. However, the progeny of worms co-injected with a mixture of CeENT1 and CeENT2 RNAs exhibited an everted vulval phenotype, suggesting that CeENT1 and CeENT2 exhibit a redundant function.

953. At least two of the three ADP-ribosylation factors (ARFs) in *C. elegans* have distinct roles in development

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ADP-ribosylation factors (ARFs) are highly conserved, 21 kDa GTPases with regulatory roles in vesicular traffic, lipid metabolism, and likely other essential cellular processes. The functions of ARFs, and the structurally related ARF-like (ARL) proteins, have been extensively explored biochemically in mammalian and yeast cells but no evidence has emerged to explain the redundancy (6 mammalian ARFs plus more than 8 ARLs) in this family. Mammalian cells express all ARF isoforms in all tissues examined. Homology searches of the worm genome have identified three predicted ARFs and 5-7 ARLs in *C. elegans*. Distinct roles in development were explored as a possible explanation for redundancy in the ARF family. Roles for the worm orthologs of human ARF1, ARF5, ARF6, and ARL1-3 were explored by the use of RNA-mediated interference (RNAi) to study disruption of functions. Injection of double-stranded RNA (dsRNA) encoding the worm orthologs of human ARF1 or ARF5 into N2 hermaphrodites produced embryonic lethality. Embryos deficient in ARF1 arrested earlier than those lacking ARF5. Injection of ARL2 dsRNA resulted in a disorganized germline and sterility in the offspring of injected N2 hermaphrodites. There was no phenotype detected after injection of dsRNA encoding the worm orthologs of human ARL1, ARL3, or ARF6.

Transgenic strains were established that expressed C-terminal GFP fusion proteins for each of the three ARFs and three ARLs. ARF1::GFP was localized in the body to pharyngeal muscles, gut, nerve ring, ventral nerve cord, and hypodermis. The fusion protein

localized to membranes in these cells, and exhibited a punctate pattern that is likely to represent binding to vesicles. In contrast, ARF5::GFP was expressed primarily in hypodermis and neurons. In addition, ARF1::GFP was expressed earlier during embryogenesis than was Arf5::GFP and expression of each was induced at a time in development that was consistent with the RNAi result. Thus, the timing and possibly the location of the expression of these two ARFs may be critical steps in embryogenesis. ARF6::GFP expression was observed in the body wall muscle, pharynx, vulva muscle cell, ventral nerve cord, gut lumen, and the excretory cell. ARL1::GFP was most evident in neurons in the head region while ARL3::GFP was observed in neurons in both the head and tail regions. There was no detectable expression of ARL2::GFP. In summary, the worm orthologs of human ARF1 and ARF5 are essential genes during different stages of embryogenesis whereas ARL2 appears to be required for fertility. The different ARF family members have different patterns of expression that may help in the identification of specialized functions. The prevalence of ARL1 and ARL3 in neurons is consistent with results from mammalian studies and may suggest roles for these genes/proteins in the nervous system.

954. Specification of the MS founder cell in *C. elegans*

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Screening our collection of maternal effect mutants on LG III we found a mutant *t1530* with obvious defects in pharynx and body wall muscle specification. Instead of the normal 21 pharyngeal cells staining with mAb 3NB12 only 6±2 are found. Body wall muscle is reduced from normally 81 to 37±10 cells. In wild-type embryos, the pharynx is formed by descendants of ABa and MS where the ABa derived pharynx is induced by the MS blastomere. The body wall muscle is derived from MS, C and D contributing 28, 32 and 20 body wall muscles respectively. A single cell is derived from the AB lineage. The observed pattern of differentiation in the mutant cannot be attributed to a loss of differentiation in a single founder cell. Using laser ablations we found that in this mutant still both ABa and MS contribute to the small pharynx cluster. This can be due to either a failure in specific sublineages derived from both founder cells or to only a partial induction of pharynx in the ABa lineage by a defective MS blastomere which can still produce some pharyngeal cells itself. Our lab observed such partial inductions earlier in studying pharyngeal induction. The lack of body wall muscle exceeds that expected if only the MS lineage is not producing any muscle. However, as shown earlier body wall muscle in the D lineage also depends on an induction from the MS blastomere. The average number of body wall muscles found in the mutant is also consistent with a partial induction of body wall muscles in D. Our current hypothesis is that *t1530* is a hypomorphic mutation in a gene primarily required for the specification of MS. Since we isolated only one allele in a huge mutagenesis experiment we do not expect to get more alleles in a simple way. Therefore we hope to define the loss of function phenotype using RNAi. Towards this end we are cloning the gene taking advantage of SNPs in the region. No previously

described gene involved in the specification of MS is found close to the genetic position of the new gene indicating that we may define a new function for specification of the founder cell MS. Currently we are testing two candidate genes. One is a T-box gene and the other a gene with a WD domain.

955. USING YEAST-TWO HYBRID AND RNA-MEDIATED INTERFERENCE TO IDENTIFY PROTEINS ESSENTIAL FOR PIE-1 ACTIVITY AND LOCALIZATION IN EARLY EMBRYOS

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The CCCH zinc finger protein PIE-1 is an essential regulator of germ cell fate that segregates with the germ lineage in early *C. elegans* embryos. PIE-1 has at least two functions in the embryonic germ lineage: 1) inhibition of mRNA transcription, and 2) promotion of efficient NOS-2 expression, a maternal protein required for primordial germ cell development. Structure-function studies have indicated that these two functions require sequences in the C-terminal region of PIE-1 (Tenenhaus et al., 2001). The C-terminus of PIE-1 is also required for asymmetric segregation of PIE-1 in dividing germline blastomeres (Reese et al., 2000). We have used the C-terminus of PIE-1 as a bait in a yeast two hybrid screen in the hope of identifying proteins required for PIE-1 activity and/or localization.

Screening of 900,000 transformants yielded 365 candidate interactors (thanks to Zheng Zhou and Bob Horvitz for their excellent library). To quickly identify functionally relevant candidates, we designed a simple protocol to test the *in vivo* function of each candidate by RNAi. We first isolate the "prey" plasmids from the positive yeast transformants by selection on an auxotrophic *E. coli* strain. The prey inserts are then PCR amplified and transferred by recombinational cloning into Lisa Timmons and Andy Fires feeding vector and transformed into *E. coli* strain HT115. Resulting transformants are used to "feed" three tester worm strains: 1) a MED-1:GFP line (thanks to Morris Maduro and Joel Rothman) to assay for defects in transcriptional repression, 2) a GFP:*nos-2* 3UTR line to assay for defects in NOS-2 expression, and 3) a PIE-1:GFP line to assay for defects in PIE-1 localization. The screen is ongoing and progress will be reported at the meeting.

956. Role of MEX-3 in Patterning PAL-1 Expression

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PAL-1 is a caudal-like homeodomain protein that is first expressed at the 4-cell stage in the two posterior blastomeres. This spatial and temporal patterning of PAL-1 expression is dependent on the putative RNA binding protein MEX-3 and the *pal-1* 3' untranslated region (UTR). A large body of circumstantial evidence suggests that MEX-3 may repress *pal-1* translation by binding to the *pal-1* 3' UTR. We are taking a biochemical approach to test this possibility directly. Current efforts focus on using reversible RNA-protein cross-linking, followed by immunoprecipitation of MEX-3, and RT-PCR analysis of the co-precipitated RNA. Positive results in this assay will be followed by experiments to investigate the mechanisms by which MEX-3 patterns PAL-1 expression.

957. Identification of factors involved the translational control of GLP-1 in the Caenorhabditis elegans early embryo

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GLP-1 is a Notch family receptor that is important in cell fate specification of the early *Caenorhabditis elegans* embryo. The translation of *glp-1* is spatially and temporally regulated through sequences in its 3 untranslated region (UTR). GLP-1 protein is translated mostly in anterior cells beginning at the two-cell stage continuing to the 16-cell stage. Several regulatory regions of glp-1 3 UTR have been identified. There is one region at the 3 end of the UTR that is essential for translational repression in oocytes and 1-cell embryos. This region is called the oocyte control region (OCR). Another region is essential for the special regulation of GLP-1 translation. This GLP-1 localization region (GLR) is both necessary and sufficient for the spatial regulation of GLP-1. The GLR contains both an element necessary for translational repression in posterior cells and another element required for activation of translation in the anterior cells. Our hypothesis is that RNA binding proteins interact with the 3' UTR of *glp-1* controlling its translation, and that these proteins contribute to anterior-posterior patterning. In order to identify these proteins, we have performed RNA precipitations to identify proteins that bind the regulatory regions of the glp-1 3 UTR. These RNA precipitations have isolated two bands which can be visualized by UV crosslinking, p58 and p30, that bind with specificity to a region within the GLR that is required for repression of translation in posterior cells. These two bands are not able to crosslink to a probe containing a 5-nucleotide mutation within this repression region. Furthermore, these bands cannot be competed off with an excess of this same mutation. We conclude that p58 and p30 are RNA binding proteins that may be involved

in the translational repression of GLP-1 expression in posterior cells. We intend to isolate enough of these polypeptides to identify them using mass spectrometry.

958. Approaches to Studying the Function of APH-1

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The aph-1 gene is involved in Notch-mediated signaling events in the C. elegans embryo. Mutations in aph-1 were isolated in the Priess lab by virtue of their embryonic lethal phenotype which showed a striking resemblance to the phenotype caused by mutations in the glp-1 gene. Further analysis of the aph-1 mutant phenotype demonstrated that the aph-1 gene product was necessary for GLP-1-mediated events at both the 4-cell and 12-cell stages of embryogenesis, thus identifying a new component of the GLP-1 signaling pathway in the early embryo. We have cloned the aph-1 gene and found that it encodes a novel protein with unknown function. Several lines of experimentation are underway to determine what is the specific function of the APH-1 protein.

One approach we have taken is to build an aph-1::GFP fusion construct to look at the cellular and subcellular localization of the APH-1 protein in embryos. We have established transgenic lines with these constructs and shown that the fusion protein can rescue the aph-1 phenotype, albeit with low penetrance. Given that the fusion has some wild type activity, we are now analyzing the fluorescence pattern obtained from these constructs.

A second approach we have taken is to search for genes that interact with aph-1. We have used a leaky allele of aph-1 to isolate extragenic suppressor mutations. Six independent mutations were found to suppress the aph-1 embryonic lethal phenotype. Two of these are dominant mutations in two different genes, and the other four mutations are recessive and identify two additional genes. Mapping data and further characterization of these four genes will be presented.

959. *C.elegans* RAB-5 is involved in asymmetric cell division in early embryogenesis

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PKC-3, a *C. elegans* homologue of atypical PKC, colocalize with PAR-3 and PAR-6 to the anterior periphery of the one-cell embryo and they cooperate together to establish cell polarity required for asymmetric cell divison. However, the mechanism determining asymmetric localization of these proteins and that leading to asymmetric cell division are still unclear.

To identify additional genes involved in the asymmetric cell divisions, we used RNAi technique and examined homologues of signal transducting genes, cell polarity genes, or genes known to regulate vesicle transport. Among 50 genes we tested, 4 gave embryonic lethality upon RNAi. Further analysis showed that the timing of embryonic cell division were disrupted in embryos depleted with a RAB5 homologue. In these embryos, intervals between the second and the third cell division of AB and P1, respectively, are elongated and they stay in 3-cell stage for 5 minutes, whereas it takes 2 minutes in wild type embryos. We also found mislocalization of P-granules and PAR-2 in *rab5(RNAi)* embryos, but the localization of PAR-3 and PKC-3 seemed to be normal. Double RNAi of *rab-5;;par-3* showed phenotype similar to that of *par-3 (RNAi)* embryo but *rab-5;;par-1(RNAi)* embryos exhibit complex phenotype; the first cell division is asymmetric, and the second division is asynchronous but doesn't delay as late as *rab-5(RNAi)* embryos. These results suggest that RAB-5 might act downstream of PAR-3 and upstream of PAR-1. Mammalian homologue of Rab5 is well known to be involved in endocytosis and many genes are identified to interact to it. However, RNAi of RAB-5 effector homologs, such as EEA1, Rabex-5, RabGDI, and Rabaptin-5 showed no embryonic lethality. These results suggest that RAB-5 may have yet unidentified function to regulate cell division.

960. A gain-of-function mutation in the *oma-1* gene results in C to EMS fate transformation

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OMA-1 and OMA-2 are two CCCH zinc-finger-containing proteins whose functions are redundantly required for oocyte maturation in *C. elegans* (see abstract by Detwiler et al). With antibodies specific to either OMA-1 or OMA-2, we have shown that both proteins are expressed at a very high level in the cytoplasm of maturing oocytes and in one-cell embryos. The embryonic expression drops to below detectable levels by immunofluorescence in 2-cell embryos. However, with an OMA-1-GFP fusion construct, we could detect GFP up to approximately the 4-cell stage. OMA-1-GFP is preferentially segregated to the germline precursors (P1 and P2) and is P-granule-associated, in addition to being cytoplasmic. We believe that detection with OMA-1-GFP is more sensitive than our antibody staining. Because *oma-1;oma-2* mutant worms are sterile, we are unable to examine the function, if any, of OMA-1 and OMA-2 in early embryos.

A gain-of-function mutation in *oma-1, zu405*, results in a temperature-sensitive, maternal-effect, embryonic lethality. We can show with antibody staining that OMA-1 protein is detected in *zu405* embryos from the 1-cell to approximately the 8-cell stage. The staining is specific to the germline precursors and is cytoplasmic as well as P-granule-associated. The level of OMA-2 protein is not changed in *zu405* animals.

Embryos from *zu405* homozygous mothersproduce extra intestinal cells as well as extra pharyngeal and body-wall muscles, tissue types normally derived from wild-type EMS. Laser ablation analysis indicates that the C blastomere adopts the fate of EMS in zu405. Instead of producing skin and muscles, it produces muscles and intestinal cells. We have also shown that C in zu405 expresses an EMS-specific marker: MED-1-GFP. When wild type EMS divides, intestine is derived from the

posterior daughter E and this specification requires a Wnt signal from P2. When C divides in *zu405* embryos, intestine is always derived from the posterior daughter Cp. We could show that the intestine formation from C is dependent on *wrm-1* activity, suggesting that a Wnt-like signaling pathway is required for C to produce gut in *zu405*.

In wild-type embryos, the C blastomere fate is dependent on the transcription factor PAL-1 whereas the EMS blastomere fate depends on SKN-1, despite the expression of both SKN-1 and PAL-1 in both EMS and C. Normally, SKN-1 level is low in 8-cell and not detectable in 12-cell embryos but PAL-1 protein lasts past 28 cells. It has been suggested that C expresses PAL-1-dependent cell fates because PAL-1 outlasts SKN-1 in C. If this model were correct, over-expression or endurance of SKN-1 in the C blastomere would allow it to develop like a wild-type EMS blastomere. Consistent with this notion, we detect a high level of SKN-1 protein up to the 8-cell and a low level in the 12-cell stage *zu405* embryos. SKN-1 protein appears to last one cell division longer in *zu405* than in wild type embryos.

In 30% of *zu405* embryos, the P2-to-EMS signaling is abnormal and both daughters of EMS produce mesoderm. The C to EMS fate transformation and the defect in P2 to EMS signaling are similar to what was described with the *gsk-3(RNAi)* embryos. The expression of PIE-1 and APX-1 appears unaffected in *zu405* embryos. We are currently investigating the expression patterns of other maternal factors in *zu405* mutants.

961. The cell biology of Pod-1 and Pod-2 during polarity establishment in *C. elegans embryos*

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A fundamental question in biology is how a single cell develops into a complex, multi-cellular organism. In <i>Caenorhabditis elegans<i>, assignment of cell fate begins with establishment of anterior/posterior polarity in the first cell cycle. As a consequence of this polarity, the first cell division produces an asymmetric two-cell embryo. Molecularly, anterior/posterior polarity is determined by the asymmetric localization of critical proteins within the embryo. Despite extensive research in several systems, the physical mechanism of polarity establishment in embryonic cells is largely unknown.

Our laboratory has identified at least two classes of osmoticallly-sensitive, polarity defective mutants that provide a new and unique perspective on the generation of polarity. The actin-binding protein POD-1 (polarity osmotic defective) localizes asymmetrically to the anterior of one-cell embryos before mitosis and to punctate cytoplasmic regions throughout early development. <i>pod-1<i> mutant embryos have abnormal egg shells and contain large membrane-bound vesicles. Furthermore, the pod-1 gene encodes a protein with regions similar to coronin, a protein predicted to play a role in actin-mediated endocytosis. These observations allow us to propose a model linking the generation of anterior/posterior polarity to asymmetric trafficking of membranes in one-cell embryos. This model predicts that POD-1 localization changes throughout the cell cycle and that cytoplasmic puncta containing POD-1 will show dynamic, flowing movements in the cytoplasm of one-cell embryos. To examine the dynamics of POD-1 in vivo, I created a fusion protein between POD-1 and the green fluorescent protein (GFP). Changes in POD-1 localization can be correlated with well-characterized developmental events such as maturation, nuclear migration, and nuclear breakdown in the oocyte and meiotic and

mitotic events in the early embryo. I will also characterize the ultrastructural localization of POD-1 with immuno-electron microscopy using antibodies against POD-1 and other markers of the endomembrane system.

Pod-2 functions in the same pathway as Pod-1 and is required for the asymmetric localization of several proteins important for polarity establishment including Pod-1, Par-1 and Par-3. Phenotypically, pod-2 mutants are similar to pod-1 exhibiting a loss of polarity in two-cell embryos and osmotically-sensitive embryos. The cause of osmotic sensitivity in <i>pod-2<i> has not been examined, but is likely to involve defects in eggshell structure. I will use transmission electron microscopy to examine the ultrastructural phenotypes of <i>pod-2<i> mutants including eggshell structure.
Understanding the cause of osmotic sensitivity in these mutants may provide some clue as to the function of the POD proteins in polarity establishment.

962. Embryonic development of the free-living marine nematode Pellioditis marina

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The complete embryonic cell lineage (until muscle contraction) of a Pellioditis marina embryo was established by means of 4D-microscopy. Pellioditis marina is a close relative of Caenorhabditis elegans, but has adapted to a marine, brackish environment. The overall lineage resembles strongly on that of C. elegans, with a few small differences.

1. The developmental tempo of the early embryogenesis (until division of E cell) is more then two times slower than C. elegans. But the primordial germline cell P4 is already present at the 15-cell stage (in C. elegans at the 24-cell stage). 2. At the stage of muscle contraction (when most cells are established), P. marina has more cells than C. elegans (ca. 590 cells and 570 cells respectively) and there are less cell deaths (60 and 87 respectively.) 3. The Caapaa-cell in the C-lineage, who dies in C. elegans, forms an extra hypodermis cell in P. marina. 4. Most tissues are formed by the same cells as in C. elegans. Only the hypodermis probably has a different structure. The systematic position of Pellioditis marina remains unsolved, whether Caenorhabditis (Andrassy, 1983) or Rhabditis (Sudhaus, 1976, Fitch, 1995) is the closest relative. The embryonic development of P. marina has resemblance with both genera. The early embryogenesis and the developmental timing are comparable with that of other Rhabditis species, while the overall cell lineage is almost identical (except the one cell death in the C-lineage) with that of C. elegans. The latter is a strong argument to place P. marina close to C. elegans in classification. In more primitive nematodes (like Halicephalobus sp.), sublineages form identical cells, which migrate to their exact location. C. elegans has adjusted these lineages to avoid these migrations*. P. marina falls in between: it has already formed two nerve cells in the Caa-lineage, but still has

not eliminated the extra hypodermis cell. * Borgonie et al., Nematology 2000, Vol 2(1), 65-69

963. Further Studies of MES-1 Localization and Asymmetric Embryonic Cell Divisions

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Asymmetric cell divisions generate daughter cells that have different cell fates. The *mes-1* gene is required for the asymmetric division of the germline blastomeres in embryos. Mutations in this gene cause the offspring of homozygous mutant mothers to develop into sterile adults. Mutant offspring are sterile because the primordial germ cell P4 develops instead as a muscle precursor, like its sister D (1). A loss of asymmetry during the divisions that generate P4 and D causes the fate transformation. Wild-type embryos use a different mechanism to generate asymmetry in P0 and P1 in comparison to P2 and P3. In these latter cells the centrosome-nucleus complex and it associated P granules rotate and migrate to an asymmetric position within the cell. In *mes-1* embryos, the centrosome-nucleus complex in P2 and P3 fails to rotate and migrate, resulting in a loss of asymmetry (2). Thus MES-1 plays a role in the P2/P3 specific events that generate asymmetry and distinct daughter cells.

In wild-type animals MES-1 is detected in 4- to 24-cell stage embryos as a thin crescent between each germline blastomere and the adjacent gut cell (i.e. between P2 and EMS, then between P3 and E; 3). The embryo stages in which MES-1 is detected correlate to the stages that exhibit defects in *mes-1* animals. Its position also correlates with the region of P2/P3 to which the centrosome-nucleus complex migrates. Antibody staining of isolated blastomeres showed MES-1 to be located on the surface of the P2 cell.

MES-1 may serve as a orientation cue or may attract intracellular components to an asymmetric site on the membrane. Its homology to receptor tyrosine kinases together with its position only between the germline and gut cells suggests a role for cell-cell contact in localizing MES-1. Results of experiments investigating MES-1 localization and the participation of germline-gut cell interactions in the asymmetric events in P2/P3 will be presented.

(1) Strome et al. Dev. 121 2961-72 (1995)

(2) Hird et al. Dev. 122, 1303-12 (1996)

(3) Berkowitz and Strome Dev. 127, 4419-31 (2000)

964. An RNAi based screen for regulators of GLP-1 translation and new factors involved in early embryonic polarity from a set of germline enriched genes.

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In *C. elegans* embryos, polarity is established prior to the first division by the segregation of maternally derived factors. Polarity in the early embryo leads to the localized expression of many developmental factors that promote distinct cellular fates. The notch protein GLP-1 is localized in anterior cells of the early embryo. GLP-1 is localized through translation control through the 3 untranslated region (UTR) of the mRNA. Reporter RNA experiments have shown that there are multiple 3' UTR elements that control GLP-1 translation. However, the factors that regulate GLP-1 and those that link GLP-1 translation to early polarity remain largely unknown.

We are using an RNA interference (RNAi) based screen to find new factors that either directly or indirectly regulate GLP-1 or function in the control of general developmental polarity. Previously we screened random clones from a cDNA library. Although some interesting genes were identified (see Barbee et. al., abstract), RNAi suppression limits the success of this screen. Thus, we have modified the screen to limit the number of factors being screened by screening a subset of genes that were shown to be germline enriched by gene chip array experiments performed in the Kim lab (Reinke et. al. 2000). We obtained primers pairs, which were kindly provided by Valerie Reinke and Stuart Kim that correspond to the both the germline intrinsic and the oocyte enriched genes. These primers were used to PCR amplify fragments from genomic DNA. Using gene information from the genome project, we have prioritized three groups of genes to screen. Since the regulation of GLP-1 is though the 3 UTR of the mRNA, the first of these contains any genes that may bind RNA (RNA binding proteins and zinc finger proteins). The second sub-set contains genes with an unknown function but with an interesting domain and/or

out of species homologue. Lastly, there is also a set of genes that have no known function or homologue but still may play vital roles in development. Following RNA synthesis of selected genes worms were injected with dsRNA either individually or in pools of three. Using immunoflourescence worms were labeled with antibodies to GLP-1 and P-granules (as a posterior marker of polarity) and screened for mislocalization. At present we have screened 39 factors and have found 14 genes that had varying levels of lethality following RNAi. Two novel genes appear to potentially mislocalized GLP-1in the embryo, although at very low penetrance. The low penetrance could be due to an incomplete RNAi affect, redundancy, or an indirect affect resulting from general disruption of embryonic development.

Reinke V, Smith HE, Nance J, Wang J, Van Doren C, Begley R, Jones SJ, Davis EB, Scherer S, Ward S, Kim SK, A global profile of germline gene expression in C. elegans Mol Cell 2000 Sep; 6(3): 605-16

965. Analyzing genetic networks that regulate the development of the early *C. elegans* embryo

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The fate of a cell is determined by its "genetic state", by all proteins (or gene products) present in the cell. The genetic state of a cell can be represented by naming each gene and its current state, which can be either 1 ("on") or 0 ("off"). Cells change their states during development, according to the interactions between the genes. For example, *end-1* is expressed as a result of the activity of the transcription factor SKN-1. A set of interactions is called a genetic network. Genetic networks can be modeled in (at least) two ways: First, as a matrix with as many rows and columns as there are genes in the network. The number in column *i* and row *j* represents the influence that gene no. *i* has on gene no. *j*. Second, as a boolean network: Each gene is assigned a logical operator, e.g. "AND" or "OR". Gene states serve as input values for other genes.
We have developed a software system that allows the user to enter and analyze genetic networks. The analysis can be done using one of the methods mentioned above. The user can edit the network via a graphical interface. New genes can be added by simple mouse clicking, or they can be dragged with the mouse from a predefined "database"-network. Interactions between genes can also be added using the mouse. Alternatively, the user can directly specify interactions between genes by typing in the boolean function.
The calculation of stable states of the network gives information about the number and kind of cell types that can be generated via this network. The user can thus check whether the genes he has entered so far are sufficient to obtain the different cell types observed in biological experiments. Furthermore, the user can enter data about the cells in the organism (e.g. radius and position), and he can define the initial states of all genes in every cell. Additional parameters, for example which gene is responsible for specifying the division ratio of a cell, can be fed in easily. The development of the worm embryo

under control of the given network can then be simulated using the CellO program [1]. The cells reside within a 3-dimensional model of the egg shell. They divide according to the rules specified by the network. As the cells have a spatial relationship in this simulation, inductive interactions between cells are also possible. The user can define some genes in the network to be "external", meaning that they influence only genes in neighboring cells. It is also possible to set the cell positions as observed in wild type embryos [2]. This may be useful to study inductive interactions that depend on correct cell positions and spatial relations.
To summarize, this piece of software can be used to (1) simply gather and sort information about genetic networks: a clear graphical view of the network is presented, (2) analyze networks: calculate stable states and watch cell and organism development, (3) make predictions about cell differentiation: by leaving out genes, the development of mutants may be predicted. We plan to implement a connection to internet databases, e.g. AceDB and WormBase, to import genetic data directly from the internet. As more and more data are collected, it may be possible to simulate the whole worm, not only the early embryo.

References:
[1] Gumbel M, Schnabel R, Meinzer HP (2000). 14th ESM Proceedings. SCS Publications, pp. 605-611.
[2] Schnabel R, Hutter H, Moerman D, Schnabel H (1997). Dev Biol. 184(2), pp. 234-265.

966. Nematode embryonic cell lineages are computationally efficient

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The fixed cell lineages of nematodes like Caenorhabditis elegans are thought to provide a particularly efficient way to build an organism. However, many aspects of the C.elegans embryonic lineage are not obviously efficient (e.g., the distribution of neurons). Here we test whether the embryonic lineages of three species of rhabditid nematodes, C. elegans, Pellioditis marina and Rhabditophanes sp., are computationally efficient in the way cell fates are specified. We define three measures of cell lineage computational efficiency: number of symmetry breaking events, number of determination events and number of sublineages. First, we find that the actual cell lineages of all species specify most cellular phenotypes, such as cell morphology, function, and position in the hatchling, significantly more efficiently than would be expected if these phenotypes were randomly distributed in the same lineage, regardless of the efficiency measure used. Second, we show that the topologies of the actual lineages, themselves, significantly improve the efficiency of cell fate specification compared to cell lineages with random topologies. Third, we find that the cell lineages of the three species, show comparable levels of computational efficiency, despite considerable differences in topology and cell fates assignments. Our results suggest that the embryonic lineages of rhabditid nematodes evolve to place the right cell in the right place in a computationally efficient way.

967. Chromosome remodeling during meiosis

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This abstract is being submitted as a placeholder for a revised abstract to be entered during the week of April 20. This delay is necessary because we would like to include data from different lab personnel, and my rotation students (currently comprising the entire lab) will be making their final lab decisions that week.

We are working on several questions regarding chromosome and chromatin remodeling. In particular, we are interested in how chromatin is remodeled during meiosis to accomplish chromosome pairing, crossing-over, and segregation.

968. t1171 is defective in chromosome segregation and cytokinesis

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Loss of function experiments with the *C.e.* Incenp homolog ICP-1, the Aurora-B kinase ortholog, AIR-2, and the survivin ortholog, BIR-1 reveals that these genes are all required for both chromosome segregation and cytokinesis. Since AIR-2 and ICP-1 associate with one another, we have proposed to call this complex the ABI complex (**A**urora-**B**/**I**ncenp). Members of the ABI complex have a dynamic localization pattern during mitosis. Recently, inactivation of these genes by RNAi in *C. elegans* have revealed an essentially identical early embryonic phenotype. Meiotic chromosome segregation doesn't occur and polar bodies are not extruded. During the first mitotic division, chromosome segregation is defective and the cleavage furrows only partly ingress and then they regress; cytokinesis fails. In the following cell cycles, multipolar spindles are formed, unequal DNA segregation occurs, and cleavage furrows ingress and regress, resulting in a multinucleate embryo.

The t1171 allele was isolated in the Schnabel laboratory. Phenotypic characterization of the mutant revealed defects in chromosome segregation and cytokinesis (Goenczy P., Glotzer M., and Hyman T., unpublished results). Reexamination of the phenotype indicates that the cellular defects phenocopy the RNAi phenotype of the ABI complex components mentioned above. We have mapped the mutation using SNP technology to within < 1 cM. Preliminary experiments indicate that AIR-2 (Aurora-B) kinase doesn't properly localize in t1171 mutant embryos. We are in the process of further characterizing the role of t1171 in cell division and its connection to the ABI complex.

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969. Roles of the dosage compensation protein DPY-28 and the cohesin protein HIM-1 in mitosis and meiosis

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SMC (Structural Maintenance of Chromosomes) complexes are large protein complexes that are involved in a variety of chromosome dynamics, including X-chromosome dosage compensation in *C. elegans*, mitotic chromosome condensation (condensin), sister chromatid cohesion (cohesin), and meiotic chromosome segregation (cohesin). Several proteins are shared by different complexes to function in separate chromosomal processes. An example of this is MIX-1, which is required for both mitosis and dosage compensation in *C. elegans*.

The loss-of-function phenotypes of DPY-28 (homolog of *Xenopus* XCAP-D2 from the dosage compensation complex) and HIM-1 (SMC-1 from the cohesin complex) indicate that both function in the mitotic germline. The gonads of mutant animals have reduced numbers of germ cells, abnormal DNA morphology, and are aneuploid, indicating missegregation of mitotic chromosomes. Localization of DPY-28 and HIM-1 in the mitotic germline in wild-type animals confirms their roles in germline mitosis. Whether these two proteins interact with each other in the mitotic germline is unknown.

DPY-28 and HIM-1 both localize to chromosomes during meiotic prophase I. In XO males, DPY-28 is present on all pachytene autosomes but not the univalent X chromosome, while HIM-1 is present on all chromosomes. This pattern indicates that DPY-28 localizes only between homologs, while HIM-1 localizes between sister chromatids (and perhaps homologs). Moreover, HIM-1 staining appears to be stronger than that of DPY-28 between sisters in diakinesis. The localization of DPY-28 between homologous chromosomes and HIM-1 between sister chromatids suggests that the proteins can have different functions during meiosis.

During embryogenesis, DPY-28 is localized to mitotic chromosomes before the onset of dosage compensation, and solely to the X chromosome later. In contrast, HIM-1 is present in the interphase nucleus throughout development, but is not localized on mitotic chromosomes. Accordingly, immunoprecipitation of HIM-1 from embryonic extracts, as well as embryonic phenotypes from RNAi experiments, reveals that HIM-1 interacts with known cohesin subunits such as SMC-3, but not with DPY-28.

We are currently investigating how HIM-1 functions in the cohesin complex, as well as DPY-28's role in the germline, and hope to gain further insight about how different members of the SMC complexes can participate in diverse chromosomal processes.

970. The HMGA proteins of *C. elegans*

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HMGA proteins are small chromatin proteins identified by the multiple presence of the palindromic AT-hook DNA binding motif [PGK]RGRP. They are 'architectural factors' of chromatin structure and regulate the expression of specific target genes. In mammals HMGA proteins are associated with cell proliferation and with cancer. HMGA proteins are present in all multicellular eukaryotes, but not in yeast. Therefore, the presence of HMGA proteins is correlated with multicellularity. We are interested to use *C. elegans* to characterize the functions of HMGA proteins in the context of development and cell differentiation.

C. elegans produces two HMGA proteins. A small 14 kDa protein (HMGA.1) with three AT hook motives is similar to the HMGA proteins of vertebrates. A second protein (HMGA.2) is larger (33 kDa) and contains five AT hook motives. This protein is similar to the D1 protein of *Drosophila melanogaster* and to the type of HMGA proteins present in plants. GFP fusions of both proteins revealed a beginning expression in later stages of embryogenesis, when morphogenesis and terminal differentiation of cells start. HMGA.1::GFP is a strictly neuronal protein and it is expressed in a subset of head neurons. RNAi with HMGA.1 revealed no phenotype. HMGA.2:: GFP is expressed in all neurons and further tissues. RNAi with HMGA.2 resulted in uncoordinated worms with a faster adaptation in head touch response, but with normal tail touch response. We therefore conclude that the potential transcriptional co-activator protein HMGA.2 may be involved in the neuronal development of *C. elegans*. A possible candidate neuron for this is AVD, as AVD laser ablation leads to a faster adaptation in head touch response. In the future we intend to identify the HMGA.2 dependent neurons by dsRNA expression of transgenes using neuron specific promotors.

971. Characterization of a Mutant with Defects in Chromosome Condensation and Centromere Organization

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Faithful chromosome segregation during mitosis requires proper organization of the centromere, which in turn directs assembly of a kinetochore. We are interested in characterizing genes involved in centromere and kinetochore organization in *C. elegans*. As part of this work we screened a collection of temperature sensitive, embryonic lethal mutants for defects in chromosome segregation. To identify mutants with defects in centromere or kinetochore structure, we stained embryos with antibodies that recognize components of the *C. elegans* centromere (*hcp-3*, a centromeric histone H3-like protein) or kinetochore (*hcp-1*). One mutant displays a marked mislocalization of the *hcp-3* antigen on the metaphase plate, along with defects in kinetochore structure. Because kinetochore assembly depends upon the proper establishment of a centromere, the kinetochore defects observed in this mutant may be secondary to a defect in centromere organization. One model to explain the malformation of the centromere is that the centromeric histones are being deposited incorrectly along the chromosome. Another possibility is that the centromeric histones assemble onto chromosomes correctly, but the chromosomes do not fold properly, resulting in their misalignment on the metaphase plate. Closer examination of mutant embryos revealed a defect in chromosome condensation throughout mitosis, suggesting that the second model is correct. We are currently cloning the gene that has been affected in this mutant.

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972. The ABI-complex is required for chromosome segregation but not for sister chromatid separation

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In *C. elegans*, inactivation of the Incenp homolog icp-1 by RNAi results in cytokinesis and chromosome segregation defects in the early embryo. The phenotype of *icp-1(RNAi)* embryos is virtually identical to that observed in embryos depleted of AIR-2, an AuroraB kinase and BIR-1, a survivin like protein (1, 2). It has been shown that at least two of these proteins, INCENP and AuroraB kinase, interact *in vivo* and *in vitro* (2, 3).

Since it appeared likely that Survivin is another component of the Incenp/AuroraB complex we performed co-immunoprecipitations using anti-AuroraB antibodies and found that indeed Survivin does interact with AIR-2 *in vivo*. For the sake of simplicity we propose to call this complex the ABI-complex (AuroraB/Bir/Incenp complex)

Using a strain that expresses GFP tagged histone H2B we studied chromosome dynamics in *air-2(RNAi)* and *icp-1(RNAi)* embryos. While DNA is separated in unequal masses from the second mitotic division on, chromosomes do not separate in the first mitosis. Instead, DNA appears streched along the spindle axis in anaphase, indicating that kinetochores and spindle microtubules are functional. FISH analysis indicates that sister chromatids separate in AIR-2 depleted embryos. These data suggest that the ABI complex is not required to release cohesion between sisters.

Recent work has shown that condensin recruitment on the chromosomes is dependent on AuroraB (4). Furthermore, genetic analysis of condensin subunits suggest this complex plays a role in chromosome segregation rather then condensation (5, 6). We are currently investigating if condensins might be involved in the chromosome segregation defects in *air-2* and *icp-1(RNAi)* embryos.

(1) Speliotes et al., 2000 (2) Kaitna et al., 2000 (3) Adams et al., 2000 (4) Giet and Glover, 2001 (5) Bhat et al., 1996 (6) Steffensen et al., 2001

973. *In vivo* studies of the nuclear function of Barrier-to-Autointegration Factor (BAF)

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BAF is an essential, novel DNA-bridging protein of unknown function. We are using *C. elegans* to determine BAF's function *in vivo*, and to understand its interactions with a family of inner nuclear membrane proteins termed LEM-domain proteins. The LEM-domain proteins that are conserved in *C. elegans* include MAN1 and emerin, loss of which causes Emery-Dreifuss muscular dystrophy in humans. To test the hypothesis that BAF interacts with LEM-domain proteins, we stained *C. elegans* embryos by direct immunofluorescence using BAF-specific antibodies. Endogenous ceBAF is distributed along the nuclear rim, consistent with its association with inner membrane proteins. However, there appeared to be additional BAF staining in the nuclear interior. By double-staining for BAF plus either MAN1, emerin, or lamins in *C. elegans* embryos, we found that BAF colocalizes at the nuclear envelope with MAN1 and emerin, and that BAF colocalizes with lamins both at the perimeter and interior of the nucleus. These results support our hypothesis, and suggest that BAF is intimately associated with the nuclear lamina. The RNAi phenotype for BAF is early embryonic lethality associated with chromatin defects such as DNA bridging, super-condensation and perhaps nuclear fragmentation. These phenotypes suggest an essential role for BAF in chromatin decondensation and perhaps chromosome segregation.

974. Characterization of LIN-5-interacting proteins

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Chromosome segregation requires proper assembly and positioning of the mitotic spindle, established through a balance between microtubule dynamics and forces generated by microtubule-associated proteins. To further dissect the complicated process of chromosome segregation, we have focused on the *lin-5* gene in *C. elegans.* Animals mutant for *lin-5* fail chromosome alignment and segregation, and show mispositioning of the spindle in early embryonic divisions. These processes are all mediated by microtubules and microtubule-associated proteins. We thus hypothesize that *lin-5,* which encodes a novel coiled-coil component of the spindle apparatus, affects spindle dynamics or force generation. However, the mechanisms by which *lin-5* functions remain unknown. To gain further insight into the function of *lin-5*, we decided to identify proteins that interact with LIN-5.

In a two-hybrid screen, LIN-5 was found to interact with itself and five other proteins. RNAi of these interactors did not produce a Lin-5-like phenotype, nor did it enhance or suppress the defects in *lin-5(ev571*ts*)* mutants. As an alternative approach, we examined the two-hybrid interactions in the context of *lin-5* mutations, since such mutations may prevent binding to key partners. We introduced two mutations previously identified *in vivo*, *ev571*ts and *e1457*, to determine whether they disrupted any two-hybrid interactions. The *e1457* product, though expressed in yeast, failed to bind any of the interactors. The *ev571*ts fusion protein showed decreased binding to three of the interactors. The loss of one or more of these interactions might be the cause of the *ev571*ts phenotype. Therefore, we tested a previously isolated intragenic *lin-5(ev571*ts*)* suppressor mutations for restoration of LIN-5 interactions. We found that predominantly binding to the interactor ZC8.4 was restored. ZC8.4 is

predicted to encode a homolog of PUMA1, a component of the mitotic apparatus in the nematode *Parascaris univalens.* The correlation between intact *lin-5* function and its ability to bind ZC8.4 suggests *in vivo* relevance of this interaction.

To further explore this possibility, we compared the localization of ZC8.4 and LIN-5. ZC8.4 staining coincided with that of LIN-5 at the centrosomes, kinetocore microtubules, and cell cortex. In contrast to the overlapping localization in mitosis, ZC8.4 also localized to the nucleus of interphase cells whereas LIN-5 remained cytoplasmic. Together, the two-hybrid data and the co-localization of ZC8.4 and LIN-5 suggest that an interaction between these two coiled-coil proteins plays a role in one or more of the mitotic functions of LIN-5.

975. The role of *cdc-25.2* and *emb-29* in the cell cycle.

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Embryonic, somatic and germ cell divisions are tightly controlled both temporally and spatially. The mechanisms that control these divisions are complex, but appear to use the same conserved regulators found in other organisms. For example, the cyclin-dependent kinase CDC-2 controls the entry into M-phase. Investigations into how CDC-2 is regulated may show how the different cell cycles are established and maintained. CDC2 is activated by a dual specificity phosphatase called CDC25. Whereas other organisms are known to have between one and three cdc25 genes, four cdc25 homologues have been identified in the C. elegans genome (called *cdc-25.1*, *cdc-25.2*, *cdc-25.3* and *cdc-25.4*). Interestingly, only the first three homologues appear to be expressed in wild-type hermaphrodites.

cdc-25.2 has two transcripts. Both transcripts have the phosphatase domain while only the larger transcript has the putative regulation domain. Only the longer transcript is expressed in embryos, whereas the shorter transcript is present in adults. Perturbation of *cdc-25.2* by RNAi causes L1 larval lethality. However, if all three *cdc-25s* that are expressed are perturbed at the same time, the cell cycle is arrested during the first meiotic division.

The mutant *emb-29* maps very close to the *cdc-25.2* locus. Indeed, two *emb-29* alleles at the restrictive temperature produce cell cycle phenotypes - *emb-29* mutants arrest at the G2/M transition [1]. Furthermore, simultaneous RNAi of *cdc-25.1* and *cdc-25.3* in either *emb-29* background causes a meiotic arrest similar to the triple cdc-25 RNAi phenotype. Unfortunately, only one *emb-29* allele harbours a point mutation in the *cdc-25.2* gene. We are determining whether this point mutation alters the activity of the CDC-25.2 protein. Nevertheless, we suspect that *emb-29* (+) may regulate CDC-25.2: any defect in *emb-29* may effect the activity of CDC-25.2 thus causing a cell cycle phenotype.

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976. *C. elegans* CKI-2 interacts with PCNA

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In *C. elegans*, two apparent Cip/Kip family CKIs are encoded by the *cki-1* and *cki-2* genes found in tandem on chromosome II. *Cki-1* and *cki-2* share 21-27% sequence identity with mammalian p21 and p27, and share 26% identity between themselves. Inactivation of *cki-2* by RNAi produces an impenetrant embryonic arrest phenotype. No apparent larval abnomalities were observed in the *cki-2* (RNAi) animals that hatch. It is unclear whether *cki-2* functions as a negative cell cycle regulator nor is it clear why *cki-2* (RNAi) is embryonic lethal. As one of several approaches to determine the role of *cki-2*, we tried a yeast two hybrid screen to identify interacting partners with the divergent *cki-2* C-terminus as bait.

From 109 primary positives from $6X10^6$ transformants, we got two true positives and sequenced them. Database searches revealed that cDNA sequences from the two positives were identical to the PCNA (Proliferating Cell Nuclear Antigen) coding sequence (*C. elegans* cDNA W03D2.4). Following this, we mapped the PCNA binding site on CKI-2 so that we may be able to use this to block the CKI-2/PCNA interaction *in vivo*. The PCNA binding region of CKI-2 is located between Asn158 and Arg163 (NYMPVR). This sequence is very different from the conserved PCNA binding site used by other CKIs. This may mediate a novel regulatory mechanism of PCNA by CKI-2. We are now trying to identify the CKI-2 binding site on PCNA for comparative purposes.

PCNA is an essential component of the DNA replication machinery, acting as the processivity factor for DNA polymerase d and e , while it is also required for nucleotide excision repair. PCNA interacts not only with DNA repair and replication enzymes, but also with cell cycle regulatory proteins such as p21. This suggests that CKI-2 may mediate the coordination between cell cycle progression, DNA replication, and DNA repair. Loss of this important regulatory function may result in chromosomal / DNA damage at low frequency

during early embryonic cell divisions when replication stress is high resulting in the *cki-2* (RNAi)-dependent embryonic arrest.

977. Investigation of CUL-2/RING finger complexes

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The ubiquitin-proteasome proteolytic pathway plays a critical role in the regulation of cell cycle progression. Ubiquitin ligases (E3s) function in this pathway to target substrates for degradation. There are two major types of E3 complexes, the APC and Cullin/RING finger complexes. The cullin/Ring finger complexes contain a cullin, a RBX1 ring finger protein, a SKP1-like molecule, and a substrate-binding subunit. Our previous study (Feng et al, Nature cell Biology, Vol.1(8), 1999) described the function of the cullin CUL-2. CUL-2 is a positive cell cycle regulator that is required for the G1-to-S phase transition, functioning in part by negatively regulating CKI-1 (a cyclin-dependent kinase inhibitor). Surprisingly, CUL-2 is also required for mitotic chromosome condensation. In humans, CUL-2 was found in an E3 Cullin/RING finger complex with the von Hippel-Lindau (VHL) tumor suppressor, Elongin C (a SKP1-like protein), Elongin B (a ubiquitin-like protein), and RBX1. This CUL-2/VCB complex functions to degrade hypoxia inducible transcription factor 1a and 2a. However, VHL does not appear to function with CUL-2 in C. elegans to regulate the cell cycle since inactivation of the vhl ortholog either by RNAi or with a deletion mutant (obtained by Gary Moulder and Robert Barstead) does not phenocopy cul-2. In addition, in humans, inactivation of VHL decreases the level of CKI p27, and overexpression of VHL increases p27. This is opposite to the negative regulation of CKI-1 by C.elegans CUL-2. We propose that there are substrate-binding components other than VHL in CUL-2 complexes that are required for cell cycle regulation. In order to gain insights into the composition of the CUL-2/RING finger complexes that regulate CKI-1 degradation and chromosome condensation, we are taking two approaches. First, we are testing if inactivation of potential components by RNAi phenocopies cul-2. RNAi of an Elongin C ortholog Y82E9BR.15 gave both germ cell arrest phenotype and embryonic phenotypes of uncondensed DNA and multiple nuclei similar to cul-2 mutants. Removal of ubc5 homolog (ubc2) by RNAi phenocopies the

cul-2 embryonic phenotypes. Therefore, we think that it is likely that they function with CUL-2 in a complex to regulate the cell cycle. Second, we are affinity-purifying FLAG-tagged CUL-2. We purified FLAG-CUL-2 from lysates of transgenic worms, using animals which do not contain the FLAG-tagged CUL-2 as a control. We have detected proteins that specifically associated with FLAG-tagged CUL-2 on two dimensional gels. We hope to identify the associated proteins by mass spectrometry prior to this meeting.

978. The regulation of cell cycle exit by cul-1 & lin-23 genes

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Hyperplasia, the excess proliferation of cells in a tissue, often precedes neoplasia in cancer development. In C. elegans, inactivation of either the cul-1 or lin-23 gene results in hyperplasia in diverse tissues, with mutant cells unable to exit the cell cycle in response to developmental cues. The exact function of cul-1 and lin-23 in the regulation of cell cycle exit is not understood. Orthologs of CUL-1 and LIN-23 in other organisms function together as components of SCF ubiquitin-ligase complexes that target proteins for degradation. We hypothesize two mechanisms by which CUL-1 and LIN-23 promote cell cycle exit:

A. CUL-1 and LIN-23 could directly target a positive cell cycle regulator for degradation, e.g., the vertebrate CUL1 has been shown to degrade cyclin E.

B. CUL-1 and LIN-23 could act in the Wnt/wingless pathway to degrade the signaling molecule b-catenin, which functions as an oncogene in vertebrates. lin-23 orthologs slmb (Drosophila) and b-TrCP (vertebrate) have been shown to function in this pathway.

To study CUL-1 and LIN-23 function, two approaches are being used: candidate substrates testing, and extragenic suppressor screens.

Genetic interactions between cul-1, lin-23, cycE, and b-catenin mutants are being studied. There are three b-catenins in C. elegans: bar-1, wrm-1, and hmp-2. We have constructed double mutants of lin-23 with bar-1 and hmp-2. If lin-23 mutants' hyperplasia phenotype was caused by elevated levels of BAR-1 or HMP-2 proteins, then inactivating them in the double mutants should rescue hyperplasia. However, we failed to observe such a rescue, indicating that BAR-1 and HMP-2 are probably not the critical substrates for LIN-23 in terms of cell cycle exit control. We are currently measuring the protein levels of cyclin E and b-catenin in cul-1 and lin-23 mutants by immunofluorescence and Western blot.

Extragenic suppressor screens are on-going to isolate genes that genetically interact with lin-23. Our strategy is to mutagenize the strain lin-23(rh293) dpy-2(e8) exc-7(rh252)/mnC1 and look for Dpy Exc non-Lin animals among the F2 progeny. Such animals would indicate a lin-23 exc-7 dpy-2 homozygote with a suppression of the Lin phenotype. Prospective suppressors include LIN-23 substrates, other components of the SCF complex, and other regulators of cell cycle exit.

I will report my results of both approaches at the meeting.

979. Securin and Separin: Roles in the C.elegans cell cycle?

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Cell division results in the precise halving of genetic material. In yeast, such partitioning requires the protease separin[1]. This protease acts at the metaphase to anaphase transition to degrade sister chromatid cohesion, which in turn permits chromosomes to segregate to opposite poles of a dividing cell . In addition to facilitating chromosome segregation, separin promotes spindle elongation in *S. cerevisiae* through the activity of a calcium-binding domain[2]. Separin activity also depends heavily on the anaphase inhibitor securin, which localizes separin to both the nucleus and spindle mid-zone, and inhibits its protease activity until anaphase.

Here, we report a requirement for the *C. elegans* homologue of yeast separin during oocyte meiosis. RNAi of this homologue, which contains both the protease and calcium binding domains of *S. cerevisiae* separin, results in multi-nucleated one-cell embryos with multiple spindles. In these embryos, the chromosomes are disorganized and the embryos fail to produce polar bodies. Taken together, these results suggest a role for *C.elegans* separin in chromosome segregation and cytokinesis in the early embryo. Currently, we are investigating if *C. elegans* separin also plays a role in exit from M-phase of the cell cycle.

Additionally, we hope to identify protein partners of *C.elegans* separin by using the protein as bait in a yeast 2-hybrid screen. This screen may prove the most effective way of identifying a *C. elegans* securin homologue, as all of the known securins from other organisms demonstrate no amino acid conservation. Also, to date, no separin interacting proteins other than securin have been identified. Results of the screen will be discussed at the meeting.

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980. Characterization of mammalian homologs related to cell-cycle checkpoint control in *C. elegans*

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ATM (ataxia telangiectasia mutated gene)-like gene and *Chk2*-like gene were identified in the genome of *C. elegans*. The putative products of the genes, termed *Ce-atl-1* (*C. elegans ATM-like 1*) and *Ce-chk-2* consist of 2514 and 450 amino acid residues, respectively. The C-terminal sequence of *Ce-atl-1*, which contains a PI-3 kinase like domain, showed good homology with the products of the gene *MEC1/ESR1* from budding yeast, the *rad3+* gene of fission yeast and mammalian *ATR* genes. The sequence of *Ce-chk-2* product shows 34.3% identity over 449 residues to human Chk2.

To study the function of the *Ce-atl-1* and *Ce-chk-2* genes, we carried out RNA interference (RNAi) experiments to repress its expression. The results of RNAi indicated that the major phenotype associated with repression of *Ce-atl-1* was lethality (approximately 20-50%) during early embryogenesis. Among the surviving progeny, males (XO animals) arose at a high frequency (2-30%). In addition, 5% of oocyte chromosomes demonstrated aneuploidy due to a defect in pre-meiotic chromosomal segregation. We also observed other variable phenotypes among the surviving F1 and F2 animals; namely, lethal morphological abnormalities in L1-L2 larvae, protruding vulvae, abnormal gonads, irregular oocytes, dumpy (Dpy) or small-sized (Sma) adults, and bursting of the vulva, but these phenotypes were not passed on to the next generation. The sensitivity to genotoxin (UV and X-ray irradiation) was also increased by the repression of *Ce-atl-1* gene. However, the phenotype of repression of *Ce-chk-2* gene was completely difference from that of *Ce-atl-1*. *Ce-chk-2* gene was dispensable for repair process of DNA double strand breaks, but essential for meiotic process such as chiasma formation and meiotic chromosome synapses. These observations indicate that *Ce-atl-1* acts as a monitoring enzyme for DNA damage checkpoint control, but *Ce-chk-2* does not,

although Chk2 of yeast and mammals functions as a downstream kinase of ATM family in checkpoint control.

981. THE TIMELESS-LIKE GENE TIM-1 IS CSG-5 AND IS REQUIRED FOR CHROMOSOME SEGREGATION

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The heterochronic gene *lin-42* encodes a protein with high similarity to the PAS domain of the *Drosophila* circadian rhythm protein PERIOD (dPER). Because the PAS domain of dPER interacts with TIMELESS (dTIM), a second circadian rhythm protein, we investigated the role of TIM-1, a TIMELESS-like protein in *C. elegans*. Unlike the *dtim* null mutation, which shows no developmental defects (1), RNAi of *tim-1* results in embryonic lethality. These embryos arrest development as a mass of cells with unevenly-sized nuclei. In early embryos, we observed multinucleate cells by nomarski optics. When stained with DAPI, these embryos showed DNA bridges between forming nuclei, lagging chromosomes, nuclei with abnormal DNA content and fragmented chromosomes. These observations are consistent with a role for TIM-1 in chromosome segregation. Animals that escape the early lethal dsRNA dose show no heterochronic defects and develop into sterile adults. In oocytes of DAPI-stained animals, we observed 11-12 fluorescent "spots", presumably univalents, in contrast to the 6 bivalents observed in wild-type animals. We are using FISH analysis to test whether these spots represent unpaired homologs.

Affinity purified antibodies raised against TIM-1 showed nuclear accumulation and enrichment in the region of the kinetochore spindles during metaphase. Preliminary analysis revealed that TIM-1 is abundant in all cells during early embryogenesis and is present in the nuclei of seam cells, the intestine and the developing germ line during larval stages.

To identify mutations in *tim-1*, embryonic lethal mutations that mapped near the *tim-1* locus on chromosome III were collected from the CGC. We analyzed candidate alleles by DAPI-staining and nomarski optics, looking for phenotypes

similar to the *tim-1* (RNAi) phenotype. We narrowed down the candidates to *csg-5 (t1545)* (chromosome segregation defective), isolated by Gonczy *et al*. in a maternal-effect embryonic lethal screen for genes involved in cell division (4). We sequenced *t1545* and found a single base transition, resulting in a Gly924Asp mutation in a region moderately conserved among *timeless* proteins. A long-range PCR product containing the *tim-1* coding region plus 3.3 kb 5'- and 1.3 kb 3'-flanking sequence fully rescued the embryonic lethality of *t1545.* These results indicate that *tim-1* is *csg-5.*

Recently, the identification of a second *Drosophila timeless* gene (dTIM2) was reported (2,3). dTIM2 is more closely related to TIM-1 and the single mammalian TIM protein than it is to dTIM. Interestingly, *timeless* knock-out mice die during early embryogenesis around a period of rapid cell proliferation (3), suggesting that mouse TIM and TIM-1 may play similar roles in the cell cycle and have functions distinct from the functions of the *Drosophila* clock protein dTIM.

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982. The *C.elegans* CIP/KIP-type cyclin-dependent kinase inhibitor CKI-1 is cell-autonomously required for postmitotic differentiation as well as cell cycle arrest.

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Although it has been long observed that some processes of differentiation, such as changes in cell shape and cell migration, are generally incompatible with cell division during animal development, the cellular biochemistry underlying such phenomena is still unknown.

In previous worm meetings, we showed that the *cki-1* gene is required to arrest the cell cycle during *C. elegans* embryogenesis. CKI-1 belongs to the conserved CIP/KIP cyclin-dependent kinase inhibitor (CKI) family. Studies in other systems have demonstrated that overexpression of CIP/KIP CKIs leads to G1 arrest by inhibiting the activity of various cyclin/CDK complexes, which positively regulate the cell cycle.

We also reported previously that expression of various postmitotic differentiation markers is diminished in *cki-1(RNAi)* embryos and adults, as seen in mice defective for CIP/KIP CKI function. These findings suggest that CIP/KIP CKIs might directly promote postmitotic differentiation as well as cell cycle arrest. Alternatively, hyperplasia caused by loss of CIP/KIP CKIs activity may disturb intercellular signaling processes required for normal differentiation. Although the former hypothesis is supported by *in vitro* studies showing that overexpression of CIP/KIP CKIs induce terminal differentiation in some mammalian cell cultures, this has not been experimentally demonstrated *in vivo*.

We have found that elimination of *cki-1* function by RNAi can prevent expression of two postmitotic embryonic gut markers, *pho-1*::GFP and *Ce-npa-1*::GFP (gifts from T. Fukushige, J. McGhee, and I. Johnstone), concomitant with gut hyperplasia. As we have also found that isolated E cells express *Ce-npa-1*::GFP, these results strongly suggest that *cki-1* is

cell-autonomously required for gut differentiation. Interestingly, *cul-1(RNAi)*, which also results in embryonic hyperplasia, eliminates expression of *Ce-npa-1*::GFP as well. We are currently testing whether: 1. elimination of cyclin/cdk activity by RNAi can rescue the differentiation defects in *cki-1(RNAi)* or *cul-1(RNAi)*, 2. *cki-1(RNAi)* disturbs other aspects of gut differentiation, and 3. exogenous CKI-1 that is expressed postmitotically can rescue loss of differentiation markers in deficiency embryos defective in the *cki-1* gene to examine whether CKI-1 acts in a cell-cycle independent process to promote cellular differentiation.

983. The *sys* genes and early gonadogenesis

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The process of organogenesis involves cell proliferation, differentiation and morphogenesis. We have chosen the gonad as a model system to study the coordination of these events. The somatic structures of the gonad develop from two cells (Z1 and Z4) in the gonad primordium. In the wild type, Z1 and Z4 give rise to non-equivalent sister cells during L1. In hermaphrodites, Z1.a and Z4.p give rise to a distal tip cell (DTC), while Z1.p and Z4.a give rise to an AC/VU precursor. In males, Z1.a and Z4.p give rise to DTCs, while Z1.p and Z4.a give rise to the linker cell (LC). In addition to these regulatory cells, somatic blast cells are also generated, which become rearranged into a somatic primordium (SP) that prefigures the development of later somatic structures.

We have isolated a collection of mutants that are defective in early gonadogenesis and are therefore sterile. Our recent focus has been on one group of these mutants that have similar early gonadal defects in hermaphrodites: loss of DTCs and the inability to form an SP. By mapping and complementation tests, we find that these mutations define six genes, designated *sys-1* through *sys-6* (*sys* for ***sy**mmetrical **s**isters*). Previous work demonstrated that the first divisions of Z1 and Z4 appear to generate equivalent cells in *sys-1* hermaphrodites -- Z1.a and Z4.p appear to adopt the fates of their sisters(1). This phenotype is similar to that described for the *frizzled* homologue *lin-17*(2). Furthermore, the two *sys-2* mutations are actually alleles of *pop-1*, consistent with the idea that the Wnt signaling pathway controls the polarity of this first division (see Siegfried abstract).

Here we report our progress on characterizing *sys-3 - sys-6*. The *sys-2 - sys-6* genes and *lin-17* not only have similar phenotypes, but they also interact genetically with *sys-1*. Thus, for example, *sys-1/+*; *sys-4/+* double heterozygotes display Sys gonadal phenotypes even though both mutations are normally recessive. These genetic interactions suggest that the *sys* genes and *lin-17* all function in a common pathway to control early gonadal development. However, with the exception of *sys-2*, the other *sys* genes do not map to any known Wnt pathway components. Therefore, the *sys* genes may identify regulators of Wnt signaling that have previously not been identified.

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984. Characterisation of an XPF endonuclease-like gene in *Caenorhabditis elegans*

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Cell cycle checkpoints ensure the faithful transmission of genetic information during cell division. Damaged or incompletely replicated DNA triggers cell cycle checkpoint proteins that delay cell cycle progression until damaged DNA is repaired or DNA replication is completed. Without checkpoint proteins, cell division would occur with damaged or incompletely replicated DNA, which could result in genetic mutation or cell death. Many cell cycle checkpoint genes have been isolated in yeast as cell division cycle defective (CDC) or radiation sensitive (RAD) mutants; however, less is known about the role of cell cycle checkpoint proteins in multicellular organisms. In addition to cell cycle arrest, DNA damage can also trigger apoptosis of the damaged cell. To further our understanding of cell cycle checkpoints in multicellular organisms, we are investigating the role of cell cycle checkpoint genes identified in yeast that have orthologues in *C. elegans.*

One such gene is the cell cycle checkpoint gene MUS81 (**M**MS and **U**V **S**ensitive **81**). MUS81 was identified in two separate yeast two-hybrid screens. One screen used the cell cycle checkpoint kinase CDS1 as bait (1) while the other screen used the recombinational DNA repair protein, RAD54 as bait (2). In yeast, MUS81 mutations result in sensitivity to UV irradiation and chronic MMS exposure but are not to X-rays (1,2). MUS81 encodes a protein with two helix-loop-helix domains flanking a domain with similarity to the XPF-endonuclease. We will present our results and discuss the role of MUS81 in *C. elegans.*

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985. Control of cell cycle withdrawal during *C. elegans* development

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Cells decide to enter a new division cycle in response to extracellular signals during the G1 phase of the cell cycle. The mechanisms that act upon the basic cell-cycle machinery to accomplish temporal control of cell division are poorly understood. We are using the highly defined formation of the *C. elegans* vulva as a model to study the regulation of cell-cycle entry during development. In wild-type worms, six vulval precursor cells (VPCs) are formed during the L1 stage and have the potential to adopt vulval cell fates. The VPCs remain quiescent until they undergo further divisions and differentiate during the late L3 stage. To identify genes necessary for arrest of the VPCs in L1 to L3 larvae, we have screened for mutations that allow additional cell divisions during this period of development. In gain-of-function mutants of *lin-12* Notch, all six VPCs adopt vulval cell fates which results in the formation of up to 6 pseudovulvae. In this genetic background, mutations that cause extra cell divisions of the VPCs result in additional pseudovulvae (Hong *et al*., 1998). We have screened EMS-mutagenized *lin-12(gf)* animals and have identified two loci whose mutation yields the enhancer of *lin-12(gf)* multivulva phenotype.

Homozygous *he118* mutants have extra cell divisions within the VPC lineage during the L2 stage, resulting in up to twice the wild-type number. The *he118* mutation has been mapped close to the *cki-1* gene, which encodes a cyclin-dependent kinase inhibitor. The phenotype of the *he118* mutant is rescued by a cosmid containing *cki-1*. In addition, RNA-mediated interference (RNAi) of *cki-1* also results in extra VPC divisions. Moreover, the extra VPC phenotype of both *he118* and *cki-1(RNAi)* is enhanced by loss of *lin-35* Rb activity (See abstract by Boxem *et al*.). Based on these overlapping phenotypes and the map position of *he118*, we believe *he118* is a *cki-1* mutation. However, the *cki-1* coding region was found to be wild type in *he118* mutants. Since

this mutation mainly affects the VPCs and, in contrast to *cki-1(RNAi)* animals, not the intestinal, gonadal or other hypodermal lineages, the *he118* mutation may disrupt a tissue specific element required for proper *cki-1* expression.

In addition to *he118* we obtained two alleles, *he117* and *he119*, of *lin-1* Ets in the screen for extra cell divisions. *lin-1(lf)* results in low penetrance extra VPC divisions during the L2 stage. Ectopic VPC divisions have also been observed in mutants of *lin-31* HNF-3, the presumed partner of LIN-1 in the VPCs (Miller *et al.*, 1993). Using a *cki-1::GFP* transgene (a kind gift from V. Ambros) we determined that *cki-1* expression is reduced or absent from the VPCs of *lin-1* or *lin-31* mutant animals. These data suggest that the extra VPC divisions in the *lin-1* and *lin-31* mutants may be a result of decreased expression of the cell cycle inhibitor *cki-1*. We propose that cell-type specific transcriptional regulation of the *cki-1* CDK inhibitor mediates the temporal withdrawal from the cell cycle during development.

986. *C. elegans* SR protein kinase, SPK-1, is required for germline proliferation and maturation.

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SR-protein kinases (SRPKs) and their substrates, the serine/arginine-rich pre-mRNA splicing factors (SR proteins), are key components of splicing machinery and are well conserved across phyla. Despite extensive biochemical investigation, the physiological functions of SRPKs remain unclear. In *C. elegans*, one SRPK gene and six SR protein genes have been reported. Functional analyses by us and other labs utilizing RNAi technique revealed that one member of SR protein family, CeSF2/ASF, is essential for embryogenesis (1,2), while other SR proteins play redundant roles (2,3); *spk-1* is required for early embryogenesis (1,2). However, we have also demonstrated that *spk-1* is required in germline proliferation and gametogenesis utilizing various RNAi protocols (1).

Here we report the isolation and analyses of a deletion mutant within the *spk-1* gene. *spk-1* mutants are comparable to Wild-Type in locomotion and body size. However, *spk-1* mutants shows recessive fully penetrated phenotype of sterility in both hermaphrodites and males. In *spk-1* hermaphrodites germlines are underproliferated and lack oogenesis. *spk-1* males have comparable number of germ nuclei in a gonadal arm, suggesting deficiency in sperm maturation. These observations indicate that zygotic expression of *spk-1* is required for germline proliferation in hermaphrodites and maturation in both hermaphrodites and males. In order to analyze the epistasis between germline proliferation deficiency in *spk-1* mutant and ectopic or tumorous proliferation of germline in *gld-2 (h292)* and *gld-1; gld-2* double mutant, we generated double and triple mutants. Certain population of *gld-2 (h292); spk-1* showed ectopic proliferation of germ nuclei in the most proximal region of the gonad, indicating that *gld-2 (h292)* is epistatic. On the other hand,

gld-2 (q497) gld-1 (q485); spk-1 showed Glp phenotype, indicating that *spk-1* is epistatic. With these characteristics, *spk-1* mutant may serve as a useful tool to analyze the molecular mechanisms in sex-determination, proliferation and maturation of *C. elegans* germline.

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987. Genetic Analysis of Early Germline Proliferation

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Little is known regarding the molecular basis for early proliferation of the primordial germ cells, Z2 and Z3. The first several rounds of germ cell division are independent of GLP-1,[1] yet cell-cell signaling appears to be required.[2] The gonad primordium contains four cells: Z1, Z2, Z3 and Z4[3,4]. Ablation of Z1 and Z4, the somatic gonad precursors, prevents Z2 and Z3 from dividing or entering meiosis.[2] Therefore, cell-cell communication is necessary for both the proliferation and meiotic competence of Z2 and Z3. The result of ablation of Z1 and Z4 differs from the *glp-1* null mutant phenotype in which Z2 and Z3 divide several times, enter meiosis early, and form mature sperm.[1] The effect of this ablation also differs from the phenotype of other germline proliferation mutants in which Z2 and Z3 divide several times but do not enter meiosis (e.g., *glp-4*,[5] and *glp-3*[6]). The molecular components of the Z1/Z4-to-Z2/Z3 signaling event are not known, and we are using a genetic approach to identify these components.

We have found several mutants in which the adult hermaphrodite somatic gonad appears normal but apparently houses no germ cells (Nog phenotype). A host of developmental defects could result in a Nog phenotype. For example, Z2 and Z3 may not be generated, may die, or their descendents may die. Alternatively, Z2 and Z3 may be generated but may not reach the gonad primordium or they may reach the primordium but fail to proliferate. Interference with cell-cell communication between the somatic and germ lineages suggested by the Z1/Z4 ablation experiment could account for the last scenario.

One of our Nog mutants, *ar228*, hatches with a normal-looking L1 gonad primordium containing four cells. During the late L1, however, at a time when the germ line of heterozygous siblings has begun to proliferate, the two central cells take on a slightly abnormal morphology under Nomarski optics. To assess

the identity and fate of the two central cells in the primordium, we are performing a time-course analysis with a germ line-specific antibody. The *ar228* mutants display no other obvious behavioral or morphological defects. Therefore, characterization of the gene defined by the *ar228* allele could provide a molecular handle on signaling that initiates the proliferation of Z2 and Z3. Alternatively, this mutation could produce a germ line-specific cell-cycle defect or otherwise affect the maintenance of the germ cell fate.

Genetic analysis indicates that *ar228* is recessive, completely penetrant and maps to the left arm of LGV. *ar228/sDf50* displays the same phenotype as an *ar228* homozygote, suggesting that *ar228* is a strong loss-of-function allele. Preliminary data also suggests that *ar228* fails to complement *e2173*, a small deletion that originally defined the *lin-40* locus. This allele deletes at least three complementation groups.[7,8] Mutants representing two of the genes within the *e2173* deletion (*s1669* and *s1611*) both complement *ar228*. Therefore, *ar228* identifies one of the other genes in this region. Experiments are underway to locate the *ar228* locus from among the ORFs in the region.

We gratefully acknowledge Bob Johnsen and David Baillie for providing stains and information pertaining to the *e2173* deletion. Thanks also to the Kimble lab for providing a GFP-tagged version of the *nT1* balancer that has proven very helpful in our experiments.

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988. Maintenance of embryonic stem cell identity in the germline blastomeres of *C. elegans*

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Sexually reproducing organisms arise from germ cells, which have the potential to recreate the whole organism. An important question is how germ cells maintain this potential, or remain totipotent. In early *C. elegans* embryos, germline blastomeres divide asymmetrically to produce one somatic and one germline daughter. Maternally provided PIE-1 protein is present in the germline blastomeres throughout early development (Mello et al.). Germline blastomeres also contain (but do not respond to) maternally supplied transcription factors that cause differentiation of somatic blastomeres. In *pie-1* mutants, germline blastomeres respond to these transcription factors and acquire somatic fates. Thus, PIE-1 is required to prevent germline blastomeres from adopting somatic fates. But what is PIE-1 actually doing?

Several lines of evidence suggest that PIE-1 inhibits accumulation of RNA polymerase II-dependent RNAs in the germline blastomeres, and the antibody H14 stains activated RNA polII in somatic cells, but not in germline blastomeres in *pie-1 (+)* embryos (Seydoux et al.). This phenomenon is referred to as transcriptional inhibition, though the underlying mechanism has not been determined. When PIE-1 is ectopically overexpressed in the somatic blastomeres of the early embryo, it is capable of inhibiting the accumulation of mRNA in those blastomeres (Seydoux et al.). The finding that PIE-1 can cause transcriptional inhibition in all cells of the embryo suggests that PIE-1 targets are present in all cells. We are interested in the possibility of using ectopic PIE-1 in screens to identify additional genes involved in transcriptional repression. We have examined the effect on late embryonic and postembryonic development of a heat shock driven *pie-1* transgene (constructed and provided by Geraldine Seydoux). Late stage embryos and adult worms subjected to heat shock express abundant PIE-1 in somatic nuclei, however these same nuclei remain positive for activated RNA polII by H14 immunostaining.

Nevertheless, heat shock causes a marked developmental delay that is not observed when larvae are treated with RNAi for *pie-1*. To examine the basis for this effect, we are screening for mutants that can suppress the developmental delay and asking whether these have affects on early embryogenesis that are consistent with PIE-1-mediated transcriptional repression.

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989. Identification of factors required for primordial germ cell development

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The germ plasm is a specialized region of cytoplasm in oocytes and early embryos that is both necessary and sufficient to direct cells to enter germline development, and thus, is believed to harbor "determinants" that singly or collectively specify germ cell fate (Ikenishi, 1998; Wylie, 1999). The identity of these determinants, however, remains elusive. With few exceptions, the factors required to specify germ cell fate are still unknown.

Among the germ plasm components identified in *C. elegans* thus far, PIE-1 and *nos-2* are the only ones with a known function in primordial germ cells (PGCs). PIE-1 is a putative RNA binding protein characterized by two CCCH fingers (Mello et al, 1996). CCCH fingers in other proteins, such as mammalian TIS11, have been shown to bind to mRNAs and have been implicated in mRNA stability (Lai et al, 2000). Work in our lab has shown that missense mutations in the second CCCH finger (ZF2) of PIE-1 result in a very specific maternal-effect sterile phenotype (Tenenhaus et al, 2001). Embryos derived from mothers that express the ZF2 mutant version of PIE-1 make abnormal PGCs, which are not associated with the somatic gonad, and often remain on the surface of the embryo. As a result, these embryos develop into sterile animals with empty gonads. These observations suggest that PIE-1 regulates PGC development by binding to and regulating specific mRNAs.

Consistent with this hypothesis, PIE-1 has been shown to be required for the efficient translation of the germ plasm mRNA, *nos-2*. *nos-2* is a maternal RNA that segregates with the germ plasm and is enriched on P granules. The NOS-2 protein is translated specifically in primordial germ cells and is required for these cells to associate efficiently with the somatic gonad (Subramaniam and Seydoux, 1999). NOS-2 protein is significantly decreased in PIE-1 ZF2 mutant embryos, suggesting that

nos-2 RNA is not translated efficiently in these mutant embryos (Tenenhaus et al, 2001). Interestingly, the PIE-1 ZF2 mutant phenotype is more severe than the *nos-2* mutant phenotype (in the latter, the primordial germ cells manage to gastrulate, but have trouble joining up with the somatic gonad). These data strongly suggest that PIE-1 regulates other germ plasm mRNAs in addition to *nos-2*.

We are attempting to identify new germ plasm components required for primordial germ cell development, using the essential germline protein, PIE-1. A biochemical method using immunoprecipitation, developed for *C. elegans* by Lee and Schedl, will be used to identify RNAs and proteins that are complexed with PIE-1 *in vivo* (Lee and Schedl, manuscript in preparation). In preliminary experiments, we have shown that PIE-1 can be efficiently immunoprecipitated from embryonic cell extracts, using an anti-PIE-1 monoclonal antibody. We have also detected several bands, using SDS-PAGE, that are unique to PIE-1 IP samples. Further progress will be reported at the meeting.

990. Suppressor analysis of FOG-1, a CPEB protein that regulates germ cell fate.

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The *fog-1* gene controls whether germ cells differentiate as sperm or as oocytes. Sequence analysis revealed that FOG-1 is a Cytoplasmic Polyadenylation Element Binding (CPEB) protein. These proteins are known to bind the 3'-UTR of messenger RNAs and regulate their translation. The *fog-1* message contains a consensus binding site for CPEB proteins. We used northern analysis and co-immunoprecipitation assays to show that FOG-1 can bind its own 3'-UTR *in vitro*, and that this binding depends on wild-type FOG-1 activity. By contrast, FOG-1 does not bind control transcripts, like that of *unc-37*. These data suggest that FOG-1 regulates germ cell fates translationally, and that one of its targets might be *fog-1*, itself.

In *Xenopus*, CPEB proteins are known to interact with maskin and CPSF to regulate translation of target messages. To isolate genes that regulate or interact with FOG-1, we performed a screen looking for mutations that suppress the temperature-sensitive allele, *fog-1(q253)*. We identified 17 recessive and two semi-dominant suppressors from approximately 40,000 haploid genomes. Eight recessive mutations form a single complementation group on chromosome III, and four compose a second group on chromosome II. We call these loci *sof-1* and *sof-2*, respectively (*sof = suppressor of fog-1)*.

Three criteria suggest that the suppressor alleles of these genes cause a loss-of-function. First, these mutations are common. Those in *sof-1* arose at a frequency of 1/5000 haploid genomes, while those in *sof-2* occurred at a frequency of 1/10,000. Second, these *sof-1* and *sof-2* alleles are recessive. Third, we isolated additional alleles from non-complementation screens, and these new alleles also suppress *fog-1(q253*ts*)*. Seven of these new *sof-1* alleles were identified (at a frequency of approximately 1/700 haploid

genomes), and one new *sof-2* allele was identified (at a frequency of 1/1500).

The penetrance of *sof-1* mutations ranges from 50-90%, while that of *sof-2* mutations ranges from 40-60%. Furthermore, mutations in *sof-1* and *sof-2* are allele specific suppressors, since they do not suppress null alleles of *fog-1*. Finally, *sof-1* and *sof-2* mutations have no detectable phenotype in a wild-type genetic background. However, *sof-2(v3); sof-1(v20)* double mutants show a significantly increased hermaphrodite broodsize. This result suggests that they might be functionally redundant genes that normally promote germ cells to differentiate as oocytes rather than as sperm.

To learn their biochemical functions, we are cloning *sof-1* and *sof-2*. So far, *sof-1* has been mapped to a region spanning approximately 83 kb, at position -0.76 on chromosome III. We are using transformation rescue and RNAi to finish cloning *sof-1*, and are employing a similar strategy to clone *sof-2*. Molecular analyses of these two genes might elucidate how CPEB proteins interact with other molecules to regulate the polyadenylation and translation of specific targets, and how translational control can specify cell fates.

991. RAS/MAP kinase signaling in meiotic prophase progression

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A RAS/MAP kinase signal transduction pathway is essential for meiotic prophase progression in hermaphrodites and males (Church *et al*., 1994). Strong loss-of-function (lf) mutations in *let-60* RAS, *lin-45* RAF, *mek-2* MAPKK, and *mpk-1* MAPK result in germ cells arrested in pachytene. This suggests that the RAS/MAP kinase cascade transduces a signal necessary for progression through pachytene and/or for the transition from pachytene to diplotene/diakinesis. To further understand germline RAS/MAP kinase signaling, we used an antibody that specifically recognizes only the doubly phosphorylated active form of MPK-1 in western analysis as well as in germline staining of wild type, lf and gain-of-function (gf) mutants in this pathway.

MPK-1 has two isoforms, 55 KD and 45 KD; the 55 KD isoform has 68 amino acids more at the N-terminus (Wu and Han, 1994; Lackner and Kim, 1998) and is expressed only in the germline, whereas the 45 KD isoform is expressed in the germline and in the soma. In strong lf mutants of *let-60* RAS and mek-2 MAPKK, the doubly phosphorylated active MPK-1 is absent or severely reduced. In wild type hermaphrodite germlines, MPK-1 is doubly phosphorylated and becomes active in two sets of cells: pachytene stage germ cells and in the most proximal diakinesis stage oocytes. Active MPK-1 first appears in the middle of the pachytene region and continues until the end of the pachytene region. To determine whether activation of MPK-1 is sufficient for progression through pachytene or for the transition from pachytene to diplotene/diakinesis, we employed the *let-60 (ga89)* RAS gf mutantion (Eisenmann and Kim, 1997), which causes early activation of MPK-1 in pachytene stage germ cells. The early activation of MPK-1 in *let-60 (ga89)* RAS gf mutants is not sufficient to drive germ cells into

diplotene earlier than in wild type. This suggests that RAS/MAP kinase signaling may be necessary for progression of germ cells from an early to a late stage of pachytene, while the transition from pachytene to diplotene is controlled by a separate process. Alternatively, the transition from pachytene to diplotene may require not only RAS/MAP kinase signaling but also the execution of additional events such as the completion of homologous recombination. However, the activation of MPK-1 and the relative proportion of pachytene stage germ cells in *spo-11* and *mrt-2* mutants (Dernburg *et al*., 1998; Gartner *et al*., 2000), which never initiate homologous recombination and are defective in monitoring homologous recombination, respectively, are not significantly different from wild type. This suggests that the completion of homologous recombination is not necessary for MPK-1 activation, or for the transition from pachytene to diplotene/diakinesis in *C. elegans*.

The degree of phosphorylation of MPK-1 decreases rapidly as germ cells make the transition from pachytene to diplotene/diakinesis. This shift in phosphorylation may be important for oocyte growth, because oocyte size is much smaller in *let-60 (ga89)* RAS gf mutants, which exhibit hyper-activation of MPK-1 in developing oocytes.

The reactivation of MPK-1 in the most proximal oocytes is dependent on an MSP signal from sperm (Miller *et al*., 2001), suggesting that MAP kinase signaling may function in *C. elegans* oocyte meiotic maturation (McCarter *et al*., 1999), as it does in other organisms. Consistent with this possibility, time lapse video analysis of conditional *mpk-1 (ga111)* mutants shows defects in maturation/ovulation.

992. Forward and Reverse Genetic Approaches to Identify Genes Involved in Initial Meiotic Entry

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The initial onset of meiosis occurs in the proximal-most part of the *C. elegans* larval germ line in the third larval stage. Proper timing and position of initial meiosis is important for fertility and fecundity. We have isolated mutants that disrupt the pattern of germline development such that the initial meiotic onset is late and shifted distally, leaving a large region of mitotic germ cells in the proximal germ line of the adult, proximal to mature gametes. This phenotype, proximal proliferation (Pro), is observed with certain alleles of *glp-1* as well as other mutants we are characterizing (see abstract by Pepper, Lo and Hubbard), and was originally described in *lin-12(lf)* mutants (Seydoux, et al., 1990). GLP-1 is a receptor of the LIN-12/Notch family, and its activity promotes mitosis and/or inhibits meiosis in the germ line (Austin and Kimble, 1987). One allele, *glp-1*(*ar202*), exhibits a temperature sensitive and highly penetrant Pro phenotype. Therefore, we are using this allele as a starting point to look for additional factors that affect initial meiotic entry.

We have begun a genetic screen for suppressors that allow maintenance of the *glp-1*(*ar202*) strain at the restrictive temperature. This approach offers several advantages. First, mutagenesis screens produce valuable alleles and pose no *a priori* constraints on the molecular nature of the gene product affected by the mutation. Second, since the starting strain can not be maintained at the restrictive temperature, the primary task is a selection and should enable us to cover many haploid genomes relatively easily. So far, we have found 15 suppressors from approximately 11,736 haploid genomes. Preliminary mapping data suggest that some of these suppressors do not map to LG III and thus do not represent intragenic suppressors. Further characterization of the suppressor loci may identify factors that act on, with, downstream of, or parallel to GLP-1-mediated signaling to specify the precise

time and position of initial meiotic entry.

Our reverse-genetic strategy uses RNAi to assess the role of candidate genes involved in this aspect of germline development. RNAi works well in the germ line and can silence genes involved in GLP-1-mediated signaling (e.g., *lag-1*, Smardon et al., 2000). This approach offers simultaneous identification of gene product and phenotype. We have identified candidates with significant sequence similarity to factors previously shown to exhibit genetic and/or biochemical interactions with the *Notch* pathway in *Drosophila* and vertebrates. The function of many of these candidates has not been characterized in *C. elegans*. We are conducting RNAi experiments in both wild-type and *glp-1(ar202)* worms. We hope that by using these two strains in parallel, we can assess the specificity of any phenotypes we observe and can detect subtle effects that may be discernible only as an enhancement or suppression of the Pro phenotype. In addition, it is conceivable that RNAi of a given gene could cause lethality in the wild type but produce live progeny in *ar202* mutants.

There are advantages and disadvantages to both the forward and reverse approaches; together, they are complementary. Mapping and molecular identification of a loss-of-function suppressor mutation may be facilitated by the previous identification of a nearby RNAi-mediated suppressor. We hope that by applying these two approaches concurrently we will identify factors that influence the timing and position of initial meiotic entry during germline development.

993. Defects in Timing and Position of the Initial Onset of Meiosis in *glp-1* Proximal Proliferation (Pro) Mutants

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Many early events in germline development are similar across phyla. The germ lineage is usually separated early in development and is often physically removed from the site of somatic gonadogenesis. During development, the cells of the germ lineage and somatic gonad lineage meet and germ cells proliferate. In many organisms, a subset of the early germ cell population enters meiosis at a certain developmental time and position. Prior to this first onset of meiosis, germ cells are presumably equivalent. Once the initial onset of meiosis occurs, mitotic germ cells are maintained as a stem cell population, the descendents of which enter meiosis and form gametes.

In the *C. elegans* hermaphrodite, the initial entry into meiosis occurs in the L3 at the proximal-most part of the germ line, the border of the gonad "arms" and the central somatic gonad (Kimble and White, 1981). The proper timing and position of this initial meiosis is critical for the future fertility of the worm. We have identified mutants in which the timing and position of the first meiosis is abnormal: meiosis occurs late and is distally displaced, stranding the proximal-most cells in mitosis. In the adult, this defect in initial meiosis manifests as a "proximal proliferation" (Pro) phenotype (originally described by Seydoux, Schedl and Greenwald, 1990). Adult Pro mutants contain a large mass of proliferating cells in the proximal-most part of the germ line, proximal to mature gametes. The adult distal-to-proximal pattern of the germ line is thereby changed from the normal "mitosis, meiosis, gametogenesis", to "mitosis, meiosis, gametogenesis, mitosis". Therefore, the germ line in these mutants is capable of executing normal cell fates, albeit in an abnormal pattern.

Three of the Pro mutants we identified in genetic screens proved to be novel temperature-sensitive *glp-1* alleles. GLP-1 is a member of the conserved LIN-12/Notch family of receptors. *glp-1* activity in the germ line maintains mitotis and/or inhibits meiosis (Austin and Kimble, 1987). The *glp-1*(Pro) lesions map to the extracelluar region of the protein within or adjacent to the LIN/Notch repeats (LNRs). Since the primary defect in these mutants is in the proximal germ line and initial meiotic entry, the phenotype differs from the previously characterized tumorous and late-onset tumorous mutant phenotype observed with the hypermorphic allele, *glp-1(oz112)* (Berry, Westlund and Schedl 1997). The *glp-1*(Pro) phenotype suggests that mechanisms exist to restrict the initial meiotic entry to a well-defined temporal and spatial pattern. Characterization of other non-*glp-1* Pro mutants we have identified should offer additional insight into these mechanisms.
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994. MOG-6 and FEM-3 expression patterns are complementary in the hermaphrodite germ line

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The three DEAH-box proteins MOG-1, -4 and -5 are required for the switch from spermatogenesis to oogenesis in the *C. elegans* hermaphrodite by repressing the *fem-3* mRNA through its 3'UTR. We have now cloned *mog-6*, a gene that codes for a divergent cyclophilin. Cyclophilins represent a family of proteins that have a peptidylprolyl *cis-trans*-isomerase activity and bind to the immunosuppressive and anti-parasitic agent Cyclosporin A. Consistent with its role in germ line cell fate specification, the *mog-6* mRNA is mainly expressed in the hermaphrodite germ line. Furthermore, the MOG-6 protein is present in many somatic and germ cell nuclei. We have generated antibodies against MOG-6 and FEM-3 and shown that the expression patterns of these two proteins are complementary in the germ line. This observation supports the idea that MOG-6 acts as a negative regulator of *fem-3* expression, but the mechanism through which MOG-6 acts on *fem-3* is still unclear. Since some cyclophilins are involved in RNA processing, we are currently testing whether *mog-6* regulates *fem-3* via pre-mRNA splicing, a possibility that is suggested by the fact that MOG-1, -4 and -5 are homologs of yeast splicing factors. We have shown that MOG-6 does not bind to the *fem-3* 3'UTR, nor to any of the other cloned MOG proteins. However, MOG-6 binds to MEP-1, a nuclear zinc finger protein that is also capable to interact with MOG-1, -4 and -5, indicating that MOG-6, MEP-1 and the other MOG proteins might act in a complex that post-transcriptionally regulates *fem-3* via its 3'UTR.

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995. *fem-3* Expression, Localization, and Interactions

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fem-3 acts as a developmental switch gene in the *C. elegans* sex dtermination pathway; its activity is necessary and limiting for male development in all tissues. We are interested in understanding how FEM-3 protein interactions and FEM-3 subcellular localization control *fem-3* activity so that male development occurs appropriately in XX and XO animals.

FEM-3 is a novel protein that interacts with the cytoplasmic domain of the predicted transmembrane receptor TRA-2A. This interaction leads to the inhibition of *fem-3* activity in the XX soma (Mehra *et. al*, 1999). FEM-3 also binds to FEM-2, a type 2C protein phosphatase (Chin-Sang and Spence, 1996). *fem-3* and *fem-2* act at the same genetic level and both are required for male somatic development in XO animals and for spermatogenesis in XX and XO animals. Therefore, we hypothesize that the FEM-3/FEM-2 interaction is required for male development in all tissues. Using a modified version of the yeast 2-hybrid system we isolated a FEM-3 mutant that is unable to bind to FEM-2 but retains the ability to bind to TRA-2A. If our hypthesis about the biological significance of the FEM-3/FEM-2 interaction is correct then a *fem-3* transgene carrying this mutation will fail to rescue male development in *fem-3(0)* animals. We are currently testing this prediction.

We have also made a fem-3::GFP transgene in order to look at the subcellular localization of FEM-3 in cells that are specified wtih female versus male fates. One model is that TRA-2A sequesters FEM-3 at the cell membrane thereby preventing FEM-3 from gaining access to its downstream targets. In this model, when *tra-2* activity is low then FEM-3 is able to act with FEM-2 in the cytoplasm to direct male development. We are making transgenic animals that carry the fem-3::GFP reporter transgene to look at FEM-3 distribution in all tissues of XX and XO animals at different developmental stages. By introducing mutations that interfere with FEM-3 binding into the fem-3::GFP transgene, we will be able to dtermine how FEM-3 binding activities control FEM-3 localization.

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996. Identification and characterization of putative male-specific sexual regulators and TRA-1 target genes

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C. elegans is highly sexually dimorphic, with about 30% of the somatic cells in the adult hermaphrodite and 40% of the somatic cells in the adult male being sexually specialized. In *C. elegans*, all aspects of somatic sexual development are controlled by TRA-1A, a zinc finger transcription factor. Sexual dimorphism in *C. elegans* affects all tissue types and is achieved through diverse mechanisms including sex-specific cell divisions and migrations, sex-specific cell death, generation of sex-specific blast cells and sex-specific changes in gene expression. TRA-1A probably directly regulates multiple genes involved in restricted aspects of sexual differentiation, and an important goal is to identify these genes.

We have identified a large number of male-enriched genes in *C. elegans* using microarray analysis with help from Stuart Kim and the Stanford Microarray Database curators. We have compared N2 XX hermaphrodites and *tra-2(ts)* XX pseudomales from the L2 through the L4 stages to generate a developmental profile of sex-specific gene expression. Many of these genes likely function as male-specific sexual regulators. We have also identified putative TRA-1A binding sites in the *C. elegans* genome using a hidden Markov model (HMM) computer algorithm and weight matrix searches. Combining these two approaches, we seek to identify and characterize male-specific TRA-1A target genes in an effort to understand the mechanisms by which *C. elegans* achieves sexual dimorphism. We are currently conducting functional analysis of these putative male sexual regulators using RNAi and GFP reporter analysis.

997. Identification and Characterization of FEM-2 interacting proteins in *C. elegans*

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Animals lacking *fem-2* activity develop as fertile females. The *fem-2* product is a protein serine/threonine phosphatase of Type 2C (PP2C) and its phosphatase activity is necessary in promoting male development. The identities of the substrate of FEM-2 and of the kinase that opposes its activity are unknown. We are using FEM-2 in affinity chromatography studies to identify substrates or other interacting proteins. By this approach we can reproduce the known interaction between FEM-2 and FEM-3. GST-FEM-2 columns specifically retained FEM-3 when loaded with lysates prepared from worms carrying a heat-shock driven Myc-FEM-3 transgene. We have observed several bands in silver stained gels specifically retained on GST-FEM-2 columns loaded with *C. elegans* lysates. We are purifying the proteins on these bands and we are identifying them by MALDI-TOF mass spectrometry. We will confirm the interactions with FEM-2 and we will use a reverse genetics approach to investigate the function of the corresponding genes and their role in sex determination.

Since interactions involving wild type FEM-2 may be weak or transient, we have generated putative substrate-trapping versions of FEM-2 in the hope that they may bind substrates more tightly thereby increasing the sensitivity of the chromatography experiments. We are currently testing whether these mutants trap FEM-2 substrates from worm lysates in affinity chromatography experiments.

998. Disruption of *tra-2* translational control results in TRA-2 protein forming large nuclear structures

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Translational control of the sex determining gene *tra-2* mRNA is required for XX hermaphrodite development. Spermatogenesis in XX animals requires repression of *tra-2* translation by two elements, called TGEs (for *tra-2* and *gli* elements), located in the 3' untranslated region. Deletion of the TGEs therefore results in increased *tra-2* translation and in XX animals making only oocytes. We have shown that this repression is mediated by the binding of the germline protein GLD-1 to the TGEs. To better understand the effect of increasing *tra-2* translation *in vivo,* we have generated antibodies to TRA-2 and assayed TRA-2 staining patterns in the germlines of wild-type and mutant hermaphrodites in which translation of *tra-2* is disrupted.

The *tra-2* gene encodes two proteins, TRA-2A and TRA-2B. TRA-2A is a transmembrane protein. TRA-2B is co-linear with the putative intracellular domain of TRA-2A, and may be cytoplasmic. The polyclonal antibody generated recognizes both TRA-2A and TRA-2B proteins, as indicated by Western analysis in which the antibody recognizes two proteins of the approximate predicted size. No protein is detected in *tra-2(null)* extracts. (For simplicity, unless otherwise indicated, the two proteins will be referred to as TRA-2). Using immunofluorescence, we find different localization patterns for TRA-2 depending on the region of the germline. For example, in the mitotic region and transition zone of the germline of wild-type XX hermaphrodite animals, TRA-2 is detectable in the cytoplasm. In the meiotic/pachytene region, TRA-2 is found in discrete 'spots' that are closely associated with the nuclear envelope, with approximately two spots per nucleus. In oocytes, TRA-2 is located in the membrane and the cytoplasm. Disruption of the TGE control alters the pattern of TRA-2, most notably in the meiotic/pachytene region. In the

meiotic/pachytene region of *tra-2(gf)* XX animals, in which the TGEs are deleted, TRA-2 protein is found in large nuclear 'rods', with one 'rod' structure per nucleus. Moreover, in *gld-1(q126fog)* animals, TRA-2 also forms nuclear 'rods'. The *gld-1(q126fog)* mutation, which causes XX animals to make only oocytes, likely disrupts *tra-2* translational control as the mutant protein does not bind TGEs. By Western blot analysis, TRA-2A and TRA-2B protein levels are increased in *tra-2(gf)* and *gld-1(q126fog)* animals, indicating a correlation between the 'rod' structures and increased protein production. In wild-type male germlines, the overall levels of TRA-2 are reduced and no 'spots' or 'rods' are detected. Our analysis suggests that TRA-2 is localized to specific subcellular regions that depend on germline location, sex, and protein levels.

999. Maternal-effect germline silencing of the sex-determining gene fem-1

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The *fem-1* gene is required for male development of germline and somatic tissues in *C. elegans*. Animals lacking *fem-1* activity develop as cross-fertile females regardless of their X chromosome dose. Following a cross to wild-type males, most *fem-1* mutants produce phenotypically wild-type *XO* male and *XX* hermaphrodite heterozygous progeny. In contrast, females homozygous for any of three deficiencies that remove all or part of *fem-1* produce cross-progeny of both sexes that exhibit germline feminization. The penetrance of this effect varies from 20% to over 90%, depending upon the deficiency, and it dramatically increases in the second generation following a backcross to females homozygous for the deficiency. Feminization appears to result from maternal absence of some product of the *fem-1* locus rather than a zygotic interaction involving the deficiency chromosome, because the paternally disomic progeny of a cross to deficiency homozygotes exhibit germline feminization. It is unlikely that maternal loss of FEM-1 protein is responsible for the effect, because alleles carrying point mutations that prevent production of FEM-1 complement the deficiencies with respect to their maternal effect on the germline. Genetic evidence suggests that the affected animals fail to express *fem-1* at normal levels in the germline, which could account for their phenotype. I suggest that a *fem-1* transcript is maternally required to license *fem-1* for expression in the germline. A similar requirement might exist for the germline expression of other genes, but it would be difficult to detect in the absence of deficiency alleles that allow homozygotes to develop into fertile adults. I am investigating the relationship between mechanisms involved in maternal-effect silencing of *fem-1* and other silencing phenomena in *C. elegans*.

1000. A new transfomer mutant in *C. elegans*

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We performed an EMS screen using the deficiency *sDf121* in order to isolate mutations in a gene, C05D2.5, involved in meiotic chromosome segregation (see Kuervers and Baillie poster). In the process of analyzing what appeared to be chromosome segregation mutants, we discovered a new transformer *(tra)* mutant. This mutant produces wild-type fertile hermaphrodites and infertile transformed hermaphrodites. The transformed hermaphrodites exhibit a variety of abnormal phenotypes including the presence of a few oocytes in an otherwise male germline, abnormal male tail formation, and abnormal gonad morphology. Interestingly, the phenotype is lost upon crossing with wild-type males but not lost when crossed to *dpy-17(e164)* heterozygous males. Therefore, the CB164 strain may bring in a hidden mutation that interacts with the tra mutation isolated in this screen to produce a transformed phenotype. Either mutant alone does not produce a transformer phenotype. We are currently attempting to determine the nature of this hidden mutation.

1001. Identification of a New X-Signal Element that Determines Sex in *Caenorhabditis elegans: ceh-39*

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In *C. elegans*, sexual fate is determined by the ratio of X chromosomes to sets of autosomes. The number of X chromosomes is signaled by the cumulative dose of several zygotically expressed X-linked genes, called X-signal elements (XSE), which act synergistically to repress the sex determination and dosage compensation master switch gene, *xol-1*. To date, genetic analysis has identified four regions of the X chromosome that harbor XSEs. Genes responsible for XSE activity in two of these regions have been identified as *sex-1* and *fox-1*. An RNA interference (RNAi)-based screen of another region of the X chromosome, called Region 2, that bears one or more unidentified XSEs has been implemented in order to identify the genes that function as XSEs in this region. Approximately seventy clones from this region have been analyzed with one positive result. *ceh-39* has a predicted sequence that is homologous to ONECUT class of homeodomain proteins. This class of proteins has been found in mammals where they are involved in the transcriptional regulation of several developmental programs. RNAi of *ceh-39* resulted in ~90-95% rescue of male viability in a XSE duplication strain that normally causes complete male lethality. The extent of rescue is comparable to that seen after RNAi of *sex-1* or *fox-1* in these animals indicating that *ceh-39* functions as an XSE. *ceh-39* also causes synergistic XX-specific lethality with *sex-1*. *sex-1* reduces XX-viability to 65% and this is reduced to ~30% after RNAi of *ceh-39.* This type of synergy is a defining characteristic of XSEs and provides strong evidence that *ceh-39* is an XSE. RNAi of wild-type hermaphrodites gave variable phenotypes of low penetrance that include embryonic lethality and swollen or lumpy posterior. Further analysis of *ceh-39* will determine if it is indeed an XSE and will elucidate its role in the sex determination and dosage compensation pathway. It is possible that there is more than one XSE in Region 2 and

therefore the RNAi screen of all the ORFs in this region will be completed.

1002. The HAT activity of CBP-1 is indispensable for its biological function during *C. elegans* embryogenesis

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CBP/p300 represent a conserved family of transcriptional cofactors possessing histone acetyltransferase (HAT) activity. This group of proteins has been shown to play critical roles in differentiation and cell proliferation in *C. elegans*, *Drosophila*, mouse and human. Despite their importance, the mechanisms by which they regulate transcription and thus exert their biological functions are poorly understood. A central question is whether the HAT activity is essential for the biological functions of these proteins. In this study, we investigated the importance of the HAT activity for CBP-1 in *C. elegans*. We show that a truncated CBP-1 protein lacking the HAT domain fails to promote differentiation, consistent with the notion that the HAT activity of CBP-1 is essential for its biological functions. To further address this question, we used a chemical that has been shown to be a specific inhibitor of the enzymatic activity of CBP/p300 HAT. Injection of this chemical into the gonads of the hermaphrodite mothers resulted in dead embryos arrested at different stages of development. Significantly, some of these embryos appear identical to *cbp-1(RNAi)* embryos in every aspect, as judged by the excess cell number, lack of endoderm and mesoderm tissues but excess neuronal differentiation (Shi and Mello, 1998). Taken together, our findings provide *in vivo* evidence that the HAT activity is not only critical, but also may be the only biochemical activity that is necessary for the biological functions of CBP-1.

Shi, Y. and Mello,C. (1998), ’A CBP/p300 homolog specifies multiple differentiation pathways in *Caenorhabditis elegans*.’ Genes Dev. 12(7), 943-55.

1003

1003. The Roles of the par Genes in Intracellular Motility Before the First Asymmetric Cell Division in *C. elegans*

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Generation of cell diversity during development is accomplished either via cell-cell signaling or asymmetric cell division. In asymmetric cell division, two distinct daughter cells are born. Between fertilization and the first cell division in the *C. elegans* zygote, central and cortical cytoplasmic flows are observed. The timing of these flows coincides with the asymmetric localization of P granules, RNAs, proteins and cytoskeletal elements.

To begin to determine whether some of the genes required for asymmetric cell division play a role in polarized intracellular motility, we asked whether the *par* genes (1) are required for cytoplasmic flows. Using DIC microscopy, we filmed embryos with mutations in the *par* genes and analyzed rates and duration of flows as well as distances traveled along the axis of the embryo, and compared them to wild-type. We found that strong loss-of-function mutations in *par-2*, *-3*, *-4*, and *-6* prevent cytoplasmic flows, whereas *par-1* mutant embryos (*b274, it51, it60*) have flows comparable to wild-type. Kemphues and colleagues (1) have placed the par genes into a pathway by analysis of their protein distributions in various genetic backgrounds. Our results reveal that, with the exception of *par-1*, the *par* pathway is required to produce polarized cytoplasmic flows.

P granules are normally redistributed to the posterior of the embryo before the first cell division. Hird et al. (2) found that P granules redistribute primarily by moving to the posterior at the same time as flows occur, and at a similar rate, suggesting that P granules might be moved by bulk flows of cytoplasm. We have filmed fluorescent P granules and cytoplasmic flows in the same embryos, and created overlays of these films. Consistent with the hypothesis that P granules move by bulk flows of cytoplasm, we have found that P granules move perfectly coincident with the flows of yolk granules (n= 6 embryos; 30/30 P granules in the central cytoplasm, 20/20 P granules in the cortical cytoplasm). This result suggests that the mislocalized P granule phenotype in *par-2*, *-3*, *-4*, and *-6* may be a result of failure to produce cytoplasmic flows. We are currently testing whether P granules fail to move in these mutants by filming mutant embryos similarly.

par-1 mutants have cytoplasmic flows comparable to wild-type embryos but the P granules do not become asymmetrically localized (1). These results, along with the posterior cortical localization of PAR-1 (3), suggest to us at least two hypotheses for the role of PAR-1 in localization of P granules. First, PAR-1 might be part of a complex that anchors P granules in place once they reach the posterior cortex, preventing further movement along the cortex, even as cortical flows continue. This hypothesis appears unlikely, since our preliminary evidence from wild-type embryos suggests that no such anchor exists: P granules in the cortical cytoplasm stop moving when cortical flows stop, and not before (3/3 embryos, 9/9 P granules). Second, PAR-1 might be required to prevent degradation of P granules, acting as a "safe haven" for P granules that reach the posterior cortex. Consistent with this, Hird et al. found that P granules in wild-type embryos that do not reach the posterior disappear, suggesting that a P granule degrading or disassembling activity is present in the anterior of the embryo (2), and Guo and Kemphues (3) have noted that P granules are missing in 2-cell *par-1* mutant embryos. Experiments testing the safe haven hypothesis are currently underway.

1. Rose & Kemphues (1998) Annu Rev Genet 32:521-45 2. Hird et al (1996) Development 122:1303-12 3. Guo and Kemphues (1995) Cell 81:611-20

1004. Is PAR-2 an E3 Ubiquitin Ligase?

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Protein degradation via the ubiquitin-proteasome pathway plays an important role in the cell cycle and development. E3 ubiquitin ligases function in this pathway to identify target proteins for ubiquitination and subsequent degradation. We are interested in determining whether the *C. elegans* protein, PAR-2, is an E-3 ligase. Analysis of *par-2* mutations indicates that this gene plays a role in establishing anterior-posterior polarity in the embryo. The PAR-2 protein contains a RING finger motif similar to ones found in some E3 ligases. Other studies of RING finger proteins have indicated that these proteins can catalyze ubiquitination of target proteins as well as become ubiquitinated themselves. We are investigating both *in vitro* and *in vivo* to determine if PAR-2 itself is ubiquitinated. For *in vitro* biochemical studies a GST:PAR-2 fusion protein is produced in bacteria. Though largely insoluble, the purified fusion protein is being used for *in vitro* ubiquitination assays. Our *in vivo* analysis of PAR-2 ubiquitination has yielded preliminary results suggesting that modified forms of PAR-2 may exist. The distribution of these forms differs between embryo and mixed stage extracts. We are currently attempting to determine if these modifications are due to ubiquitination. If PAR-2 is indeed an E3 ligase, it may act to target specific proteins for degradation in the embryo. In the future, we hope to identify these targets and to understand the nature of the PAR-2 interaction with the ubiquitin-proteasome pathway.

1005. *nop-1* is a partioning pathway gene

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We are interested in understanding how polarity is established in the early *C. elegans* embryo. *nop-1(it142)* was shown by Rose *et al.* (1995) to be a viable mutant that lacks a pseudocleavage furrow and has reduced cortical and central cytoplasmic flows. Since many of the partitioning (*par*) genes are required for cytoplasmic flows (see abstract by Cheeks and Goldstein), we wondered whether the reduced flows in *nop-1* mutant embryos might be a sign that *it142* is a hypomorphic allele of a gene in the *par* pathway. We examined the phenotype of *nop-1(it142)* embryos and found that a proportion of them had no cytoplasmic flows; these embryos failed to hatch. Interestingly, most of these defective embryos either had a Par phenotype that resembled that produced by *par-2* or *par-5* loss of function, or failed to complete first cytokinesis. The penetrance of these two phenotypes was greatly enhanced (to 95%) in embryos in which *nop-1* function was reduced by having *it142* in *trans* to a deficiency. These results indicate that *nop-1* plays an essential role in early embryonic events. Furthermore, we found that the Par phenotype of *nop-1* mutant embryos was synergistically enhanced by a mutation in *par-4*. Disruption of *par-3* or *par-6* had no effect on the penetrance of this phenotype. Therefore, *nop-1* genetically interacts with some components of the *par* pathway for the establishment of embryonic polarity. We are currently assessing whether other genes involved in polarity establishment also interact with *nop-1*. We are also cloning *nop-1* as well as generating more alleles of *nop-1* by non-complementation screen, as a step toward further defining its role in early embryogenesis. Progress toward these goals will be discussed.

Rose et al. (1995). Dev. Biol. 168, 479-489

1006. The role of Ce-BTF3 in endoderm development

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Specification of the endoderm in early *C. elegans* embryos requires multiple regulatory factors that activate the zygotically expressed *end-1* and *end-3* genes specifically in the E lineage. Both *end-1* and *-3* encode GATA-type transcription factors that redundantly specify E cell fate. To uncover additional regulators of *end-1* expression, we used mass spectrometry to identify factors that bind to a 100 bp region upstream of the transcriptional start site of *end-1*, a region that we have shown is essential for proper regulation of the gene (see also abstract by Witze et al.). One of the factors we identified is Ce-BTF3, a homologue of mammalian BTF3. BTF3 is a general transcription factor originally isolated from HeLa cells which is necessary for activation of a number of mammalian and viral promoters by RNAP II. Consistent with a possible role for Ce-BTF3 in *end-1* regulation, a GFP reporter construct revealed robust expression in the E lineage as early as the 8E cell stage. This correlates with the stage at which *end-1* expression is attenuated, consistent with a possible role for Ce-BTF3 in negative temporal regulation of *end-1* expression. Interference of *Ce-btf3* function by RNAi did not yield an obvious endoderm defect; however, additional studies will assess whether *Ce-btf3* affects expression of *end-1*. These results provide evidence that BTF3, which is thought to be a general, or "basal" transcription factor, may act in a tissue-specific and/or gene-specific manner.

1007. Role of egl-5 in EGF/Wnt signal integration in the specification of P12 fate

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The *C. elegans Hox* gene *egl-5* is most similar, based on sequence analysis, to *Abdominal-B*. Consistent with its assignment into this paralog group, *egl-5* is expressed in the posterior region of the worm. Immuno-staining results have shown that in the hermaphrodite, *egl-5* is expressed in the hermaphrodite specific neuron, body wall muscle, posterior lateral microtubule neuron, PVC interneuron, the rectal epithelial cells K, F, B, U and the P12 neuroectoblast cell.[1]

The two most posterior P cells are P11 and P12. The anterior products of their first division are both neuroblasts. P11.p fuses with the epidermal syncytium and P12.p divides again during late L1. The anterior division products, the epidermal cells P12.pa and P11.p are distinguishable by their distinct nuclear morphologies and positions.

Previous genetic analysis indicates that P12 fate specification requires the synergistic action of the EGF and the Wnt signaling pathways. Reduction - or loss of function mutations in components of the EGF or the Wnt pathway result in partially penetrant P12 to P11 or P11 to P12 transformations. Double mutants of EGF and Wnt pathway components significantly enhance the frequency of transformation. P12 is not specified in an *egl-5(lf)* mutant and overexpression of *egl-5* can rescue the loss of

P12 specification phenotype of *let-23* mutants.[2]

In order to understand how information from the EGF and Wnt pathway are integrated at a *cis*-regulatory level, we have undertaken an analysis of the *egl-5* promoter in collaboration with Scott Emmons to determine elements required for *egl-5* expression in P12.[3] Do the two pathways converge on the *egl-5* promoter or upstream of it? Beginning with a large promoter construct provided by Ferreira and Emmons, we have identified an approximately 1.3 kb. fragment of *egl-5* promoter sufficient to drive expression of a heterologous promoter in P12. This 1.3 kb fragment contains six sites of approximately 20-40 bp each which are conserved between *C. elegans* and *C. briggsae*. Specific deletion of at least two of these sites in certain combinations eliminates expression in P12, suggesting that these sites might represent P12 enhancers.

Complementary to our *cis* regulatory analysis, we have been using EMS screens to identify, and are currently characterizing, potential trans-acting factors as well as other genes which may participate in signal integration.

1. Ferreira *et al.*(1999) Dev. Biol. 207:215-228
2. Jiang and Sternberg (1998) Development 125:2337-2347

1008. Genetic Analysis of Coelomocyte Specification and Patterning

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We are interested in studying the patterning of mesodermal lineages during late embryogenesis in C. elegans. To this end, we are examining the processes which affect the specification of the coelomocytes. Coelomocytes are highly endocytic mesodermal cells that reside in the pseudocoelomic space. Four of the six coelomocytes arise late in embryogenesis from MS progeny; the other two arise from the post-embryonic divisions of the M mesoblast. The lineages and timing of coelomocyte specification present several questions about cell fate decisions. In particular, we are interested in understanding how cells choose between muscle and non-muscle fates, how mesodermal cell fate choices are linked to the cell cycle, and how two unqiue lineages can give rise to cells with the same terminal phenotypes.

W have screened for defects in coelomocyte specification by assaying for changes in tissue-specific GFP expression. We have isolated over 20 mutations and are in the process of characterizing three of these in more detail. The first mutant may be a cell cycle mutant which prevents the coelomocyte mother from dividing. The second appears to be a lineage mutant affecting only the pair of coelomocytes from the MSpp blastomere. The third mutant gives a variable reduction in coelomocyte number and has an associated withered tail defect. Further genetic and phenotypic characterization of these mutants will be presented.

During the course of these analyses, we observed that unc-39 mutants also have coelomocyte specification defects. We have set out to clone the unc-39 gene, and progress on its cloning and characterization will be presented.

1009

1009. Coupling of cell type specification and cell division: the number of division rounds in the E lineage is regulated by the activity of the E-specifying *end* genes.

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The E cell is the progenitor of the entire *C. elegans* intestine. During embryogenesis, the E daughters, Ea and Ep, ingress, marking the onset of gastrulation. E undergoes up to five rounds of division to generate the 20 gut cells. The E cell is specified by the *end-1* and *end-3* genes, which are activated by maternal SKN-1 and its zygotic targets, MED-1 and MED-2 (Bowerman et al., 1992; Zhu et al., 1997; Maduro et al., 2001). In a fraction of *skn-1* and *med* mutant embryos, differentiated gut is made, but the E daughters do not ingress normally. This observation implies that gastrulation and production of intestinal cells from the E lineage are separable processes (Bowerman et al., 1994; Maduro et al., 2001).

We have observed that many of the *skn-1(-)* and *med-1,2(-)* terminal embryos that make gut contain more than the wild-type number of gut cells, as revealed using gut-specific nuclear GFP markers. For example, among the ~50% of *med-1,2(RNAi)* embryos that still make gut, 20% contain >20 gut nuclei. In both *skn-1* and *med* mutants, expression of *end-1* and *end-3* reporters is reduced, but not eliminated (Kasmir et al, WCWM 2000; Maduro et al., 2001). Moreover, we have observed extra gut cells in embryos in which *end-3* function is debilitated by a point mutation or by RNAi. In contrast, overproduction of *end-1* causes the E lineage to arrest prematurely with too few divisions. These data suggest that the *end-1,3* expression levels determine the number of cell division rounds that occur in the E lineage: elevated *end* levels apparently inhibit cell division, while subnormal levels allow for extra divisions. This observation may explain why the *end* genes are turned off shortly after they are first activated during normal development.

We have found that mutants in which the E daughter cells fail to ingress (*gad-1* mutants; Knight and Wood, 1998, and *emb-5* mutants, Nishiwaki et al., 1993) also produce an excess of gut cells: e.g., over 60% of *gad-1(RNAi)* embryos contain 25-30 gut nuclei. We found that *gad-1* embryos show delayed onset of *med-1* expression, suggesting that *end-1,3* expression may also be delayed or reduced. Therefore, GAD-1 and EMB-5 might indirectly affect gastrulation, perhaps by regulating the timely expression of *med-1,2*. Many maternal mutants are blocked in gastrulation but nonetheless produce intestine (e.g. cited in Knight and Wood, 1998), suggesting that there may be many components to this process.

We propose that proper regulation of *end-1,3* expression is critical for (a) specification of E fate, (b) gastrulation, and (c) correct cell division patterns within the E lineage. Our observations also suggest that additional regulators of gastrulation, *end* gene expression, and E fate, might be identified by screening for mutants with elevated gut cell numbers.

1010. A screen for factors affecting the expression pattern of *lin-48*

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lin-48 encodes an Ovo-like zinc finger protein. It is important for normal development of the *C. elegans* hindgut. Genetic and expression data show that *lin-48* is a hindgut-specific target for EGL-38, a Pax transcription factor important for development of the hindgut, the egg-laying system, and structures in the male tail. The *lin-48::gfp* expression in the hindgut is significantly reduced in *egl-38* mutants. Likewise, mutations of EGL-38 binding elements in *lin-48* promoter disrupt hindgut expression. However, *lin-48::gfp* expression in other cells is unaffected in these experiments. Since EGL-38 functions and is expressed in cells in addition to the hindgut, we predict it functions in combination with another factor to regulate *lin-48* specifically in hindgut cells.

To identify additional factors regulating the tissue-specific expression of *lin-48*, we performed an F2 screen to isolate mutants with altered *lin-48::gfp* expression pattern. Following EMS mutagenesis 24 mutant lines have been isolated from a screen of 18,000 mutagenized gametes. These mutants can be classified into three major categories: 1) reduced expression in hindgut, 2) ectopic expression in vulva, 3) other altered expression patterns. Some mutant lines also exhibit altered growth rate, viability, and morphology in hindgut or vulva. Category 1 may include animals carrying mutations in genes that function with *egl-38* in the hindgut, mutations in genes important for EGL-38 activity or expression, or new mutations in *egl-38* itself. Categories 2 and 3 may include animals with mutations in genes that affect *lin-48* expression or EGL-38 activity in other ways. Currently, we are working on complementation tests and mapping to identify the genes.

1011. DNA binding properties and potential downstream targets of the cell fate determining T-box gene *mab-9*.

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We are interested in the molecular mechanisms by which the T-box transcriptional regulator *mab-9* specifies cell fate. MAB-9 is required to distinguish two cells in the developing hindgut, B and F, from their anterior neighbours Y and U. Worms lacking *mab-9* have severely abnormal male tails as a consequence of these cell fate transformations. There are also abnormalities in the hermaphrodite rectum, causing constipation. *mab-9* mutant worms are also weakly backwards Unc, suggesting a possible role for *mab-9* in the nervous system. Consistent with this, we see *mab-9* expression in the ventral cord (see accompanying poster by Pocock *et al*).

We are analysing the DNA binding properties of MAB-9. We have performed *in vitro* DNA binding site selection experiments with MAB-9 and have identified a consensus binding site. This site is almost identical to the target binding sequence for *brachyury*, the founding member of the T-box gene family. Band shift assays confirm that MAB-9 binds specifically to this consensus sequence. A MAB-9-specific antibody stabilises the interaction, indicating that MAB-9 may be part of a DNA-binding complex. We find the hypomorphic allele of *mab-9*, *e2376*, is completely unable to bind to DNA. This mutation is a single amino acid substitution (M97I) in an absolutely conserved region of the T-box DNA binding domain. Preliminary results of trans-activation experiments using mammalian cells suggest that

MAB-9 acts as a transcriptional activator rather than a repressor, as is the case for a subset of the T-box genes. We are using the MAB-9 target binding site in a bioinformatic approach to try to pull out *in vivo* targets of *mab-9* transcriptional regulation and will present the results we have obtained.

1012. Delineation of enhancer elements and trans-acting factors controlling *mab-21* activity in sensory ray differentiation.

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In *C. elegans* male tail, the major peripheral sensory structures comprise of nine pairs of ray sensilla embedded in a cuticular fan. Each ray has its own specific morphological and functional identity as defined by a group of regulatory genes. Among them, *mab-21* regulates ray 6 identity. To establish the requirement of *mab-21* in normal development, we dissect the regulatory elements in the *mab-21* locus using both rescue and reporter assay approaches. A minimal genomic fragment that can efficiently rescue the *mab-21* mutant sensory ray phenotype was defined.

Using green fluorescent protein (GFP) as the reporter system, we demonstrate that *mab-21* locus contains multiple transcriptional regulatory elements including hypodermal, neuronal and structural cell enhancer. The hypodermal enhancer lies in the 5' flanking sequence of the gene, which direct expression of the reporter in body and tail hypodermal tissue. A neuronal enhancer is located in intron 4. This element generates fluorescent signal in the neurons of pharyneal bulb, ventral nerve cord and the sensory ray. The 0.7kb enhancer residing in the 3' flanking sequence of the locus directs the expression of the reporter in the sex muscle, a subset of sensory ray neurons and structural cells of ray 2 and 6.

We present data showing that not all these enhancer elements are required for ray differentiation. Both hypodermal and neuronal enhancer are dispensable for specification of the ray 6 identity, as demonstrated by rescue experiment with *mab-21* mutants. The only *cis*-element essential for ray 6 morphology determination is the 3' enhancer. Delimitation of this enhancer element, identification of its associated *trans*-acting factors are in progress. The outcome of such experiments would provide insight about the regulation of *mab-21* gene and indication of its tissue specificity with

reference to sensory ray 6 differentiation.

1013. Clustering analysis of gene expression patterns

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In this lab, whole mount in situ hybridization has been performed using the cDNAs that have been classified in our EST project (See Thierry-Mieg et al.). Thus far, in situ analysis for some 7600 genes was finished. Minimal annotation has been given to the in situ images; 10 stages for embryogenesis, 4 stages for larval-adult stage, on average 10 patterns (cell(s), tissue, region) per stage, 3 levels of relative intensity of signals per each pattern. Using the information ((10+4)x10x3 = 420 dimensions), we have done clustering analysis of the in situ patterns. The gene expression patterns were roughly clustered by the factor analysis using the Euclid distance. This treatment classified the embryonic patterns of 5064 genes into 39 clusters that contained 5 to 3355 genes per cluster (12 clusters had more than 50 gene members). Then, we focused on several cell/lineage/stage-specific clusters; gut specific cluster (910 genes), hypodermis specific cluster (421 genes), and body wall specific cluster (72 genes). The three clusters were further classified by the Ward method. This treatment produced 7 sub-clusters along the developmental time course from the gut cluster and 6 sub clusters from the hypodermis and body wall muscle clusters.

This means that we know the time of transcription initiation of the member genes in the individual cell lineages and that the members. The same sub-cluster might be regulated by the same mechanisms. Thus, we are now trying to extract common sequence motifs, possible candidates for transcriptional regulatory signals, in the 5’- upstream region of the genes in the same sub-cluster.

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1014. Tissue-specific gene regulation by intronic elements in *C elegans*

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Neuronal cells express many genes for their specific functions such as neurotransmitter synthesis, its release and axonal outgrowth. SNAP-25 is a presynaptic protein involved in vesicle fusion at the plasma membrane of the synapse and is encoded by ric-4 in the nematode. Immunohistochemistry revealed that SNAP-25 is exclusively expressed in the nervous system of C. elegans. We investigated the cis regulatory requirements for neuron-specific expression of SNAP-25 by reporter gene assay and identified compact regulatory elements located in the 5' upstream and the first large intron of Ce SNAP-25. Fused to the basal promoter of the native SNAP-25 gene, each group of elements worked in distinct types of neuron cells in C. elegans. Most elements were sufficient for expressing the reporter gene but some were dependent on nearby elements. Introduced to a naive promoter as inverted sequences, each element also showed discrete manners. We tentatively conclude that at least one or more is an enhancer element of SNAP-25. We have also shown, by interspecies transformation experiments, that SNAP-25 is functionally conserved between C. elegans and C. briggsae. Comparative sequence analysis supports the importance of the regulatory sequence that we have identified by reporter gene analysis.

A second gene we found to be regulated by intronic elements is run. C. elegans run gene, a homolog of Runx, has been identified recently on the basis of C. elegans genome database research. C. elegans run is expressed in the nuclei of hypodermal cells and intestinal cells. Expression of run in hypodermal cells and intestinal cells began to be detected at the bean stage and L1 stage, respectively, and lasted until the L3 stage. This gene is composed of 11 exons. There is an exceptionally long intron(7.2kb) between exon 3 and 4. We thought that this unusually long intron controlled the cell type specific expression of run like SNAP-25. Therefore, to understand this specific expression pattern of run, we have examined various constructs of run fused GFP in transgenic animals. From these experiments, we have obtained results that show that the N-terminal 2kb of the long intron regulates the expression in hypodermal cells and the C-terminal 5kb of long intron regulated the expression in intestinal cells. In order to analyze the regulatory element of run, we are constructing various deletion run clones. We will characterize the regulatory motif of tissue specific expression from these experiments.

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1015. Defining Regulatory Regions In Nematode Muscle Genes By Computational And Transgenic Studies.

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We have initiated experiments designed to understand the regulatory regions of *C. elegans* genes using known muscle genes of *C. elegans* as a model. Two approaches are being used to pursue this goal.

The first approach is to computationally compare the muscle genes from *C. elegans* to the orthologous muscle sequences from *C. briggsae*. This comparison is useful because the patterns of gene regulation and regulatory elements are often conserved across species. The *C. briggsae* orthologue are found by making a probe from the *C. elegans* muscle gene and probing the *C. briggsae* fosmid filter available from Incyte. The most promising positive clones are determined by fingerprinting and these are sequenced by the Genome Sequencing Center. To compare the orthologous sequences from *C. elegans* and *C. briggsae*, we will use pairwise alignment methods like BlastZ(4) or Bayes aligner(5) to identify regions of interest. Local multiple alignment programs can then be used to search for common regulatory elements in these regions. Since the local multiple alignment methods work best with sequences which are only 1000-2000 nucleotides long, phylogenetic footprinting will be useful in identifying shorter regions from much longer regions(10,000-20,000 nucleotides).

The second approach is to use a combination of computational methods to identify potential muscle specific regulatory elements from the known set of *C. elegans* muscle genes. Local multiple sequence alignment methods like Consensus(1), Ann-Spec(2) and Co-Bind(3) are being used to identify these potential regulatory elements. Using the above method we have already identified several potential regulatory elements which show high degree of specificity for the muscle genes. The regulatory elements that these computational methods predict can then be used to screen the *C. elegans* genome for new genes that are expressed in muscle cells.

To test our results we have developed a method to examine the expression patterns of genes in *C. elegans* using gfp promoter fusions. We are including in our promoter fusions 6,000 nucleotides upstream of the start methionine, all of the first exon and all the first intron. In our initial experiments, known muscle genes tested in this manner show muscle-like expression. We can now use this method to test the requirement for regulatory regions predicted by the computational work to determine if they convey muscle specific expression. In addition, we can use this method to test genes we predict to be, but not previously known to be, expressed in muscle. Furthermore, we are developing these methods to allow for the rapid production of these promoter fusions so that ultimately, a genome wide program to categorize all *C. elegans* genes by gfp and automated lineaging can be done.

1. Hertz, G.Z., and Stormo, G.D. (1999) Bioinformatics, vol. 15, pp. 563-577 2. Workman, C.T., and Stormo, G.D. (2000) Pacific Symposium on Biocomputing, vol 5, pp. 464-475 3. GuhaThakurta, D., and Stormo, G.D. (2001) Bioinformatics, in press. 4. Schwartz, S. et.al. (2000) Genome Research, vol. 10, pp. 577-586. 5. Zhu, J., Liu, J.S., and Lawrence, C.E. (1998) vol. 14, pp. 25-39.

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1016. The *lin-26* gene is regulated by tissue-specific cis-elements

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The *lin-26* gene, which codes for a zinc-finger transcription factor, is involved in the maintenance of all epithelial cell fates in the ectoderm and mesoderm (the somatic gonad) and can induce epithelial markers when overexpressed. In order to place this gene in a differentiation pathway and to look at upstream events, we decided to perform a promoter study. By comparing *C. elegans lin-26* cis-sequences to those of *C. briggsae*, we identified seven conserved regions. To test their enhancer activity, we cloned promoter fragments in front of a minimal promoter linked to GFP. Moreover we also tested their role by looking at the expression pattern of deleted *lin-26*::GFP fusion constructs, and at the rescuing activity of deleted constructs in a *lin-26* null background. To date four major cis-elements (from 5' to 3') have been indentified that drive the expression in specific cells:

A rep cell element (3.5 kb): Only defined by enhancer properties, turns on GFP in the rectal rep cells. The expression of *lin-26* in these cells is not essential for viability.

A gonad element (445 bp): This element drives GFP expression in Z1 and Z4, the precursors of the somatic gonad in which *lin-26* is normally expressed, and later in the uterus. In addition, when the gonad element is deleted, animals are sterile and show gonad defects.

A glial cell and minor hypodermal cell element (530bp): This element drives the GFP in about 20 cells in the head (mostly glial-like cells and few cells belonging to the minor hypodermis), 6 cells in the tail (PHshL/R, Hyp8,9,10), and also in the excretory cell. Expression in the head and in the tail is driven separately by two constructs of about 270 bp, which overlap by 130 bp. Because of the division of the pattern into two different regions, we tested the possible regulation of the "tail element" by two Hox genes belonging to the posterior-group, *nob-1* and *php-3*, by silencing these genes using RNAi techniques. The number of GFP positive cells remained unchanged in RNAi animals, carrying the integrated "tail element".

A hypodermal element (2.1 kb): Sufficient to obtain a GFP expression in cells of the major hypodermis, but not necessary for *lin-26* expression in that tissue. This suggests the existence of a functionally redundant regulator for the expression in the hypodermis, located either upstream, or in the 3'UTR. The enhancer activity of a larger fragment (4 kb, comprising this 2.1kb element) requires the activity of *elt-1*, since in *elt-1*(zu180) mutants the number of GFP-expressing cells is dramatically reduced. Moreover, three GATA sites perfectly conserved between *C. briggsae* and *C. elegans* are found in that fragment, suggesting a direct regulation which we will investigate.

The 530 bp element, in both orientations, is also able to act synergistically with sequences highly conserved in *C.briggsae* (contained within the 4 kb hypodermal element) which are not able to drive any expression on their own, thus giving rise to new expression patterns including rectal cells (K, K', U, B, F and Y), and the P cells.

These data suggest that *lin-26* expression is controlled by redundant elements in a tissue-dependent manner rather than by lineage-dependent mechanisms. We are now focusing on regulators of *lin-26* expression in different tissues, starting with the gonad. A sequence of 100 bp, conserved between *C. briggsae* and *C. elegans*, was analysed by the MatInspector software. We selected four transcription factors (Sox, GKLF, Creb, GFI-1) which have homologues in *C. elegans*. RNAi techniques are currently being used in order to test the influence of these candidates in an integrated line carrying a *lin-26* gonad specific enhancer coupled to GFP. Up to date six candidates have been tested separatly (C32E12.3, F40E10.2: Sox homologues, Y38H8A.5, Y55F3AM.14, C55B7.12: GFI-1 homologues, and the GKLF homologue F54H5.4) without having any influence on the reporter expression. This work, including the promoter study, is still in progress.

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1017. The Roles of *cis*-elements in the Regulation of *C. elegans* Hox Gene *egl-5*

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Hox genes are well-known segmental identity genes that are conserved in multicellular organisms. Despite the extensive studies on the roles they play during development, how signaling pathways coordinate to activate Hox genes in the proper regions of an organism is not well described. The *C. elegans* Hox gene *egl-5* is involved in many cell fate specification events in the posterior body region. In the seam cell V6 lineage, the expression of *egl-5* is under sexual, spatial and temporal regulation. The activation of *egl-5* in the seam is male-specific, the expression of *egl-5* is restricted to a subgroup of seam cells that give rise to ray 3, 4, 5 and 6, and the earliest activation event always happens at the V6.ppp stage. Another Hox gene, *mab-5*, is required for the activation of *egl-5*. However, *mab-5* is present all through the V6 lineage in both hermaphrodites and males. Therefore the sexual, spatial and temporal specificity of *egl-5* expression in the V6 lineage remains unaccounted for.

To understand the complicated expression pattern of *egl-5*and the convergence of signaling pathways upon activation of *egl-5* in a specific lineage, we have been doing PCR-based promoter dissections of *egl-5*. A comparison of the intergenic regions upstream of *egl-5* in *C. elegans* and *C. briggsae* has revealed that there are short stretches of DNA perfectly conserved within the generally diversified non-coding regions. We presume these to be the candidate enhancers of the *egl-5* gene. We have been trying to assign them to specific lineages by introducing deletions of these conserved sequences into an *egl-5::gfp* reporter and looking for changes in the expression pattern. So far, we have roughly mapped the enhancers of rectal epithelial cells and male tail hypodermal cells. Moreover, in a tissue-specific rescue experiment, we have been able to rescue the tail retraction defect in *egl-5* mutant males with a construct expressed in the male tail hypodermal cells but not in the rectal epithelial cells or the seam cells.

We have also identified a 200bp region highly conserved between *C. elegans* and *C. briggsae* as the putative V6 lineage enhancer. This region is not only necessary for the V6 lineage expression of *egl-5*, but also gives the wild type expression pattern of *egl-5* in the V6 lineage when placed before basal *pes-10::gfp*. We have found putative binding sites within this conserved region for several relevant transcription factors, namely TRA-1, POP-1 (TCF/LEF1 homolog), and MAB-5/CEH-20 (Extradenticle homolog). Now we are in the process of making smaller deletions and point mutations within the putative V6 lineage enhancer, to identify the functionally significant motifs. Surprisingly, a small deletion of 19bp that contains a putative TRA-1 binding motif lead to ectopic expression of the V6 enhancer driven *pes-10::gfp* in anterior seam cells, ventral cord and head region in both hermaphrodites and males. The temporal regulation of *egl-5* is also disrupted. The same deletion in a full-length *egl-5::gfp* reporter also causes ectopic expression in the same groups of cells, although to a lesser extent. We hypothesize that this deletion has revealed the binding site of a widely expressed repressor. We are now trying to determine if it is TRA-1 that acts as the repressor. Meanwhile, we are taking a candidate gene approach, looking for loss or ectopic expression of *egl-5* in RNAi experiments with known Hox gene regulators such as trithorax related genes.

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1018. Identification of *Cis* -Regulatory Response Elements in Vulva Cell Fate Markers

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After induction of primary and secondary fates by the anchor cell and *lin-12*, and undergoing two rounds of cell division the final vulval cell types are specified A, B1, B2, C, D, E and F. A number of transcription factors have been identified that play a role in vulva development. However we know little about how these transcription factors interact with each other, about their interactions with the inductive pathway, or about how they are regulated. The identification of *cis*-regulatory regions that confer cell specificity and respond to the *ras* pathway would be very helpful in determining such relationships. Three such target genes are *egl-17*, *cdh-3* and *zmp-1*. These genes offer the opportunity to find response regions for multiple vulva cell types: vulE, vulF, vulC, vulD, and vulA as well as the anchor cell. In addition, *egl-17* is an early cell fate marker for the response to the inductive signal; the isolation of a *cis*regulatory element that drives this early expression and identification of genes that regulate this expression would be informative. We are analyzing these regions using a PCR based strategy to generate defined deletions to test the necessity and the sufficiency of fragments to confer regulation on the naïve promoter *pes-10*.

egl-17::gfp comes on in P6.p, P6.p daughters and granddaughters; goes off in early L4; staying off in mid-L4; on in vulC and vulD cells in mid L4 [Burdine RD, Branda CS, and Stern MJ, *Development* Mar;125(6):1083,1998]. This GFP marker strain contains 3.8 kb of *cis*-regulatory region. Since this gene is the earliest known maker in response to the inductive signal, it makes a good candidate for a immediate response element to the *ras* pathway. We have found a 0.2 kb region that is necessary and sufficient for vulC and vulD cell specificity and a 1.5 kb region that confers early expression in P6.p daughters. *zmp-1::gfp* is expressed during late L4 in vulE and vulA cells and in the L3 animals it is also expressed in the anchor cell [J. Butler & J. Krug, personal communication]. This GFP marker also contains 3.8 kb of upstream regulatory sequence. We have defined a 0.35 kb region that is necessary and sufficient vulE, vulA and anchor cell specificity. *cdh-3::gfp* is expressed during L3 in vulE and vulF cells and in L4 animals it is expressed in the vulC and vulD cells. *cdh-3* is also expressed in the anchor cell starting in the late L2 stage. The gfp marker strain contains 6.0 kb of upstream regulatory sequence. We have found a 0.6 kb region that confers specificity to the anchor cell element and 0.5 kb region that drives expression in vulE and vul F cells.

1019. Computer prediction of cis-acting elements from co-regulated genes.

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The complete nucleic acid sequence of a genome provides new opportunities to study gene regulation. cis-regulatory elements are required for the correct developmental activation (spatially, temporally as well as appropriate level of expression) of genes. Identification and analysis of these sites are crucial for advancing our appreciation of genome regulation.

The ultimate goal of the research is to construct specific promoter models containing a combination of several regulatory elements. As a first step towards this goal, it is necessary to focus on the detection of single motifs (representing transcription factor binding sites) common to the promoter sequences of putatively co-regulated genes. However, this problem is complex: putative regulatory motifs will be of unknown size with variable sequence and ill-defined position.

Analysis of gene expression using a reporter gene fusion approach [http://129.11.204.86:591] has identified groups of genes with similar expression patterns. Many if not all these genes are likely to be co-regulated and contain cis-elements recognized by a common regulatory system. The regions of these genes able to drive this expression pattern has been analyzed using two software packages, MEME [http://meme.sdsc.edu/meme/website/] and SPEXS [http://ep.ebi.ac.uk/EP/SPEXS/] to identify cis-regulatory elements. The significance of the MEME results was difficult to assess as many motifs were detected but the low sequence complexity of many of them raised questions about the significance of the output. In contrast the SPEXS output was simpler although the number of motifs generated was greater. The output is being further processed to include other characteristics in a score for identified motifs. The motifs with the highest scores should be the strongest candidates for experimental verification.

1020. Regulation of the *C. elegans* posterior *Hox* paralogs *nob-1* and *php-3*

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During metazoan embryonic development, regional differences along the anterior-posterior axis are specified by *Hox* gene products, which are highly conserved homeodomain transcription factors. We have recently described two previously unknown *C. elegans* posterior *Hox* paralogs, *nob-1* and *php-3* (Van Auken et al., PNAS 97:4499-4503, 2000), and are now undertaking a study of their regulatory regions. The two genes are 232bp apart; however, they probably do not form an operon because *php-3*, the downstream gene, is SL1-spliced. *nob-1* and *php-3* are 47% identical at the nucleotide level, and preliminary evidence suggests that they have redundant functions. A genetic null allele, *ct223*, contains a 25kb deletion upstream of *nob-1* that is likely to remove most or all of the regulatory region for both genes. We are currently making constructs to rescue *nob-1* and *php-3* together as well as individually. We have cloned the *C. briggsae nob-1* region for comparison to the *C. elegans nob-1* region and identification of putative conserved regulatory elements. We will eventually use GFP reporters to functionally test these elements and correlate them to expression patterns. Knowledge of the regulation of *nob-1* and *php-3* will provide an interesting comparison to the regulation of the mouse and *Drosophila* posterior *Hox* paralogs.

1021. Systematic identification of cell specific enhancers in *C. elegans*.

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A large numbers of GFP reporter constructs have been reported to show specific expression patterns in particular set of cells in *C. elegans*. This fact leads us an idea that comparison of the genes expressed in specific cells would reveal the core elements essential for the expression.

To identify such core elements, we are going to;

1) take a GFP reporter expressed in a target cell.

2) produce a series of deletion promoter::GFP fusion by PCR.

3) inject the PCR fragment into worms.

4) wait for two generations. (no F1 pick).

5) isolate a stable transgenic line from each injection.

6) check the expression pattern in the transgenic worms.

7) repeat step 2) - 6) until identifying the core element.

The protocol is very quick and suitable to a lazy(!) person, because we do not need to spend a long time to have GFP constructs nor to pick F1 worms. Moreover, we plan to compare the genomic sequences between *C. elegans* and other nematode such as *C. briggsae*. It would facilitate to highlight the conserved (important) regulatory elements in the promoter region of orthologous genes. Currently, we are collecting many GFP reporters expressed in a target cell, and analyze neuron-specific promoters. Thus far, we have narrowed down the promoters for the thermosensing neuron AFD. The following fragments are responsible for the AFD specific expression; ~700 bp of *ceh-14* (homeobox), ~100 bp of *gcy-8* (guanylyl cyclase), ~200 bp of *nhr-38* (nuclear receptor), and ~1 kb of *rhp-5* (7TM receptor). Further analysis will be reported at the meeting.

1022. Dissection of the upstream regulatory elements of the Inositol 1,4,5-trisphosphate receptor gene *itr-1*.

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Inositol 1,4,5-trisphosphate receptors (IP3Rs) play a central role in the organisation of calcium signals by regulating intracellular calcium flux. IP3Rs in *C. elegans* are encoded by a single gene, *itr-1* (also called *lfe-1* (1) and *dec-4* (2)). In previous work (3), we characterised the gene structure in detail and identified three putative transcriptional start sites and three alternative splice sites. Sequence comparisons to the *C. briggsae itr-1* homologue support the hypothesis that the *itr-1* gene has three promoters (pA, pB and pC), each of which gives rise to an alternative mRNA and hence unique protein. The pattern of expression directed by each promoter was determined using GFP reporter gene fusions. pA directs expression in the pharyngeal terminal bulb, the rectal epithelial cells and vulva; pB directs expression in the amphid socket cells, the PDA motor neuron and the spermatheca; pC directs expression in the spermathecal valve, uterine sheath cells, pharyngeal isthmus and intestine (4). Thus tissue-specific expression of *itr-1* variants is directed by three promoters and this results in adjacent cells in the same tissue containing different IP3R isoforms.

Analysis of the sequence conversation between promoter A of *C. elegans* and *C. briggsae* identified four short regions (pA-A to pA-D) of conservation. Deletion analysis of pA demonstrated that the region containing pA-C is required for expression in the terminal bulb and rectal epithelial cells and the region containing pA-D is required for expression in the vulva. pA-C contains sequences which show homology

to binding sites for known *C. elegans* transcription factors. We are currently identifying factors that regulate the promoter using a yeast one-hybrid screen.

1. Clandinin *et al* (1998) Cell: 92, 523-533. 2. Dal Santo *et al* (1999) Cell: 98 757-767. 3. Baylis *et al* (1999) J. Mol Biol: 294, 467-476. 4. Gower *et al* (2001) J. Mol Biol: 306, 145-157.

1023. An investigation of the role of the *Exd/Pbx* orthologs *ceh-20* and *ceh-40* in the activation of *ceh-13*

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Hox genes encode a conserved set of transcription factors that play a central role in patterning of the body axis during animal development. Recent studies in *Drosophila* and mammals have revealed that Hox proteins interact with additional factors such as the TALE homeodomain proteins EXD and HTH. HOX/EXD or HOX/EXD/HTH complexes are required for both regulation of Hox gene activity and enhancement of the binding specificity of Hox proteins to target sites.

Recent work in our lab (see abstract of Streit *et al*.) has identified a conserved 10 bp long autoregulatory element in the promoter of *ceh-13*, the *Drosophila labial*/mammalian *Hox1*-class ortholog of the *C.elegans* HOM-C/Hox cluster. In *Drosophila* and mammals, this element represents a bipartite binding site for a LAB(HOX1)/EXD(PBX1) complex. To see whether the autoregulation of *ceh-13* requires the same basic mechanisms, we investigated the role of the *C. elegans* EXD orthologs CEH-20 and CEH-40 in *ceh-13* regulation. Animals injected with *ceh-20* and *ceh-40* RNAi simultaneously exhibited synthetic embryonic lethality and early larval arrest, a phenotype, that was not observed in *ceh-20* or *ceh-40* single RNAi experiments. This shows that the two genes may interact genetically.

Expression of a GFP reporter construct containing the *ceh-13* autoregulatory element was highly reduced in a *ceh-20* null mutant background as compared to WT background. Moreover, introduction of a single point mutation in the EXD site of the autoregulatory element also abolished GFP expression, suggesting that CEH-20 is required for *ceh-13* autoregulation. To further prove binding of CEH-20, we performed an electrophoretic

mobility shift, and we investigated a possible role of *ceh-40* in the regulation of *ceh-13*. The results will be presented.

1024. Identification of genes involved in lateral inhibition during vulval cell fate specification

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During the development of the hermaphrodite vulva, three out of six equivalent vulval precursor cells (P3.p through P8.p) in the ventral hypodermis are induced by a signal from the gonadal anchor cell (AC) to adopt vulval cell fates. The AC signal activates in P6.p an evolutionary conserved RTK/RAS/MAP kinase pathway to specify the primary cell fate. A lateral signal from P6.p then activates the LIN-12/NOTCH signalling pathway in P5.p and P7.p. The lateral signal performs two distinct functions: In a first step, it inhibits the expression of the primary cell fate by P5.p and P7.p, and in a second step, it directly promotes the specification of the secondary cell fate. The lateral inhibition of the primary fate is in part mediated by the MAP kinase phosphatase LIP-1 (1). LIN-12 Notch up-regulates *lip-1* transcription in P5.p and P7.p where LIP-1 inactivates the MAP kinase MPK-1, thus inhibiting the specification of the primary fate. Since loss of *lip-1(+)* function is not sufficient to disrupt lateral inhibition, there may exist additional inhibitory mechanisms. We have used a sensitised genetic background to isolate additional genes involved in lateral inhibition. The *gaIs36[HS-mpk-1(+)]* transgene (2) produces more but not enough MPK-1 to alter vulval cell fate specification. However, loss of *lip-1(+)* function in a *gaIs36[HS-mpk-1(+)]* background disrupts lateral inhibition, causing P5.p and P7.p to adopt the primary instead of the secondary vulval cell fate (1). We have screened the F2 progeny of mutagenised *gaIs36[HS-mpk-1(+)]* animals for mutants in which P5.p and P7.p adopted the primary instead of the secondary vulval cell fate. To date, we have isolated 10 mutations that cause a strong Muv phenotype in a *gaIs36[HS-mpk-1(+)]* background and are currently performing further experiments to characterise these mutations.

(1) Berset et al. *Science* 291, 1055-58 (2001).
(2) Lackner et al. *Genetics* 150, 103-117 (1998).

1025. An Approach to Identifying the Lateral Signal in Vulval Development

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In *C. elegans*, six vulval precursor cells (VPCs), P3.p through P8.p, are capable of generating vulval cells. In wild-type hermaphrodites, several signaling events (i.e., inducing, lateral, and inhibitory signaling) specify the VPC cell fate pattern 3*-3*-2*-1*-2*-3*. P5.p, P6.p and P7.p adopt vulval fates (1* and 2*), while the other three cells adopt a non-vulval fate (3*).

LIN-12 appears to be the receptor for the lateral signal. The lateral signal itself has not been identified in genetic screens, but characterized ligands of LIN-12/Notch receptors all belong to the Delta/Serrate/LAG-2 (DSL) protein family. We have identified a set of DSL genes in the genome --F58B3.8, H02I12.4, W09G12.4, F15B9.3, and F16B12.2, as well as *apx-1* and *arg-1*--to be evaluated as candidates for the lateral signal.

The simplest expectation would be that the ligand gene would be expressed in P6.p (and perhaps other VPCs) in the L3 stage after inductive signaling and that loss of ligand activity would lead to the absence of the 2* fate. By both of these criteria, *lag-2* does not appear to be the lateral signal. Some relevant information is available for certain other DSL genes (*apx-1*, *arg-1* and F15B9.3), but the fact that mutations have not yet identified a candidate ligand may indicate that there is functional redundancy or that loss of gene function has a pleiotropic effect that has precluded its identification in vulval mutant screens. We therefore are screening all candidate DSL genes for their expression in the VPCs, and performing RNAi analysis of various combinations of DSL genes. We will report on our progress at the meeting.

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1026. ISOLATION AND CHARACTERIZATION OF NEW SYNMUV GENES, INCLUDING *lin-61*, A CLASS B GENE WITH ROLES IN CELL DIVISION AND MULTIPLE POSTEMBRYONIC DEVELOPMENTAL PROCESSES

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The regulation of vulval induction in *C. elegans* provides a useful model system for the study of genetic and molecular mechanisms involved in signal transduction and cell-fate determination. Previous work has defined two classes of genes (A and B) that act redundantly to negatively regulate the adoption of vulval cell fates by vulval precursor cells. The simultaneous elimination of the function of a member of both gene classes results in a synthetic multivulva (synMuv) phenotype. The class B synMuv genes include genes similar to members of the mammalian RB signaling pathway. By contrast, the determination of the molecular nature of the known class A synMuv genes has thus far yielded relatively little insight into the mechanism of their function.

We have cloned a new class B synMuv gene. *lin-61* is located on LG I and encodes a protein weakly similar to members of the *Drosophila* polycomb family and strongly similar to predicted human and *C. elegans* proteins of unknown function. *lin-61* reduction-of-function mutations produce a synMuv B phenotype in a variety of class A synMuv backgrounds. By contrast, elimination of both maternal and zygotic *lin-61* activity via RNAi results in early embryonic lethality as a result of a failure to complete cytokinesis beginning at the first embryonic cell division. We further examined the developmental functions of *lin-61* by injecting *lin-61* dsRNA into RNAi defective *rde-1* hermaphrodites and then mating these animals with N2 males (a technique described by Herman, 2001; Development *128*, 581-90). Cross progeny were then observed in an effort to gain an understanding of the phenotype produced after the reduction of zygotic *lin-61* activity while maintaining at least some maternal *lin-61* function. Unlike *lin-61(*RNAi*)*, this approach yields animals that reach adulthood but display a host of developmental abnormalities. Based on these results we conclude that *lin-61* functions in a variety of embryonic and post-embryonic developmental processes in addition to its role in vulval development.

We are also attempting to identify new class A synMuv genes. Previous screens to isolate class A genes would not have recovered synMuv mutations that also cause sterility or maternal-effect lethality. For this reason, we are now conducting a clonal screen to seek alleles of new class A synMuv genes. Beginning with a strain containing a strong *lin-15B* allele, *n744*, we have screened approximately 30,000 haploid genomes and isolated more than 40 mutants that display a Muv phenotype. These isolates include at least two homozygous viable mutations in new candidate class A synMuv genes and over 15 Muv and synMuv mutations that cause sterility or maternal-effect lethality as homozygotes. Complementation testing and mapping of these mutations is currently underway.

1027. FUNCTIONAL STUDIES OF THE CLASS B SYNMUVS REQUIRED FOR THE NEGATIVE REGULATION OF VULVAL INDUCTION AND CHARACTERIZATION OF A NOVEL CLASS B SYNMUV GENE

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A receptor tyrosine kinase/Ras pathway necessary for vulval induction in *C. elegans* has been shown to be negatively regulated by two redundant pathways, defined by the synthetic Multivulva (synMuv) class A and B genes. Mutations in members of either class alone do not result in a Multivulva phenotype. However, animals containing loss-of-function mutations in both a class A and a class B gene display a synMuv phenotype. While most of the identified class A genes encode novel proteins, many of the class B synMuv genes, including *lin-35* Rb, *dpl-1* DP, *efl-1* E2F, *lin-53* RbAp48, *hda-1* HDAC, and *let-418* Mi-2, have homologs in other organisms known to be involved in chromatin modification complexes.

Three mutations that define a new class B synMuv were identified in a recent screen for additional class B genes performed with Craig Ceol and Frank Stegmeier in our laboratory. We mapped these mutations to the far left of the X chromosome and are currently attempting to clone the gene by cosmid rescue.

Several mammalian homologs of the class B synMuv genes have been shown to be components of the NuRD chromatin remodelling complex. Additionally, some of the proteins in the synMuv B pathway have been shown to physically interact either *in vitro* (1,2) or in yeast two-hybrid assays (3). We are attempting to use co-immunoprecipitation experiments to expand the current knowledge of the presumptive class B protein complex in *C. elegans*. Analysis of the effects of various mutations on co-immunoprecipitation may allow us to assign functionality to some novel class B genes. Currently, there is little understanding of how the class A and B synMuvs redundantly regulate vulval induction. Class A and B synMuvs might play redundant roles in the formation of a chromatin modifying complex, and such physical interactions could also be demonstrated through co-immunoprecipitaion experiments.

(1) Ceol, C. and H.R. Horvitz (in press).

(2) Lu and Horvitz. (1998) Cell **95**: 981.

(3) Walhout et al. (2000) Science **287**:116.

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1028. What is the cellular focus of SynMuv genes?

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The synthetic Multivulva (synMuv) genes comprise two classes of genes, Class A and Class B, that act in the determination of vulval precursor cell (VPC) fates (Ferguson, E.L. and Horvitz, H.R.,1985,1989). A synthetic Multivulva phenotype is produced by two mutations, one in each of the two classes of genes. Mutations in one or more class A genes or one or more class B genes do not produce a Multivulva phenotype. It has been proposed that the class A and class B genes define two functionally redundant pathways that act to specify the non-vulval fate of the vulval precursor cells.

We have previously shown that *lin-13* behaves as a Class B SynMuv gene, a class that also includes genes encoding the tumor suppressor Rb and RbAp48, a protein that binds Rb. We have also shown that LIN-13 is a nuclear protein that contains multiple zinc fingers and a motif, LXCXE, that has been implicated in Rb binding. These results together suggested a role for LIN-13 in Rb-mediated repression of vulval fates.

A Multivulva phenotype can in principle result from activation of a pathway or process that promotes vulval fates, or the inhibition of a pathway or process that represses vulval fates. Genes that influence this process can in principle act in an external signalling cell or in the VPCs. There have been two views to date. One view is that SynMuv genes act in an external signalling cell, hyp7, to control expression of a factor that antagonizes the effects of the inductive signal or otherwise to promote nonvulval fates (Herman and Hedgecock 1990). The other view is that SynMuv genes act in the VPCs to repress transcription of genes that are involved in vulval differentation (Lu and Horvitz 1998).

The proposal that SynMuv genes act in hyp7 was based on genetic mosaic studies of a *lin-15* mutation that concomitantly removes both Class A and Class B activities (Herman and Hedgecock 1990), and was supported by mosaic analysis of the Class B SynMuv gene *lin-37* (Hedgecock and Herman 1995). Our findings that *lin-13::lacZ* and LIN-13::GFP are consistently expressed in hyp7 and are generally undetectable in VPCs at the time of VPC specification has been consistent with this view. If SynMuv genes act in hyp7 and not in the VPCs, they may promote the expression in hyp7 of an inhibitory factor that represses vulval fates.

As it remains possible that some SynMuv genes act in hyp7 while others act in the VPCs, and as expression data can be misleading as to cellular focus, we have undertaken genetic mosaic analyses of various SynMuv genes. We will report on our progress and plans concerning mosaic analysis of *lin-13* and *lin-35* and expression of SynMuv genes in specific cells.

1029. Organogenesis of the *C. elegans* vulva: *cog* mutants

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While a large body of research has provided insight into how cell fate is specified early in embryogenesis, little is known about mechanisms through which these cell fate decisions are implemented, ultimately resulting in the formation of discrete organs. *C. elegans* vulval development provides a powerful model system for elucidating the organogenesis process. Our research focuses on the aspect of how the developing vulva connects properly to the overlying uterus. We have been continuing the studies of *cog-2* and *egl-26*, which display Cog (connection of gonad defective) phenotypes. Mutation of each of these genes results in a physical blockage between the vulva and uterus at the completion of morphogenesis and an egg-laying defective phenotype.

The Cog phenotype of *egl-26* mutants is a result of adoption of an abnormal morphology by the most apical vulva cell, vulF. EGL-26, a novel protein, is expressed with a distinct subcellular localization pattern around the apical edge of the cell that neighbors vulF called vulE. Thus, we believe that EGL-26 acts non cell-autonomously in controlling cell morphology, and we are currently testing this model. The Cog phenotype in *cog-2* mutants is a result of a failure of the anchor cell to be cleared from the apex of the vulva during the fourth larval stage when it normally fuses to the uterine seam cell. COG-2 encodes a Sox (SRY-related HMG box) domain transcription factor that is expressed in the uterine seam cell. We are conducting a *cog-2* suppressor screen in order to identify genes that act together with *cog-2*, and we have developed a GFP-based screen to identify additional mutants with phenotypes similar to that of *cog-2*mutants.

1030. Studies of mutations that disrupt vulval development in C. elegans

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We are interested in studying various steps of vulval fate specification and morphogenesis. Here we report our analyses on four vulval defective mutants.

ku229 was identified in a temperature-sensitive (TS) mutant screen for mutants involved in vulval morphogenesis, although the mutation itself is not TS. About 60% of ku229 worms are Egl. Under Nomarski optics, many ku229 worms have abnormal but recognizable "Christmas-tree" structures, and seem to have numerous lineage defects. A weak lin-39 allele, n709ts, can enhance the Egl phenotype of ku229 to 95%. These results suggest that the gene defined by ku229 may be involved in vulval cell fate specification. When ku229 is placed over a deficiency, the Egl phenotype is significantly weaker (~20%), suggesting that ku229 could be a gain-of-function allele. We have mapped ku229 to a small region on LGIV and have been using several methods to clone the gene.

Our laboratory has been screening and analyzing many sterile mutants with protruding vulvae (Pvl) in the past a few years. The studies have also included many evl genes identified previous by G. Seydoux in I. Greenwald's lab (1), three of which are described here. First, evl-14 seems to have a lineage defect and dose not form a wild-type Christmas-tree structure. evl-4 is defined by a mutation that displays a Cog (connection to gonad defective) phenotype. evl-22 mutants have an abnormal vulval lineage so the vulva usually consists of less than 15 nuclei instead of 22 in wild type. evl-22 mutants also show underproliferation in somatic gonad and germ line suggesting that the gene plays a role in cell division in general. After further mapping, we have rescued all three genes either with a single cosmid/fosmid clone or, in the case of evl-22, with two overlapping cosmids.

We are in the process of determining the specific open reading frames for these genes.

(1)Seydoux, G, Savage, C., and Greenwald I. (1993). Developmental Biology 157, 423-436.

1031. DOES *lin-15* STIMULATE CELL FUSION AND ANTAGONIZE VULVAL CELL FATES?

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lin-15a/b are SynMuv genes that generate an inhibitory signal by two redundant pathways (A and B) to antagonize RAS signaling in wild type worms. In the mutant *lin-15(e1763lf)* both genes are deleted and neither of the SynMuv redundant pathways are functional (1). The result is that P3.p, P4.p and P8.p, which normally fuse to the hypodermis, adopt vulval fates leading to a **Mu**lti-**V**ulva (Muv) phenotype.

In order to study the role of *lin-15* in cellular events during vulva morphogenesis, we constructed the strain *lin-15(e1763lf); jam-1::gfp.* We have followed vulva formation in this strain, by observing the fluorescent adherens junctions of the vulva precursor cells in L3-L4 worms, using confocal microscopy. We will summarize the cellular events leading to real vulva formation and then the pseudo-vulvae formation. Two different types of "real" (functional) vulva structures were observed. First, a wild-type like vulva, and second, a vulva primordium that contains twice as many cells in the central region resembling a primary-primary pattern of sublineages (TTTTTTTT) at early L4 stage, and a super vulE-vulF structure containing up to 16 nuclei instead of 8 in the wild type at late L4 stage. We also found primordia showing that the daughters of P5.p and P6.p divided T (transverse) instead of L (longitudinal). We hypothesize that these early abnormal transverse divisions could be the cause of super vulE-vulF structure formation. In *lin-15lf* mutants, P(3,4,8).p continue to divide instead of fusing with the hyp7 resulting in the formation of a variable number of protruded pseudovulvae. We have found some variability in the patterns of pseudo-vulvae formation. This variability is observed in at least three parameters: The patterns of sublineages each pseudovulva exhibits, the number of the pseudovulvae each worm has and the pseudo-vulvae position relative to the real vulva. We also found, that

these pseudovulvae are connected to the real vulva and to their neighboring pseudovulvae by cellular extensions. These filopodia extensions arise from precursor cells that fail to migrate to the forming vulva, and therefore do not participate in the final structure of each vulva. We also characterized another migration abnormality in the *lin-15lf* mutants. Ectopic cellular connections are formed by the migration of Pn.p descendants towards the lateral epithelial (seam) cells. We observed this type of connections in 2% of *lin-15lf* worms in comparison with 30% in the background of *rol-6(dm);jam-1::gfp.*To test whether *lin-15* activity may stimulate cell fusion between specific vulval cells, we analyzed the fusion fates within the *lin-15* vulvae in comparison to the wild type. We have seen fewer fusions within vulval C cells in *lin-15lf* animals. Inappropriate fusion patterns may also explain the formation of cell extensions connecting pseudo to real vulva.

In order to further investigate the role of *lin-15* in cellular events leading to vulva morphogenesis we have constructed a double mutant *lin-15(e1763lf);eff-1(hy21). eff-1(hy21)* is a mutant defective in all epidermal fusion events that was recently isolated in our lab (2). In spite of the fact that *eff-1(hy21)* and *lin-15(e1763)* are defective in VPCs fusion, *lin-15* has a highly penetrant Muv phenotype while *eff-1(hy21)* is 100% Pvl (**P**rotruded **vul**va) and 3% Muv . *eff-1(hy21)* is epistatic to *lin-39* (3) which was found to be a suppressor of *lin-15* (4). Further studies of the double mutant will reveal a direct or indirect effect of *lin-15* activity on fusion and migration events during development.

1. Clark et al. (1994). *Genetics* 137: 987-997; Huang et al. (1994) Mol. Biol. Cell **5**: 395-412.

2. Shemer et al. (2001)13th international C. elegans meeting.

3. Shemer and Podbilewicz (2001)13th international C. elegans meeting.

4. Clark and Horvitz (1999) Worm Breeder's Gazette 11: 107.

1032. The EGL-18/ELT-5 and ELT-6 GATA factors regulate cell fates and fusion during vulval development

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Development of the vulva is a complex process regulated by several signaling pathways. Two of these regulatory inputs, Ras and Wnt, converge on *lin-39*, which is essential for proper regulation of cell fates and fusion during vulval development. We previously reported that the *elt-5* and *elt-6* genes may be involved in development of the vulva as well as in seam cell development (KK and JR, WCWM, '00). Here we report that *egl-18*, a gene previously identified in genetic screens, is identical to *elt-5*, and that *egl-18/elt-5* and *elt-6* may function in the vulva in a manner similar to that of *lin-39*.

elt-5 and *elt-6* are adjacent genes encoding single-finger GATA factors that appear to be transcribed both monocistronically and as a dicistronic operon. Injection of concentrated *elt-5* dsRNA into adults results in fully penetrant late embryonic or L1 lethality. Seam cells in affected animals do not differentiate properly and often undergo inappropriate fusion with the epidermal syncytia. Injection of *elt-6* dsRNAs does not cause any observable phenotype, and co-injection of *elt-5* and *elt-6* dsRNAs does not enhance the *elt-5(RNAi)* single mutant phenotype. These results can be explained by proposing that while *elt-5* dsRNA eliminates both monocistronic *elt-5* transcripts and dicistronic *elt-5/6* transcripts, *elt-6* dsRNAs interfere with only the dicistronic transcripts.

Both *elt-5* and *-6* are expressed in the developing vulva, seam cells, and many other cells. Their transcription is controlled by separable enhancer elements that direct expression to different groups of cells. When ELT-6 is driven by a partial promoter of *elt-5* that does not include the vulval enhancer region, the early lethality and seam cell defects

resulting from *elt-5* dsRNA are rescued. However, the surviving animals show Vul, Pvl, and Egl phenotypes. These phenotypes are similar to those of mutations in *egl-18*, which maps to the vicinity of *elt-5*. We found that *egl-18* mutant phenotypes can be rescued by a transgenic *elt-5* gene. Moreover, three *egl-18* alleles (*n162*, *n474*, and *n475*) are nonsense mutations in the *elt-5* sequence, all of which are predicted to terminate translation prior to the DNA binding domain. Thus, *egl-18* encodes the ELT-5 GATA factor. *elt-6(RNAi)* in *egl-18* chromosomal mutants cause penetrant late embryonic/early larval lethality. Collectively, these results imply that *egl-18/elt-5* and *elt-6* are functionally redundant and that only *egl-18*/*elt-5* activity, but not *elt-6* activity, is affected in *egl-18* chromosomal mutants.

Preliminary results suggest that *egl-18/elt-5* and *elt-6* are expressed in the vulval precursor cells in a graded pattern: i.e., most strongly in P6.p, at an intermediate level in P5.p and P7.p, and weakly in P3-4.p and P8.p. This expression pattern is similar to that of *lin-39*, suggesting that like *lin-39*, *egl-18/elt-5* and *elt-6* may be upregulated upon Ras signaling. In addition, in *egl-18/elt-5(RNAi)* animals, vulval precursor cells often fuse in the late-L2/early-L3 stage or undergo only one or two cell divisions, similar to the defects seen in *lin-39(rf)* mutants. We are currently performing experiments to assess the relationship between *egl-18/elt-5*, *elt-6*, and *lin-39* in the pathway for vulval development.

1033. Evolution of vulva induction: studies in *Oscheius / Dolichorhabditis* sp. CEW1

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We study the evolution of development by comparing vulval patterning mechanisms between different species of nematodes. We are focusing on Oscheius / Dolichorhabditis sp. CEW1, a nematode of the same family as C. elegans. In Oscheius, as in C. elegans, the vulva precursor cells (Pn.p) adopt an induced fate (central (1°) fate or external (2°) fate) versus a non induced fate (3°). However in Oscheius sp. CEW1 the anchor cell first induces the Pn.p to adopt a 2° fate and specifies the 1° fate of the central Pn.p by a second induction on P6.p daughters. Our aim is to understand if the same pathways are involved in both species although the mechanisms of induction have not been conserved. We have three different approaches to study vulval induction in Oscheius.

We performed genetic screens to obtain vulva mutants. We found three mutant categories that concerns vulva formation. The largest one affects division number of the vulva precursors P(4-8).p without changing their fate. The second category concerns mutants in which the competence of the Pn.p cells is affected. Finally, we are studying mutants in which the pattern of induction of vulval fates is abnormal. We found five mutants (three genes) that are hyperinduced for the first induction: P4.p or P8.p adopt a 2° fate instead of a non induced fate 3°. We also found a hypoinduced mutant in which the 1° fate is never observed and the cells adopt a 2° or a 3° fate suggesting that both inductions are affected in this mutant. We are now studying the epistatic relationships between induction mutants. We are also studying relationships between induction and competence (surprisingly the hyperinduced mutants are epistatic to a competence mutant mf 53) and the relationships

between induction and division. We also work on reverse genetic approaches in Oscheius. As the standard C. elegans protocols for injection and for RNAi do not work in Oscheius, we are trying to create transgenic lines by using the Mos 1 transposon. This transposon has been shown to efficiently integrate in the C. elegans genome. We also consider using microparticle bombardment to create transgenic lines. We are finally using an inhibitor of Mek kinase (U0126): in C. elegans, this inhibitor blocks totally or partially the induction. In two species of the Oscheius genus, both inductions are affected suggesting that the Mek kinase (a downstream effector of the RAS pathway) could play a role in the two steps of vulval induction. We envisage using the inhibitor in different mutant contexts and in different nematode species to study the conservation of the Ras pathway requirement in vulval induction during evolution.

1034. *Pristionchus pacificus* mutants defective in vulva induction

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We are studying the evolution of cell fate specification, using vulva development as a model system. Comparative analysis between *C. elegans* and *Pristionchus pacificus* revealed that the specification of the vulva is highly diverse at the cellular level between these two nematodes. Vulva formation in *P. pacificus*, for instance, requires an induction by multiple gonadal cells, whereas in *C. elegans* the single anchor cell is sufficient for inducing the vulva. To study the molecular mechanisms underlying vulval pattern formation and evolution, we carried out several TMP/UV and EMS genetic screens for mutants defective in vulva formation. We isolated and characterized a new class of induction vulvaless mutants, in which P6.p differentiates as a normal 1° cell, but P5.p and P7.p adopt a non-vulval fate (3°). In wild type *P. pacificus*, P6.p adopts the 1° fate, whereas P5.p and P7.p generate the 2° lineage as in *C. elegans*. These mutants represent three different complementation groups. Interestingly, *C. elegans* has only one mutant with a similar phenotype, *sem-4*. Linkage analyses using genetic and molecular markers revealed that two of three complementation groups are closely linked and lie on *P. pacificus* chromosome IV. Positional cloning of these mutations is currently in progress.

1035. Cell fate specification during vulva formation in *Pristionchus pacificus*

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We compare vulva development between *Caenorhabditis elegans* and *Pristionchus pacificus* to study the evolution of developmental processes. Cell fate specification during vulva development differs in these two nematodes. In *C.elegans*, P8.p is part of the vulva equivalence group and adopts a 3° fate under wild-type conditions. In contrast, several experiments indicate that P8.p in *P.pacificus* represents a new cell type. First, P8.p is unable to respond to an inductive signal from the gonad and fuses with the epidermis, but can respond to lateral signal from P6.p and form vulval tissue. Second, P8.p provides a lateral inhibitory signal that prevents P5.p and P7.p to adopt a 1° fate. Third, P8.p provides a negative signal that inhibits gonad-independent vulva formation of P(5-7).p. To study P8.p specification we screened for mutants with P8.p specification defects and identified three candidate mutants in which P8.p behaves like a normal VPC. In one of the mutants, named *tu151*, the nucleus of P8.p has an abnormal size. Cell ablation experiments in *tu151* revealed that P8.p is able to respond to the inductive signal from the gonad to form vulval tissue. Also, P8.p in this mutant cannot provide the lateral inhibitory signal so that after P6.p ablation, P5.p and P7.p can adopt the 1° fate. In addtion, P8.p loses the function of providing negative signal in *tu151* mutant animals since gonad-independent vulva formation occurs. Positional cloning of *tu151* is currently in progress.

1036. TWO BIZARRE VULVAE: *MAB-5* in *PRISTIONCHUS PACIFICUS*

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Recently, *Pristionchus pacificus (Ppa)* became a satellite model organism for the study and the comparison of developmental processes that were described for *C. elegans (Cel)*. Here we focus on vulva morphogenesis events in *Ppa-mab-5* and we compare these events to vulva formation in *Ppa-wt*, *Cel-wt* and *Cel-mab-5*. Our interest in *Ppa-mab-5* was risen by two facts: 1) *Cel-mab-5* hermaphrodites do not express altered vulva phenotypes, while *Ppa-mab-5* does. 2) Another Hox gene (*lin-39)* in both species represses different cell processes (fusion in *Cel* versus apoptosis in *Ppa*) to establish the vulva equivalence group (VPCs) early in development: P(3-8).p in *Cel* and P(5-8).p in *Ppa* [1].

P(5-7).p in wt divide yielding 22 cells in *Cel* and 20 cells in *Ppa* that eventually will form the vulva. A stack of ring shaped syncytial cells form the final vulva structure: 7 rings in *Cel* and 8 rings in *Ppa* [2]. To trace the sequence of events during vulva formation we visualized the epithelial cell's adherens junctions by immunofluorescence with MH27 and 3D-confocal reconstructions (See figure).

We have previously described the complete events during *Ppa-wt* vulva formation and compared them to vulva formation in *Cel-wt* [2]. In *Ppa-mab-5*, the posterior pseudovulva is generated as a result of fate switch in P8.p cell: fusion to the hyp7 is transformed to the generation of 6-8 cells. We divided the resulting real/pseudo vulva structures to 5 distinct groups according to their structural fates. We propose that this variability results from the activity of multiple organizers, cells that determine the location of the invaginated vulva. In general the real vulva remained intact in terms of final shape and the order of morphogenetic events. We suggest that in *Ppa* as in *Cel* ring formation is an autonomous event, because even one cell can form a ring. We have also found unfused 1-2-cell rings derived from P11.p in 14% of

mab-5 mutants.

[1] Sommer R.J. et al. (1998) Development125: 181

[2] Kolotuev and Podbilewicz (2000) WBG 16(3): 18

Figure: MH27 staining of adherens junctions of the vulva. *Ppa-mab-5*, young J3 stage.

1037. Regulation and function of *mab-9* and other T-box genes in *C. elegans*.

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The *mab-9* T-box transcriptional activator is a key regulator of cell fate in the *C. elegans* hindgut. Loss of *mab-9* function results in cell fate transformations of the B and F male specific blast cells, which take on the fates of their anterior neighbours, Y and U. Consequently, *mab-9* mutants have severely abnormal male tails. *mab-9* function is also required in the hermaphrodite hindgut, to maintain the structural integrity of the rectum. Hermaphrodite *mab-9* mutants have weak constipation phenotypes consistent with this role. *mab-9* mutants of both sexes are also weakly backwards Unc, suggesting that *mab-9* may also have functions in the nervous system. We have analysed the expression pattern of *mab-9* using GFP reporter constructs and find expression in nuclei of the B and F hindgut cells as well as in posterior hyp 7 nuclei and in the ventral cord. Mis-expression of *mab-9* in the wrong tissues has severe developmental consequences, indicating that control of *mab-9* expression is crucial for proper development. We have begun a screen for mutants which miclocalise *mab-9* with the aim of identifying *mab-9* regulators and will present results we have obtained. In addition, we have started to dissect the *mab-9* regulatory region and have begun to define enhancer elements responsible for different components of the expression pattern. We have preliminary evidence that *mab-9* may be autoregulatory.

We are also interested in whether any of the other *C. elegans* T-box genes have roles in male tail development, or in the nervous system, perhaps acting in concert with *mab-9*. We are therefore screening through these genes by RNAi in *him* mutant strains (to assess any potential male phenotypes) and in a *mab-9* background (to reveal any potential redundant functions) and by examining their expression patterns.

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1038. The Mind of a (Male) Worm

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In 1986, John White and coworkers published their reconstruction of connectivity in the nervous system of the C. elegans hermaphrodite. Since that time, only limited further reconstruction has been carried out. This has included determination of some posterior circuitry governing male mating behavior (Sulston et al., 1980) and outgrowth patterns in the developing embryonic nervous system (Norris et al., 1997 International Meeting). Complete connectivity remains unknown for the adult male and for all larval and embryonic stages. Full knowledge of connectivity is becoming increasingly important as studies of nervous system development and function advance and attempts are made to understand how the nervous system generates and controls complex behaviors. Sexual dimorphism of the nervous system is of particular interest. As part of the efforts in many areas to provide a complete description of the worm, we have undertaken to define additional circuitry within the nervous system.

For nervous system reconstruction, the course of neuron processes and the chemical and electrical contacts that they make are determined by following processes through a series of electron micrographs of serial thin sections of fixed worms. In the original work, although a computerized system was developed for analyzing the electron microscopic images and compiling the data (J. White, PhD Thesis, 1974), it did not prove to be greatly superior to hand reconstructions, which were used for much of the analysis. Times have changed computerwise since the early 1970s, and we plan to return to a computer-aided approach and attempt to accelerate the reconstruction process by computerizing as many steps as possible. Serial thin sectioning and electron microscopy will continue to be carried out as before, and identification of neurite profiles and contacts in the images will also be done by visual inspection, but images will be digital and all steps subsequent to process and contact identification will be entirely in digital format. We hope that use of the computer will speed the reconstruction not only by promoting facile data storage, handling, and retrieval, but also by allowing simultaneous reconstruction and data entry that would make it possible for the computer to perform a kind of grammar checking on new data (e.g. no process can have greater than or less than one cell body) and consistency checking (e.g. with known pathways derived from previous reconstructions and GFP reporters).

We plan to develop hardware and software and test it by attempting initially to reconstruct the preanal ganglion of the adult male. This ganglion contains circuitry serving to program male mating behavior that is as complex as that found in the nerve ring. The male tail contains 48 sensory neurons with axon processes targeted to this ganglion. Liu and Sternberg (1995) defined the relationship of inputs from these sensory neurons to the sequential steps of copulatory behavior. In our reconstruction, we hope to determine the targets of these sensory neurons and trace neuronal pathways to the copulatory muscles. If we are successful in making extensive additional reconstruction feasible with reasonable time and effort, our long term goal will be to trace male-specific interneurons into the nerve ring and determine how they influence central circuitry, as well as to undertake reconstruction of the L1 larva and other important stages.

1039. Cell fate specification during male hook development

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The hook and hook sensillum, which aid in male mating, are derived from three multipotent hook precursor cells (HPCs), P(9-11).p. Male P(9-11).p cells keep unfused at the L1 stage, and later from the mid-L3 stage each cell exhibits one of the three fates with a invariant pattern of 3*-2*-1* in intact wild type animals. To elucidate the cell interactions among 1*, 2*, and 3* fate cells, following up an experiment of Michael Herman, cell ablations were performed within the P(9-11).p group. The 2* fate in an isolated P10.p is not altered by the disruption of 1*-fated P11.p in the early L3 stage after P10.p migrated to a posterior position close to the cloaca. Within the 2* lineage, ablation of the hook socket cell or its parent cell P10.ppa, but not the hook neurons, has a negative effect on hook structure. To examine whether HOM-C gene *mab-5* has more roles in HPC patterning other than inhibiting the P(9-11).p cell fusion during L1 stage, we use a heat shock-inducible transgenic line *muIs9* encoding *hs-mab-5* to manipulate the MAB-5 activity. Overexpression of *mab-5* during mid-L2 to early-L3 stage led to a high percentage of hook abnormalities in adult males, including no hook, misshapen hook, or anterior hook. *hs-mab-5* can block formation of all three hooks in *lin-12(gf)* males. Activated LET-23 pathway by *let-23(gf)* and *let-60(gf)* mutations induces cell division in P9.p. However, additional hook neurons marked by GFP had never been seen. It is not clear if there is any ectopic HPC 2* fate transformation in P9.p or activated LET-23--RAS signaling actually promotes P9.p to adopt a vulval-like fate.

1040. Identification of a Plexin as a Genetic Enhancer of *Semaphorin-2a* in *C. elegans*

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Semaphorins act as cues in axon guidance and are expressed during axon outgrowth in vertebrates and invertebrates. Several members of the semaphorin family have demonstrated the ability to redirect extending growth cones of specific neurons *in vitro* and *in vivo*.

Two semaphorin subfamilies exist in *C. elegans*. The Semaphorin I subfamily consists of membrane-bound members, *semaphorin-Ia* and *-Ib*, whereas the Semaphorin II subfamily consists of a single, diffusible, *semaphorin-2a* member. Mutations in *semaphorin-2a/mab-20* demonstrate defects in ventral enclosure, axon fasciculation and morphogenesis of the body and male tail (Roy *et al*., 2000, *Development* 127:755-67; Baird *et al*., 1991, *Development* 113:515-26). In order to identify components that may signal through the *semaphorin-2a* pathway, a screen for genetic enhancers of male tail sensory-ray fusions was conducted using a putative hypomorphic allele of *mab-20*. Mutations in five loci were isolated and are presently being cloned and characterised. One enhancer that was isolated contained a mutation in the *C. elegans* plexin gene located on the right arm of LGII (gene is present on cosmid K04B12). Plexins are a family of transmembrane proteins possessing an extracellular semaphorin domain and three c-Met proto-oncogene-related sequence (MRS) motifs homologous to those found in the Scatter Factor Receptor family. The intracellular domain of plexin is novel and shows no known homology to any other protein yet identified providing little insight to its catalytic function. It was recently shown in *Drosophila* and vertebrates that plexins function as receptors for semaphorins. Mutant phenotype, genetic and expression analysis suggest that the plexin homolog encoded by K04B12.1 may act as a candidate receptor for *semaphorin-2a* signaling in *C. elegans*.

1041. A mutation affecting phasmid function and tail morphogenesis

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mh21 was isolated in a screen for mutations that cause phasmid dye-filling defects. The phasmids are a pair of bilateral sensory structures found in the *C. elegans* tail. Each phasmid is comprised of two neurons, a sheath cell, and two glial support cells, called socket cells, which serve to provide a pore for phasmid neurons to sense the outside environment. When animals are soaked in fluorescent dyes, such as DiO, the phasmid neurons fill with dye. Phasmid socket cells are derived from the posterior daughter of the first asymmetric division of the T blast cell, T.p. Despite a highly penetrant phasmid dye-filling defect, amphid dye-filling is normal in *mh21* animals and most have phasmid socket cells. These observations suggest that either the phasmid sockets or phasmid neurons are rendered non-functional in *mh21*animals. Expression of the *srb-6::gfp* phasmid neuron reporter was normal in *mh21* animals, which indicates that *mh21* phasmid socket cells are defective. The *mh21* mutation is pleiotropic. *mh21* animals display reduced fecundity, uncoordinated movement, constipation and an egg-laying defect. *mh21* males have abnormal tail morphologies; sensory rays 3 and 4 are often fused and spicules are short or crumpled and fail to protract. The multiple defects caused by this mutation suggest that the gene identified by *mh21* has several morphogenetic roles. *mh21* has been physically mapped to a small region on LG IV by mapping breakpoints of non-complementing deficiencies relative to nearby polymorphisms. Transformation rescue has been achieved with a YAC clone found within the *mh21* interval. Rescue experiments aimed at cloning *mh21* are underway and their progress will be reported.

1042. Male Mating Behavior and the Role of UNC-55, A Nuclear Receptor

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unc-55 encodes a transcription factor that is a member of the nuclear receptor gene family. unc-55 mutant males are unable to mate successfully. The relatively mild defect in coordination is unlikely to be responsible for the defect in male-mating behavior. Using an unc-55::gfp transgenic strain, gfp expression was observed in one neuron, a muscle and the four spicule sheath cells in the male tail. The spicule sheath cells, which normally have a dumb-bell shape, are stretched when the spicules are extended. The pattern of expression in unc-55 mutant males was indistinguishable from wild-type males. Observation of unc-55 mutant male behavior revealed that males exhibit normal chemotaxis in locating hermaphrodites, move backward along the body of hermaphrodites and make appropriate turns but mutants do not copulate. Further observation of unc-55 males revealed that often the paired spicules are extended in the absence of hermaphrodites, this is rarely observed in wild-type males. One approach that we are using to investigate the relationship between the cells expressing unc-55::gfp and the control of spicules during copulation is to identify target genes regulated by UNC-55, a putative transcription factor. We compared the mRNA populations of two him-5 strains, one homozygous for unc-55(+) and the second homozygous for unc-55 (e1170) using Representational Differentiation Analysis. We have sequenced 48 different clones representing 36 different cDNAs. The transcription of each of the cDNAs appeared to be activated by UNC-55. Attempts to identify cDNAs suppressed by UNC-55 did not yield any products. The cDNAs fell into five categories: muscle specific (4), cytoskeletal (5), protein synthesis (9) signal transduction (5) and novel (4). The remaining represented flanking fragments of products that had already been identified. Reverse northern blots will be used to confirm the differential expression of the mRNAs in the two populations. We are encouraged by the correlation between the cell types, the potential function in copulation and the categories of genes that are potentionally

regulated either directly or indirectly by UNC-55 in those cell types.

1043. Positive and Negative Regulation of her-1 Expression.

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The *her-1* gene is required for male somatic development in *C.elegans*. It contains two tandem promoters named P1 and P2 which drive the expression of four exons to form two male-specific transcripts. A rare 1.2 kb transcript containing all four exons is generated from the P1 promoter while a relatively abundant 0.8 kb transcript containing exons three and four is generated from the P2 promoter. Three loss-of-function *(lf)* mutations in the *her-1* promoter have been isolated; they occur within nine bp, 45 bp upstream of the transcriptional start site and cause variable feminization of XO animals. This cluster of *lf* mutations defines a site required for *her-1* expression in XO animals. Two gain-of-function *(gf)* mutations, occurring two bp upstream of the transcriptional start site, causing variable masculinization in XX animals have also been identified. This represents a negative regulatory site of *her-1*. To identify *trans*-acting factors that regulate *her-1*, we used two short oligonucleotides from the *lf* and *gf* sites in the P1 promoter to screen a *C.elegans* cDNA yeast expression library. The oligonucleotide from the P1 *lf* site selected a putative general transcription factor containing three zinc fingers. The *gf* site binds specifically to the worm homologue of p66, a subunit of Mi-2, suggesting a chromatin remodeling complex is involved in the negative regulation of *her-1*.

Gel mobility shift assays to identify either single proteins or multi-protein complexes binding to either the *gf* or *lf* sites in P1 using nuclear embryonic extracts have been carried out. DNaseI footprinting experiments are being done to identify other *cis*-acting elements in the *her-1* promoter and to precisely localize the binding sites previously identified by gel shift analysis.

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1044. FROM BIRTH TO ADULTHOOD: CELL FUSION IS NEEDED FOR PROPER MORPHOGENESIS

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Although cell fusion plays an important role in the development of organisms, no fusogens directly involved in cell-cell fusion have been found. In order to find new genes that participate in this complex process, we have concentrated on epithelial cell fusion in *C. elegans*, which involves almost one third of all the somatic cells in this organism [1]. Since vulva formation in *C. elegans* involves twenty cell fusion events, we have started with primary screening for vulval defects. Pvl (protruded vulva) and Muv (multivulva) mutants were subjected to a secondary fluorescence-based screening leading to the isolation of *eff-1(hy21)* [**e**pithelial **f**usion **f**ailed].

eff-1(hy21) was discovered to be defective not only in vulval fusion, but also in general epithelial cell fusion, as all the epithelial cells in this mutant remained unfused [3]. This cell fusion failure led to gross morphological defects. Organogenesis of the vulva and the male tail was abnormal, resulting in low fertility (Egl phenotype). Defective hypodermal cell fusion also led to severe elongation problems during embryogenesis and larval development (expressed as the Dpy phenotype). The mutant animals exhibited irregular body form with lumpiness during larval stages and bulged tail throughout development.

The postembryonic defects in *eff-1(hy21)* were more severe at 25°C than at 15°C. We have found that the temperature sensitive periods (TSP) of the elongation and fertility defects were almost identical to the TSP of the fusion defects (at 12h-33h post fertilization), thus correlating the resulting phenotypes directly to lack of cell fusion.

Our results, along with preliminary data on the gene structure [2] and its regulation [3] lead us to propose that *eff-1* is the first potential fusion effector, directly involved in the actual process of cell fusion.

1. Shemer and Podbilewicz (2000) Dev. Dyn.

2. Mohler et al. (2001) 13th International *C. elegans* meeting

3. Shemer and Podbilewicz (2001) 13th International *C. elegans* meeting

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1045. GENETIC INTERACTIONS BETWEEN TWO CELL FUSION MUTANTS

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Hypodermal cells undergo programmed cell fusion to generate multi-nucleated cells during embryonic development in *C.elegans*. Analysis of mutants that have epithelial cells that fail to fuse provides an opportunity to clone the genes involved. *duf-1*(*zu316cs*) (Dorsal UnFused) is a zygotic lethal mutant that arrests at 1.5 to 2-fold stage of elongation with fewer dorsal hypodermal cell fusions (1). Strains expressing *jam-1*::GFP fusion protein that is localized to the adherens junctions of epithelial cells were used for fusion analysis on living animals that were also analyzed by Nomarski optics. *eff-1 (hy21)* is a recessive viable mutant with lumpy, Dpy, Pvl, and Egl phenotype that is defective in all the epithelial cell fusion processes (e.g. vulva, embryonic hypodermis, Pn.p cells, seam cells.) (2)

To investigate the possible genetic interactions between *eff-1* and *duf-1* we constructed and analyzed an *eff-1*(*hy21ts*) homozygous *duf-1*(*zu316cs*) heterozygous strain. We found that *duf-1*/+; *eff-1* segregates 25% *duf-1;eff-1* embryos that arrest with all hypodermal cells unfused and 75% *eff-1* viable unfused animals. Since the *duf-1*; *eff-1* arrested embryos had a 100% unfused hypodermis (both dorsal and ventral) and *eff-1*; *duf-1*/+ were viable unfused identical to *eff-1*, thus, *eff-1* is epistatic to *duf-1* concerning hypodermal dorsal fusion while *duf-1* is also independently required for embryonic viability.

In summary, *duf-1* embryos arrested with partially unfused dorsal hypodermis while the *duf-1; eff-1* embryos arrested with completely unfused hypodermis. Both *eff-1; duf-1*/+ and *eff-1* worms were viable with the Eff (Epithelial Fusions Failed) phenotype. We propose that *duf-1* controls dorsal epidermal fusions by regulating the activity of *eff-1* that may be a general Effector of Fusion active in all epithelial cells.

1. Gattegno T. and Podbilewicz B. IWM (1999) Abstract, p. 328.

2. Shemer et al. Mohler et al. (2001) 13th International *C. elegans* Meeting.

***duf-1* ------> *eff-1* ------> dorsal hypodermal fusion**

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1046. The *lin-26* gene can trigger epithelial differentiation without conferring tissue specificity

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How epithelial cell fates become specified is poorly understood. We have previously shown that the putative zinc-finger transcription factor LIN-26 is required for the differentiation of ectodermal and mesodermal epithelial cells in *Caenorhabditis elegans*. To further investigate the role of *lin-26*, we analysed the consequences of ectopic *lin-26* expression under a heat-shock promoter.

Ectopic LIN-26 expression at a specific time-window, during early gastrulation, transforms most blastomeres into epithelial-like cells. Specifically, overexpression of LIN-26 induced an overexpression of the three following epithelial markers: i) the apical junction protein JAM-1 which is recognised by the MH27 antibody, ii) DLG-1, a protein of the MAGUK family, which is essential for the assembly of JAM-1 at junctions, and iii) CHE-14, a transmembrane protein involved in apical vesicle trafficking. Furthermore, ultrastructural studies revealed that ectopic LIN-26 expression induced the formation of apical-like junctions both in external and internal cells. However, ectopic *lin-26* expression did not confer any tissue-specific cell fate, such as the hypodermal cell fate, as evidenced from the observation that several hypodermal-specific genes were not induced.

Conversely, we have shown that in *lin-26* mutants, JAM-1 and CHE-14 patterns display some polarity defects, and that about half of the apical junctions were abnormal in the hypodermis, consistent with a role of *lin-26* in epithelial differentiation. The persistent expression of *jam-1* and *che-14* in *lin-26* null mutants suggests that these two genes are controlled by parallel pathways including *lin-26* and presumably by genes that has been shown to control ectodermal differentiation, such as GATA factors *elt-1* and *elt-3*.

Finally, we conclude that *lin-26* can induce epithelial differentiation without conferring any tissue specificity and that epitheliogenesis is not a default pathway in *C. elegans*.

1047. Prolyl 4-hydroxylase - essential function in cuticle collagen modification and development

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Prolyl 4-hydroxylase is an enzyme complex of the ER that modifies proline residues in the Gly-X-Y repeats of procollagen molecules. Nematode cuticles are predominantly composed of collagen and are required for maintenance of nematode body shape. Mutation of specific cuticular collagens of C. elegans result in alterations in body shape.

We have studied the genes encoding subunits of the pro-collagen modifying prolyl 4-hydroxylase (P 4-H) complex from C. elegans. The prolyl 4-hydroxylase alpha-subunit encoding genes, phy-1 and phy-2, and the beta-subunit gene, protein disulphide isomerase 2 (pdi-2), were examined. RNA-mediated interference (RNAi), transgenic promoter/reporter gene studies and semi-quantitative RT-PCR were used to determine the function and expression of these genes. Additionally the strain dpy-18 was identified as a phy-1 mutant by transgenic repair of phenotype and from cloning of phy-1 alleles from three dpy-18 strains. Results showed that phy-1, phy-2 and pdi-2 are expressed in the nematodes collagen synthesising tissue at times consistent with the production of collagen and function as part of a complex which is essential for development and maintenance of body shape in C. elegans. We are currently characterising further the central role of prolyl 4-hydroxylase complexes in ECM formation in free-living and parasitic nematode species.

1048. Hemicentin Polymer Assembly in the Extracellular Matrix of *C. elegans*

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The product of the *him-4* gene is hemicentin, a large (5198 a.a.) extracellular protein, whose main structural features are a stretch of 48 tandem Ig and 3 EGF modules flanked by unique ends that are highly conserved in 2 vertebrate orthologs. Hemicentin is secreted by body wall muscle cells and proximal gonadal leader cells and assembles into long, line shaped polymers at distant cell junctions. Sites of assembly include junctions where cells (e.g. uterine utse cell and mechanosensory neurons ALM and PLM) make intimate attachments to the cuticle that are mediated by epidermal hemidesmosomes and sites where cells make dynamic extracellular attachments (e.g. male linker cell, syncytial germ cells). The ability of hemicentin to assemble at secondary sites suggests that these cell junctions have a mechanism to recruit hemicentin that is likely to involve a cell surface receptor. To identify the putative hemicentin receptor, a screen is being conducted for mutants with defects in recruitment of GFP-hemicentin to mechanosensory neuron and germ cell surfaces. So far, we have identified novel alleles of *him-4, mec-1* and several genes yet to be identified. In addition, we are attempting to define the function of different structural modules within hemicentin. Deletion constructs missing the C-terminal domain are still recruited to the correct cell surfaces, but have a punctate distribution. This suggests that the highly conserved N-terminal domain may have cell-binding activity, and the C-terminal domain may be involved in hemicentin polymer assembly.

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1049. *gly-3* through *gly-11* are glycosyltransferases critical for embryo morphogenesis

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Glycoproteins and proteoglycans may be distinguished by the type of sugars that are O-linked to specific amino acid side chains. Mutations in some glycosyltransferases revealed that the sugar chains are essential for modulation of notch signaling and embryo polarity in the fly. Because the functional significance of most cell surface sugars are not know, we have begun to systematically characterize glycosyltransferase gene families in *C. elegans*. The ppGaNTase gene family encodes enzymes responsible for the initiation of mucin type O-linked glycosylation. Previously, our lab has shown that 11 unique sequence homologs exist in *C. elegans: gly-3, gly-4, gly5a, gly-5b, gly-5c, gly-6a, gly-6b, gly-6c, gly-7, gly-8,* and *gly-9.*[1] Completion of the *C. elegans* genome sequence yielded two more putative members of the family, *gly-10* and *gly-11*. *Gly-10* and *gly-11* cDNAs were obtained by screening a lambda phage mixed-stage N2 cDNA library. Sequencing the cDNAs confirmed the presence of a highly conserved catalytic domain that is essential for glycosylation activity. Recombinant GLY11 protein showed catalytic activity in vitro using mammalian apo-mucin peptides as substrates, while GLY10 showed no significant activity above background. Confident that the family is known in its entirety we used *gly*-promoter::(lacZ or GFP) constructs for each family member to produce transgenic animals. Expression patterns showed that family members had unique expression patterns both temporally and spatially throughout *C. elegans* development and into adulthood. There was overlapping expression and biochemical activity for some members of the family. Redundancy between some of the family members was suggested by RNA interference (RNAi) studies, in that knockdown of mRNA expression did not reveal any significant loss-of-function phenotype for most of the isoforms. *Gly*-4 RNAi, however, resulted in 30% embryonic lethality. We also used promoter driven antisense constructs to knock down gene expression of multiple glycosyltransferases in a tissue-specific manner. Co-injection of multiple *let-653* promoter::*gly*-antisense cDNA constructs revealed an embryonic lethal phenotype. In addition, we also observed arrested 2-fold larva at hatching and a potential adhesion defect in the anterior hypodermal cells. These data along with unique expression pattern support the theory that the glycosylation machinery is differentially regulated on a cellular basis during development and is critical for cell-cell interactions. To validate these studies, we are developing inhibitory single chain antibody reagents against GLY11 and other glycosyltransferases and glycoproteins.

[1] Hagen and Nehrke (1998) cDNA cloning and expression of a family of UDP-N-acetyl-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase sequence homologs from *Caenorhabditis elegans*. J Biol Chem. 273:8268-77

1050. Chemical and Genetic Inhibition of Sulfation in C. elegans

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In order to examine the process of sulfation in C. elegans, sulfation was inhibited chemically using sodium chlorate, and genetically using the process of RNA-mediated interference (RNAi). Sodium chlorate inhibition during early larval stages resulted in a dose-dependant developmental delay. BLAST searches of characterized sulfotransferases against the worm genome resulted in the identification of 4 putative sulfotransferases: C34F6.4 and F08B4.6 (previously identified: [1] and [2]), F40H3.5, and Y34B4A.e. RNAi of the putative N-deacetylase/N-sulfotransferase F08B4.6 resulted in "stacking" of eggs in the gonad, along with eggs laid at the 2- and 4-celled stage. RNAi of the putative hexuronic 2-O sulfotransferase C34F6.4 resulted in a shortened, bulbous gonad. These initial results indicate that sulfation may be important during development of C. elegans.

[1] Shworak, NW, Liu, J, Fritze, LMS, Schwartz, JJ, Zhang, L, Logeart, D, Rosenberg, RD. JBC 272: 28008-19 (1997).

[2] Kobayashi, M, Sugumaran, G, Liu, J, Shworak, NW, Silbert, JE, Rosenberg, RD. JBC 274: 10474-80 (1999).

1051. Molecular characterization of heparan sulfate/heparin N-deacetylase/N-sulfotransferase in worms

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Heparan sulfate binds and activates a large variety of growth factors, enzymes and extracellular matrix proteins. These interactions largely depend on the specific arrangement of sulfated moieties and uronic acid epimers within the chains. These oligosaccharide sequences are generated in a step-wise manner, initiated by the formation of a linkage tetrasaccharide which is then extended by copolymerization of alternating a1,4GlcNAc and b1,4GlcA residues. As the chains polymerize, they undergo a series of sulfation and epimerization reactions. The first set of modifications involves the removal of acetyl units from subsets of GlcNAc residues, and the addition of sulfate groups to the resulting free amino groups. These reactions are catalyzed by a family of enzymes designated as GlcNAc N-deacetylase/N-sulfotransferases (NDST), since they simultaneously. Four members of the family have been identified in vertebrates, with single orthologs present in Drosophila and C. elegans. We have revealed tissue-specific expression pattern and unique enzymatic properties of these four isozymes1,2). In fly, loss of NDST (sulfateless) results in unsulfated chains and defective signaling by multiple growth factors and morphogens. I reconstituted cDNA for worm NDST from EST clones and 5’ RACE products. Enzymatic activities will be discussed.

1) Aikawa, J. & Esko, J. D J. Biol. Chem. 274, 2690-2695 (1999) 2) Aikawa, J., Grobe, K., Tsujimoto, M. & Esko, J. D J. Biol. Chem. 276, 5876-5882 (2001)

1052. Molecular cloning of the *sma-5* gene in *C. elegans*

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What makes an animal to have the size as we observe? In order to study the mechanism of body size determination, we have been trying to clone the gene responsible for *sma-5(n678)* mutation. The *sma-5* mutant is small, and grows very slowly. We also found that its intestinal granules were distributed irregularly. *sma-5(n678)* had been mapped on linkage group X and on the right of *mes-1*(6.35) and near or to the left of *nuc-1*(7.01) (ACeDB). We found that DNA clones YAC Y75H1 and cosmid R03B7 rescued those three abnormal phenotypes of the mutant. R03B7 contains four predicted genes (ORFs). PCR fragments of one of them, W06B3.2, also rescued. RNA interference with W06B3.2 dsRNA causes phenotypes similar to those of *sma-5*. On the basis of these results, we think W06B3.2 is the most probable candidate of the *sma-5* gene. W06B3.2 encodes a predicted Ser/Thr kinase, and we found two types of mRNAs. We are looking for a mutation site in this gene of the *sma-5* mutant.

1053. Cloning and characterization of a cyclic GMP-dependent protein kinase controlling body size in *C. elegans*

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The body size determination is one of the most fundamental aspects of development but its mechanism remains to be largely unknown. A mutant *gig-1* (*ks16*) with a body volume of up to twice in adults was isolated previously in our laboratory by Ikue Mori. To elucidate the mechanism of body size determination, we have analyzed this mutant. The *gig-1* is grossly normal in morphology and growths except for weak egg-laying defects, dark intestine and low male mating efficiency. We have mapped *ks16* mutation to a region that is about 50 kbp on the left arm of chromosome IV using single nucleotide polymorphorisms between N2 and CB4856. This region contains a single gene, F55A8.2 encoding a cGMP-dependent kinase (PKG) of 780 amino acids. We were able to rescue the phenotypes with a YAC encoding the PKG and identified a nonsense mutation in this mutant. *ks16* map position was near that of *egl-4*. Both *egl-4* and *ks16* show similar phenotypes, such as egg-laying defects and large body volume. We were not able to complement *egl-4* (*n477*) with *ks16*, and found missense (*n478* and *n612*) and frameshift (*n477*) mutations in the PKG gene of *egl-4* mutants. These results suggest that *gig-1* gene encodes PKG and is the same gene as *egl-4*. The PKG gene have two different start codons; the upstream one for PKGa and the downstream one for PKGb. PKGa is predicted to contain a glycine rich region, a cyclic nucleotide binding domain, and a kinase domain that is highly conserved in *Drosophila* and human with about 55 % amino acid identity. GFP fused with PKGa promoter was predominantly expressed in many head neurons and hypodermis except for seam cells, while GFP fused with PKGb promoter was expressed in body wall muscles. To investigate PKG function in detail, we produced an anti-PKG antibody that specifically recognizes an 80 KDa protein in a *C. elegans*

lysate. Recently, it was suggested that TGF-b pathway is involved in body size determination, and it was reported that some phenotypes of *egl-4* are suppressed by *daf-3* which is a component of TGF-b pathway (Daniels *et. al*., *Genetics* 156, 123-141, 2000). It is also shown that insulin-like growth factor (IGF) transduces signals that positively regulate growth in *Drosophila* and mammals. In body size determination, cGMP signaling might crosstalk with TGF-b and IGF pathways. We are trying to analyze the functions of PKG to address the mechanisms of body size determination.

1054. *lon-3* encodes a regulator of body length in *C. elegans*

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Body length in *C. elegans* is regulated by a conserved signalling pathway activated by a protein in the TGF-beta superfamily, DBL-1. Loss-of-function mutations in genes encoding components of the pathway cause a marked decrease in body size, the Sma phenotype. Conversely, constitutive activation of the pathway results in a dramatic increase in body length. The molecular mechanisms by which the TGF-beta pathway affects body length are not yet known however. One of us (A.L.) has previously reported that mutations in *sma-2* and *daf-4* reduce the extent of endoreduplication of hypodermal nuclei. We found that hypodermal ploidy was also reduced in *dbl-1(0)* mutant hermaphrodites. Ploidy was not increased, however, in worms that overexpressed *dbl-1* indicating that while it is a regulator of body length, *dbl-1* is permissive but not instructive for hypodermal ploidy. A mutation in *lon-3*, *e2175*, gives rise to a Lon phenotype that is strikingly similar to that caused by overexpression of *dbl-1*. In a screen of 50,000 haploid genomes for new Lon mutants, we isolated three new *lon-3* alleles, *sp5*, *sp6* and *sp23*. The strongest alleles cause worms to be more than 20% longer than wild type. All alleles are recessive to wild type and behave genetically as though they reduce or eliminate gene activity. We cloned *lon-3* by transformation rescue and showed that it is predicted to encode a collagen with a structure typical of that of cuticle collagens. Consistent with the genetic analysis, characterization of the sequence changes associated with *lon-3* mutant alleles suggests that loss-of-function phenotype is Lon. *sp6* and *sp23* are associated with sequence changes that introduce stop codons very early in the open reading frame. Analogous mutations in other collagen genes, such as *sqt-1*

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and *rol-6*, behave as null alleles. Interestingly we found that while the loss-of-function phenotype is Lon, higher than wild-type levels of *lon-3* cause a Dpy phenotype. Thus *lon-3* can function as a regulator of body length. The hypodermal ploidy in both *lon-3(0)* mutants and worms overexpressing *lon-3* was wild type indicating that *lon-3*, like *dbl-1*, can regulate length independently of hypodermal endoreduplication. Defects in *lon-3* expression have no effects on dorso-ventral patterning of the male tail or on dauer development, processes that are controlled by the TGF-beta ligands DBL-1 and DAF-7 respectively. In addition, a *lon-3-lacZ* fusion gene is expressed in many hypodermal cells; cells that are known to secrete the cuticle. Thus it seems likely that *lon-3* regulates worm length by altering the shape or elasticity of the cuticle rather than by altering the activity of genes in the TGF-beta pathway. Previously it has been shown that mutations in *sqt-1*, which also encodes a cuticle collagen, can also affect body length. However *sqt-1* does not seem to be a prime regulator of body length since mutations in *sqt-1* giving rise to longer or shorter worms appear to be neomorphic, and the *sqt-1* null phenotype is wild type. LON-3 may nevertheless function together with SQT-1 to regulate body length: the *lon-3* overexpression phenotype is completely suppressed by *sqt-1(0)*, and *sqt-1(0)* strongly suppresses the Lon phenotype of *lon-3(0)*. Since *sqt-1* null mutations do not suppress the Dpy phenotype caused by mutations in any of the known collagen genes we have tested, overexpression of *lon-3* cannot cause a Dpy phenotype simply by interfering indiscriminately with the activity of the proteins encoded by these genes. The relationship between *lon-3* and the TGF-beta pathway regulating body length is presently unclear. The expression of a *lon-3-lacZ* reporter is not affected by mutations in genes in the TGF-beta pathway. Experiments with a LON-3 antiserum have revealed that LON-3 protein levels are altered in worms lacking *dbl-1* or that overexpress the protein. However, LON-3 protein levels do not change in a simple reciprocal fashion in response to changes in *dbl-1* activity. Thus *lon-3* could either function as one (of two or more) targets downstream of the TGF-beta pathway to regulate body length or in a parallel pathway. To learn more about how body length and body size are regulated in *C. elegans* we are presently characterizing four new Lon genes that we identified in our screen. *lon-4* maps between *egl-29* and *lin-29* on chromosome II, whereas *lon-5*, *lon-6* and *lon-7* all map to the X chromosome.

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1055. Mutants with larger body size and elongated life-span in *C. elegans*

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In order to analyze the mechanisms for body size determination, at least ten larger body size mutants were isolated after EMS mutagenesis. Four of them had up to twice as much volume as that of the wild type in adults.These four mutants have similar phenotypes which are larger body size, weak egg laying defect, dark intestine, reduced brood size, abnormal male tail structure, and elongated life span. Complementation tests showed that the four mutations (*ks16, ks 60, ks61* and *ks62*) are allelic with one another, and the corresponding gene encodes a cyclic GMP-dependent protein kinase (see abstract by Takashi Hirose and Yasumi Ohshima).

To reveal biological roles of this gene in the body size determination and life span, we have analysed mutant phenotypes mainly on body size and life span. As a basis to study the body size, we developed a system to measure body length and diameters, and to estimate volume, of a worm by an automatic image analysis. Using this, we obtained growth curves of the four mutants. They showed that large mutants continue to grow in adults to be approximately twice as big four days after becoming adults. Measurements of the life span of four large body size mutants and wild type N2 showed that, all the four large size mutants have elongated lifespan. Especially, in a condition without FUDR, N2 had 14.28 ± 0.56 days mean life span and 29 days maximam life span (n=71). On the other hand, *ks61* mutant had 20.4 ± 0.97 days mean life span and 43 days maximam life span (n=75). However, in a condition with FUDR which inhibits DNA synthesis, mean lifespan and maximam lifespan of this mutant were shorter than those without FUDR. Also, body length and volume were smaller than the animals grown without FUDR. These results suggest that long life span and large body size of the mutants requrire DNA synthesis. How much body size do the aging mutants have? We measured the body size of *daf-16* mutant and *daf-2* mutants. *daf-16* lives shorter than wild-type and *daf-2* lives longer. Although both of them had the same body length as wild-type N2, *daf-2* was thinner and less in volume than wild type, and *daf-16* was wider and more in body volume. This relation between body size and life span is oposite to the case of our large mutants. Furthermore, a double mutant of *daf-16* and *ks61* was much larger than each of the single mutants. These results suggest that body size penotypes of large mutants are independent of *daf-16* dependent size determination. Also, role of life span regulation of this gene may be independent of IGF signal dependent life span regulation.

1056. Nuclear Powered Growth in Nematodes.

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Most of the hypodermis of a Rhabditid nematode such as Caenorhabditis elegans is a single syncitium. The size of this syncitium (as measured by body size) has evolved repeatedly in the Rhabditid nematodes. Two cellular mechanisms are important in the evolution of body size: changes in the numbers of cells that fuse with the syncitium, and syncitial growth that is independent of cell division. Thus nematodes differ from mammals and other invertebrates in which body size evolution is caused by changes in cell number alone. The evolution of syncitial growth without cell division in nematodes is associated with changes in the ploidy of hypodermal nuclei. These nuclei are polyploid as a consequence of iterative rounds of endoreduplication, and the extent of polyploidy has evolved repeatedly. The association between syncitial growth and endoreduplication is not only seen among species, but also within species with the smaller males of sexual taxa being hypo-endoreduplicated relative to the female or hermaphrodite. Furthermore, this association is also seen in C. elegans with mutations which interrupt TGF-beta signalling and which result in dwarfism and deficiencies in hypodermal ploidy. The TGF-beta pathway is a candidate for being involved in nematode body size evolution.

1057. *enc-1* functions redundantly with the *C. elegans* protocadherin *cdh-3* and is required for embryonic elongation.

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The protocadherin CDH-3 has been shown to be widely expressed during *C. elegans* development. A deletion allele, *cdh-3(pk87)*, results in morphogenic defects in a tail epithelial cell (1) as well as disrupting formation of the excretory system (2). The defects caused in the excretory system results in the animals dying with a Clr (Clear) phenotype. We have previously reported the identification of a mutation *enc-1(fe2)* that interacts redundantly with *cdh-3(pk87)* and in combination with this allele causes a 25-fold increase in the Clr phenotype at 20ºC (3).

At 25ºC *enc-1(fe2)* confers a temperature-sensitive Mel phenotype that is independent of the *cdh-3(pk87)* allele. *enc-1(fe2)* animals raised at the non-permissive temperature appear wild-type until the onset of elongation. However, during elongation they arrest at the 1.5-fold stage with protrusions on their dorsal surface. We have determined that the ventral hypodermal cells change shape and elongate in a wild-type manner, however, the dorsal hypodermal cells do not appreciably change shape leading to the observed dorsal protrusions. Both dorsal and ventral hypodermal cells in *enc-1(fe2)* homozygotes fuse as normal, suggesting that *enc-1* has a specific role in hypodermal elongation. The phenotype observed in *enc-1(fe2)* homozygotes raised at the non-permissive temperature resembles the Hmp phenotype caused by mutations in the genes encoding the *C. elegans* α- and β-catenins (4).

We have mapped *enc-1* to LG III, where it lies to the left of *daf-2*, and we are currently carrying out RNAi with candidate genes from this region to phenocopy and clone *enc-1*. We hope to present data on the molecular characterisation of the gene encoded by *enc-1*.

To further understand which genes are involved in hypodermal elongation we have carried out a further genetic screen. We have used a modified version of the Kemphues and Priess screen, by incorporating the *jam-1::GFP* insertion allele into an *egl-23* background, to identify maternal-effect genes involved in elongation. Incorporation of *jam-1::*GFP allows direct scoring of the adherens junctions in potential candidate mutants, allowing us to distinguish between enclosure or elongation mutants. Three mutants have been recovered from this screen and each mutant has been characterised at the phenotypic level, all appear to arrest due to elongation defects. We have now begun to map these mutants to their respective linkage groups, one of which, *fe7*, which arrests at the 2-fold stage, maps to the mid-region of LG II. We hope to present further molecular characterisation of this allele.

1) Pettitt, Wood and Plasterk (1996). Development 122, 4149-4157.

2) Hodgson and Pettitt 1999 International *C. elegans* Meeting, 402.

3) Hodgson and Pettitt 2000 European *C. elegans* Meeting, 55.

4) Costa *et al*., (1998) J. Cell Biol. 141, 297-308.

1058. Regulation of body size in worms

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Many animals such as Drosophila can regulate their body size independently of cell number, and often do in response to environmental signals. What about worms? In C. elegans, adult growth is associated with endoreduplication of nuclei in the hypodermal syncitium and both growth and endoreduplication are controlled by a TGF-beta signalling pathway. Why have worms adopted this peculiar mode of growth? Why not just add cells? We think that somatic polyploidization may be a very flexible way of altering the body size of a worm to changing environmental or developmental circumstances. We have found at several such circumstances. When L3's are raised on low food rations, they grow up very small and have low ploidies. We have also found that Tetraploid worms have nearly wild type hypodermal ploidies (half of what they should have). We think that this regulation explains why Tetraploid worms are not twice as big as wildtype. Finally, we are testing the effect of ablating hypodermal nuclei on body size. Again, we have found signs that worms know how big they should be regardless of how many cells they have. We are now testing the idea that the TGF-beta pathway has a role in each of these kinds of body size regulation.

1059. A *C. elegans* TGF-beta DBL-1, Controls the Expression of an Evolutionarily Conserved Protein, LON-1, that Regulates Polyploidization and Body length

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Using cDNA-based array analysis combined with double stranded RNA interference (dsRNAi), we have identified yk298h6 as a target gene of *C. elegans* TGF-beta signaling. Worms overexpressing *dbl-1* , a TGF-beta ligand, are 16% longer than wildtype. Array analysis shows yk298h6 to be one of several genes suppressed in such worms. Disruption of yk298h6 function by dsRNAi also resulted in long worms, suggesting that it is a negative regulator of body length. yk298h6 was then mapped to, and shown to be identical with, *lon-1* , a known gene that affects body length. LON-1 encodes a 312 amino acid protein with a motif sequence that is conserved from plants to humans. Expression studies confirm that LON-1 is repressed by DBL-1 suggesting that LON-1 is a novel downstream component of the *C. elegans* TGF-beta growth regulation pathway. Consistent with this, LON-1 is expressed mainly in the larval and adult hypodermis and has dose-dependent effects on body length associated with changes in hypodermal ploidy, but not hypodermal cell proliferation.

1060. Identification of UNC-112 interacting molecules.

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Myofilaments within *C. elegans* body wall muscle cells are organized and anchored by dense bodies and M-line structures. These structures are both analogous and homologous to vertebrate integrin mediated adhesion plaques. Recently we described the gene *unc-112* which encodes a member of the FERM family as a novel player in dense body organization and placement (JCB 150:253). Uncharacterized mammalian homologs of this gene have also been identified.

To help clarify the molecular functions of UNC-112, we performed two hybrid screens for interacting molecules with UNC-112. We used two forms of UNC-112 as bait. One consists of the C-terminal half of UNC-112 and contains a PH domain and half of the FERM domain. The second bait consists of a full length UNC-112 molecule. From screening with the C-terminal half bait, we identified the N-terminal half of UNC-112, suggesting that intramolecular interactions occur between the N- and C-terminus. This type of interaction is also found in mammalian ERM proteins. Using the full-length version of UNC-112 as bait, we identified eight interacting proteins. One interacting protein contains a putative Rho GEF domain, an activator of Rho family small GTPases. We found that the Rho GEF domain is required for interaction with UNC-112. Further two hybrid analysis revealed an interaction between this Rho GEF and *C. elegans* Rho GTPases (CED-10 and CeCdc-42), suggesting that it functions as an activator of these Rho GTPases. In mammalian cells, Rho family small GTPases are involved in the assembly of integrin based adhesion plaques. We propose that UNC-112 functions as a linker between integrin and the Rho family of small GTPases at adhesion plaques.

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1061. *ceh-13* shares overlapping functions with *lin-39*, *mab-5* and *egl-5* during development

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The genome of *C. elegans* contains a total of six Hox genes. Four of them, *ceh-13*, *lin-39*, *mab-5* and *egl-5*, are organised in a loose cluster in the center of chromosome III, whereas *nob-1* and *php-3*, two *AbdB* homologs, map to a separate location on the same chromosome. Interestingly, *ceh-13* is the only gene of the cluster that is required for embryogenesis and viability. Most of the homozygous *ceh-13(lf)* animals die during embryogenesis or at various larval stages, but about 3% are able to reach adulthood and are fertile. *lin-39*, *mab-5* and *egl-5*, however, appeared not to be essential and had no obvious function during embryogenesis. Worms can grow to adulthood without the action of these three Hox genes and the phenotypes of the corresponding null-mutants only become apparent after embryogenesis, during larval stages.

Performing a functional characterisation of *ceh-13*, surprisingly, we found that it genetically interacts with each of the other HOM-C/Hox genes of the cluster. *ceh-13(lf);lin-39(lf)* double mutants exhibit a complete embryonic arrest, whereas *ceh-13(lf);mab-5(lf)* and *ceh-13(lf);egl-5(lf)* animals die at embryonic or larval stages. These results reveal that *ceh-13* shares overlapping functions with *lin-39*, *mab-5* and *egl-5* during development. Functional redundancy between *ceh-13* and the other clustered Hox genes is also indicated by our finding that overexpression of LIN-39 significantly suppresses the Ceh-13 phenotype. We are currently generating chimeric contructs containing promoter and coding regions of the other *C. elegans* Hox genes to study the level at which they can substitute for each other.

Using GFP reporter genes and antibody co-staining, we found that the expression domain of *ceh-13* overlaps with that of each other HOM-C/Hox gene. Thus, *ceh-13* is a unique member of the *C. elegans* HOM-C cluster in that it is expressed and acts all along the anteroposterior axis of the worm rather than in a distinct domain. In this way it may act, at least partially, redundantly with the other Hox genes and thereby masking their embryonic and other essential developmental functions, that have not been detected so far in single mutants.

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1062. The Ephrin EFN-4 may function independently of the VAB-1 Eph Receptor in morphogenesis

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Eph receptor tyrosine kinases bind membrane bound ligands (ephrins) to regulate a variety of processes, including embryonic pattern formation, angiogenesis, and axon guidance. The *C. elegans* genome encodes one Eph receptor, VAB-1, and four ephrins, EFN-1 to EFN-4. We showed previously that EFN-1 is a ligand for VAB-1 and functions in neurons to regulate epithelial and neuronal morphogenesis (Chin-Sang et al., Cell 99: 781). We reported previously that mutations in the *mab-26* gene, initially identified as a ray morphology mutant (Chow and Emmons, Development 120:2579), affect the fourth ephrin, EFN-4. Although VAB-1 encodes the only Eph receptor in the worm genome, several lines of evidence suggest that EFN-4 does not function only through the VAB-1 receptor. First, we tested whether cell-surface expressed EFN-4 binds VAB-1 using a soluble VAB-1-AP fusion protein as an affinity reagent. Our preliminary results suggest that the affinity of EFN-4 for VAB-1-AP is extremely low compared with the EFN-1/VAB-1 affinity. Second, the embryonic and larval phenotypes of *efn-4* mutants differ from those of *vab-1* and *efn-1* mutants, although they show some overlap (see abstract by Moseley and Chisholm). This overlap in phenotype is reflected in the *efn-4* expression pattern. Using EFN-4-GFP transgenes and anti-EFN-4 antibodies we have found that EFN-4 is expressed in the developing nervous system in a pattern that appears to overlap both VAB-1 and EFN-1 expression domains. Finally, *efn-4* alleles display strong synthetic lethality with either *vab-1* or *efn-1* mutations. *vab-1; efn-4* double mutants display fully penetrant embryonic lethality, a phenotype that is stronger than the *vab-1* null phenotype. These data are inconsistent with *efn-4* acting exclusively in a VAB-1 dependent pathway and suggest that EFN-4 may act independently of the VAB-1 receptor. To identify genes that may be involved in this independent pathway we have begun suppressor screens of the *vab-1;efn-4* synthetic lethality and have also screened for mutations that show synthetic lethality with weak *vab-1* alleles. Characterization of our suppressors and enhancers will be presented.

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1063. Novel and Atypical Receptor Tyrosine Kinases in Morphogenesis

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Morphogenesis is a process used throughout development in the generation of many tissues and organs. Morphogenesis is also extremely important for medical reasons, including wound healing, nervous system regeneration and cancer metastasis. During morphogenetic events, cells must interpret positional information from the external environment, and subsequently act upon that information by altering cytoskeletal dynamics. The study of morphogenesis using *C. elegans* genetics has been fruitful, particularly in development of the nervous system, whose function is largely dispensable in the laboratory. However, essential genes exist that are involved more generally in morphogenetic events, including early formation of the embryo and organogenesis, and only rare viable alleles in this class would have been identified in most screens. Since transmembrane receptors can function in both signal transduction and adhesion, we reasoned that novel receptors are good candidates for unknown and essential components of morphogenesis. We noticed that there are several receptor tyrosine kinases (RTKs) in the worm genome that have atypical or novel extracellular domains without the distinctive sequence motifs characteristic of growth factor receptors. These extracellular domains probably bind novel ligands, perhaps components of the extracellular matrix or other positional cues. We studied six such receptors and found that over-expression of 5 of the 6 caused lethality, at least some of which is probably due to gross hypodermal morphology defects. RNAi of 4 of 6 genes also caused apparent defects in morphogenesis. Our observations support our basic hypothesis, and we have chosen two genes for further study.

T10H9.2 encodes an RTK with extensive similarity in the kinase domain to the human Leukocyte Tyrosine Kinase and Anaplastic Lymphoma Kinase RTKs, whose functions are unknown. Some extracellular motifs that may mediate protein-protein interactions are still present in both, but appear more diverged than the cytoplasmic domains. Over-expression of T10H9.2 causes lethality, probably due to morphogenetic defects, and RNAi causes variable gross morphological and nervous system defects, including wandering axons and defasciculation. We constructed a deletion library, have isolated two deletion alleles in this gene, and are proceeding to characterize them.

T14E8.1 encodes an RTK that is similar to the human HGF Receptor/c-met proto-oncogene in the cytoplasmic domain, but has a novel ectodomain. T14E8.1 may be encoded by *mab-19*, a gene originally characterized by Sutherlin and Emmons (Genetics 138: 675). Though we have not found the DNA lesions corresponding to the two *mab-19* alleles, four lines of evidence support the model that T14E8.1 is encoded by *mab-19*: *mab-19* maps to a very small physical interval containing the RTK, a PCR product of T14E8.1 alone rescues the Mab phenotypes, a T14E8.1 RNA-foldback construct driven by the *hsp-16-2* promoter causes Mab phenotypes similar to those of *mab-19*, and a T14E8.1 promoter::GFP construct is expressed in several cell types in the animals, including a subset of male tail sensory rays. Emmons and Sutherlin found that *mab-19*/*Df* caused embryonic lethality,
suggesting that the two alleles are hypomorphic. The lethality appears to be due to failure of the dorsal hypodermis to migrate ventrally and enclose the animal. Our deletion library will be screened for null alleles of T14E8.1.

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1064. STEROL FUNCTIONS IN *C. ELEGANS*

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C. elegans is known to require dietary sterol, because it is unable to synthesize the sterol ring. The most commonly used sterol, cholesterol, can undergo extensive enzymatic modification in C. elegans to form unique sterols of unknown function*. The existence of this pathway, and the fact that only minute amounts of sterol are required, suggests that these compounds may have specific, hormone-like actions, perhaps mediated by one or more of the 270 nuclear receptors identified from the C. elegans genome.

We are characterizing cholesterol deprivation in C. elegans, in order to identify essential genes in the cholesterol utilization pathway. We report the following observations: (1) Successful reproduction requires cholesterol. Egg-laying is reduced in the first generation of cholesterol deprivation (CF1), and completely abolished in the second (CF2). The presence of 10 ng/ ml of cholesterol in the medium in CF2 results in greatly delayed production of approximately half the normal number of progeny. The presence of 30 ng/ml results in the production of the normal number of progeny, with 1-2 days delay compared with higher concentrations. There are no significant differences in rate or number of progeny using varying amounts of cholesterol from 100 ng/ml to 10 mg/ml. (2) Normal growth requires cholesterol. Animals in CF1 are smaller than controls starting on day 4 at 20o. CF2 animals arrest at various larval stages, apparently reflecting the point at which cholesterol is required, but the stored cholesterol is exhausted. (3) A wide variety of sterols can substitute for cholesterol, but vertebrate or insect steroid hormones cannot. (4) Uptake is often rate-limiting for sterol function. This is evidenced by an initially slowed rate of growth on high-cholesterol medium following cholesterol deprivation, and by the observation that addition of detergent is necessary for effective utilization of certain sterols. Microinjection studies are in progress in order to distinguish sterol uptake from downstream effects.

Microscopic studies using the cholesterol-specific stain filipin revealed that cholesterol accumulates in five specific cells in both larval and adult hermaphrodites. These include two amphid socket cells, two phasmid socket cells and the excretory gland cell, which contains secretory granules and makes connections with both the excretory canal and the pharyngeal nerve ring. These observations, taken together, suggest a hormonal role for one or more sterol metabolites.

*Chitwood, D.J. 1999. Crit. Rev. Bichem. Mol. Biol. 34: 273-284.

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1065. Comparative study of *lin-48* gene in *C. elegans* and *C. briggsae*.

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Evolutionary change, from a gene regulation point of view, is an interesting issue in comparative developmental biology. Previous studies showed that, in *Caenorhabditis elegans*, *lin-48* encodes a C2H2 zinc finger protein and it is expressed in hindgut cells, sensory neuron support cells and the excretory duct cell. We found that in *C. briggsae*, the *C. elegans lin-48* promoter was able to drive *gfp* (green fluorescent protein) expression in the same hindgut cells and neuron support cells as it does in *C. elegans*. However, expression in the excretory duct was essentially eliminated. This result indicates that there is a difference between *C. elegans* and *C. briggsae* in either the function or regulation of *lin-48*. To investigate the molecular nature of this difference, we have isolated the *C. briggsae lin-48* gene. Our preliminary data showed that a *C. briggsae lin-48* 6 kb Sal I subclone containing the *lin-48* gene (*Cb-lin-48*) could rescue the hindgut defects in *C. elegans lin-48*(*sa469*) mutants. We are constructing *gfp* reporter transgenes to investigate the expression pattern of *Cb-lin-48* in *C. elegans* and *C. briggsae*. We have also investigated whether there is any difference in the sequence of the *lin-48* regulatory region that might be responsible for expression differences between the two species. In *C. elegans*, *lin-48* requires the *Pax* gene *egl-38* for its expression. Two *egl-38*-responsive elements in the *lin-48* promoter (defined as lre1 and lre2, *l*in-48 regulatory element 1 and 2) that are necessary for its expression have been identified. lre1 and lre2 are similar to the consensus sequence for Pax binding sites. By comparing the sequences, we found that two nucleotides CG in *C. elegans* were replaced by AA in *C. briggsae* lre1, whereas lre2 is identical in the two species. To test whether the sequence changes are responsible for the differences in *lin-48* expression in two species, we are planning to mutate the lre1 in *C. elegans* promoter to the *C. briggsae* sequence and vice versa, and test the expression pattern of the mutant transgenes in each species.

Our study on the regulation of *lin-48* in two closely related species, *C. elegans* and *C. briggsae*, will shed a light on the mechanism underlying alteration in gene expression pattern between species during the evolutionary changing process

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1066. Evolution and function of the serpentine receptors of the secretin family

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The secretin family of serpentine receptors is an ancient family that has members in C. elegans, Drosophila, and vertebrates . Our alignment data suggests at least five member genes in the common ancestor of these lineages. Further duplications of each of the genes within the individual lineages lead to further expansion of the family . The five subfamily branches with their C. elegans members are flamingo-cefla(f15b9.7), latrophilin(lat-1(b0457.1) and lat-2(b0286.2)), calcitonin receptor(c13b9.4), corticotrophin releasing factor receptor(zk643.3), and secretin receptor(c18b12.2). Most of the studied members of this family are involved in regulated secretion in response to a peptide ligand. We are currently attempting to understand the organismal biology of this family in C. elegans by expression and phenotypic analysis and by linking these receptors to the peptide ligands that activate them.

One of the aspects that we are focusing on is the latrophilin subfamily. Lat-1 expression starts after gastrulation and continues into the adult. It is expressed in the hypodermal, pharyngeal and some neuronal cells in the embryo. During larval development it is expressed in mechanosensory and interneurons in the head and tail, gland cell in pharynx, gonadal sheath cells, spermatheca, uterine epithelial cells and intestinal cells. We are focusing on the following aspects of the expression pattern: expression in alternating dorsal hypodermal cells during intercalation, expression and localization to the apical surface in the pharyngeal primordium cells, expression in ventral cord neurons and gland cell in the dauer, and expression in the reproductive organs. Phenotypes from RNAi have correlated with some of these expression patterns. These include elongation defects, abnormal pharyngeal development and attachment, small eggs, and ovulation defects in the injected animal. Lat-2 is expressed in the g1 gland cell and arcade cells in the head in which it localizes to the syncitial end of the process, and the expression cycles with the molts.

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1067. Molecular Analysis of *daf-21*, a multi-faceted Hsp90 gene, from Plant-parasitic Nematodes

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Many plant-parasitic nematodes hatch out of their eggs as a developmentally arrested pre-infective juveniles (L2) that share functional similarity to *C. elegans* dauers. Nothing is known about the genetic programming of development in these nematodes or how it may be linked to environmental cues. Nevertheless, it is likely that plant-parasitic nematodes use molecules similar to those defined by the *C. elegans* dauer pathway to regulate developmental arrest, lifespan, and chemosensation. The long-range goal of this research is to determine the molecular mechanisms controlling plant-parasitic nematode development in order to discover new targets for safe, specific and biologically based methods for controlling these damaging pathogens.

The *daf-21* gene from *C. elegans* encodes a molecular chaperone of the Hsp90 class (Birnby, *et al.,* 2000). Mutation of *daf-21* is dauer-constitutive, and has been shown to cause defects in chemosensory responses mediated by several classes of chemosensory neurons. The precise function of *daf-21* in the dauer pathway is unclear. However, Hsp90 chaperones are known to refold denatured or misfolded proteins, especially under conditions of stress. In *C. elegans*, transcription of Hsp90 is increased 15-fold in dauers versus non-dauers, which would be consistent with this type of protective function (Dalley and Golomb, 1992). Under non-stress conditions, Hsp90 has been shown to guide the proper folding of specific target proteins, including nuclear hormone receptors and protein kinases. Possible targets of Hsp90 in *C. elegans* include the *daf-11* transmembrane guanylate cyclase, the *daf-5* putative Sno transcription factor, and the *daf-12* nuclear hormone receptor. Additionally, the *C. elegans* aryl hydrocarbon receptor, AHR-1, has been shown to bind to Hsp90 (Powell-Coffman, *et al*., 1998), suggesting *daf-21* involvement in the response to environmental pollutants.

We have focused on the cloning and characterization of Hsp90 (*daf-21*) from several species of plant-parasitic nematodes of economic concern to agriculture. We have obtained full-length cDNA and partial genomic sequence from the soybean cyst nematode, *Heterodera glycines*, and are in the process of examining expression of the gene and its protein product. In addition, we have developed a set of PCR primers that amplify a portion of the Hsp90 gene from single nematode extracts. This technique has enabled us to isolate partial Hsp90 sequence from a wide range of agriculturally important nematodes, including cyst, root-knot, and lesion nematodes. We have identified variation in intron number and position in this amplification segment within the root-knot nematodes. This variation may comprise a useful diagnostic for several nematodes of agricultural concern, so we are now expanding this study to include more taxa. Hsp90 is a highly conserved protein whose sequence has been extensively used in determining the evolutionary relationships between eubacteria, archaebacteria, and eukaryotes (Gupta, 1998). Mutations in Hsp90 have been shown to unmask hidden genetic variation in fruit flies (Rutherford and Lindquist,1998) and therefore may play a critical role in regulating the evolutionary potential of an organism. Whether this phenomenon holds true for nematodes is unclear, however, we hope to gain insight into the function of plant-parasitic nematode Hsp90s by examining the sequences from a phylogenetic perspective.

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Rutherford and Lindquist, 1998. Nature 396: 336-342.

1068. Large scale analysis and genetic characterization of dumpy mutants in *Pristionchus pacificus*

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Pristionchus pacificus has been established as a satellite organism for studying developmental processes and comparing them to *C. elegans*. Cell fate specification and gene function during vulva formation have evolved substantially between these two nematodes. Most vulva defective mutants in *P. pacificus* cannot easily be assigned for gene functions from the knowledge of *C. elegans*. To facilitate the cloning of these mutants we will generate genetic and physical maps of *Pristionchus pacificus*. Our work presented here is focussing on the construction of a genetic map using *Dpy* mutants. We performed a large scale EMS mutagenesis screen for dumpy mutants. We screened 50,000 gametes and isolated 177 dumpy mutants originally, 123 of which were re-identified. In order to map these *Dpy* mutants to the six chromosomes in *Pristionchus pacificus* we are using various *Unc* and vulva phenotypes as markers, i.e. the *unc-22* homolog *Ppa-unc-1* that defines chromosome IV and *mab-5* that defines chromosome III. Crosses are ongoing. After mapping these mutants to the various chromosomes, we will perform complementation tests. The various complementation groups will be mapped onto the molecular genetic map using AFLP based linkage studies with the polymorphic strain *P. pacificus* var. Washington. The data emanating from the genetic and physical map projects will facilitate further genetic and molecular studies in *Pristionchus pacificus*. Specificially, the genetic *dpy* map will allow in-depth genetic characterization of mutants in *Pristionchus pacificus* isolated in various screens, thereby establishing it as a second general nematode model system.

1069. The *Onchocerca volvulus* glutathione S-transferase 3 (*Ov-GST-3*) confers an increased resistance to oxidative stress in transgenic *Caenorhabditis elegans*

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Onchocerca volvulus is a parasitic nematode which is infectious for humans, resulting in the disease called River Blindness or Onchocerciasis. Onchocerciasis is the worlds second cause of blindness, due to an infection. A total of 18 million people are infected with this parasite, 120 million live at risk, and roughly half a million are completely blind due to this disease. The adult parasites live for many years in nodules under the skin of the human host and produce large numbers of offspring called microfilariae. These microfilariae migrate in the subcutaneous tissue, can be swallowed by a blood sucking black fly and mature to juveniles. During another blood meal of the mosquito, the larval parasites can enter the human host to continue the life cycle.

Like all aerobic organisms *O. volvulus* must defend itself against cellular oxidative stress generated by metabolism and incomplete respiration. Therefore, protective enzymatic and non-enzymatic antioxidants exist that prevent, repair or eliminate oxidative damage. Detoxification enzymes, like the glutathione S-transferases (GSTs), minimize the concentration of oxidatively damaged cellular components, particularly oxidized DNA, proteins and lipids. Nevertheless, the parasite is able to persist for a long time in the human host even though it is heavily attacked by the

immune system of the human host. This attack includes the release of a variety of reactive oxygen species from stimulated host phagocytic cells which cause an external oxidative stress. This means that *O. volvulus* must have evolved additional mechanisms to protect itself against the immune response of the human host to ensure its own survival.

A glutathione S-transferase of *O. volvulus* (*Ov*-GST-3), shown to be upregulated in response to oxidative stress, is suspected to be involved in the defense against the host's immune system. To have the chance to investigate the function of this molecule in more detail, transgenic *C. elegans* (AK1) containing the *Ov*-GST-3 gene under the control of a *C. elegans* promotor (let-858) were generated. The comparison of AK1 worms and control worms on plates with artificially produced oxidative stress revealed significant higher survival rates of the transgenic line. The extent of lipid peroxidation, one type of damage caused by oxidative stress, is also reduced in the transgenic worms as determined by the measurement of the level of malondialdehyde (MDA). These findings clearly demonstrate that the overexpression of *Ov*-GST-3 confers an increased resistance to oxidative stress in the transgenic AK1 worms.

1070. ENHANCEMENT OF *STEINERNEMA CARPOCAPSAE* DESICCATION TOLERANCE BY GENETIC IMPROVEMENT

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The entomopathogenic nematode (ENP) *Steinernema carpocapsae* , is an important biological control agent for a wide range of soil dwelling insect pests. However, the field efficacy of this ENP is limited by its sensitivity to high drought and salinity conditions. We report efforts to improve the desiccation tolerance of *S. carpocapsae* by transforming it with the trehalose-6-phosaphate synthase (tps1) and glycogen synthase(gsy1)genes. Trehalose-6-phosphate synthase and glycogen synthase are enzymes involved in the biosynthesis of trehalose, a disaccharide that accumulates to stabilize the lipid biomembranes in many organisms when in response to stress.

To increase desiccation tolerance by genetic modification, we have cloned gene tps1 from yeast and *C. elegans* into expression vectors pJJ436 and pPD95.67, respectively. In addition, we also cloned gsy1 from *Steinernema feltiae* into expression vector pJJ436. Vector pJJ436 contained the Ce sq-1 promoter, whereas pPD95.67 contained the promoter of the tps1 Ce gene. All vectors contained the gfp transformation gene which was used as a selection marker. Vector constructions (yeast: pJYeTr.1; *C. elegans* : pP67CeTr.2; *S. feltiae* : pJSfTr.1) were microinjected independently into young *S. carpocapsae* females (48 h from infective juvenile stage). Injected females were mated with noninjected males for 2-4 days and progeny were screened for gfp expression. After selecting and retaining gfp expressing individuals for three generations, F3 progeny were tested for desiccation tolerance. We will present details of our methodology and results in our presentation.

1071. Cloning and characterization of plant parasitic nematode *Heterodera glycines* genes by using *Caenorhabditis elegans* genome information.

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The increased use of molecular biology techniques to study parasitic nematode physiology and biochemistry as well as parasite/host interactions opens up a number of possibilities to develop novel forms of effective and durable nematode controls. The genome of the soybean cyst-nematode *Heterodera glycines*, the major pathogen of soybean in the USA and world-wide, is similar in size and complexity (92.5 Mb, 82% unique) to the *Caenorhabditis elegans* genome, but less than 0.001% of it has been sequenced thus far. Using the large resource of *C elegans* genetic information we have isolated several *H glycines* genes with a conserved counterpart in *C elegans*, known to play important roles in nematode development and adaptability. Here we describe three representatives: 1) actin, an intracellular component of cytoskeleton, ubiquitous and expressed at high level; 2) heat shock protein 70, a molecular chaperone, expression of which depends on environmental stress reactions; 3) proprotein convertase 2, a neuropeptide and hormone precursor processing endoprotease, known as a highly selective enzyme located in neuroendocrine cells with expression regulated during development. All three clones, isolated from a juvenile *H glycines* cDNA library, exhibit high homology to corresponding genes of *C elegans* as well as other invertebrates. These new *H glycines* sequences were used to further study nematode diversity, and evolutionary relationships among eukaryotes on the molecular level.

1072. Secreted protein products shared between *C. elegans* and parasitic nematodes

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The venom allergen-like proteins (VAPs) constitute a family of secreted proteins widely found in invertebrates. In parasitic nematodes, VAPs have been implicated in the transition to parasitism and early stages of host infection. In the most well characterized example, the *Ancyclostoma caninum* (dog hookworm) VAP, ASP-1, has been detected in infective larvae secretions. Immunization with ASP-1 protects against subsequent infection in a mouse model. Given the potential importance of this protein family in parasitic nematodes, we have begun to analyze the function of the *C. elegans* members of the VA protein family. A search of the *C. elegans* genome has revealed over twenty *vap* genes. The majority of VAPs contain either one or two signature VA domains, characterized by highly conserved cysteine residue spacing. *vap-1* and *vap-2* encode predicted proteins that contain a pair of VA domains, whereas the remainder of the *C. elegans vap* genes encode predicted proteins that contain a single VA domain. *vap-1* and *vap-2* were selected for further analyses because of their similarity to ASP-1. Our study of *vap-1* and *vap-2* included evaluation of expression by GFP and myc reporter constructs, and phenotypic analysis of a deletion mutant and of animals subjected to RNAi. Both genes were found to be expressed in cells specialized for secretion, and the secretion of a VAP-1 fusion protein was detected by Western blot analysis.

1073. The regulation of gut development in the parasitic nematode Haemonchus contortus; a comparative study with C.elegans

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The extent to which developmental mechanisms elucidated in C.elegans can be extrapolated to parasitic nematodes is at present unclear. We are interested in studying the regulation of gut development in the parasitic nematode Haemonchus contortus and determining the extent to which it is it is conserved with that of C.elegans. As one approach to this we are attempting to isolate Haemonchus contortus orthologues of genes that are key regulators of gut development in C.elegans. We have performed PCR using degenerate primers and isolated an orthologue of the C.elegans GATA factor elt-2 , a gene necessary for normal gut development and sufficient to activate a program of endodermal differentiatiation (Fukushige et al. 1998 Dev. Biol 198 : 286-302). We have obtained the full length cDNA sequence of the Haemonchus gene and it appears to be SL1 trans spliced. Overall the level of identity is low; 17% amino acid identity outside the DNA binding domain. However the C-terminal DNA binding domain is highly conserved between the C.elegans and Haemonchus genes ; 23 out of 25 residues are identical in the C-terminal Zinc finger. Interestingly the putative N-terminal finger is also conserved (50% identity). This is a very divergent zinc finger domain, the function of which is unknown, but its conservation in both the C.elegans and H.contortus elt-2 genes argues for functional significance. Similarly there are small conserved domains at the N and C-terminus. We are generating antibodies to the Haemonchus elt-2 polypeptide in order to study its expression pattern in the parasite. We have also cloned the cDNA into the pPD49-78 expression vector to determine the extent to which it can activate gut specific gene expression when force

1074. Bt toxins are effective against phylogenetically diverse nematodes

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Bacillus thuringiensis (Bt) delta-endotoxins, a family of crystal (Cry) proteins, are widely used as insecticides in agriculture. Recently, we described two *Bt* toxins (Cry5B and Cry6A) that are effective at killing *C. elegans*. Here, we test whether *Bt* toxins are generally nematicidal. We analyzed the toxicities of eight Cry proteins (Cry 5A, 5B, 6A, 6B, 12A, 13A, 14A, and 21A) on five free-living nematodes species (*C. elegans, Pristionchus pacificus, Panagrellus redivivus, Acrobeloides sp.*, and *Distolabrellus veechi*). The results of health assays, morphology, and brood size indicated that four out of the eight proteins were nematicidal and were able to kill multiple nematodes species. One nematode species was resistant to all toxins. *Bt* toxin was toxic to all four of the other free-living nematodes tested, including one species closely related to plant-parasitic nematodes (PPNs). Structure function studies indicate it can be trimmed to a small 42 kD active core that retains full toxicity. Given its effects on many free-living nematodes and that PPNs are able to exclude large proteins from their diet, this small toxin holds promise for controlling plant-parasitic nematodes. To test this directly, we are transforming *Arabidopsis* plants and tomato hairy roots with the *Bt* toxin under control of three different promoters. Currently, successfully transformed plant lines are being isolated and our nematode infection assays are being perfected. We are growing Arabidopsis plants in modified Knop medium and are infecting them with active *Meloidogyne incognita* J2's under sterile conditions. We hope to soon assay whether expression of the toxin adversely affects infection rates and survival of *M. incognita*.

1075. Experimental testing of potential new antinematode drug targets using RNA interference in *Caenorhabditis elegans*

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The technique of RNA interference (RNAi) has the potential to expedite the search for new drug targets in parasitic nematodes, and to assist in their validation. The sugar trehalose is claimed to have important functions in the physiology of nematodes whereas it is considered to be unimportant in mammals. In this study we are assessing trehalose metabolism in nematodes, using *C. elegans* as a model.

We have shown that trehalose is synthesised by the nematode and is present at all life cycle stages; highest concentrations were observed in dauer larvae and eggs. The enzyme trehalase, which converts trehalose to glucose, is active at all stages of the life cycle; we have identified three biochemically distinct trehalase activities, each with different properties, in preparations from *C. elegans*. Four genes encode putative trehalases: F57B10.7, T05A12.2, W05E10.4 and F15A2.2. Using Northern analysis and RT-PCR we have shown that each of these genes is expressed at all life cycle stages. The synthesis of trehalose is catalysed by two enzymes, trehalose 6-phosphate synthase (TPS) and trehalose 6-phosphate phosphatase (TPP). Two genes putatively encoding TPS are present in *C. elegans*: ZK54.2 and F19H8.1. We have shown by RT-PCR analysis that both genes are expressed at all stages of the life cycle. The gene(s) putatively encoding TPP have not yet been identified. We are using RNAi to examine the effects of temporarily knocking down the expression of these genes, individually and in combination.

This investigation has received financial assistance from the Australian Research Council and the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases.

1076. Molting in free-living and parasitic nematodes: a role for nuclear receptors?

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Nematode development is punctuated by a series of molts. In parasitic nematodes, molting occurs in response to the movement of worms from one host to the next. Thus, an understanding of how molting is controlled may lead to new ideas for drug development. The signals required for molting to occur and the genes involved in interpreting these signals are not well understood. However, there is some evidence that at the genetic level molting in nematodes may be controlled by some of the same genes that control molting and metamorphosis in *Drosophila*. For example, two *C. elegans* genes that encode members of the nuclear receptor superfamily (*nhr-23* and *nhr-25*) show molting defects when inactivated by dsRNAi (Kostrouchova, M., et al. 1998. Development. 125:1617-1626; Gissendanner, C.R. and Sluder, A.E. 2000. Developmental Biology. 221: 259-272). Both of these genes have putative homologs in *Drosophila* (*DHR3* and β*FTZ-F1*, respectively) that have known roles in controlling gene expression during metamorphosis. We have identified a gene in the parasitic nematode *Dirofilaria immitis* that encodes a putative homolog of the *Drosophila E75* gene. In *Drosophila*, *E75* is required for molting and metamorphosis. The protein encoded by the *D. immitis E75* homolog, *dinhr*-6, is 83% identical to the E75A protein in the DNA binding domain. Northern blot analysis suggests that *dinhr-6* encodes multiple isoforms and is female-specific in adults. Putative homologs of *dinhr-6* exist in both the human parasite *Brugia malayi* and the free-living nematode *C. elegans*. We are currently working on characterization of all three *E75* homologs. In particular, we are making use of the tools available in *C. elegans* to ask questions that are difficult to ask in parasites. Expression studies suggest that *nhr-85* is expressed in hypodermal cells, as would be expected for a gene involved in molting. We are currently using dsRNAi to try to knock out the

function of the *nhr-85* gene. We discuss the potential of using *C. elegans* to help understand parasitic nematode development.

1077. Expression of a *C. elegans* Mucin-Like Gene

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Based on the amino acid sequence of a cDNA encoding the major larval surface protein of the parasitic nematode *Toxocara canis* [1], plus studies of *T. canis* surface proteins, it is likely that nematode surface proteins are structurally similar to the mucins that line and protect epithelial cell layers in vertebrate animals. Mucins have two characteristic features: 1. domains rich in serine and threonine, which are extensively O-glycosylated; 2. domains rich in cysteine, which form inter- or intra-molecular disulfide bonds. These features confer special physical and biochemical properties on mucins, such as high solution viscosity and resistance to proteolysis. The epitope recognized by monoclonal antibody M38 is detected on the surface of live *C. elegans* L1s, and the timing of its expression is altered in *srf-6*mutants, which display it in stages L1 through L4. We demonstrated that the M38 epitope is carried by a 30 kD antigen detected in extracts of *C. elegans* L1s [2]. The antigenicity of this protein is destroyed by pretreatment with O-glycanase, suggesting that it is an O-linked glycoprotein, like the *T. canis* TES-120 surface antigen.

A nematode-specific gene family in *C. elegans* has been described, members of which encode adjacent serine-threonine-rich and cysteine-rich (SXC or six-cysteine) domains [3]. Because of the likelihood that these encode surface proteins, we have begun to characterize their expression by RT-PCR. For initial studies, we have chosen the predicted genomic coding sequence F41G3.10, which contains 10 exons, each of which encodes a ser-thr-rich sequence followed by an SXC domain. In initial experiments using gene-specific primers, we have amplified a small cDNA fragment spanning 3' terminal exons 9 and 10 in total RNA from mixed stages of *C. elegans*. We plan to study the stage-specificity of expression of this RNA, as well as map a full-length cDNA representing this transcript.

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1078. Worm Locomotion Described: A New Method for Extracting Quantitative Data on Movement Parameters

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We have developed an automated worm tracking system that enables us to record moving worms. Videos of recorded worms are analyzed by an automated worm recognition system that extracts quantitative data describing body posture as a function of time and then computes metrics for several parameters of locomotion. These metrics include frequency, which reflects muscle contractions per second, and amplitude, reflecting the degree of muscle contraction. The metrics are mathematically related, but not identical to the wavelength and amplitude of the sinusoidal track left by worms on bacterial lawns. Comparison of metrics from anterior regions of the worm to those from posterior regions (the time delay) indicates the speed of propagation of the movement wave along the length of the worm.

We are currently testing a variety of mutants affected in movement to both establish the limits of reliability of our analysis and to further our understanding of worm movement. For example, analysis of goa-1 loss-of-function mutants showed increased amplitude and increased frequency as is readily observed simply by looking at these worms. Our movement analyzer also quantitated the increase in reversal frequency seen in the mutant animals. Comparison of the time delay between goa-1(lf) and N2 revealed that the wave propagates faster in the mutants. This difference is not easily detected without sophisticated quantitation. unc-54 mutants displayed reduced amplitude as expected, but also showed a reduced frequency. This observation is more consistent with a model in which the wave

propagation involves feedback dependent on muscle contraction (as originally proposed by John White) rather than relying solely on a central pattern generator.

1079. Differentiation, RNA interference and patch clamp electrophysiology in cultured neurons and muscle cells derived from *C. elegans* blastomeres

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C. elegans is a powerful model system for defining the genetic basis of neuromuscular function and development and associated whole animal behavior. However, relatively little is known about the electrical properties and intracellular signaling pathways that control *C. elegans* cell excitability. We have now developed robust cell culture methods that allow detailed characterization of neuronal and muscle cell functional properties.

In their recent study of excretory cell development, Buechner et al. (*Dev. Biol.* 214: 227-241, 1999) noted that isolated *C. elegans* blastomeres differentiate *in vitro* into neurons and muscles. We have made similar observations. Freshly isolated blastomeres plated onto glass substrates coated with agents that promote adhesion undergo striking morphogenesis. Within 2-3 h after plating, blastomeres begin sending out neurite-like growth processes. Differentiation is largely complete 24 h after plating. Differentiated cells survive well for at least 2 weeks in culture. Blastomeres plated onto non-coated glass substrates survive for at least 1 week but fail to undergo obvious morphological differentiation.

To examine differentiation in more detail, we cultured blastomeres from worm strains expressing various cell-specific GFP reporters (e.g., *unc-4*, *myo-3*, *gcy-5*, *opt-3*, *daf-7*, *unc-119*, *del-1*, *dat-1*, *mec-7*). GFP reporter expression *in vitro* was remarkably similar to that of fully developed embryos. For example, *unc-119* is expressed in all neurons and neuronal precursor cells. Newly hatched L1 larvae have 222 neurons, which represent 40.4%

of all somatic cells. Approximately 45% of cells in culture express *unc-119*::GFP. All cultured GFP-expressing cells derived from the *unc-119*::GFP worm have a neuron-like morphology.

The ability to perform functional studies on cultured neurons and muscles would provide critically important information required for a complete molecular description of the *C. elegans* neuromuscular system. Cultured cells can be readily loaded with ion-sensitive fluorescent dyes such as fura-2 and studied using quantitative fluorescence imaging methods. In addition, cultured cells can be patch clamped readily in the whole-cell and cell-attached modes. We have begun to carry out detailed electrophysiological characterization of several excitable cell types in culture. For example, in *gcy-5*::GFP-expressing ASER neurons, we observed strongly outwardly rectifying whole-cell K^+ currents that inactivated rapidly at depolarizing voltages. These currents are remarkably similar to those described for ASER neurons patch clamped *in vivo* (Goodman et al. *Neuron* 20: 763-772, 1998). Body muscle cells in culture express ion currents similar to those described *in vivo* by Richmond and Jorgensen (*Nat. Neurosci.* 2: 791-797, 1999).

Targeted gene expression in cultured cells is dramatically reduced by addition of double stranded RNA (dsRNA) to the culture medium. Using western analysis, we observed that *unc-54*dsRNA reduced UNC-54 protein levels >90% within 4 days after exposure. GFP dsRNA reduced the number of *unc-4*::GFP-positive neurons >90%. This knockdown effect was maximal 3 days after dsRNA exposure. In cultures derived from *myo-3*::GFP-expressing worms, GFP fluorescence was reduced by dsRNA to a similar extent. The knockdown effect in muscle was maximal within 2 days of treatment with GFP dsRNA.

The ability to make direct physiological measurements on cultured cells and readily disrupt gene expression *in vitro* with dsRNA offers important new opportunities for defining complex cellular processes in *C. elegans* at the molecular level.

1080. Differentiation of *unc-4*-expressing embryonic motor neurons *in vitro*

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A-type motor neurons (DA, VA, SAB) are innervated by specific command interneurons (AVA, AVD, AVE). The UNC-4 homeoprotein is expressed in all A-type motor neurons where it functions to maintain normal levels of synaptic vesicles. UNC-4 is also required in VA motor neurons to regulate presynaptic specificity. In *unc-4* mutants, inputs to VA motor neurons from AVA, AVD, and AVE are replaced with connections from AVB. In an effort to define the mechanism whereby UNC-4 regulates A-type motor neuron synaptic differentiation, we have established methods for culturing *unc-4*-expressing motor neurons *in vitro*. *unc-4*::GFP is initially detected midway through embryonic development (~400 min) and is ultimately expressed in 13 embryonically-derived motor neurons (9 DAs, 3 SABs, 1 I5). GFP-positive cells are rarely seen among blastomeres of freshly dissociated *unc-4*::GFP embryos but are easily detected after several hours in culture. The delay in *unc-4*::GFP expression is consistent with the observation that older embryos are not dissociated in our preparations and that early blastomeres are capable of differentiating *in vitro*. *unc-4*::GFP expressing cells exhibit neuronal-like morphology with elongated processes. Direct counting in the microscope as well as quantitation by FACS shows that *unc-4*::GFP neurons comprise 2-4% of all cells after 1-2 days in culture. This proportion is comparable to the fraction of *unc-4*::GFP cells present in the mature embryo (i.e. 13 out of 550 total cells). Antibody staining detects synaptic vesicle proteins Synaptotagmin and UNC-17 which indicates that *unc-4*::GFP cells express specific differentiated traits of cholinergic motor neurons. VA motor neurons, which are postembryonically derived, do not appear to differentiate in culture, however, as evidenced

by the lack of *del-1*::GFP expression *in vitro* in a genetic background in which *del-1*::GFP is expressed in VA motor neurons *in vivo*. The nAChR subunit gene, *acr-5*, is negatively regulated by UNC-4 in DA motor neurons in L1 larvae. We will determine if UNC-4 regulates this known target gene in vitro by asking if *acr-5*::YFP is co-expressed with *unc-4*::CFP in cells isolated from an *unc-4* mutant. In that event, it is reasonable to assume that UNC-4 also controls other downstream genes in these cultures and that these UNC-4-regulated targets could be detected by microarray experiments with FACS sorted *unc-4*::GFP cells from wildtype vs *unc-4* mutant backgrounds. Blastomeres prepared from embryos expressing the AVA-specific reporter, *opt-3::*GFP, give rise to GFP-positive neurons with elongated processes. It will be interesting to determine if these presumptive AVA-like interneurons retain synaptic specificity for A-type motor neurons in culture.

1081. *Mos1* transposon excision provides new tools to engineer the *C. elegans* genome

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We have recently shown that the *Mos1* transposon of *Drosophila mauritiana* can be mobilized in the *C. elegans* germline. 58 *Mos1* insertions have been localized in the genome. They are distributed on the six chromosomes in exonic, intronic and intergenic regions. No strong consensus sequence could be demonstrated for the insertion sites apart from a TA dinucleotide. The first application of *Mos1* transposition is insertional mutagenesis (see Williams *et al.*, IWM 2001). Even non mutagenic mutations can be used to modify the locus that contains a *Mos1* copy. Specifically, we are developing tools based upon *Mos1* remobilization first to generate deletion mutants and second to engineer mutations by gene conversion.

Mos1 transposase expression in the germline was achieved using a *glh-2* expression vector. In this context, an integrated transgene containing multiple copies of *Mos1* was excised at very high frequency (up to 20% of the chromosomes). We then demonstrated that a single *Mos1* insertion could be remobilized. Excision frequency was 1 out of 1000 haploid genomes. Various DNA lesions were generated at the excision site including large deletions. We hypothesize that the actual excision rate is underestimated because most of the DNA breaks are probably repaired using the sister chromatid as a template, thus regenerating the initial *Mos1* copy. We are currently testing this hypothesis by measuring excision rates when the insertion site is placed over a deficiency.

It has also been shown previously that DNA double strand breaks (DSB) generated by *Tc1* excision can be repaired using an extrachromosomal array as a template (Plasterk and Groenen, 1992). As a result, polymorphism contained in the array was copied into the genome. However, the frequency of these events was very low. This could in part be explained by the low excision rate of *Tc1*. Thus we propose to

use *Mos1* reexcision to generate DSB at higher frequency. We expect that this strategy will provide a way to engineer genomic modifications of a locus containing a *Mos1* insertion.

1082. *Mos1.0* Mediated Mutagenesis

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To rapidly screen for mutants and clone the relevant genes, we mobilized the *Drosophila* transposable element *Mos1* in *C.elegans*. Our strategy involves two extrachromosomal arrays. The enzyme array contains the *Mos* transposase driven by a heat shock promoter, while the substrate array contains multiple copies of the *Mos* transposable element. Because *Mos* is a heterologous element, it provides a unique sequence tag at the insertion site. Furthermore, we can control transposition by inducing expression of the *Mos* transposase by heat shock application.

Not surprisingly, transposition frequency (defined as the percentage of F1s that contain at least one insert) depends on the stability of the substrate array. Previous arrays were unstable and transposition rates were quite low. Our current arrays are 75% stable in the absence of heat shock. Presence of the array in the germ cell nucleus is probably essential: F1s that contained the substrate array displayed a higher transposition frequency (49%) than F1s without the substrate array (18%). On the other hand, the transposition frequency of F1s with the enzyme array (32%) is not significantly different from transposition in the absence of the enzyme array (30%) suggesting that the transposase can diffuse between germ cell nuclei in the syncytial gonad. Peak transposition frequency levels of 35% were observed in F1s laid 24-30 hours after heat shock.

We have used *Mos* in forward genetic screens and isolated 19 mutants. Of these 7 (37%) lacked a *Mos*insertion and were likely to be caused by hit and run events. The other 12 either contained an insert in a known gene, or the mutation segregated with a *Mos* insertion.

Mapping and rescue experiments confirmed mutagenic insertions. Insertion sites were determined by single worm inverse PCR (SWIP). Mutagenic *Mos* insertions were located in promoters, exons, and intron splice sites. However, an insert in an intron never caused a mutant phenotype, although many such events were observed by direct analysis of progeny after heat shock. This is not surprising, since insertions that occur in introns are not expected to disrupt gene function. In an effort to make *Mos* more mutagenic, we constructed *Mos1.1*, which contains the *unc-54* polyA signal sequence in the middle of *Mos*. Insertions of this engineered element should disrupt transcription of the inserted gene even if the insert is located in an intron. The transposition frequency of *Mos1.1* was identical to wild-type *Mos1.0*, indicating that addition of DNA fragments into *Mos* does not affect transposition. We have not yet compared the mutagenic properties of *Mos1.1* to other mutagens.

1083. Identification of embryonic lethal temperature sensitive mutants using a novel selection screen.

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The short life cycle and rapid development of the nematode C. elegans make it an attractive organism for embryological studies. For example, genetic screens over the past decade have identified numerous factors involved in the control of cell polarity and cell fate in the early embryo. However, despite this progress many steps have yet to be defined by mutations. We know of at least two reasons for these missing steps, one of these is redundant genes that compensate for each other and thus preclude identification by single mutant events. The second reason involves individual genes that have multiple functions where disruption of an earlier function might preclude analysis of the developmental event of interest. There are no perfect solutions to these problems but at least one possible way to identify genes with multiple functions is to search for conditional alleles. Therefore we have recently initiated a screen for temperature sensitive (TS) embryonic lethal mutants. This screen utilizes the famous Jim Priess Egl mutant trick to trap mutant embryos inside their mother, as well as several novel selection methodologies to facilitate the identification new mutants. To facilitate genetic mapping of new alleles we have used the SNP rich C. elegans isolate from Hawaii as the starting genetic background. Following chemical mutagenesis of L4 animals, F1 and F2 progeny were reared at permissive temperature (15°C). A synchronous population of potentially homozygous F2 animals were shifted as late L4 stage animals to the restrictive temperature (25*C) and allowed to accumulate eggs. After 36 hours gravid F2 adults were returned to the permissive temperature and incubated for one more day to permit the fertilization and development of a few additional embryos if possible. These F2 animals were then subject to a brief hypochlorite treatment to kill all of the adults and any hatched larvae. Adults filled with

unhatched eggs were then separated from other types of animals by sucrose flotation (we are not sure why this works but it does). Candidate TS mutants were then identified by looking for viable L1 larvae bracketing a collection of dead eggs. To date, we have screened the progeny of 6.4X106 mutagenized P0 animals and selected 2056 candidates. Approximately 16% (325) of these candidates actually harbor confirmed TS-embryonic lethal alleles. These TS-alleles have all been frozen and are categorized according to their restrictive temperature phenotypes. The majority of the mutants display a cell division and/or a tissue differentiation defect. Morphogenesis defects are also common, with the smallest class of mutants being those involved in cell fate decisions. Preliminary mapping and complementation tests suggest that at least a few of these define new mutant loci. There are also many new alleles of previously identified mutants. For example our gutless mutants include four lit-1 alleles, three mom-4 alleles, two par-2 alleles and two par-4 alleles. Our goal is to collect at least ten times the current number of ts mutants and then to assess whether or not it will be possible to saturate the C. elegans genome for TS-alleles. In the future, well mapped TS mutants from this and other similar screens conducted elsewhere, could provide a valuable resource to C. elegans researchers.

1084. Enrichment of cDNA Libraries for Clones Representing Rare mRNAs.

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Identification of recombinant clones corresponding to rare mRNAs requires large-scale screening of cDNA libraries. We have developed a method to remove abundant sequences from cDNA libraries based on the second-order kinetics of DNA reassociation. It is simpler and more direct than existing methods. First, we cloned the same set of cDNAs (unnormalized cDNAs) into two vectors - lambda gt11 and lambda ZapII. Second, the cDNA inserts of recombinant lambda gt11 molecules were amplified by PCR and used for preparation of an abundant cDNA pool. The preparation of this pool involved denaturation of the double-stranded cDNA inserts, partial reassociation, and selection of the double-stranded fraction. Single-stranded unnormalized cDNAs were prepared from the lambda Zap II library and mixed with an excess of the abundant cDNA pool. After denaturation and partial reassociation, the single-stranded fraction was selected for preparation of the rare cDNA library.

We have characterized rare cDNA libraries from dauer larvae and adults. Abundant sequences (e.g., Hsp90 and Cytochrome C oxidase subunit I) generally became undetectable, whereas rare clones increased in concentration up to 197-fold. Sequence analysis of 40 clones from the two subtracted libraries showed that 19 corresponded to genes that previously lacked ESTs.

This method should improve the tools for gene discovery in any organism, especially vertebrate systems in which accurate gene predictions from genomic sequences are difficult. It should also have applications in microarray technology, since EST-based microarrays currently lack sequences expressed at low levels. In addition, use of subtracted libraries for hybridization with microarrays should greatly improve the sensitivity of that technology.

1085. A new method of analyzing tissue-specific transcripts in *Caenorhabditis elegans*

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The nematode *C. elegans* is a model organism that is ideal for the study of development, neuronal function, and other biological phenomena. The genome of this organism has been mostly sequenced, and existence of more than 19, 000 genes has been predicted. Genome-wide gene expression analysis provides a number of valuable clues to understanding biological processes. Transcripts that are rarely expressed or expressed in limited number of cells are often difficult to detect when mRNA is prepared from whole worms. We are developing a new method to purify the transcripts from an organ of interest or from small number of cells in the nematode. The poly(A) binding protein, a protein that specifically binds poly(A) RNA, was used to co-purify mRNA from worm cells. Nematode strains that express tagged poly(A) binding protein (Tg-PABP) under the control of tissue-specific promoters were generated. The poly(A) RNA transcribed in cells that harbor the Tg-PABP were then isolated by immunoprecipitation using an antibody against the tag. So far, we have found that the combination of crosslinking mRNA to Tg-PABP, immunoprecipitation with high concentrations of NaCl, and the addition of poly(A) oligonucleotides has increased the specificity of purification. We have started a comparative gene expression analysis of motor and sensory neurons using this technique.

1086. Tetracycline-inducible gene expression system in *Caenorhabditis elegans*

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A significant portion of mutations in *Caenorhabditis elegans* genes cause lethality. To study late effects of a lethal mutant, an inducible promotor system is desireable. In addition, ectopic expression of a candidate gene is a useful way to learn about the (mis-)function of a gene. Ectopic expression in *C. elegans* can be achieved by using a heat shock inducible promotor or by using different endogenous promotors. However for most of these promotors the time of expression and the expression level cannot be controlled. In contrast, inducible promotor systems based on chimeric transcription factors have been widely used to regulate gene expression in many different eukaryotic systems (e.g. insects, mice, yeast and plants). In order to test the efficiency of the tetracycline (Tc) responsive regulatory system in *C. elegans* we generated lines expressing the tetracycline-controlled transactivator proteins (tTA2/rtTA2). These proteins bind to DNA and therefore activate transcription in the presence (rtTA2) or absence (tTA2) of the antibiotics tetracycline or doxycycline (a more potent tetracycline agonist). We have crossed these lines with lines containing a tTA2/rtTA2 responsive promotor fused to a reporter gene and showed that gene expression could be induced (rtTA2) or inhibited (tTA2) by doxycycline treatment. We are constructing different promotor-transactivator fusions to test the ability of this system to direct gene expression in specific cells.

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1087. Flp-mediated site specific recombination

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Flp is a site-specific recombinase encoded by the *S. cerevisiae* 2 micron plasmid. It efficiently catalyzes recombination between two copies of its specific 34 bp recognition site (called the Flp recognition target (FRT)). This reaction works *in vitro* as well as *in vivo* in both prokaryotic and eukaryotic cells. In *Drosophila,* this system is used to generate a variety of recombination events. For example, it has been used to generate tissue specific mosaics by catalyzing mitotic recombination between homologous chromosomes. It has also been used to generate chromosomal deletions, translocations, and inversions. Finally, it has been used as the basis for gene targeting strategies in flies.

One major obstacle to the use of the flp/FRT system in *C. elegans* has been the lack of a simple way to insert single FRT sites into the worm chromosome. I solved this problem by inserting an FRT site into the *Mos1* transposon. Germ-line transposition of this element inserts a single, randomly located FRT site into the genome. I currently have 28 insertions of this element in the genome. We have demonstrated that heat-shock driven flp can catalyze recombination in somatic cells between FRT elements on a transgene. In order to express flp in the germline, I have made transgenic arrays containing complex DNA. I now plan to use these reagents to test the feasibility of using flp/FRT to generate heritable rearrangements in worms.

We are exploring several possible applications of the flp/FRT system in *C. elegans* including:

1. Generation of a library of small, molecularly defined chromosomal deletions. These deletions would be extremely useful in genetic mapping. Further, these could be screened for haploinsufficient genes, or for early lethal phenotypes when homozygous.

2. Generation of new balancers. Currently most balancers are translocations; only a few inversion balancer chromosomes presently exist. Flp/FRT can be used to generate inverted chromosomes for balancers.

3. Mitotic chromosome segregation. *C. elegans* has holocentric chromosomes. This means that centromere function is distributed along the entire length of the chromosome. We will use flp-mediated recombination to explore how holocentric chromosomes segregate after the induction of mitotic recombination.

4. Tissue- and time-dependent transgene control. If a rescuing transgene is generated such that part of the ORF is flanked by tandem FRT sites, the ORF can be specifically disrupted by expression of the flp recombinase. The recombinase can be expressed either by tissue-specific or heat-induced promoters to eliminate gene function in a specific cell type or at a specific time. Conversely, FRT containing constructs can be designed so that expression of a gene can be induced by flp expression.

5. Inserting single copy transgenes. flp/FRT can catalyze the transfer of a piece of DNA that is flanked by tandem FRT sites into a single FRT site on another molecule. This reaction could be used to move a region of a transgene into an FRT located on a chromosome, generating a single copy transgene. This approach has the added benefit that different DNA sequences could be individually recombined into the same chromosomal FRT site, allowing for identical chromosomal environment of different insertions.

6. If you have any other ideas please drop by my poster.

1088. A SURVEY OF CHEMICALS AFFECTING HABITUATION

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C.elegans is a desirable model organism to inquire into the role of chemicals on the nervous system. Numerous chemicals influencing on *C.elegans* behavior have been studied. On the other hand response to mechanical touch or tap stimuli remains to be described. We are now developing a method to access the role by systematic survey of drugs affecting habituation and its retention. TV camera images of *C.elegans* behavior under a stereomicroscope are captured to a personal computer by using tapping pulses as tirgger. A future target is to develop the image processing of sequential images.

We applied the apparatus to pharmacological screening affecting on tap response, especially habituation and recovery from the habituation by changing ISI, pulse or tap number. We have begun with the method to study actions of various drugs including baclofen, barbital, caffeine, chlorpromazine, chlorzoxazone, diazepam, haloperidol, imipramine, mephenesin, perphenazine, phenobarbital, theophylline and (RS)-CPP. Of these, we found that mephenesin causes rapid habituation but slow recovery from habituation.

We are especially interested in the long term habituation. Therefore, in parallel with the work, we are now trying to extend time at the habituated state.

1089. Fully Automated Instrumentation for Analysis in *C elegans*

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The completion of the genomic sequencing of *C. elegans* and *D. melanogaster* enormously advances the understanding of these model organisms. Both organisms are ideally suited for broad system-wide experiments, like transcriptional profiling using DNA microarrays, as well as gene function analysis and their use in chemical screening for drug discovery. However, certain types of experiments would benefit from the development of technologies for high-speed automation and sensitive instrumentation. We have developed the COPAS *BIOSORT* instrument which can accurately select and dispense user-specified numbers of *C. elegans* at specific stages, ranging from embryo through adult, into multiwell microtiter plates. In addition the COPAS *BIOSORT* is coupled to a robotic arm for microtiter plate handling (Zymark Twister), allowing for the analysis of very large numbers of populations. The contents of each well can be analyzed at a later time for changes in developmental stage or gene expression, with the use of COPAS *ReFLX*, an add-on module to the COPAS *BIOSORT*. Analysis is based on the optical properties of the animals. Besides detecting the natural light-scattering properties of *C. elegans*, the COPAS *BIOSORT* can also be used with transgenic animals expressing jellyfish- or reef coral-fluorescent proteins from worm promoters. The system simultaneously performs a quantitative size, autofluorescence, and reporter fluorescence analysis on each organism within a population contained in an individual well (for example, 20 organisms per well) in less than 20 seconds. The data are displayed, collected, analyzed, and stored for later retrieval. This instrumentation has broad applications for genetic screening (for mutants) and chemical screening (for drugs) that affect an optical phenotype. The instrument can also be used for more specific applications such as identifying chromosomal integrants and live-dead screening assays. We will present data

from several reconstruction experiments using the COPAS *BIOSORT* instrumentation.

1090. An analysis of gene redundancy based on the *mec-8* gene

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A surprise of functional genomics in worms and yeast is the extent to which gene knockouts confer no obvious phenotype. Such genes may contribute to fitness in subtle ways, but they may also contribute to essential functions redundantly. Adding to the confusion, genes with overlapping or redundant functions need not be related by DNA sequence. We are using classical genetics and the *mec-8* gene to explore the issue of gene redundancy. MEC-8 is a putative RNA-binding protein that is required for processing of *unc-52* mRNA. Null mutations in *mec-8* confer mechanosensory and chemosensory defects but not highly-penetrant lethality. The combination of a *mec-8* loss-of-function mutation, however, with a viable mutation in any of six *sym* (synthetic lethal with *mec-8*) genes (or with a viable allele of *unc-52*, the prototype analysis for this study) results in embryonic or early larval lethality. *sym-1 X* has been previously shown to encode a hypodermally-secreted, leucine-rich repeat protein that, in the absence of MEC-8, is required for attachment of body muscles to the cuticle. Thus, *sym-1* and *mec-8* compose an example of redundant genes whose products are not related in structure and function, leading to the suggestion that *sym-1* overlaps in function with a gene whose mRNA needs to be processed by MEC-8 (Davies *et al.* 1999; Genetics **153:** 117). With the aim of identifying the hypothesized *mec-8* target and other genes that overlap functionally with *sym-1*, we have been screening for mutations that are synthetically lethal with *sym-1(mn601)*. Candidate *sly* mutations are being genetically characterized.

We are interested in understanding the nature of the overlap between *sym-2 II* and *mec-8*. Rescue experiments have suggested that ZK1067.6 is *sym-2*. DNA sequence analysis has now confirmed this supposition: *sym-2(mn617)* correlates with a change of a tyrosine to an asparagine codon at position 163 in the

618-codon open reading frame. SYM-2 contains three copies of an RNA recognition motif, suggesting that SYM-2 is an RNA binding protein that overlaps with MEC-8 in the processing of an unknown gene transcript that is essential for viability. The structure of SYM-2 will be compared with MEC-8 and with heterogeneous nuclear ribonucleoproteins (hnRNPs) from mammals.

1091. Developing a genetic map of *Caenorhabditis briggsae* emphasizing the *mip-1* region of *LGD*

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Our labs are developing a genetic map of visible markers in *Caenorhabditis briggsae* in order to do comparative studies with the related nematode, *Caenorhabditis elegans*. The two nematodes, though morphologically similar, are evolutionarily separated by approximately 25-40 million years. The genome of *C. briggsae* is currently being sequenced by the Sanger center.

We have isolated 300 visible mutations including the *C. briggsae* versions of dumpy, roller, uncoordinated, multivulva, egg-laying defective, small, and other phenotypes from wild-type *C. briggsae*. These visible phenotype mutants will be used for the construction of a genetic map. In addition to the large-scale genetic map of visible mutations, we are constructing a high-resolution genetic map of the *mip-1 r*egion on chromosome D of *C. briggsae*. The *mip-1* region of chromosome *D* was chosen because the genetic organization of the region surrounding its putative homologue in *C. elegans*, *unc-22 IV*, has been extensively studied in terms of essential gene saturation. The *unc-22 IV* region is the 2 map unit area deleted by *sDf2*.

In *C. briggsae* a large BAC/fosmid contig has been constructed which covers the *unc-22 IV* region (Jacquie Schein, BC Genome Sequencing Centre, Vancouver, BC). BAC DNA plus a dominant marker gene will be injected into *C. briggsae* mutant worms to create transgenic lines in order to test for phenotypic rescue. This will help align the physical map with the growing genetic map of *C. briggsae.* Gene knockouts are being generated for all the genes in *C. elegans*, with human homologues, in the *unc-22* region (Don Moerman, *C. elegans* Knockout Centre, Vancouver, BC). This will give us a powerful tool for comparison with *C.*

briggsae mip-1 region.

In order to assist in the maintenance of lethals, balancers were generated using low dosage X-irradiation. Balancers were isolated that suppressed recombination between the linked markers *cby-7* and *mip-1*. *mip-6*, which lies between *cby-7* and *mip-1*, was used to mark the other *LGD*. Our study indicates that 1500R X-irradiation on *C. briggsae* yields a 3.7% induction rate of mutations that balance the 6.3 map unit interval between *cby-7* and *mip-1*. A total of 36 putative balancers were isolated from this screen.

A preliminary 0.025M EMS mutagenesis screen was carried out to produce lethal mutations within the balanced region of *sC5*. This yielded a lethal induction rate of 7.1%. We have since isolated a more well-behaved balancer (*sC6*) that we are using to induce lethal mutations in the *mip-1* region.

1092. Construction of GFP Tagged Chromosomes

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We have constructed 17 *C elegans* strains carrying GFP or YFP expressing transgenes integrated randomly in wild type chromosomes. The fluorescent transgenes will be useful as benign dominant markers in crosses to replace chromosomes and as weak balancers for mutations in closely linked essential genes. The integrated transgenes began life as an extrachromosomal array, with GFP fused to promoters for *myo-2* and *pes-10* or a gut-specific enhancer from F22B7.9. The arrays were integrated using standard methods (1). The resulting strains were outcrossed four times to wild type. Presently, we are mapping the location of each transgene. We will describe their map positions and phenotypes.

1. Mark Edgley, Jun Kelly Liu, Don Riddle, Andy Fire. Worm Breeder’s Gazette 15(5): 20 (February 1, 1999).

1093. Toward a Genome-Wide RNAi Screen

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In order to fully exploit the *C. elegans* genome sequence, a method must be developed to allow systematic analysis of gene function. To this end, we are constructing a reusable feeding library of bacterial clones capable of targeting *C. elegans* predicted genes by RNAi; our ultimate aim is to produce a library covering the entire genome. We have been using this library to screen genes on each chromosome by RNAi in a wild-type N2 background, scoring for grossly observable phenotypes such as sterility, embryonic or larval lethality, slowed post-embryonic growth rate, and abnormal morphology or behavior of surviving progeny. At present we have completed screening of ~90% of genes on chromosomes I and II, and we anticipate that we will have similarly screened chromosome X by the time of the meeting. Construction and screening of libraries for other chromosomes are also in progress. Thus far, our screening has allowed us to assign phenotypes to ~13% of the genes analyzed. We will present current data from our screen, including the types and numbers of phenotypes observed and a comparison with previously detected forward-genetic loci as a measure of our efficiency in detecting phenotypes. We will also present preliminary analyses of our data with respect to genome content and organization, gene conservation, and functional redundancy.

1094. Automation of PCR Primer Design for the *C. elegans* Gene Knockout Project.

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In preparation for the scale-up of the gene knockout project, oligonucleotide primers for the polymerase chain reaction are required to amplify all known and predicted genes from the *C. elegans* genome. Current gene-knockout protocols (see Barstead, et al. and Edgley et al., this meeting) require two or more nested sets of PCR primers to detect deletions affecting coding sequence. As the number of genes being targeted for knockouts grows, the time consuming process of designing primers becomes a significant factor. Large-scale primer design for various applications has been accomplished using a primer design program developed at the Sanger Centre. A database of primer sets for *C. elegans* genes is currently available at http://elegans.bcgsc.bc.ca/cgi-bin/oligodb.pl.

To meet the specialized needs of PCR primer design for the gene knockout project, we have re-written the program to fully automate primer design. The program uses AcePerl (Lincoln D. Stein and Jean Thierry-Mieg, 1998, Genome Res. 8: 1308-1315.) to extract target-specific information from ACeDB, then assembles a GFF object (Richard Bruskiewich, 1999, Sanger Centre, Wellcome Trust Genome Campus, Cambs, UK). Gene feature information is used to configure primer design so that PCR products sequentially target each exon for a predicted coding region. Based on the amount of coding DNA amplified, the quality of the PCR primer pairs and, in the case of "poison primers" (Edgley et al., this meeting), the exon being targeted, a ranked list of primers is produced. Primers are evaluated by scanning the entire available *C. elegans* genomic sequence for spurious PCR products due to false priming or unwanted primer combinations. Each primer set on the list is evaluated in turn until a set passes quality control. Empirical data from PCR with some 400 existing primer sets are being used to refine the quality control parameters.

The primer-design process requires approximately one month of CPU time to do all predicted *C. elegans* genes. This process is accelerated using parallel processing with a network of Linux servers at the Genome Sequence Centre. Genome-wide primer design for regular and "poison primer" knockout protocols are currently underway and primer information will be made available. To accommodate different primer design criteria on a gene by gene basis, the program is also user-configurable for primer design parameters and the stringency of quality control. The current version can be accessed at ko.cigenomics.bc.ca/oligos.shtml.

1095. A "Poison Primer" Technique for Enhancing Detection of Small Deletions by PCR

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Targeted PCR protocols to detect rare deletions in DNA from complex populations of mutagenized worms typically depend on a large size differential between the wild-type product and the deletion product. These protocols are used with libraries in which deletion representation is about 1 in 20,000. Recent work has shown that a significant proportion of deletions induced by trimethyl psoralen (TMP) treatment followed by UV irradiation are small, on the order of 50 to 600 base pairs (Gilchrist et al., in preparation). Detecting these deletions via PCR presents a problem because deletion amplicons are not favored over wild-type products if the amplicons are similar in size. Rare events will go undetected.

To circumvent this problem we have developed a "poison primer" technique for nested PCR (D'Souza et al., unpublished data). This method works because the poison allows the formation of deletion products but titers out full-sized products. One or two extra primers, lying internal to both the regular external and internal pairs, are included in the external round as competitors to one or both external primers. These "poisons" are designed from coding sequence and are placed roughly in the center of the amplicon. The products they make with one or the other of the external primers are much shorter than the normal external product, and are thus produced in larger amounts. However, products from any deletion that removes sequence corresponding to the poison primers are made normally, with the result that deletion products are enriched relative to wild type. Since only wild-type and deletion products from this round can be amplified by the internal

primers, formation of deletion products is greatly enhanced.

Test reactions using sequenced deletions in the *dim-1* gene and single poisons indicated that this method potentially improves sensitivity to one deletion chromosome in 5,000 wild-type chromosomes. We have isolated new deletions in more than eight genes from mutant libraries using poison primers, at complexities of 1/240 and 1/1200. Sequence data from the first three (deletions of 341, 295, and 78 base pairs) confirms that in all cases at least the 3' end of the poison primer is deleted, and control reactions on library DNA without poisons demonstrates that the poison primer was essential for detection. We are now using this system routinely for library screening, in addition to standard screens for larger deletions, to maximize the yield from each library. The specfic targeting feature of this technique should allow screens for small designer deletions, for example deletions of alternatively spliced exons or regulatory sequences.

1096. Application of the COPAS (Complex Object Parametric Analysis and Sorting) dispenser to functional genomic studies.

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The completion of the *C.elegans* genome sequence has facilitated the identification of most genes at the sequence level. This genomic data can be used to investigate how physiology and development are regulated globally . Investigators rely on the development of functional genomic tools that aid quicker identification of gene function after sequence discovery. One emerging technology in the area of functional genomics is the development of a unique sorter/dispenser for *C.elegans* available from Union Biometrica Incorporated, Somerville, MA, USA.

The COPAS (complex object parametric analysis and sorting) dispenser is capable of sorting a heterogeneous population of worms based on size (developmental stage), shape (phenotype, based on density by signal extinction), or fluorescent properties (GFP reporter expression, for example). Funding to obtain a COPAS machine for our laboratory has been provided by the Wellcome Trust. Our facility will be made available as a resource to the UK *C.elegans* research community.

The COPAS worm sorter has many applications relevant to functional genomic studies. These include;

1. The production of highly enriched, specifically staged worms. Accurately staged worms can be used for expression studies using Northern blots, RT-PCR or DNA microarray profiling.

2. The ability to detect fluorescent signals. It is possible to sort animals expressing (or mis-expressing) a fluorescent reporter, even if the reporter is only expressed during a narrow period of development.

3. The production of highly enriched single sex populations using GFP reporters in embryos and young larvae or light extinction properties in adults.

4. Dispensing of individual nematodes after mutagenesis into microtitre wells to facilitate large scale clonal screening and to generate a deletional mutagenesis library. Thus it is now conceivable to screen tens of thousands of worms with the hope of approaching mutational saturation for specific traits.

In addition to the uses described above our laboratory is taking advantage of the COPAS dispenser to generate GFP labelled genetic balancers and chromosomes. The examples above have been provided to illustrate the utility of the worm flow sorter; it is anticipated that innovative uses for the device will be developed by individual researchers to address their particular experimental needs.

1097. Automated Sorting of *C. elegans* Muv Mutants According to Pseudovulva Number

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The UBI COPAS *BIOSORT* system disperses and maintains nematodes in liquid suspension, straightens them, and flows them one-by-one through a laser beam. The system analyzes and sorts each animal according to both size and fluorescence without harm.

For this study the system was modified such that positional information was retained as the nematodes were scanned along their longitudinal axis. Rather than averaging the fluorescence signal over the length of the animal, the signal was measured at approximately 100 points along the length of adult *C. elegans*. Profiling algorithms were developed that determined the location and the number of fluorescent features of the animals. The prototype was used to characterize and sort multivulva (Muv) *C. elegans* according to the number of vulvae.

The Muv strains MT309 lin-15 and MT388 lin-12 (both obtained from the *C. elegans* Genetics Center, University of Minnesota, St. Paul), as well as wild-type strain N2, were stained using NEMASELECT *Male/Herma* stain (Union Biometrica, Inc.) to mark the location of their vulvae. Electronic profiles were collected with the new signal processing electronics and single nematodes were deposited in individual wells of a 96-well plate. Nematodes and their associated profiles were visually compared. Close correlation between the number of stained vulva and the number of fluorescence peaks in the profiles was observed. The prototype software accurately scored and sorted the animals according to vulva number.

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1098. Thermokinesis: A New Associative Learning Paradigm

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Thermotaxis represents one of a limited set of C. elegans behaviors that lends itself to the study and manipulation of behavioral plasticity. As the name implies, thermotaxis involves a directed movement, which is dependent on the previous thermal experience of the worm (Hedgecock and Russell, PNAS, 1975 Oct;72(10):4061-5). Here, we demonstrate that the ability of worms to thrash in liquid media [previously described as a high-frequency locomotory behavior (Miller et al., PNAS, 1996 Oct 29;93(22):12593-8)] is modulated by temperature and cultivation experience. We name this behavior thermokinesis and define it as the temperature and experience modulated movement of an animal with no specified body-axis orientation.

Worms are initially grown at a pre-determined temperature. In contrast to thermotaxis where worms are placed on solid media containing a temperature gradient, thermokinesis involves placing individual animals into solution of a set temperature and counting the number of thrashes per minute. Wild type worms modify their thrashing rate in a way that depends on their previous thermal experience. If placed into a solution of the same temperature as their cultivation temperature (e.g. 20°C), N2 worms will thrash at an intermediate rate. If placed into a solution warmer than their cultivation temperature (e.g. 25°C), worms will increase their thrashing rate relative to that intermediate rate. Conversely, worms will slow down if placed into colder solution (e.g. 15°C). This response is more than a metabolic or neuromuscular reaction to temperature as ttx-3 mutants, which affect AIY and AIA interneuron differentiation, are defective in this behavior. These mutant worms thrash slowly at all assay temperatures regardless of their cultivation temperature.

ceh-14, tax-2, tax-4 and tax-6, which are impaired in thermotaxis due to defects in AFD thermosensory neuron development and function, have an unexpected thermokinesis phenotype. These strains are still able to perceive the assay temperature and therefore have the wild type profile of fast, intermediate and slow rates at 25°, 20° and 15°, respectively. However, they cannot vary this profile based on their cultivation temperature. Thus, this class of mutants is able to modify thrashing rates depending on the assay temperature but this modification is independent of cultivation temperature. We conclude from this result that thermokinesis utilizes two distinct thermosensory mechanisms: one is the classical AFD-mediated pathway and the other is a novel mechanism.

In addition to cultivation temperature, thermokinesis also depends on the worm’s previous growth conditions revealing the associative nature of this behavior. When wild type worms are starved they modify their thrashing rates so as to slow down at the assay temperature corresponding to the starvation temperature but speed up at all other temperatures. Taken together, thermokinesis can be separated into three genetically distinct processes: 1) Perception of cultivation temperature, 2) Perception of temperature encountered during the assay, and 3) Perception/integration of food/starvation signals during cultivation.

We will describe various classes of mutants that are defective in one or more of these components including a homeobox gene, calcium channel components, dopamine signaling and serotonin signaling mutants. We will also describe our efforts to identify the cellular components of this behavior based on laser ablation studies.

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1099. Characterization of translation initiation factor eIF4E in *C. elegans* development

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Translational control of maternal and zygotic mRNAs is a critical mode of gene regulation during embryonic development. The control generally involves regulated recruitment of mRNAs to ribosomes. Recruitment begins with the recognition of the mRNA 5' cap by translation initiation factor 4E (eIF4E). To understand how translational control occurs during development, we are studying the regulation of eIF4E and mRNA cap recognition.

The majority of *C. elegans* mRNAs contain an unusual trimethyl-guanosine (TMG) cap structure as a result of *trans*-splicing in the nucleus. The remaining mRNAs contain a canonical monomethyl-guanosine (MMG) cap. We have previously characterized five eIF4E isoforms (IFE-1, -2, -3, -4, and -5) expressed in *C. elegans*, which share 54-80% amino acid sequence similarity [*J. Biol. Chem. 275*, 10590-10596 (2000)]. IFE-1, -2 and -5 bind to both TMG and MMG caps, while IFE-3 and IFE-4 recognize only MMG caps. This suggests that IFE's are involved in the translation of both conventional and *trans*-spliced mRNAs.

Three IFE's (-1, -3 and -5) are enriched in the adult gonad. IFE-1 is specifically associated with the RNA-binding protein PGL-1 in P granules during germline development. Depletion of IFE-1 causes defects in spermatogenesis but not oogenesis (see Amiri, *et al.* abstract). Another germline-enriched isoform, IFE-3, is most similar to human eIF4E and is essential for embryonic viability in the worm. Homozygous *ife-3(ok191)* embryos arrest in early division stages of embryogenesis, indicating that it performs a unique function early in development. At least one of the TMG-binding isoforms is also required for embryo viability, suggesting that one eIF4E of each specificity is essential for translation of *C. elegans* mRNAs. IFE-2 is found primarily in somatic tissue. Given the differences in cap-binding specificity, tissue-specific expression and functional requirement, eIF4E isoforms may provide a basis for translational control mechanisms used in animal development.