2002 West Coast Worm Meeting Program

GENOMICS
Amy Pasquinelli, chair
Saturday, 10 August 2002, 7-11

1. The C. elegans Knockout Consortium: Interim Report
   Robert Allan, Robert Barstead, Mark Edgley, Keiko Gengyo-Ando, Jeff Holmes, Meghan Hughes, Steven Jones, Yuji Kohara, Martin Lansdale, Lucy Liu, Jason Maydan, Sheldon McKay, Etsuko Machiyama, Shohei Mitani, Don Moerman, Gary Moulder, Maimun Nisha, Sachiko Noguchi, Jamie Osborn, Anna Rankin, Nadereh Rezania, James Robertson, Beth Rogers, Bin Shen, Miwa Tamura, Malini Viswanathan

2. THE C. ELEGANS ORFEOME CLONING PROJECT : VERSION 1.0
   Philippe Vaglio, Jérôme Reboul, Jean-François Rual, Philippe Lamesch, Laurent Jacotot, Monica Martinez, Christopher Armstrong, Nicolas Bertin, Alban Chesneau, Rekins Janky, Troy Moore, Jim Hartley, Mike Brasch, Hongmei Lee, Lynn Doucette-Stamm, David E Hill, Marc Vidal

3. WormBase, a growing source of worm information
   WormBase Consortium

4. Functional Proteomics of the Mammalian Midbody Reveals Conserved Cell Division Components
   Ahna Skop, Hongbin Liu, John Yates III, Barbara Meyer, Rebecca Heald

5. Discovery of DAF-16 Targets and Novel Lifespan Genes: Microarray Analysis of the C. elegans DAF-2 Pathway
   Coleen T. Murphy, Steven A. McCarroll, Hao Li, Cynthia J. Kenyon

6. Genome-wide RNAi analysis of C. elegans fat regulatory genes
   Kaveh Ashrafi, Francesca Y. Chang, Jennifer L. Watts, Andrew G. Fraser, Ravi S. Kamath, Julie Ahringer, Gary Ruvkun

7. COMPARATIVE FUNCTIONAL GENOMICS: USING CONSERVATION TO UNDERSTAND GENE EXPRESSION PROGRAMS
   Steven A. McCarroll, Coleen T. Murphy, Sige Zou, Scott D. Pletcher, Yuh Nung Jan, Cynthia Kenyon, Cornelia I. Bargmann, Hao Li

8. Deciphering the Combinatorial Code Controlling Pharyngeal Gene Expression
   Jeb Gaudet, Susan Mango

9. Targets of Homeobox Genes in C. elegans
   Andreas Eizinger, Tibor Vellai, Fritz Müller, Stuart K. Kim

10. An RNAi screen to identify regulators of pharyngeal development.
    Pliny A. Smith, Susan E. Mango

11. Functional analysis of the C. elegans genome by RNA-mediated interference
    Andrew Fraser, Ravi Kamath, Yan Dong, Peder Zipperlen, Gino Poulin, Monica Gotta, Natalie le Bot, Sergio Moreno, Julie Ahringer

12. Check out my Profile! Isolation of chemotaxis defective mutants with altered str-1 expression levels using automated, high-sensitivity fluorescence profiling.
    Anthony A. Ferrante, Britta Moellers, Jennifer Kean, Gregory O'Connor, Vance Chang, Bruce Holcombe, Peter Van Osta, Steven Alam

13. Functional Characterization of the Conserved Set of C. elegans Nuclear Hormone Receptors
    Marc R. Van Gilst, Keith R. Yamamoto

CELL BIOLOGY
Janet Richmond, chair
Sunday, 11 August 2002, 8:30-12:15

14. Relationship between the synaptonemal complex and progression of meiotic recombination in C elegans
    Monica P. Colaiacovo, Amy J. MacQueen, JoAnne Engebrecht, Cinzia Rinaldo, Adriana La Volpe, Anne M. Villeneuve

15. pom-1 is a Conserved Regulator of Cell Polarity and Cell Division in the C. elegans Embryo and Fission Yeast
    Jason Pellettieri, Valerie Reinke, Stuart Kim, Geraldine Seydoux

16. ICD-1, a putative target of CED-3, protects cells from programmed cell death
    T. Bloss, E. Witze, J.H. Rothman

17. C. elegans tubulin genes tbb-2 and tba-2 are required for microtubule severing of the MEI-1/MEI-2 katanin complex during meiotic spindle formation.
    Chenggang Lu, Francis J. McNally, Paul E. Mains

18. The C. elegans TIMELESS homolog is an essential regulator of chromosome cohesion.
    Raymond C. Chan, Annette Chan, Mili Jeon, Tammy F. Wu, Ann Rougvie, Barbara J. Meyer

19. Mutations in alpha and beta tubulin genes affect spindle orientation in the one-cell stage C. elegans embryo
    Jennifer B. Phillips, Gregory C. Ellis, Rebecca Lyczak, Bruce Bowerman

20. Glycosyltransferases mediate Bt toxin action in C. elegans
    Joel S. Griffitts, Lisa D. Marroquin, Raffi V. Aroian

21. The egl-41 gene, identified by gf mutations that cause partial sexual transformation, is identical to sel-10, a negative regulator of lin-12
    Ning Pan, Elizabeth Kimberly, Beryl Hatton, Ute Meisel, Manisha Dudley, Ding Xue

22. The Roles of ham-1 and hlh-14 in Neuroblast Divisions
    C. Andrew Frank, Nancy Hawkins, Gian Garriga

23. C. elegans evl-20 Gene Encodes a Functional Homologue of Human Small GTPase ARL2 and Regulates Cytoskeleton Dynamics during Cytokinesis and Morphogenesis
    Igor Antoshechkin, Min Han

24. Proper GABAA receptor trafficking depends on correct synapse formation
    Aaron M. Rowland, Jason G. Olsen, Bruce A. Bamber

25. A role for cbp-1, the worm homolog of the histone acetyl transferases CBP and p300, in vulval induction.
    Dennis Eastburn, Min Han

26. Identification of CHE-13, a novel IFT protein required for cilia formation
    Courtney J. Haycraft, Jenny C. Schafer, Qihong Zhang, Patrick D. Taulman, Bradley K. Yoder

NEUROBIOLOGY
Miriam Goodman, chair
Sunday, 11 August 2002, 7-11

27. A BK potassium channel mediates the behavioral effects of ethanol in C elegans
    Andrew G. Davies, Jonathan T. Pierce-Shimomura, Hongkyun Kim, Tod R. Thiele, Antonello Bonci, Steven L. McIntire

28. GPB-2 Interacts With Two Muscarinic Acetylcholine Receptors In The Pharynx
    Kate A. Steger, Leon Avery

29. A GABA-gated non-selective cation channel in C. elegans.
    Asim A. Beg, Erik M. Jorgensen

30. Molecular Mechanism of Synaptogenesis
    Kang Shen, Cori Bargmann

31. Identifying the role of endophilin in synaptic vesicle endocytosis
    Janet Richmond, Kim Schuske, Daniel A. Rube, Dawn Signor Matthies, Warren Davis, Steffen Runz, Alexander M. van der Bleik, Erik M. Jorgensen

32. Dense core vesicle trafficking in neurons visualized in vivo
    Tobias R. Zahn, Joseph K. Angleson, Margaret A. MacMorris, John C. Hutton

33. Mutations that Activate the Gs alpha Pathway Bypass the Neurotransmitter Release Blockade in ric-8(md303) and Reveal Another Branch of the Synaptic Signaling Network
    Nicole K. Reynolds, Lisa Coclazier, Jayabarathy Rajaiya, Claudia M. Dollins, Kenneth G. Miller

34. Cellular physiology of cultured C. elegans mechanosensory neurons
    Laura Bianchi, Jian Xue, Monica Driscoll

35. Analyses of mechanosensory transduction by in vivo calcium imaging
    Hiroshi Suzuki, Rex Kerr, Christian Froekjaer-Jensen, Dan Slone, Jian Xue, Monica Driscoll, William Schafer

36. Intracellular Ca++ increase in ASH sensory neuron in response to water-soluble chemical repellents.
    Massimo A. Hilliard, Rex Kerr, Hiroshi Suzuki, Alfonso J. Apicella, Paolo Bazzicalupo, William R. Schafer

37. Dissecting the Neural Circuit Controlling Chemotaxis in C. elegans
    Jesse Gray, Cori Bargmann

38. The DAF-7 TGF-beta signaling pathway regulates chemosensory receptor gene expression in C. elegans
    Katie Nolan, Trina Sarafi-Reinach, Jennifer Horne, Adam Saffer, Piali Sengupta

39. unc-37/Groucho and cog-1/Nkx6.2 Act to Specify Left/Right Asymmetric Cell Fate in a Set of Chemosensory Neurons
    Sarah L. Chang, Robert J. Johnston, Oliver Hobert

40. Serotonin modulation of a chemosensory circuit in C. elegans
    Michael Y. Chao, Hidetoshi Komatsu, Rhonda Hyde, Heather M. Dionne, Anne C. Hart

DEVELOPMENT
Noelle L'Etoile, chair
Monday, 12 August 2002, 8:30-12:15

41. CED-10/Rac and UNC-34/Enabled mediate distinct pathways in UNC-6/Netrin-dependent axon attraction
    Zemer Gitai, Tim Yu, Erik Lundquist, Marc Tessier-Lavigne, Cori Bargmann

42. Three miscellaneous axon guidance genes
    Catherine Chiu, Ray Squires, Scott G. Clark

43. Axon guidance at the ventral nerve cord by UNC-71, a disintegrin and metalloprotease protein
    Xun Huang, Peng Huang, Matthew K. Robinson, Michael Stern, Yishi Jin

44. mig-21 Encodes a Novel Putative Transmembrane Protein Required for the Asymmetric Q Neuroblast Migrations
    Lisa Williams, Cynthia Kenyon

45. Regulation of Epidermal Cell Fusion in C. elegans
    Scott Alper, Cynthia Kenyon

46. VAB-19, a novel conserved protein involved in epidermal elongation in C. elegans
    Mei Ding, Wei-meng Woo, Andrew D. Chisholm

47. Nicotine adaptation at the molecular level - the dynamics of nAChR expression and localization
    Alexander Gottschalk, William R. Schafer

48. Characterization of the class A synMuv proteins LIN-56 and LIN-15A
    Ewa M. Davison, John DeModena, Linda S. Huang, Paul W. Sternberg, Bob Horvitz

49. LIN-12 downregulation in response to Ras activation in P6.p requires endocytosis and is necessary for lateral signaling
    Daniel Shaye, Iva Greenwald

50. lin-17, lin-18 and patterning of the P7.p lineage
    Takao Inoue, Rashmi Deshpande, Russell Hill, Paul W. Sternberg

51. The transcriptional Mediator complex functions in Wnt, Notch and Ras signaling pathways and may integrate the pathways during the vulval development
    Akinori Yoda, Hiroko Kouike, Hideyuki Okano, Hitoshi Sawa

52. Mutational Analysis of the Sex Determining Protein FEM-1
    Usha Viveganantha n, Ian Chin-Sang, David Lum, Andrew M. Spence

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

AGING and DISEASE MODELS
Andrew Dillin, chair
Monday, 12 August 2002, 1:30-3:30

54. Searching for mechanisms of neuronal synchrony in the convulsing worm
    Allyson V. McCormick, James H. Thomas

55. Constitutive promotion of muscle protein degradation by FGF is prevented by a DAF-2 signaling pathway
    Nate Szewczyk, Brant Peterson, Sami Barmada, Leah Parkinson, Lew Jacobson

56. A conserved p38 MAP kinase pathway in C. elegans innate immunity
    Dennis H. Kim, Rhonda Feinbaum, Genevieve Alloing, Fred E. Emerson, Danielle A. Garsin, Hideki Inoue, Miho Tanaka-Hino, Naoki Hisamoto, Kunihiro Matsumoto, Man-Wah Tan, Frederick M. Ausubel

57. Gustatory and olfactory neurons regulate C. elegans longevity
    Joy Alcedo, Cynthia Kenyon

58. C. elegans - a new model for the human peroxisomal disorders
    Oleh I. Petriv, David B. Pilgrim, Richard A. Rachubinski, Vladimir I. Titorenko

59. A model of cholesterol trafficking and NP-C1 disease in C. elegans
Jie Li, Gemma Brown, Michael Ailion, James H. Thomas

60. Molecular Identification of Transcriptional Targets of the DAF-16 Winged Helix Transcription Factor
Joshua J. McElwee, Kerry Bubb, James H. Thomas

61. ANC-1 tethers nuclei by connecting the nuclear envelope to the actin cytoskeleton
Daniel A Starr, Min Han

BEHAVIOR
Bruce Bamber, chair
Monday, 12 August 2002, 4-6

62. Identification of tph-1 regulators reveals a role of TRP channels in serotonin synthesis
Shenyuan Zhang, Gabriela Blanco, Kai-Xuan Shi, Ji Ying Sze

63. Pharyngeal synchrony in Rhabditida
Boris Shtonda, Leon Avery

64. Evolution of Nematode Feeding Behavior
Mark Steciuk, Boris Shtonda, Leon Avery

65. slo-1 modulation of neuronal activity in the pharynx
Alan Chiang, Leon Avery

66. Long-term memory requires non-NMDA excitatory receptor function and produces decreased GLR-1::GFP expression
Jacqueline, K., Rose, Sylvia, H., Chen, Catharine, H., Rankin

67. Genetic Analysis of the Neuromodulatory Control of Search Behavior in C. elegans
Thomas T. Hills, Fred Adler, Andres Villu Maricq

68. Fluoxetine response genes in C. elegans
John M. Kemner, James H. Thomas

69. Different pathways function at different times to regulate longevity of C. elegans
Andrew Dillin, Ao-lin Hsu, Douglas Crawford, Andrew Fraser, Ravi Kamath, Julie Ahringer, Cynthia Kenyon

EMBRYOGENESIS
Andrew Fraser, chair
Tuesday, 13 August 2002, 8:30-12

70. Characterization of nmy-1, a suppressor of mel-11
Alisa J. Piekny, Gwendolyn D. Cham, Jacque-Lynne F. Johsnon, Paul E. Mains

71. Nuclear POP-1 asymmetry between A-P sisters is regulated by a 14-3-3 mediated nuclear export mechanism
Miao-Chia Lo, Rueyling Lin

72. The Role of par Genes in the Regulation of POP-1 Asymmetry
Raanan S. Odom, Rueyling Lin

73. LET-99 determines spindle position and is asymmetrically enriched in response to PAR polarity cues in C. elegans embryos
Meng-Fu Bryan Tsou, Lesilee S. Rose

74. New Insights into the Mechanisms Underlying the A-P Polarity Brought by pod Genes
Akiko Tagawa, Chad A. Rappleye, Raffi V. Aroian

75. Mutants that affect gamete function at fertilization
Andrew Singson, Indrani Chatterjee, Emily Putiri, Pavan Kadandale, Brian Geldziler, Marty Nemeroff

76. Development and fertility in C. elegans clk-1 mutants depends upon transport of dietary coenzyme Q8 to mitochondria
Tanya Jonassen, Beth N. Marbois, Kym F. Faull, Catherine F. Clarke, Pamela L. Larsen

77. FRK-1: Roles of a Fer-type non-receptor tyrosine kinase in cadherin and integrin cell adhesion systems and Wnt signaling
Aaron P. Putzke, Joel H. Rothman

78. AIR-2 Regulates the Selective Release of Chromosome Cohesion during Meiosis I
Eric Rogers, John D. Bishop, James A. Waddle, Jill M. Schumacher, Rueyling Lin

79. HIM-17, a novel protein required for proper initiation of meiotic recombination in C. elegans
Kirthi Reddy, Anne Villeneuve

80. Characterization of HCP-6, a C. elegans protein required to prevent chromosome twisting
Jeffrey H. Stear, Mark B. Roth

81. TEP-1, a C. elegans telomere binding protein, is required for chromosome stability
Soon Baek Hwang, Seung Hyun Kim, In Kwon Chung, Junho Lee

POSTERS
Sunday, 11 August 2002, 1-6
Workshops 1:30-5:30

82. An RNAi-based screen for genes involved in the regulation of lifespan by the reproductive system
Jennifer Ramond Berman, Nuno Arantes-Oliveira, Andrew Dillin, Malene Hansen, Allen Hsu, Cynthia Kenyon

83. Measuring C elegans Sensory Functions Across Lifespan
Charles Glenn, Catherine A. Wolkow

84. Gene expression changes in clk-1 and caloric-restricted worms.
James Lund, Stuart K. Kim

85. Protein Repair Deficient Worms Reproduce and Age Normally
Kelley L. Banfield, Pamela L. Larsen, Steven G. Clarke

86. Showing sensitivity to Bacillus thuringiensis toxins
Karen Chien, Adam Boutin, Joel Griffitts, Raffi V. Aroian

87. C. elegans as a model host for the Bacillus thuringiensis toxin Cry5B
Danielle L. Huffman, Raffi V. Aroian

88. Functional characterization of a structural motif in UNC-11 that has been implicated in phosphoinositide binding.
Kesheng Liu, Ravit Golan, Kondury Prasad, Andrea M. Holgado, Suping Jin, Eileen M. Lafer, Aixa Alfonso

89. cen-1 is required for centrosome maturation in the early Caenorhabditis elegans embryo.
Bruce Nash, Bruce Bowerman

90. Identification of Genes Involved in Sterol Metabolism in C. elegans
Neal Freedman, Jen-Chywan Wang, Keith R. Yamamoto

91. Intermediate Filament IFB-1 Functions in Epidermal Morphogenesis and is a component of trans-epidermal attachments
Wei-meng Woo, Andrew D. Chisholm

92. let-711: A GENE INVOLVED IN SPINDLE POSITIONING
Leah R. DeBella, Lesilee S. Rose

93. A protein phosphatase 4 homologue, PPH-4.1, is essential for centrosome maturation in mitosis and sperm meiosis in C. elegans
Eisuke Sumiyoshi, Fumio Motegi, Asako Sugimoto, Masayuki Yamamoto

94. Mutations affecting mitotic spindle position in the C. elegans embryo.
Morgan Goulding, Bruce Bowerman

95. Analysis of the Intraciliary Transport Motor, OSM-3
Regina S. Walker, Jonathan M. Scholey

96. A Genetic Analysis of Neuronal Polarity in C. elegans
Miriam R. Kaplan, Cornelia I Bargmann

97. glo-1 is necessary for lysosome-related organelle biogenesis in C. elegans
Greg J. Hermann, Caroline A. Hieb, James R. Priess

98. Synaptic vesicle kinesin and synapse development
Ewa Bednarek, Christelle Gally, Jean-Louis Bessereau, Erik M. Jorgensen

99. slr-2 and slr-8, two genetic modifiers of lin-35/Rb in C. elegans
Aaron M. Bender, David S. Fay

100. Germline precursor cells arrest at G2/prophase during embryogenesis and dauer diapause
Masamitsu Fukuyama, Joel H. Rothman

101. Developmental control of cell cycle in C. elegans
R. Mako Saito, Sander van den Heuvel

102. cep-1/p53-Independent Activation of Apoptosis in the C. elegans Germline
Tak Hung, Kevin Keegan, Mark Lackner, Mark Maxwell, Garth McGrath, Lisa Moore, Carol O'Brien, Dianne Parry, Mike Costa

103. Identification of candidate genes that function in CED-3-independent programmed cell death
Thomas A. McCloskey, Mark Dorfman, Joel H. Rothman

104. ceh-16: the C. elegans ortholog of the evolutionary conserved engrailed gene
Giuseppe Cassata, Ralf Baumeister

105. A New Mutation that Affects Distal Tip Cell Migration
Tsai-Fang Huang, Yi-Chun Wu

106. mig-29 encodes a SEC34 vesicle trafficking protein required for cell migration in C. elegans .
Yukihiko Kubota, Norio Suzuki, Kiyoji Nishiwaki

107. Extending the unc-53 pathway - Part 1: Yeast two hybrid reveals interactors
Adetayo O. Adeleye, Samantha J. Grainger, David L. Baillie, Eve G. Stringham

108. Extending the unc-53 pathway- Part II: Isolation of genetic suppressors.
Ardelle C. Stauffer, Nelvia Van Dorp, Eve G. Stringham

109. A Wnt gradient may guide HSN migration.
James B. Endres, Shaun Cordes, Nancy Hawkins, Gian Garriga

110. Identification of proteins interacting with MIG-13 in Q cell migration
Yung S. Lie, Scott Anderson, John R. Yates, Cynthia Kenyon

111. Mechanisms of odor discrimination
Makoto Tsunozaki, Cori Bargmann

112. Imaging neuronal activity in AWC chemosensory neurons
Jami Milton Dantzker, Cori I. Bargmann

113. Modulation of Olfactory Adaptation in C. Elegans
Gloriana V. Gallegos, Noelle L'Etoile

114. Visualizing OSM-9::GFP in AWC during odor exposure
Aimee Warren, Amanda Kahn-Kirby, Cori Bargmann, Noelle L'Etoile

115. Helping Chromosomes Pack Up and Split: The Role of Condensin in Mitotic and Meiotic Chromosome Segregation
Kirsten Hagstrom, Hongbin Liu, John Yates, Barbara Meyer

116. Cohesin proteins regulate the reorganization of the holocentric chromosome during mitosis in the C. elegans embryo
Landon L. Moore, Mark B. Roth

117. Proteomic approach identifies a single-stranded telomere binding protein in the nematode Caenorhabditis elegans
Kyu Sang Joeng, Junho Lee

118. Posterior body contraction - novel fast signaling
Maureen A. Peters, Paola Dal Santo, Asim Beg, Erik M. Jorgensen

119. Wormatlas: A web-based behavioral and structural atlas of C. elegans
David H. Hall, Thomas Boulin, Maurice Volaski, Michael Zhang, Constantinos Polydorou, Zeynep F. Altun

120. The Incyte Genomics BioKnowledge LibraryTM: A comprehensive, integrated resource for the analysis of functional conservation between model organismal and mammalian proteins
Angela R. Winnier, Marek S. Skrzypek

121. DPR-1, a membrane-associated nuclear hormone receptor, prevents dauer recovery
Erin Newman-Smith, Gina Broitman-Maduro, Marina Len, Joel H. Rothman

122. bug-3, a new regulator of asymmetric cell division in C. elegans
Shaun Cordes, C. Andrew Frank, Gian Garriga

123. Genetic analysis and molecular cloning of nsy-2
Chiou-Fen Chuang, Alvaro Sagasti, Cori Bargmann

124. Specification of Individual Olfactory Neuron Function
Marc E Colosimo, Piali Sengupta

125. Sensory Neuron Cell Fate Determination
Miri K VanHoven, Sarah B Huang, Cori I Bargmann

126. Dissecting the C. elegans ray developmental pathway with DNA microarrays
Douglas S. Portman, Scott W. Emmons

127. Genetic analysis of the C. elegans Pax-6 locus and characterization of its functions in epidermal morphogenesis
Hediye Nese Cinar, Andrew D. Chisholm

128. The roles of EFN-4 in embryonic morphogenesis and oogenesis
Sarah L. Moseley, Andrew D. Chisholm

129. The temperature sensitive lethal mutation or566 may define a new gene that functions in early morphogenesis
Sabrina G. C. Shore, Sarah L. Moseley, Andrew D. Chisholm

130. Identification of the downstream genes of lon-1
Kiyokazu Morita, Min Han

131. Identifying genetic loci that control early pharynx morphogenesis
Michael F. Portereko, Susan E. Mango

132. PAR proteins and cell polarity after the one-cell stage
Jeremy Nance, James R. Priess

133. Studies of mutations involved in vulval development in C. elegans
Fang Wang, Igor Antoshechkin, Mitsunobu Hara, Min Han

134. Identifying genetic loci that control early pharynx morphogenesis
Michael F. Portereiko, Susan E. Mango

135. Ephrin/Eph Receptor Kinase Signaling in Caenorhabditis elegans
Simona Ghenea, Ian Chin-Sang

136. slr-5 and slr-9 function with lin-35/Rb to control pharyngeal morphogenesis in C. elegans.
Monica Darland, David S. Fay

137. Regulation of the FoxA homologue pha-4 in organ development
Dustin L. Updike, Susan E. Mango

138. Characterizing ELT-7, an apparent redundant partner with ELT-2, in intestinal organogenesis
Keith Strohmaier, James D. McGhee, Joel H. Rothman

139. A system for intestine-specific RNA interference
Benjamin Leung, James R. Priess

140. Analysis of pel-2, a gene required for embryonic pharyngeal differentiation and body morphogenesis
Kelly A. Harradine, Joel H. Rothman

141. Dimers of GLD-1 bind to a single TGE repeat of the tra-2 mRNA 3'-untranslated region
Sean P. Ryder, Dana L. Abramovitz, Elizabeth B. Goodwin, James R. Williamson

142. Knockout analysis of putative germline-related genes in C. elegans.
Takeshi Karashima, Tetsuo Yasugi, Yasuko Sakurai, Ikuma Maeda, Masayuki Yamamoto

143. Vulval development and glycosaminoglycan biosynthesis
Sara Olson, Ho-Yon Hwang, Jeffrey D. Esko, H. Robert Horvitz

144. A role for ephrin signaling in vulval development?
Martin L. Hudson, Andrew D. Chisholm

145. SITE-DIRECTED MUTANTS OF THE C. elegans TRANSCRIPTION FACTOR LIN-31 REVEAL DISTINCT FUNCTIONS.
Corey A. Morris, David B. Doroquez, E. Lorena Mora-Blanco, Yvonne Yang, Leilani M. Miller

146. Genome-wide search for LAG-1 target genes that function in the AC/VU decision and VPC specification
Andrew S. Yoo*, Carlos Bais*, Han J. Yu, Iva Greenwald

147. Genetic analysis of the vulval mutants in C. briggsae
Bhagwati P. Gupta, Jennifer X. Li, Shahla Gharib, Paul W. Sternberg

148. Deficiencies in C20 polyunsaturated fatty acids cause behavioral and developmental defects in C. elegans fat-3 mutants
Jennifer Watts, John Browse

149. Analysis of learning, short-term and long-term memory in the presenilin mutant strains sel-12 and hop-1
Catharine, H., Rankin, Jacqueline, K., Rose

150. Toward the identification of novel regulators of b-catenin signaling in C. elegans
Steve Gendreau, Changyou Chen, Damian Curtis, Emery Dora, Lorena Mora-Blanco, Mike Costa

151. sbp-1, a homolog of SREBP, controls lipid metabolism in the worm
Jennifer Huber, Lisa Kadyk, Mike Costa, Kim Ferguson, Carol O'Brien, Jennifer Watts, Sarah Elson, Tak Hung, Steve Doberstein, Cynthia Seidel-Dugan

152. A Genetic Selection for Amphetamine Resistance
Miles Trupp, Steven L. McIntire

153. A Genetic Study of Muscular Dystrophy in C. elegans
Hongkyun Kim, Steven L. McIntire

154. Mapping chromosomal targets of the dosage compensation complex
Györgyi Csankovszki, Barbara J. Meyer

155. An XO-lethal mutation identifies a gene with interesting friends.
Tammy Wu, Christian Hassig, Bobby Tsang, Paola Dal Santo, John Plenefisch, Barbara Meyer

156. dsh-2 is required to polarize asymmetrically dividing cells.
Nancy C. Hawkins, Gregory Ellis, Bruce Bowerman, Gian Garriga

157. Identification of Embryonic Mesoderm Genes Using Microarrays
Morris F. Maduro, Gina Broitman-Maduro, Joel H. Rothman

158. Genetic Analysis of Asymmetric LET-99 Localization
Jui-ching Wu, Meng-Fu Bryan Tsou, Lesilee S. Rose

159. Mapping and cloning of generation vulvaless mutants of Pristionchus pacificus
Jagan Srinivasan, Ralf J. Sommer

160. Global synteny between C. briggsae and C. elegans
Todd W. Harris, Jason Stajich, Marc Sohrmann, Lincoln D. Stein

161. Analysis of Dauer Formation Mutations in C. briggsae
Hannah Kim, Shirley Phan, Takao Inoue, Paul W. Sternberg

162. Selection shapes patterns of neutral polymorphism in C. elegans' genome
Asher D. Cutter, Bret A. Payseur

163. Microarray analysis of ethanol effects in the nematode Caenorhabditis elegans identifies ethanol-sensitive genes
JaeYoung Kwon, Mingi Hong, Minsung Choi, Seol hee Im, Kyle Duke, Stuart Kim, Junho Lee

164. Analysis of Matrix Metalloproteinases in C. elegans
David H. Lum, Zena Werb

165. Identifying male fertility genes by recombinant inbred mapping
Marc Hammarlund, Shawn Olsen, Erik M. Jorgensen

166. Sperm competition and reproductive interactions in C. elegans
Gillian M. Stanfield, Anne M. Villeneuve

167. Systematic analysis of post-embryonic roles of embryonic lethal genes by RNAi-by-L1-soaking
Naoko Sakumoto, Rika Maruyama, Nobuko Uodome, Yumi Iida, Yuji Kohara, Asako Sugimoto

168. Identification of intestine-specific genes using mRNA-tagging
Pei-Jiun Chen, Peter J. Roy, Stuart K. Kim

169. Toward the identification of genes expressed in C. elegans GABAergic neurons
Hulusi Cinar, Yishi Jin

170. Identification of new endoderm regulators using an RNAi based genome wide screen
Cricket Wood, Andrew Fraser, Ravi Kamath, Julie Ahringer, Joel Rothman

171. Polyunsaturated Fatty Acids are Important for Normal C. elegans Cuticle Function
Trisha J. Brock, Jennifer L. Watts, John Browse

172. Characterizing MEL-26, the post-meiotic inhibitor of MEI-1/MEI-2 microtubule severing.
Jacque-Lynne F. Johnson, Martin A. Srayko, Paul E. Mains

173. Spindle Movements during Female Meiosis
Hsin-ya Yang, Karen L. McNally, Francis J. McNally

174. hal-2 , a gene involved in pairing of homologous chromosomes during meiotic prophase
Natasha L Miley, Amy J MacQueen, Anne Villeneuve

175. Finding new components necessary for correct meiotic chromosome pairing
Enrique Martinez-Perez, Anne Villeneuve

176. Direct screening for meiotic homologous pairing mutants in C. elegans
Kentaro Nabeshima, Anne M. Villeneuve

177. Using a Combination of Two Recombinases to Create Targeted Single-copy Genomic Insertion in C. elegans
Phillip Grote, Matthias Lauth, Michael Meyer, Barbara Conradt

178. Developing new techniques
M. Wayne Davis, Jeff Gritton, Erik M. Jorgensen

179. A System for Measuring Worm Movement; Improvements and Applications.
Christopher Cronin, Jane Mendel, Young-Mee Kim, Saleem Mukhtar, Jehoshua Bruck, Robert Stirbl, Paul Sternberg

180. Worms on the International Space Station
Nate Szewczyk, Melissa Kirven-Brooks, Cassie Conley

181. Visualizing synapses in the motor neuron circuit.
Jessica Rivera, Christina Gleason, Sam Wells, David M. Miller, III

182. Alae Mutant Defects Assessed by SEM and TEM
Carolyn Marks, Tylon Stephney, Adam Hartley, Hui Feng, Min Ren, Charles S. Rubin, David H. Hall

183. Modeling of human mitochondrial diseases in the nematode Caenorhabditis elegans
Leslie I. Grad, Bernard D. Lemire

184. The endophilin-related protein ERP-1 regulates mitochondrial division
Daniel A. Rubé, Ashley P. Wright, Alexander M. van der Bliek

185. Search for Proteins Affecting Division of the Mitochondrial Inner Membrane
Ayako Hasegawa, Alex van der Bliek

186. Nematodes with Mitochondrial Diseases
William Y. Tsang, Bernard D. Lemire

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

Robert Allan[1], Robert Barstead[2], **Mark Edgley**[1], Keiko Gengyo-Ando[3], Jeff Holmes[2], Meghan Hughes[2], Steven Jones[4], Yuji Kohara[5], Martin Lansdale[2], Lucy Liu[1], Jason Maydan[1], Sheldon McKay[1], Etsuko Machiyama[3], Shohei Mitani[3], Don Moerman[6], Gary Moulder[2], Maimun Nisha[1], Sachiko Noguchi[3], Jamie Osborn[2], Anna Rankin[1], Nadereh Rezania[1], James Robertson[2], Beth Rogers[2], Bin Shen[1], Miwa Tamura[5], Malini Viswanathan[2]

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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 three years, the consortium has received 1437 requests for targeted gene disruptions. From this request list, consortium labs have eliminated the function of approximately 500 genes using a chemical mutagenesis approach [as of April, 2002]. 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 knockouts requested by individual labs and on nematode homologs of human genes.

We will report on our progress and on our evolving strategy for high throughput production of knockouts, coordination with WormBase and the CGC.

2. THE C. ELEGANS ORFEOME CLONING PROJECT : VERSION 1.0

Philippe Vaglio[1], Jérôme Reboul[1], Jean-François Rual[1], Philippe Lamesch[1], Laurent Jacotot[1], Monica Martinez[1], Christopher Armstrong[1], Nicolas Bertin[1], Alban Chesneau[1], Rekins Janky[1], Troy Moore[2], Jim Hartley[2], Mike Brasch[2], Hongmei Lee[3], Lynn Doucette-Stamm[3], David E Hill[1], Marc Vidal[1]

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In addition to the draft of the human genome sequence, the genome sequences of an increasing number of model organisms are now available. This sequence information is expected to revolutionize the way biological questions can be addressed. Molecular mechanisms should now be approachable on a more global scale in the context of (nearly) complete sets of genes, rather than by analyzing genes individually. However most protein-encoding open reading frames (ORFs) predicted from these sequencing projects have remained completely uncharacterized at the functional level. For example, out of 19,000 ORFs predicted from the C. elegans genome sequence, the function of approximately 1,200 has been experimentally characterized during the last 30 years. Functional genomics and proteomics address this limitation through the simultaneous annotation of large numbers of predicted ORFs. Despite the urgent need for large-scale functional annotation projects, functional genomics approaches have remained relatively undeveloped in multicellular organisms, primarily because of the lack of suitable methods to clone large numbers of protein-encoding ORFs into many different expression vectors. Indeed, most strategies developed in these projects are based upon the expression of large numbers of proteins in exogenous settings and in fusion with relevant tags. In order to facilitate these different proteome-wide projects, a complete set of ORFs (or ?ORFeome?) will need to be cloned multiple times into many different expression vectors for each model organism of interest. To achieve this goal, one solution is to clone an ORFeome of interest once and for all in a "resource" vector allowing a convenient transfer to various expression vectors. To clone the C. elegans ORFeome into various expression vectors, we use a recombination cloning technique referred to as Gateway. This technique allows both the initial cloning of ORFs and their subsequent transfer into different expression vectors by site-specific recombination in vitro.

We have now finished the first part of the C. elegans ORFeome project which was to attempt to clone the ~19,000 predicted ORFs. We will present the success rate in cloning of the ORFs and the overall quality of the ORFeome to date. We will also describe how the ORFeome was used as a new approach to construct a ~100% normalized yeast two-hybrid library. Finally we will show how we could transfer thousands of ORFs from the resource clones into a dozen different expression vectors for uses in large-scale functional genomic and proteomic projects such as gene inactivation by RNAi, protein interaction mapping by yeast two-hybrid, protein production for structural genomics etc.

3. WormBase, a growing source of worm information

WormBase Consortium
WormBase-Caltech, California Institute of Technology, 156-29, Pasadena, CA 91125

Since its inception in 2000, WormBase has been striving to meet the needs of our users--the *C. elegans* research community and anyone who is interested in worm biology and genomics. As an information database, our goal is to provide useful information and to make it easier for users to find it.

Highlights of WormBase features:

Genome browser: tools to search the genome for a sequence, *C. briggsae* alignment, SNP, transposon insertion or Gene Knockout Consortium allele.

Gene information: mutant phenotype, expression pattern, microarray analysis, expression map, RNAi experiment, description and functional annotation, mapping, marker and strain.

Cell information: cell, lineage, pedigree and neuronal wiring.

Other: batch download (FASTA, GFF, ACeDB), paper and meeting abstract, author information, *C. elegans* community news.

During the meeting, we will discuss our progress on database content and WormBase web site navigability. We will run a workshop to provide hands-on tutorials. Most importantly, we will be there to listen to your suggestions, requests and complaints.

We will also announce the winner of our logo contest at the meeting. Please make your submissions at <http://www.wormbase.org/about/logo_contest.html>.

4. Functional Proteomics of the Mammalian Midbody Reveals Conserved Cell Division Components

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Understanding how an exact copy of the genome is transmitted to daughter cells during mitosis is a key question in biology. A molecular characterization requires the efficient identification of all the factors involved. One fundamental element of the cell division apparatus is the midbody, a protein-dense microtubule-based structure contained in the persisting intercellular bridge of dividing animal cells. We isolated mammalian midbodies to provide an accessible and enriched source of cell division proteins, and identified 128 of them by tandem liquid chromatography and mass spectrometry (LC/LC-MS/MS). The function of each midbody protein in cell division was then systematically evaluated in C. elegans using RNA-mediated interference (RNAi) and in vivo time-lapse video microscopy. We found that 100% of the midbody proteins identified were conserved between mammals, and nematodes and that all of those tested were required for cell division. The major category of identified factors consisted of vesicular trafficking and secretory proteins, including molecular motors and actin-binding proteins. Depletion of the C. elegans homologues by RNAi affected cytokinesis and/or cellularization, meiotic or mitotic events including spindle formation or alignment, and chromosome segregation. Thus, a functional proteomic analysis of subcellular assemblies provides a highly efficient method to identify novel factors participating in a complex cellular process.

5. Discovery of DAF-16 Targets and Novel Lifespan Genes: Microarray Analysis of the *C. elegans* DAF-2 Pathway

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The *C. elegans* DAF-2 IGF/insulin-like receptor pathway controls dauer formation, progeny production, stress response, and lifespan through its regulation of the DAF-16 HNF/forkhead transcription factor. While Daf-2 pathway components upstream of *daf-16* have been well studied, few targets of DAF-16 have been identified, and the cellular and biochemical processes that are needed to prolong lifespan through this pathway have not yet been explored. We used microarray analysis of mutants and dsRNA-interference-treated worms to identify genes downstream of DAF-16. At the transcriptome level, general aging effects outweigh *daf-2*-specific changes, indicating that transcriptional differences in a relatively small number of genes and cellular processes are required to achieve the profound physical differences that result in the long lifespan of *daf-2* mutants. At the single gene level, we found that reducing the activity of putative DAF-16-induced targets shortens the lifespan of *daf-2* worms, but not of wild type worms, while activity reduction of genes with the opposite transcriptional profile lengthens lifespan. Thus, with this approach we have been able to analyze the transcriptional output of DAF-16 at the transcriptome, biochemical process, and individual gene levels, and we have used the predictive power of transcriptional profiling to discover new lifespan-determining genes.

6. Genome-wide RNAi analysis of *C. elegans* fat regulatory genes

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Regulation of body fat storage involves control of metabolism as well as signals from fat storage sites to feeding regulatory circuits in the nervous system to control the intake of food. An imbalance between energy intake and energy expenditure causes diseases such as obesity, atherosclerosis, and diabetes. We have used the dye Nile Red to visualize fat droplets in intestinal cells of living *C. elegans.* Differences in fat content in mutants previously known to have elevated levels fat content, were readily detectable by this assay. Using the Nile Red assay in a forward genetic screen, we identified mutants with increased body fat, reduced body fat, or altered fat droplet morphology. Moreover, we conducted a genome-wide analysis of fat regulatory pathways using RNA-mediated interference (RNAi) to disrupt the expression of each of 16,757 worm genes and score for altered fat storage. This comprehensive genetic analysis identified 326 genes that cause reduced body fat and 115 genes that cause increased fat storage when inactivated by RNAi. Many of these newly identified worm fat regulatory genes have mammalian homologs, some of which have demonstrated roles in mammalian fat regulation. More importantly, the many *C. elegans* fat regulatory genes that are conserved across animal phylogeny, but not previously implicated in regulation of fat storage, offer new paradigms for body weight regulation.

7. COMPARATIVE FUNCTIONAL GENOMICS: USING CONSERVATION TO UNDERSTAND GENE EXPRESSION PROGRAMS

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Functional genomics offers detailed portraits of an organism's physiological programs, but identifying the key components of those portraits is challenging. In studying protein sequences, the principle of conservation across species has led to powerful automated ways of discovering analogies and relationships (such as BLAST) and of identifying essential modules (by aligning protein sequences to find conserved features). We have developed a general method for using conservation to understand gene expression programs, by combining DNA microarrays with computational comparative study of gene sequences. Phylogenetic analysis is used to define complete sets of candidate ortholog relationships between two organisms, which are then used to integrate gene expression data across organisms. In validating this method, we have studied the extent to which phenomena like aging and environmental stress produce shared gene expression signatures in highly diverged organisms.

We have found that adult worms (*Caenorhabditis elegans*) and adult flies (*Drosophila melanogaster*) mobilize a shared transcriptional program as they age. This program includes many genes shared by all eukaryotes, and an approximately equal number of genes found only in metazoan animals. Much of this program is implemented early in adulthood. Regulation of orthologous mitochondrial, ATPase, DNA repair and peptidase genes is especially conserved.

These results reveal that aging in highly diverged animals incorporates broad similarities in gene regulation, and suggest that functional genomics can be used to discover and explore analogies among the physiological programs of diverse organisms.

8. Deciphering the Combinatorial Code Controlling Pharyngeal Gene Expression

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During organ formation, cells acquire distinct fates in response to a host of regulators, many of which encode transcription factors. These factors function combinatorially, to regulate the expression of target genes during precise times and in particular cell types within a developing organ (Ghazi and VijayRaghavan, Nature 408: 419 (2000)).

We have taken a genomics approach to decipher the combinatorial code that governs gene expression within a simple organ, the pharynx (or foregut) of the nematode *C. elegans*. We previously demonstrated that many, perhaps all, genes selectively expressed in the pharynx are under direct control of the forkhead box transcription factor PHA-4 (Gaudet and Mango, Science 295: 821 (2002)). However, PHA-4 is expressed in all pharyngeal cells and at all times, suggesting that other transcription factors cooperate with PHA-4 to refine expression of individual genes to a particular developmental stage or a particular pharyngeal cell type.

To determine the regulatory circuits that govern pharyngeal gene expression, we have taken advantage of 338 candidate pharyngeal genes identified by microarray experiments. These genes provide a powerful reagent to identify additional cis-acting regulatory sequences using a computational approach.

Our strategy to identify potential regulatory elements is first to cluster candidate pharyngeal genes into smaller groups that are likely to be co-regulated within the pharynx. For one set of analyses, we divided our microarray positives into two temporal classes (early vs. late, using expression data from Y. Kohara's Nematode Expression Pattern Database, http://nematode.lab.nig.ac.jp/db/index.html). For the second set of analyses, we divided the genes according to their location on a *C. elegans* 'expression map'. On this map, genes that are clustered together have highly correlated expression across hundreds of *C. elegans* microarray experiments (Kim, et al. Science 293: 2087 (2001)). Analysis of these clusters suggests that they reflect differences in both spatial and temporal expression. Next, we used an expectation maximization algorithm to search the promoters of our gene groups for possible motifs (A. Zahler and J. Kent's Improbizer, http://www.cse.ucsc.edu/~kent/ improbizer/). Finally, we determined which of these motifs were enriched in the promoters of pharyngeal genes compared to non-pharyngeal genes.

Using this approach we have identified 16 candidate pharyngeal elements. We have begun our analysis of these motifs to determine which are important for pharyngeal expression in vivo. We have identified biological activity for six of eight motifs examined so far. One motif appears to function as a late expression element by repressing early pharyngeal expression. Five other motifs act as enhancers of expression, three of which appear to be specific to a subset of pharyngeal cells. Importantly, our analysis also identified the binding sites for three known pharyngeal factors: PHA-4, CEH-22, and PEB-1. These findings indicate that we can successfully identify regulatory elements for pharyngeal expression, including temporal and spatial control elements.

Once we have identified several regulatory elements, we plan to test interactions between these elements to try to understand how their combined actions can produce distinct pharyngeal expression patterns. Additionally, we are using the microarray to search for the relevant trans-acting factors.

9. Targets of Homeobox Genes in *C. elegans*

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We are interested in identifying target genes of homeobox genes using full genome DNA microarrays in *C. elegans*. Homeobox genes play an important role in specifying anterior-posterior identity in all metazoa. In *C. elegans* they are involved in anterior organization in the embryo, and vulva development, hermaphrodite and male tail development in the postembryonic lineage. In spite of their highly conserved importance during development less is known about which genes are regulated by different homeobox genes. We decided to take a genomic approach to identify target genes using our full genome DNA microarrays. We are currently focusing on three homeobox genes, *lin-39, ceh-13* and *mab-5*.

To simultaneously induce these homeobox genes in all cells we used strains in which the homeobox genes are driven by a heat shock promoter. We isolated RNA from heat shocked embryos and compared the expression level of all genes to RNA from non-heat shocked embryos to identify genes that change their expression after induction of a homeobox gene. In order to subtract genes that respond to the heat shock treatment we compared RNA levels in wildtype embryos before and after heat shock treatment. Every experiment was repeated four times. Statistical analyses (t-test and 2-way ANOVA) were used to select genes that change their expression after the expression of the homeobox genes. A preliminary list of homeobox target genes for *lin-39, ceh-13* and *mab-5* will be presented at the meeting.

10. An RNAi screen to identify regulators of pharyngeal development.

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To build complex organs, embryos have evolved mechanisms that integrate the development of multiple cell types. These cells typically arise from distinct progenitor populations that assemble into a primordium; the primordium undergoes patterning and morphogenesis to produce the mature organ. To identify genes required for organogenesis and understand how they function, we have chosen to study a simple organ, namely the pharynx (foregut) of *C.elegans*.

We have taken a genomics approach to identify new regulators of pharyngeal development. Genes expressed in the pharynx were identified with microarray chip by comparing embryos with excess pharyngeal cells (*par-1*; [1]) vs. embryos with no pharyngeal cells (*skn-1*; [2]) [3]. Since the function of most of the pharyngeal genes was unknown, we developed a sensitive RNAi screening approach to determine their loss-of-function phenotypes. We chose 295 genes for this analysis, based on their expression pattern and homology with other genes. We are currently conducting a general RNAi survey of these genes. In a typical experiment, we introduce double-stranded RNA (dsRNA) into worms by microinjection and assess the phenotype of the progeny 2-3 days later. To increase the sensitivity of the screen, we are using a worm strain that is weakly compromised for pharyngeal development.

From 85 genes surveyed so far, 40% are associated with a phenotype and 19% have an obvious pharyngeal phenotype. Some dsRNAs appear to affect pharyngeal function (e.g. slower pump rate, quivering pharynx). Others are associated with phenotypes that may reflect cell fate specification defects. For example, RNAi of 4 genes results in unattached pharynx, another 4 result in the reduction/increase of the size of the anterior pharynx, 2 cause disorganization or extra pharyngeal cells, and 2 result in arrhythmic contractions / quivering of the pharynx. Our goal is to complete the RNAi survey and initiate an in-depth analysis of those genes that encode likely regulators of pharyngeal development.

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11. Functional analysis of the *C. elegans* genome by RNA-mediated interference

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Although there is a phenomenal quantity of sequence data available, biological function has only been assigned to a small percentage of predicted genes in any metazoan. Understanding how genetic information relates to biological function at the level not only of a single gene but of an entire genome is thus a key problem in modern biology. One approach is to analyse loss-of-function phenotypes of every predicted gene in the genome -- this is the approach that we have taken in *C. elegans*. We have generated a reagent that uses RNA-mediated interference (RNAi) to individually inhibit ~90% of all ~19,000 predicted genes in the *C. elegans* genome. Using this reagent, we examined loss-of-function phenotypes for ~90% of all predicted genes and have found ~1700 genes to have detectable loss-of-function phenotypes. This is the first systematic functional analysis of a metazoan genome. We find striking differences in the functions encoded on different chromosomes, particularly the X chromosome, which has very few essential genes. In addition to differences between chromosomes, we find evidence for the clustering of genes of similar functions in multi-megabase regions of individual chromosomes. Genes in these regions tend to share transcriptional profiles, suggesting that the physical clustering we observe may facilitate transcriptional co-regulation. We also discuss the future prospects for the technique of genome-wide RNAi screening in the worm.

12. Check out my Profile! Isolation of chemotaxis defective mutants with altered *str-1* expression levels using automated, high-sensitivity fluorescence profiling.

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Autofluorescence background, the natural fluorescence from biological materials, reduces sensitivity of detection of localized fluorescence in multicellular organisms. For example in the case of a transgenic *C. elegans* that expresses GFP in two cells, that fluorescence can be masked by the autofluorescence of the remaining 1000 somatic cells. We have solved this problem with an add-on to our COPAS analysis and sorter technology that allows the instrument to measure and store the pattern of fluorescence along the length of the animal. That information was used to restrict fluorescence analysis to the region of the animal that contained the fluorescent cells thus improving the signal to autofluorescence ratio more than 20-fold.

We used the Profiler system to perform a genetic screen in which we isolated mutants of PY1089 (kindly provided by Piali Sengupta), a transgenic *C. elegans* that expresses GFP in the AWB sensory neurons under control of the *str-1* promoter. Proper expression of the odorant receptor, STR-1, in the AWB sensory neurons is required for appropriate chemotactic response to the repulsive odorant 2-nonanone. Without the Profiler technology the COPAS system was unable to distinguish PY1089 from N2 wild type animals, and it could not be used in a genetic screen for mutants with reduced GFP expression. With the addition of Profiler we were able to clearly distinguish PY1089 from N2 and were further able to sort animals that had either reduced or increased GFP expression in the sensory neurons.

F2 progeny of mutagenized PY1089 were analyzed and sorted using the COPAS *BIOSORT* with prototype Profiler hardware. First, 100 animals were run and their peak fluorescence intensities were determined. That information was used to set a range of normal peak heights in the Profiler. The Profiler was instructed to dispense those animals whose peak heights were outside of the normal range, either higher or lower, one per well into 96-well plates. Mutants were confirmed by regrowing the sorted animals and running them through the Profiler a second time. Approximately 50,000 animals from 8 separate pools were screened and 1,000 were dispensed to wells in the first pass. We have retested 109 lines from the sorted animals to date. We have isolated 5 independent mutant lines with decreased GFP expression, 5 independent lines with increased GFP expression and 2 lines with a mix of increased, wild-type, and decreased GFP expression. So far, we have tested 5 mutant lines for their chemotactic response to 2-nonanone. Of these, one line has demonstrated defective chemotaxis.

13. Functional Characterization of the Conserved Set of C. elegans Nuclear Hormone Receptors

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Nuclear Hormone Receptors comprise a large class of ligand-regulated transcription factors found in metazoans. NHRs are important regulatory factors that "sense" their environment through interaction with small lipophilic molecules and translate these signals into precise transcriptional regulation of target genes. The activity of an NHR is also modulated by the type of DNA response element to which it binds and by the composition of its protein cofactors in the cell. This complex regulatory system enables NHR regulation to be important for numerous biological processes including development, sexual differentiation, lipid and fatty-acid metabolism and xenobiotic resistance.

C. elegans contains a remarkably large number of NHRs, with nearly five fold more receptors than humans and other vertebrates. The function and origin of this expanded family is still not clear. Sequence analysis has revealed that C. elegans NHRs fall into two basic classes, a "conserved" set of receptors that are clear orthologs of receptors commonly found in other metazoans and a much larger group of "diverged" NHRs that have thus far only been found in nematodes. Despite the large number of NHRs in C. elegans, very little is known about the molecular function and regulatory networks of worm nuclear hormone receptors. Our work is focused on investigating the structural and functional origin and evolution of the conserved and diverged sets of NHRs in C. elegans and other nematodes. We have found that many worm NHRs behave as transcriptional activators in yeast and in mammalian cell culture transcription assays, two of the most common systems employed to study vertebrate NHR function. Because of the lack of an analogous cell culture system for worms, we have successfully exploited the yeast and mammalian assays to carry out structure-function studies on worm NHRs, including the examination of DNA binding-site specificity and protein cofactor interaction. These systems should also prove useful for the discovery of compounds that can serve as ligand regulators of worm NHR activity and of protein cofactors that modulate receptor function. Using our yeast and mammalian assays, we have conducted a comprehensive investigation of the transcriptional properties of the orthologous set of worm NHRs to determine the level of functional conservation between these receptors and their vertebrate counterparts. We have found that while the majority of these C. elegans NHRs employ highly conserved DNA binding mechanisms, others including DAF-12 and its related receptors, have evolved novel DNA binding preferences that may be specific to nematodes. The results of our studies indicate that the methods of target gene localization and regulation are important enough to be highly conserved throughout evolution. Therefore C. elegans can provide an excellent model for understanding how response elements control NHR regulatory function in a tissue and developmentally specific manner in live animals. We are currently developing systems for studying worm NHR transcriptional regulation by GFP reporter assay in worms. Finally, our findings demonstrate that the conservation of DNA binding site specificity across metazoans may allow the quick identification of NHR orthologs from other species, for which genome information is not yet available, by using yeast one-hybrid assays.

14. Relationship between the synaptonemal complex and progression of meiotic recombination in *C elegans*

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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 paired and 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 function(s) 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 during *C elegans* meiosis. Upon entry into prophase in *syp-1* and *syp-2* mutants, chromosomes load meiosis-specific axis protein HIM-3, undergo normal spatial reorganization and homologs do initially pair. However, pairing is not stabilized and homologs dissociate prematurely; further, polarized nuclear organization, normally lost upon completion of synapsis, persists for a prolonged period. All three SYP proteins contain extended coiled-coil domains and localize at the interface between paired aligned pachytene homologs. Based on criteria including TEM analysis and the kinetics of their localization to and dissociation from chromosomes, we conclude that SYP-1 and SYP-2 are structural components of the SC central region; persistence of chromosome-associated SYP-3 beyond diakinesis suggests that SYP-3 may be a component of chromosome axes. We suggest the SC is important for stabilizing intimate chromosomal associations subsequent to initial pairing, and that synapsis may directly promote or provide a signal for redistribution of chromosomes out of a polarized nuclear organization.

syp-1 and *syp-2* mutants fail to form crossovers and chiasmata; they also exhibit a recombination initiation-dependent increase in germ cell apoptosis, indicative of triggering the pachytene checkpoint in response to persistent meiotic recombination intermediates. Further, an antibody generated against RAD-51, which catalyzes strand invasion and strand exchange steps between homologous chromosomes during meiotic recombination, was used as a visual marker for recombination initiation. We observed a prolonged persistence of RAD-51 foci on *syp-2* chromosomes indicating that recombination is initiated early on in the meiotic program but is not efficiently completed. Some *syp-2* meiocytes escape apoptosis and complete meiosis having regenerated intact chromosomes, suggesting that delayed repair, which is also RAD-51-dependent, can occur as transitions in chromosome organization allow use of a sister chromatid as a template for repair. We observe a similar prolonged persistence of RAD-51 foci in *him-14(msh-4)* and *msh-5* mutants normally required for crossing over and chiasma formation. Our results argue that SYP-2, MSH-4 and MSH-5 all promote completion of initiated recombination events as crossovers by acting at or proximal to the strand invasion step to facilitate conversion of DSBs into stable post-strand-exchange intermediates.

15. *pom-1* is a Conserved Regulator of Cell Polarity and Cell Division in the *C. elegans* Embryo and Fission Yeast

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Polarization of the *C. elegans* zygote depends on cortically enriched proteins (e.g. the evolutionarily conserved PARs) and two cytoplasmic proteins (MEX-5/6) that function together to localize cytoplasmic determinants (e.g. PIE-1) in response to a polarity cue associated with the sperm asters.

To identify new players in this process, we have conducted an RNAi screen for genes required for the localization of a PIE-1:GFP fusion. Because PIE-1 asymmetry is visible in the zygote before the first cleavage, this approach avoids biases against genes that function in both cell polarity and cell division. Indeed, our screen identified one such gene - F49E11.1, which we call *pom-1* (see below).

During pronuclear migration, *pom-1(RNAi)* embryos fail to localize PIE-1 and P granules. Surprisingly, however, the PAR proteins and MEX-5/6 localize normally. Cytoplasmic flow and pseudocleavage are also unaffected. During mitosis, *pom-1(RNAi)* zygotes exhibit defects in cell division: the mitotic spindle often aligns perpendicularly to the long axis of the zygote, and cytokinesis usually fails. Histone:GFP movies have revealed that there are also defects in chromosome segregation in the absence of *pom-1* function. A *pom-1* deletion (kindly provided by Bill Raich) exhibits phenotypes identical to those observed by RNAi. We conclude that 1) *pom-1* regulates both cell polarity and cell division in the 1-cell embryo, and 2) *pom-1* identifies a new step downstream of MEX-5/6 that is essential for PIE-1 and P granule asymmetry.

Preliminary evidence suggests that POM-1's role in division may be separable from its role in polarity. Weak *pom-1(RNAi)* embryos undergo a normal first cleavage, but still do not localize PIE-1 or P granules. The distribution of the POM-1 protein is consistent with two potentially distinct functions. It is uniformly cortical in oocytes and newly fertilized embryos, but coalesces into discrete cortical foci just prior to the onset of PIE-1 and P granule localization. During pronuclear migration, these foci begin to disappear, starting in the posterior and progressing to the anterior. Shortly thereafter, POM-1 appears on the centrosomes and the spindle midzone as the embryo enters mitosis.

POM-1 is a member of the DYRK (dual-specificity tyrosine kinase) family. These kinases, conserved from yeast to man, include Pom1p, which regulates cell polarity and cell division in *S. pombe* (Bahler and Pringle, 1997). Thus, our findings reveal an unexpected link between A/P polarity in *C. elegans* and cell polarity in *S. pombe* that may extend to other organisms as well.

16. ICD-1, a putative target of CED-3, protects cells from programmed cell death

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The core pathway for programmed cell death (PCD) in C. elegans controls initiation and implementation of PCD during development. The functions of proteins targeted by the CED-3 caspase, the terminal effector in the cell death pathway, are not well-understood. We have identified a C. elegans protein, ICD-1 (Inhibitor of Cell Death-1), that may define a post-CED-3, PCD-suppressing step in the PCD pathway.

icd-1(RNAi) leads to a marked increase in PCD, evident during mid-embryogenesis and subsequently in many stages and tissues throughout development. Corpses are observed in embryos, larvae, and adults in the regions of the nerve ring, pre-anal ganglion and ventral nerve cord, and sporadically in other tissues. Widespread apoptosis is also observed in the germline: while germline apoptotic death is normally observed specifically in the pachytene region of the germline, icd-1(RNAi) worms contain cell corpses in the proximal and distal regions of the gonad as well. Moreover, cell corpses are seen in the germline of males, which normally does not undergo PCD. These corpses result in the elimination of cells, since we observe diminished numbers of nuclei in the ventral nerve cord and nerve ring, missing rays in the male tail, and the absence of a specific identified cell (the pharynx M4 neuron) with the expected associated phenotype ("stuffing" of the anterior pharynx).

Unlike the extra corpses resulting from loss-of-function mutations in ced-9, a gene that represses PCD by acting upstream of CED-3 activation, those in icd-1(RNAi) worms are not eliminated, and the missing cells are not restored, by a strong ced-3 mutation. ~33% of ced-3; icd-1(RNAi) larvae contain cell corpses in their ventral nerve cords (2-8 cell corpses per nerve cord). These corpses are not the result of the general death of the larvae per se, since the corpses are often engulfed and larvae often survive for extended periods well after the corpses are observed, showing variable defects in morphology and behavior. Thus, the increase in cell death associated with loss of ICD-1 function may be independent of CED-3 caspase activity.

Several observations suggest that the cell deaths observed in icd-1(RNAi) worms arise from an apoptotic-like process rather than another type of death, such as necrosis. These deaths stain for SYTO12 and strongly resemble apoptotic rather than necrotic cell deaths both by Nomarski and electron microscopy. Moreover, overexpression of ICD-1 results in decreased embryonic cells corpse numbers and extra surviving cells in the anterior pharynx (an average of ~4 extra survivors per anterior pharynx), demonstrating that ICD-1 is a potent suppressor of CED-3-activated developmental PCD.

ICD-1 is the worm homologue of mammalian BTF-3, a protein that was first identified based on its role in transcriptional activation of a number of promoters in vitro. BTF3 contains a nascent polypeptide association complex (NAC) domain, implicating it in translational control. While it has not previously been shown to participate in apoptotic regulation, BTF3 is altered in human Burkitt lymphoma cells and Jurkat T cells undergoing apoptosis. These observations, and our localization data, suggest that ICD-1 may function in the cytoplasm. Immunoreactive ICD-1 is predominantly cytoplasmic, with only faint nuclear staining. ICD-1 is expressed throughout the entire development of the worm, including in the nerve ring, pre-anal ganglia, and germline, where most PCD occurs, as well as in the gut, where it never normally occurs. Weaker expression is also detected in muscle, pharynx, and ventral nerve cord.

We hypothesize that ICD-1 protects cells from undergoing PCD and that this activity is abrogated by CED-3. BTF3 has been shown to be cleaved by caspase-3 in vitro and ICD-1 contains a caspase cleavage site at the same location as the caspase-3 cleavage site in human BTF3, consistent with its possible cleavage by CED-3. Further evidence for a possible interaction between ICD-1 and a caspase is the presence of a predicted caspase recruitment domain (CARD) in the ICD-1 sequence. This domain might be the site through which CED-3 binds ICD-1 to inactivate it. If this hypothesis is correct, ICD-1 is the first direct target of CED-3 involved in repression of PCD in C. elegans.

17. *C. elegans* tubulin genes *tbb-2* and *tba-2* are required for microtubule severing of the MEI-1/MEI-2 katanin complex during meiotic spindle formation.

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Female meiotic spindle formation requires two meiosis-specific genes, *mei-1* and *mei-2*, which encode the *C. elegans* subunits of the microtubule severing complex katanin. In addition to their sequence similarities to katanin, MEI-1 and MEI-2 disassemble interphase microtubules when coexpressed in Hela cells.[1] In wild-type embryos, MEI-1 and MEI-2 localize to the female meiotic spindle and are inactivated after meiosis, likely through a UBQ-like *Nedd-8* pathway.[2] MEI-1 and MEI-2 likely restrict the length of meiotic spindle fibres to maintain the unique morphology of the anteriorly-localized, barrel-shaped meiotic spindle. Therefore, the ectopic persistence of MEI-1/MEI-2 activity into the following mitosis is disastrous. This is seen in a *mei-1* gain-of-function (*gf*) mutant where MEI-1 and MEI-2 localize ectopically to mitotic spindles resulting in small, misoriented spindles similar to those seen after treating embryos with microtubule destabilizing drug, nocadazole.[3] This is consistent with our idea that an ectopic microtubule severing activity is present in the mutant mitosis.

Here we report analysis of three extragenic suppressors of this *mei-1*(*gf*) mutant. These suppressors show semi-dominant suppression of the *mei-1*(*gf*) defect, but are phenotypically wild type by themselves. One suppressor is a missense allele of the ² tubulin gene, *tbb-2* (C36E8.5). A TBB-2 specific antiserum showed that the protein is ubiquitously expressed through out worm development and RNAi studies revealed that *tbb-2* is functionally redundant with *tbb-1*. Two other *mei-1*(*gf*) suppressors are missense alleles of an ± tubulin gene, *tba-2* (C47B2.3). All three suppressors genetically behave as if they generally inhibit microtubule severing: they suppress the mitotic phenotype resulting from ectopic *mei-1* activity while enhancing meiotic defects seen when *mei-1* severing activity is compromised during meiosis. Experiments are in progress to determine if this correlates with *in vitro* sensitivity of MT to severing.

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18. The C. elegans TIMELESS homolog is an essential regulator of chromosome cohesion.

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Faithful segregation of chromosomes during cell division is essential for the inheritance of equivalent genetic information by each daughter cell. One mechanism to ensure the fidelity of chromosome segregation during mitosis is to maintain the tight association of sister chromatids from DNA replication until chromatid separation at anaphase. A conserved mitosis-specific multimeric protein complex, termed cohesin, is largely responsible for maintaining sister chromatid cohesion. The core of this complex consists of a heterodimer of Smc1 (structural maintenance of chromosome) and Smc3. At least two additional proteins are found within this complex; these include the Scc3p/SA (stromal antigen) and Scc1p/Mcd1p/RAD21 protein families. In meiosis, chromosomes undergo two rounds of division following a single round of replication in order to generate haploid gamates. To accommodate this specialized and highly regulated dispersal of meiotic chromosomes, there is also a meiosis-specific cohesin complex. A conserved difference between the mitotic and meiotic cohesin complexes is the substitution of the mitotic Scc1p/RAD21 protein by the meiotic REC-8 protein. Although the worm Scc1p homologs and their meiotic ortholog have been identified, the larger cohesin complexes, as defined by the four cohesin proteins, had not been fully characterized. Here we report the characterization of the mitotic and meiotic cohesin complexes in order to examine 1) the composition of the worm complexes relative to their counterparts in other eukaryotes and 2) the regulated localization of these proteins to meiotic chromosomes.

During our studies, we discovered an unexpected role for the worm homolog of the Drosophila TIMELESS protein regulator of biorhythm, TIM-1, as a regulator of cohesin function. Based on biochemical, immunocytochemical and functional analyses, we found that TIM-1 interacted specifically with the mitotic cohesin complex and that *tim-1(lf)* mutation causes mitotic chromosome segregation defects indistinguishable from those caused by reduction of cohesin function. Furthermore, *tim-1* was also required for proper chromosome segregation in the germline. We showed that a *tim-1* mutation causes defective nuclear organization and chromosome synapsis through meiotic prophase. Moreover, the *tim-1* mutation preferentially affects the localization of the non-SMC proteins to meiotic chromosomes. These results provide the first evidence that members of the cohesin complex may be differentially loaded in meiosis. With the completion of the Drosophila genome sequence, it was apparent that the fly genome encodes a second TIMELESS homolog, termed TIMEOUT/TIM-2. By contrast, the worm and mammalian genomes each contains only one *Timeless*-like gene which shares greater homology with *Timeout* than *Timeless*. Intriguingly, the *Timeout* knockout mice arrested early in embryonic development indicting that the murine *Timeout* is also essential for viability. Taken together, the data are consistent with a conserved role for *Timeout* in chromosome segregation in metazoans.

19. Mutations in alpha and beta tubulin genes affect spindle orientation in the one-cell stage *C. elegans* embryo

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In a screen for embryonic lethal mutations, we isolated several mutations affecting P_0 spindle orientation. Two of these, *or346ts* and *or362ts*, exhibit severe defects in microtubule dependent events: Pronuclear migration, centration, and rotation of the nucleocentrosomal complex fail in these mutants, resulting in a posterior, transverse P_0 spindle. Although polarity markers such as P-granules are properly localized prior to the first division, the abnormal orientation of the spindle and plane of cleavage result in the mis-segregation of P-granules to both daughter cells. Embryos produced by heterozygous *or346* hermaphrodites exhibit the same spindle orientation defects as those seen in embryos from homozygous *or346ts* parents, indicating that this allele is dominant. We mapped *or346ts* to LG I, between *nob-3* and *lin-11*, placing it very near the α-tubulin gene *tba-1*. The second gene, *or362ts,* fails to complement a gene previously known as *rot-2 (t1673*)(Gönczy et al, 1999), and both alleles of this gene were subsequently mapped to the left arm of LGIII in the same region as the β-tubulin *tbb-2*. We sequenced these loci in our mutants and discovered missense mutations in each. Immunofluorescent analysis using antibodies specific to TBB-2 shows that this protein is present at greatly reduced levels in the P_0spindle of *tbb-2 (or362ts)* embryos. We also obtained a deletion allele from the *C. elegans* gene knockout consortium*, tbb-2 (gk129*). *tbb-2 (gk129*) exhibits temperature-dependent embryonic lethality and mild defects in stability of the P_0 spindle. However, pronuclear migration, centration, and rotation are normal, and the strain is homozygous viable, suggesting that *tbb-2* function is partially redundant with other β-tubulins in the early embryo. As expected, TBB-2 staining is completely absent in *tbb-2(gk129*) embryos. *tba-1* shares 97% homology at the amino acid level with another α-tubulin, *tba-2*. Microinjecting the full-length *tba-1* dsRNA into wild-type worms results in a severely reduced mitotic spindle, presumably due to a depletion of multiple α-tubulins. To specifically target *tba-1*, we generated *tba-1* 3-prime UTR dsRNA. When this is microinjected into wild-type worms, early embryonic divisions are normal and most embryos hatch. However, when 3-prime UTR dsRNA is injected into homozygous *tba-1(or346ts*)hermaphrodites, pronuclear migration, centration and rotation defects are rescued, resulting in a normal first spindle and restoring viability at the restrictive temperature. We conclude that for both α- and β-tubulin, the embryonically expressed isotypes are partially redundant in function. Moreover, we find that a partial disruption of these genes is more deleterious to microtubule-dependent processes than the total absence of either protein in the early embryo.

We would like to thank Chenggang Lu and Paul Mains for providing antibodies to TBB-2, and the *C. elegans* Reverse Genetics Core Facility at the University of British Columbia in Vancouver for providing *tbb-2 (gk129).*

Gönczy et al. (1999). JCB **144**: 927-946

20. Glycosyltransferases mediate Bt toxin action in *C. elegans*

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Mutants resistant to *Bacillus thuringiensis* Cry toxins (called Bre for Bt toxin resistant) have been isolated in *C. elegans*. These mutants identify five genes (*bre-1*through *bre-5*), each of which is required for *C. elegans*susceptibility to Cry5B, a member of the major family of insecticidal Cry proteins. Two of these genes, *bre-3*and *bre-5*, have been identified, and their predicted functions have been investigated. *bre-5* encodes a putative glycosyltransferase, belonging to a family which incudes beta-1,3 Gal- and beta-1,3 GlcNAc transferases. Members of this family can be found in nematodes, insects, and mammals. The predicted product of *bre-3* is remarkably similar (60% identical) to a Drosophila protein of demonstrated importance for insect development, but unknown biochemical activity. BRE-3 shares distant kinship with other beta-glycosyltransferases from bacteria. Given that BRE-3 and BRE-5 resemble glycosyltransferases, we have investigated the possibility that these genes are involved in the biosynthesis of a specific glycoconjugate. To demonstrate genetically that these enzymes act in a common pathway, we have quantified resistance in *bre-3* and *bre-5* loss-of-function single and double mutant strains. Early results from an assay based on growth inhibition indicate that dose response curves for the single mutants, as well as the double mutant, are virtually superimposable, suggesting that these genes control a single pathway. Analysis of genetic mosaics, along with ectopic expression and immunostaining experiments demonstrate that *bre-3*and *bre-5* are necessary and sufficient in the cells of the gut epithelium to mediate susceptibility, and that their protein products localize to cytoplasmic puncta which may be in the secretory pathway. These results are consistent with a role for BRE-3 and BRE-5 in the synthesis of a glycan structure required for the binding of toxin to the apical surface of gut epithelial cells. The evidence obtained so far for the inability of *bre-3* and *bre-5*mutant animals to bind toxin is based on the observation that these mutants are highly resistant to endocytosis of dye-labeled toxin into their gut cells, while normal animals exhibit rapid uptake of toxin under identical conditions. We have observed striking defects in the glycoconjugate profile of *bre-3* and *bre-5* mutants, which suggest an epistatic relationship between the genes, and are currently working to identify the precise substrates of BRE-3 and BRE-5 activity and their relationship to Bt toxin action.

21. The egl-41 gene, identified by gf mutations that cause partial sexual transformation, is identical to sel-10, a negative regulator of lin-12

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Programmed cell death (PCD) plays an important role in many biological processes including generating sexual dimorphism. In *C. elegans* there are two sets of sex-specific neurons: two hermaphrodite-specific neurons (HSNs) and four male-specific cephalic companion neurons (CEMs). The HSNs innervate the vulval muscles and stimulate egg-laying in the hermaphrodite; the CEMs are believed to play a role in the chemotaxis of the male to the hermaphrodite for mating. Both sets of neurons are born during embryogenesis in both sexes, however the unnecessary set undergoes sex-specific PCD: the HSNs die in males and the CEMs die in hermaphrodites. The regulation of sex-specific PCD in *C. elegans* offers an ideal paradigm for understanding the mechanisms that activate PCD in specific sets of cells.

The *egl-41* gene (egg-laying deficient) was originally identified by three dominant mutations *(n1069, n1074, n1077)* that cause inappropriate HSN cell deaths in hermaphrodites (1,2). In addition, the CEMs improperly survive, suggesting that *egl-41* may play an important role in regulating sexually dimorphic apoptosis in *C. elegans*. To determine the loss-of-function phenotypes of *egl-41* and to facilitate its cloning, we performed an *egl-41(n1074)* suppressor screen, looking for hermaphrodite mutants that are non-Egl and without CEMs. We screened 21800 haploid genomes and identified 12 recessive suppressors (*sm122-sm125*, *sm165-sm168*, and *sm170-sm173*). Among them, *sm171* is the strongest suppressor. Four-factor mapping of *sm171 n1074* revealed that *sm171* and *n1074* are very closely linked, suggesting that *sm171* might be an intragenic suppressor of *n1074*. Complementation tests between *sm171* and the other suppressors indicate that 9 other suppressor mutations are allelic to *sm171*.

We have conducted several consecutive SNP mappings of *egl-41(n1074),*narrowing down its position to a very small interval of 4 cosmids on LG V. We found that injection of these four cosmids can counteract the Egl and CEM survival phenotypes of the *egl-41(n1074)* mutation, suggesting that the normal activity of *egl-41* antagonizes the likely altered function of the EGL-41 mutant product. Using this characteristic, we performed further rescuing experiments and found *egl-41* to be identical to the previously characterized gene *sel-10*(suppressor/enhancer of *lin-12*). *sel-10* is a negative regulator of *lin-12* (lineage-abnormal) and encodes an F-box/WD40 repeat-containing protein (3,4). The *sel-10(ar41)* mutant displays no obvious defects in either HSNs or CEMs, similar to what we have observed in our *egl-41(n1074)* intragenic suppressors. In addition, injection of a 6.9 kb PCR product derived from the *sel-10* region of the *n1074*animals into either wild-type or *sel-10* animals mimicked the *egl-41(n1074)* mutant phenotypes.

We have identified the molecular lesions corresponding to two *egl-41*alleles, *n1074*and *n1077*(both are a G567E missense mutation in SEL-10), and four *n1074* intragenic suppressors. *sm122* and *sm170* are missense mutations (R472Q and S467F, respectively) in the sixth WD40 repeat of SEL-10; *sm170* is a splice site mutation before exon 10; *sm123* is a missense mutation in the first WD40 repeat of SEL-10.

Currently we are sequencing other suppressor mutations to determine if and how they affect the *sel-10* ORF. In addition we are doing experiments to determine what proteins interact with SEL-10 and how these interactions are affected by the gain-of-function and loss-of-function mutations in *sel-10*. These experiments should provide crucial insight into how *sel-10* and its interacting partners affect the life vs. death fates of the HSNs and CEMs in *C. elegans*.

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22. The Roles of *ham-1* and *hlh-14* in Neuroblast Divisions

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Asymmetric cell division is a primary mechanism for generating neuronal diversity in *C. elegans*. The HAM-1 protein is asymmetrically distributed in many mitotic embryonic cells and participates in the divisions of several different neuroblasts. In the HSN/PHB lineage, for example, the HSN/PHB neuroblast (ABpl/rapppap) divides asymmetrically. In wild type, its anterior daughter dies and its posterior daughter becomes the HSN/PHB precursor. However, in loss-of-function *ham-1* mutants, both daughters can adopt an HSN/PHB precursor-like fate resulting in extra HSN and PHB neurons. This phenotype is incompletely penetrant; often the daughter cell fated to die will still die in *ham-1* mutants, but aspects of its death may be abnormal such as delayed onset or inappropriate corpse persistence.

Recent lineaging work using a nonsense mutant, *ham-1*(*gm279*), has allowed us to identify additional defects in the HSN/PHB neuroblast division. The daughter cells of this division often end up oriented abnormally along the A/P axis in *ham-1*(*gm279*) embryos. Additionally, when the anterior daughter does die, its corpse is aberrantly large. Taken together, these data suggest that HAM-1 may control the positioning of the HSN/PHB neuroblast spindle. We also note that many of the other corpses in *ham-1*(*gm279*) embryos are unusually large, suggesting that *ham-1* plays a global role in asymmetric cell division at this stage of development.

With the overall goal of identifying additional components involved in the proper execution of the HSN/PHB lineage, we conducted genetic screens for mutants with abnormal numbers of HSN and PHB neurons. Using a phasmid neuron GFP reporter, mutants with the extra PHB neurons were identified, including new alleles of *ham-1* (novel), *ced-3* (cell death/ICE), *egl-5* (homeodomain), *egl-27* (MTA1/NURD complex), and *lin-32* (bHLH transcription factor). Mutants with missing PHBs were also identified. This class of mutants is interesting because this is the phenotype one would expect for the loss of a cell fate determinant in the HSN/PHB lineage. We have focused on one such mutant, *gm34*.

gm34 mutants are missing HSNs and PHBs, a phenotype that is epistatic to *ham-1*, *ced-3*, and *lin-32* mutations. Cloning showed that the defective gene is C18A3.8 which encodes basic helix-loop-helix protein previously designated HLH-14. *hlh-14* mutant animals have a number of visible defects including lumpy, disorganized posteriors, occasional ventral coiling, and a high degree of larval lethality. On a cellular level, *hlh-14* mutants show abnormal expression of a number of nervous system GFP reporters, consistent with HLH-14 playing a proneural role in development. Directly relevant to our studies, *hlh-14* mutants seem to be missing PVQ neurons. Since PVQ is lineally related to the HSN and PHB, it is possible that HLH-14 is necessary to specify the fate of their common precursor, ABpl/rapppa. Alternatively, HLH-14 may be required to specify the fates of the both daughters of ABpl/rapppa independently. Preliminary lineage analysis suggests that *hlh-14* mutants fail to generate and HSN/PHB neuroblast daughter that dies, perhaps because the HSN/PHB neuroblast or its precursor fails to divide.

To discern which cells may require HLH-14 function, we generated a rescuing *hlh-14-gfp* transgene and examined its expression pattern. In wild-type embryos, at approximately 270 minutes after fertilization, the HSN/PHB neuroblast has been born but has not yet divided. At this stage, transgenic embryos express HLH-14-GFP in several anterior cells, but in only two cells on each side of the ventral posterior. We are currently performing lineage analysis to positively identify these posterior cells; their position is consistent with HLH-14-GFP being expressed in the HSN/PHB neuroblast, and perhaps its sister, the PVQ precursor.

23. *C. elegans evl-20* Gene Encodes a Functional Homologue of Human Small GTPase ARL2 and Regulates Cytoskeleton Dynamics during Cytokinesis and Morphogenesis

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Little is known about the *in vivo* functions of ARF-like (ARL) members of the Ras superfamily of GTPases. Here, we describe the analysis of *C. elegans evl-20* gene that encodes a functional homologue of human ARL2. Elimination of function *evl-20* results in abnormal vulval, gonad, and male tail development and disrupts embryonic proliferation, hypodermal enclosure, and elongation. Using GFP tagged beta-tubulin, we demonstrate that loss of *evl-20* function causes a specific defect in the microtubule cytoskeleton, which likely is at least one of the molecular mechanisms that underlie the observed defects. EVL-20 is closely associated with both the cell cortex and astral microtubules suggesting that it may directly interact with microtubule structures at those locations. Using yeast two hybrid system, we identified several proteins that directly interact with the activated, GTP-bound form of EVL-20 suggesting that EVL-20 may also participate in actin cytoskeleton remodeling. Together, our data indicate that EVL-20 functions in the cytoplasm and at the plasma membrane to regulate cytoskeletal dynamics during cytokinesis and morphogenesis.

24. Proper $GABA_A$ receptor trafficking depends on correct synapse formation

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$GABA_A$ receptors are important inhibitory neurotransmitter receptors in *C. elegans* and mammals. The strength of the postsynaptic response to the neurotransmitter, GABA, is proportional to the number of receptors in the postsynaptic membrane. Our lab is interested in determining how $GABA_A$ receptors are trafficked to, and localized at synapses, using *C. elegans* as a model system. The *C. elegans* $GABA_A$ receptor is composed of two subunits, UNC-49B and UNC-49C, and is required for coordinated locomotion. This receptor is expressed in muscles and localized to the neuromuscular junctions along the dorsal and ventral nerve cords; an UNC-49B::GFP full-length translational fusion recapitulates this expression pattern. To identify genes required for receptor trafficking and localization, we screened for mutants with altered patterns of UNC-49B::GFP fluorescence, using a dissecting microscope. We isolated five mutant alleles (*gr 1, 2, 5, 6* and *8*) representing at least two complementation groups. All mutants have GFP fluorescence in vesicular bodies within muscle cells, a phenotype we have termed **G**ABA **r**eceptor **m**islocalized (Grm). Preliminary data, using a pH sensitive dye (Lysotracker), suggests that these vesicles may be acidified compartments. An interesting possibility is that they are endosomes, containing UNC-49B::GFP subunits that have entered into the degradation pathway.

We have determined that *gr5* is an allele of *unc-3*. UNC-3 is a transcription factor that is transiently expressed in neurons during development. *unc-3* mutants have severely disrupted nervous system structure, characterized by defasciculated ventral and dorsal nerve cords, and ectopic synapses. Both the presynaptic (UNC-47::GFP) and postsynaptic (UNC-49B::GFP) GABAergic markers appear more severely affected dorsally: Large gaps between synapses appear in the dorsal nerve cord, and the majority of UNC-49B::GFP containing vesicles (80%) are found within the dorsal muscles. Because UNC-3 acts presynaptically and the severity of the Grm phenotype correlates with the severity of the synapse spacing defect, we hypothesize that the stability of the $GABA_A$ receptor within the postsynaptic membrane depends upon the proper establishment of the synapse. We tested this hypothesis by examining the localization of UNC-49B::GFP in another mutant with a disrupted nervous system structure, *unc-5*. *unc-5* encodes an UNC-6/netrin receptor, expressed in neurons, which is required to guide axonal projections to the dorsal nerve cord. *unc-5* mutants also display the Grm phenotype, and the majority of the GFP-containing vesicles (80%) are found within the dorsal muscles. These data suggest that a signal from the presynaptic cell is required to recruit and stabilize postsynaptic $GABA_A$ receptors. Analyzing the other mutants from our screen may identify this signal, or the proteins that convey this information to the $GABA_A$ receptor trafficking machinery.

25. A role for *cbp-1*, the worm homolog of the histone acetyl transferases CBP and p300, in vulval induction.

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The C. elegans vulva is induced by an EGF like signal that activates the highly conserved 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 ectopically induced vulval precursor cells (VPCs). By initiating suppressor screens of activated *let-60* ras, many previously unknown components of this pathway have been identified. One suppressor, *ku258*, is a semi-dominant mutation isolated in a screen for temperature sensitive mutations that suppress the *let-60(n1046)* allele. While homozygous *ku258* can suppress *let-60(n1046)* from 80% Muv to 5% Muv, *ku258/* + heterozygotes also suppresse the *let-60(n1046)* phenotype to 10% Muv. Additional genetic analysis, such as the ability of *ku258* to suppress the Muv phenotype of *lin-1(e1275)*, has suggested that *ku258* acts at a late step in the Ras/MAPK signaling pathway. Interestingly, *ku258* is not able to suppress the muv phenotype caused by a *lin-31(lf)* mutation. Animals carrying the *ku258* mutation are also semi-sterile, display lethality in 25% of their embryos and show defects in other developmental processes. *ku258* was fine mapped using single nucleotide polymorphisms (SNPs) to a 20 kb region on LGIII. A lesion corresponding to the *ku258* mutation was found in an open reading frame coding for the worm homolog of CBP/p300, *cbp-1*. Further genetic, molecular and biochemical analysis is currently being performed to better understand the role of *cbp-1* in ras signaling and vulval induction.

26. Identification of CHE-13, a novel IFT protein required for cilia formation

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Cilia and flagella are organelles found on diverse cell types in eukaryotes where they function in fluid and cell movement, developmental patterning, and sensory perception. Studies in *Chlamydomonas* have identified a mechanism for the formation and maintenance of cilia and flagella termed intraflagellar transport (IFT) for which several of the proteins have yet to be identified. Homologs of the known IFT proteins have been identified in organisms as diverse as *Chlamydomonas*, *C. elegans* and mouse. Of the complex B IFT proteins identified in *C. elegans* to date, all are regulated by the transcription factor DAF-19 through a conserved X-box found in the promoter. We used this conserved sequence to search the genome and identified a DAF-19 regulated gene *F59C6.7/9* that is located near the *che-13* locus. *F59C6.7/9*is expressed in ciliated sensory neurons in a DAF-19 dependent manner and is required for *C. elegans* cilia formation. This is evidenced by its disruption in *che-13* mutant worms and the transgenic rescue of *che-13* cilia defects with expression of *F59C6.7/9*. In agreement with a role in cilia formation, fluorescent tagged CHE-13 protein concentrates at the base of cilia and moves along the axoneme typical of other IFT proteins. Furthermore, the *che-13* mutation differentially affects the localization of two known IFT components supporting its role as an IFT protein. Intriguingly, CHE-13 shares strong homology with the mammalian protein Hippi. When bound to Hip-1, Hippi activates the neuronal apoptotic pathway associated with the Huntington pathology. Expression analysis of the *C. elegans* homologs of *Hip-1* (*ZK370.3a/b*) and *Hippi* (*che-13*) indicate that they are not expressed in the same cells and are unlikely to form a complex. Furthermore, the tissue and spatial expression profile of mouse *Hippi* is similar to that of *Tg737*, a known IFT protein. These data, along with the conservation of the IFT process, suggest that mouse Hippi may function as an IFT protein in addition to its proposed role in neuronal apoptosis in disease conditions.

27. A BK potassium channel mediates the behavioral effects of ethanol in *C elegans*
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The acute behavioral effects of ethanol are often apparent as euphoria and incoordination at low doses and incoherency and sedation at higher doses. Despite being a widely used drug of abuse, the fundamental mechanism responsible for ethanol's intoxicating properties is unclear. Ethanol has been reported to have effects on over 20 different membrane proteins *in vitro*; whether any of these effects are biologically relevant or relate to the intoxicating properties of ethanol is unknown. Ethanol, like other small chain alcohols, can have highly non-specific effects on the activity of neuronal membrane proteins when tested *in vitro*.

Ethanol has intoxicating effects on *C. elegans* including inhibitory effects on locomotory and egg-laying behaviors. Although high exogenous concentrations of ethanol are required to induce these behavioral changes, the tissue concentration in these intoxicated animals is the same (20-25 mM) as that required to produce intoxication in mammalian systems. To identify the neuronal proteins required for the sensitivity to ethanol, we carried out two independent genetic screens for mutants that are resistant to the inhibitory effects of ethanol on locomotion or egg laying. Mutations in the *slo-1* gene were found to produce the strongest level of resistance to ethanol for each behavioral effect. *slo-1* encodes a calcium-activated potassium (BK) channel (Wang *et al*. Neuron 32:867 2001).

The *slo-1* mutants show a dramatically reduced sensitivity to ethanol compared with wild-type animals. We restored ethanol sensitivity to a *slo-1* null mutant by expressing *slo-1(+)* only in neurons, suggesting that ethanol is acting on the nervous system to bring about its effects. *slo-1* mutants display hypersensitivity to aldicarb, an indication of their neuronal hyperactivity. This does not appear to be the cause of the ethanol resistance because other aldicarb-hypersensitive mutants that we tested do not show a comparable level of ethanol resistance.

We tested the possibility that ethanol mediates its effects through the SLO-1 potassium channel by performing electrophysiology of identified neurons in *C. elegans*. We show that ethanol increases the activity of the native SLO-1 channel at physiologically relevant concentrations of ethanol in multiple neurons. Effects, that were absent in the neurons of *slo-1* mutants, were observed at doses that cause mild intoxication in humans (20 mM) as well as at doses that cause sedation (100 mM). To determine whether ethanol can activate SLO-1 in the absence of cytosolic factors, we recorded the single-channel activity of SLO-1 in patches excised from neurons. Ethanol reversibly increased the probability of channel opening.

Together, the genetic and electrophysiological results demonstrate that BK channels mediate the major *in vivo* effects of ethanol in *C. elegans*. As such, we predict that activation of BK channels represents a fundamental mechanism by which ethanol causes intoxication in *C. elegans* and other systems.

28. GPB-2 Interacts With Two Muscarinic Acetylcholine Receptors In The Pharynx

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GPB-2 is a g-protein beta subunit that interacts with RGS proteins to regulate signaling through g-protein coupled receptors. GPB-2 acts in both G_{alpha}o and G_{alpha}q coupled pathways, influencing behaviors such as egg laying, locomotion and defecation. In addition, *gpb-2* mutants are hypersensitive to the muscarinic agonist arecoline: in the presence of 5mM arecoline, *gpb-2* mutants fail to grow past the L1 stage and their pharynxes pump infrequently. This observation has led to the hypothesis that GPB-2 is necessary to regulate signaling through a muscarinic receptor in the pharynx. However, screens to isolate mutants that suppress the arecoline hypersensitivity of gpb-2 mutants have not generated muscarinic receptor mutants. We have found that two muscarinic receptors, *gar-1*(C15B12.5) and *gar-3*(Y40H4A.1), are expressed in the pharyngeal muscle, and might function redundantly in GPB-2-linked pathway. Therefore, we conducted a screen in a *gpb-2; gar-1* background, in which one muscarinic receptor is disrupted by a deletion. In this screen, we isolated arecoline resistant worms that carry a mutation in *gar-3*.

gar-3(vu78) worms carry a mutation that changes a conserved alanine to a threonine. While over-expression of wild type *gar-3* renders wild type worms hypersensitive to arecoline, over-expression of *gar-3(vu78)* does not, suggesting that the *vu78* mutation reduces the function of GAR-3. We believe this is strong evidence that disruption of two muscarinic receptors, *gar-1* and *gar-3*, is necessary to overcome the arecoline hypersensitivity of *gpb-2* mutants.

In order to elucidate the function of a muscarinic signaling pathway in the pharynx, we are now comparing *gpb-2* arecoline hypersensitive worms with resistant *gpb-2, gar-1; gar-3* worms. We have found that, even in the absence of arecoline, mutations in muscarinic receptors increase the growth rate of *gpb-2* mutants. In the presence of arecoline, triple mutants are able to pump more rapidly than *gpb-2* single mutants. We have also found that the pharynxes of *gpb-2* single mutants tend to be hypercontracted during treatment with arecoline. However, the electrical activity of *gpb-2* mutant pharynxes appears fairly normal, even in the presence of arecoline. We therefore hypothesize that muscarinic signaling in the pharynx alters the relationship between excitation and contraction of the muscle.

29. A GABA-gated non-selective cation channel in *C. elegans*.

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γ-aminobutyric acid A receptors are members of the ligand-gated ion channel superfamily that mediate inhibitory neurotransmission in both vertebrates and invertebrates. GABA binding to these receptors causes the opening of an anion-selective channel that predominantly conducts chloride ions, thereby hyperpolarizing cells. However, there are numerous mechanisms by which GABA can serve as an excitatory neurotransmitter. To date, the molecular identity of an excitatory ionotropic GABA receptor has remained elusive.

In *C. elegans*, GABA is inhibitory to locomotory muscles but is excitatory at enteric muscles. GABA release from the VD and DD motor neurons exerts an inhibitory effect on body wall muscles through the chloride-selective *unc-49* GABA receptor, ensuring coordinated locomotion. By contrast, the action of GABA on the enteric muscles appears to be excitatory. The GABA motor neurons AVL and DVB synapse directly onto the enteric muscles and are necessary for their enteric muscle contraction (1), allowing for expulsion of gut contents. Furthermore, *unc-25* mutants, which are deficient in the biosynthetic enzyme glutamic acid decarboxylase (2), fail to have enteric muscle contractions, suggesting GABA mediates this excitatory function. We are interested in the molecular nature and physiology of the excitatory GABA receptor that mediates enteric muscle contraction.

Of six genes known to be required for GABA function in *C. elegans*, *exp-1* is the only gene specifically required for the excitatory function of GABA at the enteric muscles. *exp-1* mutants lack enteric muscle contractions and are phenotypically constipated (3). *exp-1* was mapped to an interval between *lin-4* and *lin-23* on chromosome II. A fosmid within this interval, H35NO3, was injected into *exp-1(sa6)* mutants which rescued the mutant phenotype. The open reading frame H35N03.1 on this fosmid exhibits significant similarity to GABA receptors. Transgenes containing the H35N03.1 gene were sufficient for rescue of the *exp-1* phenotype and all six *exp-1* alleles contained mutations in this open reading frame.

The distribution pattern of the EXP-1 protein was visualized utilizing GFP. EXP-1::GFP reporter expression was observed in the enteric muscles. Importantly, puncta are localized to the pre-anal ganglion where AVL and DVB contact the enteric muscles, suggesting a synaptic localization of EXP-1. Analysis of a cDNA that encodes the complete EXP-1 protein suggests that EXP-1 is similar to members of the GABA receptor family. Interestingly, EXP-1 differs significantly from all known GABA receptor subunits within the M2 domain, which lines the ion channel pore and determines ion selectivity. In this domain, EXP-1 contains many residues that have been implicated in cation-selectivity among ligand-gated ion channels.

We examined the functional properties of EXP-1 expressed in *Xenopus laevis* oocytes using two-electrode voltage clamp recording. Oocytes injected with EXP-1 cRNA exhibit a robust GABA-evoked current, suggesting EXP-1 forms a GABA-gated homo-oligomeric receptor. To determine which ionic species permeate the EXP-1 channel, we performed current-voltage relationships in a number of solutions where Na^+, K^+ and Cl^- were manipulated. These experiments demonstrate that EXP-1 is an atypical ionotropic GABA receptor, in that EXP-1 is permeable to the cations Na^+ and K^+, and impermeable to the anion Cl^-. To date, this is the first identification and characterization of a non-selective cationic GABA receptor. Remarkably, EXP-1 resembles GABA receptors, yet is cation-selective and serves as an excitatory GABA receptor at postsynaptic membranes. The localization and ion-selectivity of the EXP-1 channel suggests that EXP-1 mediates GABA induced enteric muscle contractions in *C. elegans*.

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30. Molecular Mechanism of Synaptogenesis

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Synapse formation is an essential step in the development of nervous systems. Most of our understanding of synapse formation comes from studies on a special type of synapses, the vertebrate neuromuscular junctions. Little is known about the cellular and molecular mechanisms mediating the development of other types of synapses such as neuron-neuron synapses and *en passant* synapses. Using C. elegans as a model, we studied *en passant* synapses formed by an excitatory neuron, HSNL *in vivo*. We observed that ablation of the vulval epithelial cells, but not the postsynaptic VCs and vms, results in a defect in synaptic vesicle localization. Together with mutant analysis, these findings suggest that cells other than the pre and postsynaptic neurons can play important roles in synapse formation. Previously, the vulval tissue has been shown to play a role in HSNs and VCs branching, as well as HSN axon guidance and fasciculation. Our findings provide further evidence that the vulval cells are essential in the formation of the egg laying neuronal circuit.

In order to identify the molecular nature of the vulval signal, we isolated and characterized the mutant *syg-1(ky652)*, which has defects in synapse formation that are similar to those observed following ablation of the vulval epithelial cells. In *syg-1* mutants, HSN synaptic vesicles failed to accumulate at normal synaptic locations, and form ectopic clusters instead.
SYG-1(synaptogenesis) encodes an Immunoglobulin super family protein, Syg-1, which appears to act in the presynaptic HSN axon as the receptor for the epithelial signal. In the HSN of wild-type animals, Syg-1 localizes at synapses during the course of synaptogenesis. These findings suggest that immunoglobulin superfamily proteins play important roles in initiating synapse formation and might also specify individual connections between cells.

31. Identifying the role of endophilin in synaptic vesicle endocytosis

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Endophilin is an SH3 domain-containing protein which binds the proline-rich domains of synaptojanin and dynamin. Both dynamin and synaptojanin play a major role in clathrin-mediated endocytosis. Recent studies on the Drosophila endophilin mutant, D-endoA/endo, demonstrated that mutant animals have a depletion of synaptic vesicles in nerve terminals, indicating a defect in vesicle recycling (1,2). Endophilin is encoded by the unc-57 gene. We are characterizing unc-57 mutants to determine 1) if endophilin is required for synaptic vesicle endocytosis in C. elegans, 2). if the role of endophilin is to bind and localize synaptojanin and/or dynamin to sites of endocytosis, and 3). if the lipid modifying activity of endophilin is required for its function.

Consistent with a role in endocytosis,unc-57 is expressed in many, if not all neurons and in the spermatheca. In addition, GFP-tagged UNC-57 protein is present at synapses. Physiological recordings demonstrate that unc-57 mutants have reduced evoked and endogenous activity. This is consistent with a defect in either exocytosis or endocytosis. However, synapses fatigue more rapdily in mutants than wild-type animals. These data are consistent with endophilin playing a role in synaptic vesicle recycling. Moreover, proteins normally endocytosed into vesicles are diffusely distributed on neuronal processes. Specifically, a GFP-tagged synaptic vesicle protein, synaptobrevin (VAMP), is diffusely localized in the unc-57(ok310) mutant background rather than punctate as is seen in wild-type worms. This result is similar to the phenotype of other endocytosis mutants such as unc-26, dyn-1, unc-11, and snt-1. Finally, proof of an endocytosis defect will require an ultrastructural analysis; these experiments are in progress.

It has been suggested that endophilin is required to bind and localize dynamin and synaptojanin to sites of endocytosis. To test this we are looking at the localization of DYN-1::GFP and UNC-26::GFP in an unc-57 mutant background. Our data suggests that at a gross level, DYN-1:GFP is correctly localized in unc-57 mutants. We do not yet know if UNC-26 is localized.

Schmidt et al. recently showed that endophilin has a lipid modifying function - specifically, lysophosphatidic acid acyl transferase activity (LPAAT) (3). This lipid modifying activity converts lysophopatidic acid to phosphatidic acid. It was proposed that a specific lipid composition may be required for plasma membrane invagination during synaptic vesicle endocytosis. We are considering a variety of approaches to determine if C. elegans endophilin has LPAAT activity and if that activity is required for its role in synaptic vesicle endocytosis.

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32. Dense core vesicle trafficking in neurons visualized in vivo

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In mammals, the closely related protein tyrosine phosphatase-like type 1 transmembrane proteins ICA512 and phogrin 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. IDA-1 is the single C. elegans ortholog of ICA512 and phogrin and is also expressed in a subset of neurons and the neuroendocrine-like uv1 cells. Immunocytochemistry showed that IDA-1 is localized intracellularly to small punctate organelles identified as DCVs. An IDA-1::GFP fusion protein proved a useful tool to investigate the in vivo trafficking of DCVs in C. elegans neurons. Real-time imaging demonstrated that vesicle movements occurred bidirectionally with frequent saltatory movements and occasional reversals. Transport was blocked by nocodazole and azide, suggesting the involvement of distinct microtubule-based molecular motors of the kinesin and/or dynein superfamilies. Movements were complex and occurred at multiple distinct velocities of up to 4 um/s, implying the presence of multiple motor proteins with different properties. Furthermore, transport velocities were different for axons and dendrites, demonstrating a polarized distribution of the molecular transport machinery.

33. Mutations that Activate the Gs alpha Pathway Bypass the Neurotransmitter Release Blockade in *ric-8(md303)* and Reveal Another Branch of the Synaptic Signaling Network

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During synaptic transmission neurons exchange information with each other, or with other cells, at specialized structures known as synapses. The amount of synaptic transmission that occurs at synapses is highly regulated, and understanding this regulation should provide insights into how the nervous system establishes, maintains, and modifies behavior. With this goal in mind, we are investigating the molecular signaling pathways that regulate neurotransmitter secretion.

Genetic studies in *C. elegans* have revealed a network of signaling proteins, involving GOA-1 (Goα) and EGL-30 (Gqα), that appears to regulate synaptic transmission, in part by regulating diacylglycerol levels. 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 extends beyond the known components. We therefore undertook 2 genetic screens to identify some of the missing components. In the first screen we looked for mutations that suppress the nearly paralyzed phenotype of *ric-8(md303)* mutants. RIC-8 (synembryn) is a novel, conserved protein whose precise function is a mystery, but genetic studies suggest that it functions upstream of EGL-30 (Gqα), or in a parallel intersecting pathway. In the second screen we looked for additional mutants that exhibit the hyperactive locomotion phenotype caused by excessive EGL-30 signaling.

The combined screens yielded a number of interesting mutants, including a group of 7 mutants that are strikingly strong suppressors of *ric-8(md303)* and exhibit a similar spectrum of phenotypes in a *ric-8(+)* background (including hypersensitivity to aldicarb and hyperactive locomotion). Through high resolution SNP mapping, candidate gene sequencing, and transformation rescue (where possible), we found that the 7 mutations define 3 genes: 3 of the mutations are dominant gain of function mutations in *gsa-1* (Gsα), 2 are dominant gain of function mutations in *acy-1* (adenylyl cyclase; a.k.a. *sgs-1*), and 2 are recessive, reduction of function mutations in *kin-2* (regulatory subunit of protein kinase A). All of the mutations change conserved residues and are predicted to result in constitutive activity of either GSA-1, ACY-1, or KIN-1 (catalytic subunit of protein kinase A).

Our results suggest that the inferred neurotransmitter release blockade in *ric-8(md303)* mutants results from strongly reduced activity of the GSA-1 pathway, or of a parallel intersecting pathway. One candidate for a parallel intersecting pathway is the EGL-30 (Gqα) pathway. We are currently using the new Gsα pathway mutants to investigate the relationship between the Gsα and Gqα pathways. These studies may provide new insights into the molecular circuitry that turns synapses ON and OFF.

34. Cellular physiology of cultured *C. elegans* mechanosensory neurons

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In *C. elegans* the response to the gentle touch delivered by an eyelash is mediated by six specialized sensory neurons, the mechanosensory neurons. Analyses of *mec* mutants (*mec*hanosensory abnormal) identified many genes that are important for the normal function of these neurons including those encoding the DEG/ENaC ion channel subunits *mec-4* and *mec-10*, *mec-7* and *mec-12* that encode for β and α tubulins respectively, the collagen gene *mec-5*, the EGF-rich *mec-9* and the stomatin-like gene *mec-2*. MEC-4 and MEC-10 are coexpressed exclusively in the six touch neurons and form a heteromeric channel postulated to constitute the core of a mechanosensory ion channel. Localized tension is postulated to be administered by tethering the extracellular MEC-4/MEC-10 channel domains to extracellular matrix proteins such as MEC-5 and MEC-9 and anchoring intracellular channel domains to a 15-protofilament microtubule network exclusively assembled in the touch receptor neurons by MEC-7 and MEC-12.

C. elegans has provided an extremely powerful model system for the identification of the molecular determinants of the mechanotransduction complex, but has had limitation for the direct electrophysiological characterization of the postulated mechanically gated ion channel complex. In addition, a recent report (Goodman et al., *Nature* 2002 415, 1039 - 1042) has shown that while functional MEC-4/MEC-10 channels can be reconstituted in *Xenopus* oocytes, in this expression system they are not mechanically gated possibly due to the lack of some important interacting proteins. Recently a method has been developed for the isolation and culture of *C. elegans* embryonic cells (Christensen et al., *Neuron* 2002 33: 503-514) that allows the application of standard electrophysiological techniques to isolated touch neurons.

Of the six touch neurons, four (ALML/R and PLML/R) develop during embryogenesis and are likely to be present among isolated embryonic cells. We cultured embryonic cells isolated from *Pmec-4*::GFP-expressing worms. Within 24 h after isolation, GFP expression was detected in ~2.0% of cells in culture. All cells expressing GFP developed only one neuronal process that increased in length with further days of culture. Cultured touch neurons expressed, as *in vivo*, acetylated α tubulin and underwent necrosis when expressing the deadly *mec-4* mutant subunit harboring the A713V substitution (*mec-4(d)*). Cell death was rescued in cultured touch neurons by maneuvers known to rescue *mec-4(d)* induced cell-death *in vivo*, namely addition of amiloride or dantrolene to the culture media or the deletion of calreticulin (Xu K. et al., *Neuron* 2001 31:957-71). These results suggest that touch neurons in culture differentiate and behave similarly to what is observed *in vivo*.

In the attempt to identify and study the mechanosensitive ion channel complex we patched clamped in the cell-attached and inside-out modes cultured touch neurons. We identified a strongly outwardly rectifying potassium channel that displays a conductance of ~13 pS and a stretch-activated sodium channel that functions independently of *mec-4*. We are presently carrying experiments to identify the molecular determinants of the stretch-activated channel and to determine the localization of MEC-4 channels within the touch neurons using specific antibodies.

We thank Dr. K. Strange for sharing with us preliminary observation on cultured touch neurons.

35. Analyses of mechanosensory transduction by *in vivo* calcium imaging

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In *C.elegans*, genetic analyses of gentle body touch sensation have lead to identification of the molecular components required for the mechanosensory transduction. However, characterizations of molecular events carried out by these molecules have been limited by the lack of techniques to detect such events *in vivo*. Direct electrophysiology is precluded by the tough cuticle and the unique environment surrounding the touch neurons, which also limit pharmacological characterization.

We have developed imaging methods to monitor in vivo calcium transients in neurons and muscle cells by using a gene-encodable calcium indicator, yellow cameleon (Miyawaki, et al., *PNAS* 96:2135-2140, Kerr et al., *Neuron* 26:583-594). We can monitor *in vivo* calcium transients by measuring the fluorescent emission of yellow cameleon through the transparent cuticle.

Several improvements to our method have been made since our preliminary study (IWM 2001, abstract 659). 1) reconstruction of *mec-4* promoter/cameleon reporter 2) integration of the transgene and repeated outcrossings 3) employment of Piezo electronic devices for our mechanical stimulator to precisely deliver gentle touch stimuli 4) programming the movement of the stimulator (speed, traveling-distance, interstimulus interval, etc)

Delivery of a stimulus consisting of a 10 micron deflection lasting 150msec(75msec forward travel, 75msec backward travel) on the sensory process of a touch neuron (ALM, AVM or PLM) evokes a rapid increase in intracellular calcium followed by a gradual decrease. A variety of other "gentle touch stimuli" with different movements were tried, and the size of the calcium transients corresponded to the duration of motion during stimulation. However, a motionless probe pressed against the worm failed to cause or maintain a calcium transient. These observations suggest that touch neurons primarily sense the motion of the applied mechanical stimulation.

Several types of "gentle touch stimuli" were applied to *mec* mutants (*mec*hanosensory abnormal) identified by the genetic analyses. We have tested *mec-4* (for a candidate mechanically-gated ion channel), *mec-2* (for a stomatin-like protein that has been shown to potentiate MEC-4 channel activity) and *mec-6* (neeed for stable MEC-4 channel expression) and none of the mutants have responded to the "gentle touch stimuli".

We have also employed a recently developed primary culture method of *C.elegans* embryonic cells (Christensen et al., *Neuron* 33: 503-514). The cells were depolarized with high potassium saline, and the calcium transients were imaged the same way as the intact worms. The cultured mechanosensory neurons from the wild-type and *mec-2* and *-4* mutants showed essentially indistinguishable calcium transients, confirming the intactness of the indicator and the calcium influx/ release mechanisms in the mutant worms. These observations also suggest that MEC-2 and MEC-4 are required to depolarize the touch neurons in response to mechanical stimulation, presumably by sodium influx via MEC-4 channel potentiated by MEC-2.

Voltage-gated calcium channels (VGCC) are good candidates for the calcium entry in response to mechanosensation. Apparently wild-type calcium transients have been observed in *unc-2* (non L-type VGCC) null alleles. Other sources of cytosolic calcium transients are under investigation, including egl-19 (L-type VGCC), *unc-68* (ryanodine receptor) and *itr-1* (inositol 3-phosphate receptor).

We are grateful to Dr. Atsushi Miyawaki for his kind supply of recent versions of cameleons.

36. Intracellular Ca^{++} increase in ASH sensory neuron in response to water-soluble chemical repellents.

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C.elegans is able to detect and avoid water-soluble chemical repellents in the environment. This behaviour is mainly mediated by few sensory neurons of the amphid, of which ASH is the principal player. We are interested in understanding the molecular mechanisms underlying the detection and transduction of the different chemical stimuli by ASH, as well as characterizing the activity of the neuronal circuit downstream the sensory cells.

Recently, optical recordings of neuronal and muscle cell activity have been made in *C.elegans* using cameleon, a novel calcium sensor (Kerr et al. 2000). Cameleons are genetically-encoded fluorescent indicators for Ca^{++} in which a short-wavelength mutant of green fluorescent protein (GFP), calmodulin (CaM), a CaM-binding peptide (M13), and a long-wavelength mutant of GFP are tandemly fused. Binding of Ca^{++} to the CaM causes it to grab the M13 peptide, thus increasing FRET (fluorescence resonance energy transfer) from the short wavelength mutant GFP (donor) to the long-wavelength one (acceptor).

Using a cell specific promoter, we have generated lines expressing camaleon in the ASH sensory neuron (*sra-6*::YC2.12) and the activity of this neuron has been recorded after in vivo stimulation with different chemical repellents including high osmotic strength, copper ions, SDS and quinine. ASH showed a strong increase of intracellular Ca^{++} following stimulation with all four repellents used. We found that ASH responded to sustained stimuli -more than 15 sec- by maintaining high level of intracellular Ca^{++}. We also noticed that repetitive stimulation of ASH with Cu^{++} can induce reversible decrease in Ca^{++} responses; the time scale of the desensitization observed in ASH seems to be comparable with that observed during habituation experiments on the plate. Finally, we have started to use mutant animals (*osm-10*, *osm-9*, *tax-6* and *eat-4*) to investigate the role that these ASH-expressed signaling molecules play in the Ca^{++} response.

Kerr R, Lev-Ram V, Baird G, Vincent P, Tsien RY, Schafer WR: Optical imaging of calcium transients in neurons and pharyngeal muscle of *C.elegans*. Neuron 2000, 26: 583-94.

37. Dissecting the Neural Circuit Controlling Chemotaxis in C. elegans

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The behavior of humans and other animals is generated by neural circuits. Elucidating the logic of information processing in circuits is an important step in understanding how behavior is generated. We are studying the C. elegans chemotaxis circuit, a simple circuit that nevertheless carries out complex behaviors.

Previous work has demonstrated that C. elegans can chemotax to water-soluble attractants sensed by the ASE neurons via a biased random walk (1), during which periods of relatively straight movement are interrupted by sharp turns (or "pirouettes," which consist of reversals and omega bends). When worms detect an increase in attractant, they suppress pirouettes, and when they detect a decrease in attractant, they stimulate pirouettes. We have created temporal gradients of odors to demonstrate that a similar biased random walk strategy can also be used by the AWC neurons during chemotaxis to volatile odors. Because the circuit downstream of AWC and ASE has been mapped anatomically, this circuit offers a unique opportunity to ask how a neural circuit can direct a biased random walk.

We are addressing this question by using a laser to ablate neurons in the circuit and assessing the effects on turning and on chemotaxis. Interestingly, we found that the frequency of pirouettes in the absence of odorant varies inversely with starvation. Worms removed from food pirouette frequently for about twenty to thirty minutes but pirouette far less often after that. Surprisingly, a single class of sensory neurons, AWC, is required for the elevated pirouette frequency seen imediately after removal from food.

Because AWC is also required for chemotaxis to several volatile odorants, we have used this new "removal from food" assay as a starting point for dissecting the neural circuit downstream of AWC. We find a strong role for several interneuron classes, including AIY and AIB, in mediating pirouette frequency upon removal from food. These same neurons have very little effect on the short reversals typical of worm behavior on food. We are currently testing these interneurons for their effects on pirouettes in gradients of attractant.

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38. The DAF-7 TGF-beta signaling pathway regulates chemosensory receptor gene expression in *C. elegans*

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Animals sense chemicals in their environment using the olfactory and gustatory sensory systems. Although olfactory plasticity regulated by environmental conditions and developmental cues has been described in both insects and vertebrates, the underlying molecular mechanisms and sites have not been fully defined. *C. elegans* provides an excellent model system in which to study the mechanisms by which olfactory behaviors are modulated. Worms exhibit robust and sensitive olfactory responses to multiple chemicals using a small and well-defined number of chemosensory neurons. Chemosensory responses are altered under environmental conditions of overcrowding or starvation, long-term exposure to a chemical, or upon pairing of a normally attractive chemical with aversive stimuli.

Similar to other organisms, nematodes sense odorants via G protein-coupled receptors. However, in contrast to both *Drosophila* and rodents, *C. elegans* expresses multiple, partially overlapping sets of receptors in each chemosensory neuron type. Moreover, the behavior of the animal is determined not by the receptor itself but rather the neuron in which a particular receptor is expressed. For example, ODR-10 expression in AWA mediates attractive behavior, but when ODR-10 is misexpressed in the AWB neurons, which mediate aversive behavior, worms avoid diacetyl. Thus, alterations in chemoreceptor expression can have a strong effect on behavior in *C. elegans*.

Here we describe a role for the *daf-7* TGF-β pathway in the regulation of expression of a subset of chemoreceptors in *C. elegans*. DAF-7 TGF-β signaling regulates the expression of chemoreceptor genes in the ASI chemosensory neurons and a receptor gene in the ASH sensory neurons via distinct mechanisms. We describe a novel role for this pathway in maintaining receptor gene expression in the adult and demonstrate that the DAF-4 Type II TGF-β receptor functions cell-autonomously to modulate chemoreceptor expression. Furthermore, alteration of receptor gene expression in the ASI neurons by environmental signals, such as dauer pheromone, appears to be mediated via a DAF-7-independent pathway. Our results suggest that the expression of individual chemoreceptor genes in *C. elegans* is subject to multiple modes of regulation, thereby ensuring that animals exhibit the response most appropriate for their developmental stage and environmental conditions.

39. *unc-37/Groucho* and *cog-1/Nkx6.2* Act to Specify Left/Right Asymmetric Cell Fate in a Set of Chemosensory Neurons

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While the overall body plan of *C. elegans* is grossly bilaterally symmetric, there exist certain left/right asymmetries at both the anatomical and cellular levels. We have been focusing on the establishment and maintenance of left/right asymmetry in a set of bilaterally symmetric chemosensory neurons, ASEL and ASER, at the cellular level. While ASEL and ASER share many of the same features (i.e. axonal and dendritic morphology, synaptic connectivity and cell position), they show specific gene expression asymmetries. Three putative sensory receptors of the guanyl cyclase family, *gcy-5*, *gcy-6*, and *gcy-7* were found to be asymmetrically expressed in ASEL (*gcy-6, 7*) and ASER (*gcy-5*) [1]. It has also been previously shown that disruption of the *lim-6* transcription factor, which is exclusively expressed in ASEL, leads to the ectopic expression of *gcy-5* in ASEL [2]. This suggests that *lim-6* is responsible for mediating certain aspects of the left/right asymmetry that is seen in the ASE neurons. However, *lim-6* cannot be solely responsible for this asymmetry since it itself is already asymmetrically expressed. We have performed a genetic screen to uncover mutants that display symmetrization of *lim-6* expression and, consequently, symmetrization of otherwise ASEL- and ASER-specific features. We have gone on to identify 5 genes from this screen, but will focus our presentation on two complementation groups, one defined by the alleles *ot59* and *ot61* and the other by the alleles *ot28, ot38, ot62, ot76* and *ot80*. We found the first complementation group to be allelic to the transcriptional corepressor *unc-37/Groucho* and the second group allelic to *cog-1*, a recently identified homeobox transcription factor that is most similar to vertebrate *Nkx6.2* (Inoue and Sternberg, personal communication). Through analysis of promoter subfragments and comparison to *C. briggsae* sequences, we have defined stretches of homology between the *lim-6* and *gcy-7* promoters and it is possible that COG-1 binds directly to one or all of these sequences to mediate its repression in ASER. COG-1/Nkx6.2 possesses a conserved amino acid motif that is similar to the core region of the engrailed homology-1 (eh1) domain present in *Drosophila* Engrailed, a transcriptional repressor. Previous studies by Muhr et al. [3] have shown that chick Nkx6.2 interacts with Groucho *in vitro* via its eh1 domain to ensure maximal repression of inappropriate cell type-specific gene transcription as a means for restricting cell fate. We speculate that COG-1 may directly interact with UNC-37 via a similar mechanism.

A *cog-1* transcriptional reporter is symmetrically expressed in ASEL and ASER and it has previously been shown that *unc-37* is expressed ubiquitously [4]. This raises the question as to how symmetrically expressed genes can regulate asymmetric features within two bilaterally symmetric cells. One possibility is that the COG-1 and UNC-37 proteins are activated in ASER, but not in ASEL. Another possibility is that COG-1 and UNC-37 are post-translationally modified in ASEL such that they no longer function as repressors of *lim-6* and *gcy-7*. A third possibility is that they are active in both cells, but require other factors that are not present in ASEL in order to repress the expression of *lim-6* and *gcy-7*. There is evidence in *Drosophila* that activation of a receptor tyrosine kinase signaling pathway permits gene expression by antagonizing Groucho-mediated repression [5]. We have other uncloned mutants from our screen whose phenotypes suggest that they may carry mutations in the gene(s) that provide the inductive signal responsible for establishing asymmetric UNC-37 and COG-1 function in the ASE neurons. Based on the genetic interactions of the mutants retrieved from our screen as visualized by transcriptional gfp reporters, it appears that the final fates of ASEL and ASER are established by a series of transcriptional repressive interactions that are layered upon what is likely to be a symmetric ground state.

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40. Serotonin modulation of a chemosensory circuit in *C. elegans*

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C. elegans responds to the volatile repellant 1-octanol by moving backwards. To study this chemosensory response we screened for mutants that do not respond to octanol, and found that a subset of the mutants obtained were defective only in the absence of food. Because the presence of food is thought to increase serotonin (5-HT) signaling, we investigated whether 5-HT is involved in this food-dependent regulation of octanol detection. Exogenous 5-HT restores the ability to respond to octanol in the absence of food in most of these mutants. Furthermore, at least two of these mutants were also partially resistant to 5-HT-induced paralysis, suggesting that they may be defective in 5-HT signaling in motor neurons as well. Because *eat-4* animals (which are defective for glutamatergic signaling) are mutant for octanol avoidance, but *tph-1* animals (which lack 5-HT biosynthesis) and *dgk-1* animals (which are defective for 5-HT signaling) are only defective in detecting dilute octanol, our results suggest that 5-HT modulates glutamatergic signaling in this circuit. We are currently further characterizing these mutants in the hope of identifying genes involved in 5-HT modulation of synaptic signaling.

We investigated the roles of ASH, ADL, and AWB, three amphid sensory neurons previously found to be important for responding to volatile chemorepellants (1), in this food-dependent modulation. Laser ablation experiments revealed that ASH is the primary sensory neuron for octanol detection in the presence of food, while ADL and possibly AWB are important for octanol detection off food. Exogenous 5-HT largely mimicked the presence of food, providing further evidence that 5-HT directly modulates synaptic activity in this circuit. In one possible scenario 5-HT could inhibit octanol detection in ADL and AWB, resulting in only ASH being active on food; however, in *tph-1* animals, ablation of ASH results in a strong defect in octanol avoidance both on and off food, indicating that ADL and AWB are inactive even in the absence of 5-HT biosynthesis. Therefore, we propose an alternative model in which "silent synapses" between the sensory neurons in the off-food circuit and command interneurons are silent when 5-HT is absent, inhibited at high levels of 5-HT (*i.e.*, when animals are well-fed), and become active when 5-HT levels drop below a certain threshold (*i.e.*, when animals are starved). This is reminiscent of silent glutamatergic synapses in the rat spinal cord dorsal horn, in which synapses are active at low levels of 5-HT but are silent when 5-HT levels are absent or high (2). Our data suggest a possible conserved cellular mechanism by which 5-HT could modulate sensitivity in the nervous system.

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41. CED-10/Rac and UNC-34/Enabled mediate distinct pathways in UNC-6/Netrin-dependent axon attraction

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UNC-6/Netrin promotes axon outgrowth and turning through the DCC/UNC-40 family of receptors. In order to identify signaling events downstream of UNC-40, we have generated a constitutively active UNC-40 molecule by replacing the extracellular and transmembrane domains with a myristoylation signal. MYR::UNC-40 expression causes axon guidance and branching defects, as well as excessive axon and cell body outgrowth. This excessive outgrowth is suppressed by loss-of-function mutations in three genes: *ced-10*, a Rac GTPase; *unc-34*, an Enabled homolog; and *unc-115*, a putative actin-binding protein. Analysis of loss-of-function mutants suggests that *ced-10, unc-34*, and *unc-115* also function in the endogenous *unc-40* pathway. UNC-40 has two conserved cytoplasmic motifs that mediate two distinct downstream pathways: CED-10, UNC-115 and the UNC-40 P2 motif act in one pathway, and UNC-34 and the UNC-40 P1 motif act in the other. Thus, UNC-40 might act as a scaffold to deliver several independent signals to the actin cytoskeleton. Futhermore, our results indicate that, in addition to its known role in axon repulsion, Enabled can function as an effector of axonal attraction.

In order to understand how UNC-6/Netrin elicits axon outgrowth and turning, we are currently examining the effects of presenting UNC-6/Netrin to primary cultures of C. elegans neurons from wild type animals and *unc-40, unc-34, ced-10* and *unc-115* mutants. Preliminary work in collaboration with Kevin Strange (Vanderbilt University) indicates that purified vertebrate Netrin-1 can stimulate axon outgrowth and turning of cultured *C. elegans* neurons.

42. Three miscellaneous axon guidance genes

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To understand further the molecular mechanisms involved in growth cone guidance, we performed several screens to identify new genes involved in axon extension and pathfinding. Mutants with a variety of axon outgrowth, branching and pathfinding defects as well as mutants with either axonal degeneration, cell fate, cell migration, cell position or programmed cell death defects were recovered. Over 160 mutants were isolated that define over 40 genes (see abstract by Chiu, Sabater, Squires & Clark). We cloned five of these genes: *zag-1, tba-1, klp-7, mig-11* and *wly-1*.

zag-1 mutations alter the expression pattern of several neuronal reporters and cause defects in axon branching and fasciculation, suggesting that *zag-1* functions in neuronal differentiation and axonal development. ZAG-1 contains two clusters of Zn finger domains and a single homeodomain and is similar in sequence and structure to the products of the *Drosophila zfh-1* and vertebrate deltaEF1 and SIP1 genes. ZAG-1 likely functions as a transcriptional repressor like other deltaEF1/zfh family members, as it also shares a conserved sequence motif required for association with the CtBP corepressor protein. A *zag-1::gfp* transcriptional reporter is expressed in both neuronal and nonneuronal cells. A high level of *zag-1::gfp* expression is observed in ventral cord motoneurons in adult *zag-1* mutants yet not in wildtype adults. A similar expression pattern is seen using a *zag-1* promoter construct containing a mutated putative *zag-1* binding site, suggesting that ZAG-1 directly regulates its own expression.

Microtubules are needed for growth cone extension, but their role in guidance is less well defined. *tba-1* is one of nine alpha tubulins present in the genome. Axons terminate their extension prematurely and are often misdirected in the *tba-1(zd109)* mutant. Expression of a *tba-1::gfp* reporter is widespread during early embryogenesis and becomes restricted largely to neurons and neuroblasts as development progresses. These observations suggest that high levels of the TBA-1 alpha tubulin are needed to promote the correct, directed growth of axons.

klp-7 encodes a homologue of the mitotic centromere-associated kinesin (MCAK), which is needed for anaphase chromosome separation. MCAK belongs to the Kin I (I for internal, as the conserved motor domain is located in the middle of protein) family of kinesins and has microtubule depolymerization activity. *klp-7(rnai)* has been reported to cause embryonic lethality. We found a *klp-7* mutation that causes embryonic lethality as well as Unc and Him phenotypes. In *klp-7(zd159)* animals, specific neurons can extend a long, wandering ectopic axon. The normal axon from these neurons appears unaffected. These results indicate that *klp-7* function is needed beyond mitosis and meiosis, as the reduction of *klp-7* microtubule depolymerization activity leads to the generation of ectopic axons in neurons.

mig-11, which causes CAN migration defects when mutated, was found to be the same gene as *ceh-10* and *wly-1*, which causes axonal degeneration when mutated, is described in the abstract by Yam, Chiu & Clark.

43. Axon guidance at the ventral nerve cord by UNC-71, a disintegrin and metalloprotease protein

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We are interested in how the ventral cord type D motor neurons, including 6 DDs and 13 VDs, are guided to their targets. Each D neuron first extends a longitudinal axon along the ventral nerve cord. At a defined point, the axon branches a commissure, which is repelled by UNC-6/netrin to grow circumferentially to reach the dorsal cord. The growth of the commissures has a striking left-right preference such that 17 commissures of the D neurons exit to the right side of the ventral cord and only two exit to the left. The longitudinal axons of DD neurons fasciculate tightly with those of VDs. Studies by R. Durbin showed that the interneuron AVG plays a pioneering role in guiding the longitudinal elongation and branching pattern of the D neuron axons (1). However, the underlying molecular mechanisms are not well understood.

From a genetic screen for D neuron axon guidance mutants, we isolated 8 *unc-71* alleles (2). *unc-71* is known to regulate axon fasciculation, left-right asymmetry of the D neuron commissures, and sex myoblast (SM) migration (3, 4). We found that in *unc-71* mutants the axon guidance for the DA and DB classes of ventral cord motor neurons is only slightly affected. We cloned *unc-71* and showed that it encodes a disintegrin and metalloprotease protein, also known as ADM-1 (5). Identification of the molecular lesions in *unc-71*alleles indicates that the extracellular disintegrin and cysteine-rich domains are critical for UNC-71 function in vivo. Different from the reported expression pattern of ADM-1, we found that an *unc-71* promoter-driven GFP is expressed in the excretory canal, some epidermal cells, and a selected set of neurons that do not appear to include the D neurons. A functional UNC-71::GFP is localized to cell membranes.

To address where *unc-71*function is required for the guidance of D neuron axons, we performed genetic mosaic analysis and tissue-specific rescue studies. We found that *unc-71* is required in the AB lineage, and that a neural and epidermal promoter *unc-115*driving *unc-71* fully rescues the D axon defects. However, the axon defects of D neuron can not be rescued by D neuron specific promoters driving *unc-71,* instead, are partially rescued by a *glr-1* promoter driven *unc-71,* which is expressed in AVG and other interneurons. Exogenous promoter-rescue experiments have also suggested a cell non-autonomous role for UNC-71 in SM migration guidance. We conclude that *unc-71* functions cell non-autonomously to provide guidance information for both D neuron axon outgrowth and sex myoblast migration.

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44. *mig-21* Encodes a Novel Putative Transmembrane Protein Required for the Asymmetric Q Neuroblast Migrations

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The QL and QR neuroblasts are born in bilaterally symmetric positions along the A-P body axis in *C. elegans* (QL on the left and QR on the right). Shortly after hatching, the two cells undergo characteristic, stereotypical L/R asymmetric migrations. QL extends a long process to the posterior and its nucleus then migrates into this projection. The descendants of QL remain in the posterior. Conversely, QR extends a process to the anterior and QR and its descendants subsequently migrate anteriorly.

In *unc-40* or *dpy-19* mutants, polarization of both Q cells is randomized and the Q nuclei fail to undergo their migrations [1]. *unc-40* encodes the C. elegans *unc-6*/netrin receptor; however, neither *unc-6* nor *unc-5*, a co-receptor for *unc-40*, is required for Q cell migration. This raises the possibility that a novel signaling pathway may be instructing the Q cells to polarize using the *unc-40* netrin receptor. In order to identify additional components of this pathway, we have isolated several asymmetry mutants from a large scale Q cell migration screen. These genes, *qid-1*, *hch-1*, and *qid-3*, are all required for the initial asymmetry of the Q cells. Mutations in both *qid-1* and *qid-3* result in randomized Q cell polarizations reminiscent of *unc-40* and *dpy-19* mutants. The Q nuclei also fail to migrate in *qid-1* and *qid-3* mutants. Complementation tests revealed that *qid-1* is allelic to *mig-21*, a gene previously identified as being required for Q cell migration [2]. Epistasis analysis suggests that *mig-21* may act in parallel with *unc-40* in guiding the Q cell migrations.

In order to determine how the initial L/R asymmetry of the Q cells is established, we have cloned *mig-21* and found that it encodes a novel protein with a putative transmembrane domain and two thrombospondin (TSP) type I repeats. TSP type I domains are thought to mediate cell-cell or cell-substrate interactions. *unc-5* also contains two TSP type I domains, raising the possibility that *mig-21* may be interacting directly with *unc-40*, by analogy with the *unc-5* - *unc-40* interaction. *unc-40* is expressed in the Q cells during their migrations, and is thought to act cell-autonomously. To test whether *mig-21* may be acting cell-autonomously, we plan to build promoter fusions to define where it can function to direct the migrations of the Q cells. We will also use transcriptional and translational GFP reporters to determine its endogenous expression pattern.

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2. **Du, H and Chalfie M.** (2001). Genes regulating touch cell development in *Caenorhabditis elegans*. *Genetics* **158** 197-207.

45. Regulation of Epidermal Cell Fusion in *C. elegans*

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The *C. elegans* epidermis is composed of several large multinucleate syncytia that are generated by the fusion of mononucleate 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. Late in L1, some of the 12 Pn.p cells that line the ventral surface of the worm fuse with the hyp7 syncytium. However, it is important that certain Pn.p cells remain unfused because they generate sex-specific mating structures.

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. MAB-5 activity is inhibited in hermaphrodites by two other transcription factors, REF-1 and EGL-27. 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, they neutralize each other's activities, so that P7.p and P8.p fuse with hyp7.

We identified several mutations that affect the pattern of Pn.p cell fusion and have been characterizing mutations that affect Pn.p cell fusion by altering Hox protein activity. In *ref-2(mu218)* mutants males, P7.p and P8.p fail to fuse with hyp7 because LIN-39 and MAB-5 fail to inhibit each other. *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 altering expression of this transcription factor. 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-inducible 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. REF-2 functions with the Hox proteins to prevent Pn.p cell fusion. *ref-2* may also be a transcriptional target of the Hox proteins.

46. VAB-19, a novel conserved protein involved in epidermal elongation in C. elegans

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Trans-epidermal attachments (also known as fibrous organelles) are composed of intermediate filaments and hemidesmosome-like structures and are important for epidermal elongation in *C. elegans*. However, their exact function in controlling cell shape is not well understood. Previously, we reported that the gene *vab19* is required for normal epidermal elongation and encodes a novel conserved protein. We further explore the function of *vab-19* in epidermal elongation as reported here.

The original *vab-19* allele, *e1036*, is a cold-sensitive allele. At 15 °C, all *e1036* animals arrest elongation at the two-fold stage after which they develop deformations in the epidermis (the twofold lumpy phenotype). These animals typically hatch but do not develop further. We performed a semi-clonal screen for new two-fold lumpy mutants. We screened 8000 haploid genomes and isolated one new *vab-19*allele, *ju406*, and over 40 additional mutations that define several new complementation groups. We are currently mapping and characterizing these new genes.

*vab19(ju406)*mutants display fully penetrant lethality at all temperatures; the *ju406* phenotype is indistinguishable from that of *e1036* at 15°C. Using 4D Nomarski microscopy analysis, we found that *ju406* mutants have normal muscle contraction movements but fail to elongate beyond the two-fold stage, and no obvious defects in earlier embryogenesis. These data are consistent with our previous finding that the temperature-sensitive period of *vab19(e1036)* is during elongation.

vab-19 encodes a novel, conserved protein with four ankyrin repeats. VAB-19::GFP is expressed in epidermal cells; expression of VAB19 under the control of the epidermal *ajm1*promoter rescues *vab-19*mutant phenotypes, indicating that VAB19 functions cell autonomously within the epidermis. Within epidermal cells, VAB-19::GFP is localized to trans-epidermal attachments and progressively colocalizes with intermediate filaments during embryonic morphogenesis. In *vab-19* mutant animals, the initial development of trans-epidermal attachments is normal, suggesting that *vab-19* may be involved in maintenance of trans-epidermal attachments.

Loss of function mutations in the β_H-spectrin *sma-1* suppress the embryonic lethal phenotypes of *vab19(e1036* and *ju406*. Suppressed animals resemble *sma1* mutants. *sma1*mutations do not suppress other elongation-defective mutants such as *let-805* (Myotactin), *vab-10* (Plectin), *pat-3*, (Integrin) or *vab-9*. The specific suppression of *vab-19* by *sma-1* suggests that VAB-19 and SMA-1 may play antagonistic roles in the epidermal cytoskeleton.

Currently, we are also using a yeast two-hybrid screen to identify *vab-19* interacting proteins and preliminary results will be presented in the meeting.

47. Nicotine adaptation at the molecular level - the dynamics of nAChR expression and localization

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We are studying the molecular changes underlying adaptation of *C. elegans* to nicotine and other cholinergic agonists. Therefore, we have assessed the dynamics of expression of nAChRs and their localization to the plasma membrane that are occuring in worms subjected to nicotine. Worms expressing translational GFP fusions of Lev-1 and Unc-29, two subunits of the main muscle ("levamisole"-) receptor, show an overall strong reduction of their expression levels in muscles and neurons after nicotine treatment. This was also found for Des-2::GFP, a mainly neuronal nAChR; in contrast, expression of the $GABA_A$ receptor subunit Unc-49 remained unchanged. Thus the effect of nicotine on expression levels is specific for nAChRs, but may be independent of their agonist specificity, since Des-2 was shown to be more sensitive for choline rather than acetylcholine [Yassin et al. (2001), MCN 17, 589-99].

Expression of GFP driven by the promoters of the levamisole-receptor subunits Unc-29, Unc-38, Lev-1 and Unc-63, was not influenced by nicotine treatment; neither was GFP-expression driven by p acr-5. Thus, downregulation is not achieved at the transcriptional level, but must be controled either at the level of *de-novo* translation of receptors, or by enhancing a turnover mechanism that quickly degrades existing and newly synthesized nAChRs.

To study only those receptors exposed at the plasma membrane, we developed a novel method for immunostaining of cell surface-exposed proteins in live *C. elegans*. Worms expressing Lev-1 or Unc-38 with epitope tags at their (extracellular) C-termini, were injected into the pseudocoelom with dilute solutions of fluorescently labeled antibodies specific for these epitope tags. The anti-bodies readily accessed the extracellular epitopes, but did not stain intracellular receptors. After 5-6 hours, the scavenger cells had filtered unbound antibodies from the pseudocoelomic fluid, eliminating background staining. We could thus clearly visualize sites containing surface-exposed nAChRs in live worms. These sites are most likely postsynaptic densities of neuromuscular junctions and neuronal synapses, since they are juxtaposed, but strictly not overlapping, with sites containing presynaptic VAMP::GFP. Further experiments showed that co-expressed Unc-29::GFP and epitope tagged Lev-1 and Unc-38 are found in the same punctate sites along the ventral and dorsal nervecords, in the nerve-ring and on nose muscles, confirming genetic evidence that these proteins are part of the same receptor. However, we found Unc-38 also in a secondary nerve bundle running in parallel to the main ventral cord, that contained neither Lev-1 nor Unc-29. This indicates functions of Unc-38 in receptors different from the levamisole receptor. Consistently, we found Unc-38 in many more neurons than Lev-1 or Unc-29.

Surprisingly, over-night nicotine treatment, that leads to behavioural adaptation and overall downregulation of the receptors, did not result in any gross change in the numbers and density of nAChRs exposed at those postsynaptic sites. This was true even after 48 hours on nicotine and was independent of the sequence of nicotine exposure and antibody injection. Since nAChRs are not removed from postsynaptic sites, we propose that adaptation to nicotine is mainly achieved by desensitization of these receptors. The reason for the global receptor reduction may thus be to prevent insertion of new, non-desensitized receptors into the plasma membrane.

Further, we studied whether mutations in proteins known to be involved in nAChR expression, maturation and clustering have effects on the expression of receptors at the cell surface. We studied a deletion mutant of C. elegans rapsyn, a mutant of kin-8/cam-1, the strongest C. elegans MuSK homolog, agrin-RNAi worms, ric-3 mutants, and a nicotine-hypersensitive mutant isolated in our lab, nic-1. In untreated worms, only ric-3 mutants showed significantly different receptor densities at NMJs, as compared to wild type: those animals had strongly reduced levels of receptors at the plasma membrane, in agreement with the proposed function of ric-3 in receptor maturation. Ric-3 animals were also strongly resistant to nicotine. The other mutants tested showed no difference, even though kin-8 mutants were also moderately nicotine resistant. When we subjected the worms to nicotine, we observed that the mutants did not show significant changes, with the exception of nic-1 animals. These worms exhibited an increase in receptor numbers, that could explain the hypersensitivity of the nic-1 mutants to nicotine.

48. Characterization of the class A synMuv proteins LIN-56 and LIN-15A

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The receptor tyrosine kinase/Ras pathway essential for vulval induction in *C. elegans* is negatively regulated by the functionally redundant class A and B synthetic Multivulva (synMuv) pathways. Hermaphrodites mutant in only one of these two pathways appear wild-type for vulval induction. Hermaphrodites mutant in both pathways exhibit the 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 genetic 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 readily apparent 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.

We have cloned *lin-56*and found it to encode a novel acidic protein. Although no standard motifs are evident in the LIN-56 protein sequence, it does contain a C_3H Zn^{2+}-finger-like motif of atypical spacing and shares this motif with LIN-15A. Immunohistochemistry using antibodies directed against LIN-56 indicates that this protein is localized to the nuclei of many, if not all, cells throughout development. LIN-15A is also nuclear and broadly expressed. The wild-type LIN-56 expression pattern is maintained in *lin-8* and *lin-38* mutant animals. By contrast, LIN-56 protein, but not *lin-56* mRNA, is greatly reduced in *lin-15A* mutants, indicating a role for LIN-15A in the translation or stability of LIN-56 protein. Overexpression of a *lin-56* cDNA under the control of the heat-shock promoters fails to rescue the *lin-15A* synMuv phenotype despite production of LIN-56 protein. Preliminary experiments suggest that the level of LIN-15A protein may be reduced in a *lin-56*, but not in a *lin-8* or a *lin-38*, mutant background. We favor a model wherein LIN-56 and LIN-15A normally coexist in a functional complex, with absence of one of the subunits resulting in the instability of its binding partner(s).

49. LIN-12 downregulation in response to Ras activation in P6.p requires endocytosis and is necessary for lateral signaling

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Three Vulval Precursor Cells (VPCs), P5.p, P6.p and P7.p, give rise to the vulva. As a consequence of inductive and lateral signalling events, these cells adopt a 2°-1°-2° pattern of fates. The inductive signal, LIN-3, produced by the gonadal 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 an unknown lateral signal, believed to originate in P6.p, that activates LIN-12 in P5.p and P7.p, leading these cells to adopt 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 necessary and sufficient to cause LIN-12::GFP downregulation. Furthermore, function of *sur-2* and *lin-25*, two genes that appear to be targets of the Ras pathway and are necessary for proper lateral signalling, is required for LIN-12::GFP downregulation 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 have investigated the mechanism of LIN-12 downregulation in response to Ras activation and the role of LIN-12 downregulation in VPC fate 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.

By deletion analysis of LIN-12::GFP we identified a downregulation targeting signal (DTS) within the intracellular domain of LIN-12. The DTS includes a di-leucine motif that is necessary for downregulation. Di-leucine motifs are well-characterized sorting signals involved in the endocytosis and downregulation of various proteins. We have observed a marked difference in subcellular localization between LIN-12(+)::GFP and LIN-12(deltaDTS)::GFP, consistent with the idea that the DTS mediates LIN-12 endocytosis. These results suggest that endocytosis of LIN-12 plays a role in Ras mediated downregulation.

Hermaphrodites that express LIN-12(deltaDTS)::GFP in all the VPCs or in P6.p alone have normal 1° fates, but exhibit defects in lateral signalling such that P5.p and P7.p do not become 2° and instead adopt non-vulval fates. These results suggest that when LIN-12 is stabilized in P6.p by a deletion of the DTS, it inhibits the expression and/or activity of the lateral signal.

Our results have identified a novel mechanism of LIN-12/Notch regulation that influences the routing of endocytosed LIN-12 and demonstrate that such regulation of LIN-12 plays an important role in the appropriate specification of cell fates *in vivo.*

50. *lin-17, lin-18* and patterning of the P7.p lineage

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We are interested in patterning of the 2° (secondary) vulval lineages P5.p and P7.p. In the wild-type, P5.p and P7.p produce stereotyped ABCD and DCBA patterns respectively. In *lin-17* and *lin-18* mutants, the polarity of the P7.p lineage becomes altered. We examined the P7.p lineage in *lin-17* (encoding Frizzled Wnt receptor), *lin-18* (encoding RYK receptor tyrosine kinase-related protein; W. Katz and P.W.S.), and double mutant using POP-1 (TCF/LEF) antibody staining and cell fate markers *ceh-2::yfp* and *cdh-3::cfp*.

In the wild-type, POP-1 is expressed in an asymmetric pattern with posterior daughters of P7.p and P7.px expressing a higher level than their respective sisters. In the *lin-17* mutant, the asymmetry among P7.p daughters is often reversed but the asymmetry among P7.px daughters is not strongly affected (R. Hill and J.R. Priess, pers. comm.). We found that in the *lin-18* mutant, as in the *lin-17* mutant, the asymmetry is often reversed among P7.p daughters but not P7.px daughters. We also found that in the double mutant, the asymmetry is reversed in both P7.p daughters and P7.px daughters.

In the mid-L4 stage, *ceh-2::yfp* labels vulB cells (P7.ppax) and *cdh-3::cfp* labels vulC and vulD (P7.paxx). vulA cells of the 2° lineage can be distinguished by non-expression of either markers and adherence to the ventral cuticle in the mid-L4 stage. We found that in *lin-17* and *lin-18* mutants, P7.pap (presumptive vulC) cells were transformed to the vulA fate. In the double mutant, P7.paa (presumptive vulD) cells were transformed to the vulA fate. These results are consistent with reversals of POP-1 expression patterns, and indicate that the lineage that had high POP-1 levels in both P7.px and P7.pxx stages correlate with the cells that adopt the vulA fate.

The mechanism by which Ryk receptor signals is not known. The extracellular domain of LIN-18 and Ryk shows homology to Wnt binding protein WIF-1, suggesting a possible mechanism as an alternative Wnt receptor or a Wnt co-receptor. Since a probable *lin-17* null mutation is enhanced by the *lin-18* mutation, LIN-18 can signal independent of LIN-17. Furthermore, a *lin-18::gfp* fusion construct lacking the kinase domain can rescue the *lin-18* mutant, suggesting that LIN-18 functions differently than receptor tyrosine kinases activated by phosphorylation. It is also possible that *lin-18* functions in a separate non-Wnt pathway also involved in P7.p polarity.

51. The transcriptional Mediator complex functions in Wnt, Notch and Ras signaling pathways and may integrate the pathways during the vulval development

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The Mediator complex can transmit activities of various transcription factors to RNA ploymerase to regulate transcription *in vitro*. However, its function in animal development is unclear. We have identified mutants in *dpy-22* and *let-19* by their defects in asymmetric cell divisions regulated by *lin-44/wnt* and *lin-17/frizzled*. We found that these genes encode homologs of TRAP230 and TRAP240 that are components of the Mediator complex. Weak mutations of *dpy-22*/TRAP230 were reported previously as *sop-1* mutations that suppress *pal-1* mutants but do not have appparent phenotypes by themselves (1). *dpy-22* and *let-19* mutants also show defects in cell fusion regulated by *bar-1*/beta-catenin, indicating that these genes functions in the Wnt signaling pathway. In addition to their defects in Wnt signaling, *dpy-22* and *let-19* mutants also show multivulva (Muv) phenotype. The induction of vulva is regulated by *lin-12/notch* and *let-60/ras*. Ras signaling promotes a cell fate called primary while Notch signaling induces the secondary cell fate. In *let-19* mutants, secondary lineages are ectopically induced, suggesting that *let-19* functions in *lin-12/notch* pathway. In contrast, another components of Mediator, SUR-2 and LET-425/MED6, was shown to function downstream of *let-60/ras* (2, 3). Furthermore, we found that *let-425* but not *sur-2* can suppress gain-of-function mutations in *lin-12* as well as *let-60(gf)*. These results indicate that both Ras and Notch signals are transmitted to Mediator to regulate three cell fates during vulval induction. We will discuss functions of Mediator in the integration of the signaling pathways.

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52. Mutational Analysis of the Sex Determining Protein FEM-1

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Sex determination in *C. elegans* occurs through a well-defined genetic pathway that consists of series of negative regulatory interactions. Molecular and biochemical data indicate that the sex determination pathway is a signal transduction pathway. The three *fem* genes, *fem-1*, *fem-2* and *fem-3*, act within this pathway to promote male development in all tissues. Of the three FEMs, FEM-1 is the most poorly understood in terms of biochemical activity. We have undertaken a genetic and molecular analysis of several loss of function alleles of *fem-1* in order to study its defined role in sex determination in more detail.

FEM-1 is a 656 amino acid protein that has seven copies of the ANK motif at its N-terminus and a single degenerate ANK repeat at its C-terminus. ANK repeats mediate protein-protein interactions. Missense mutations cluster to the N-terminal ANK repeats of FEM-1 indicating that they are critical for FEM-1 function. The majority of the missense that map to the FEM-1 ANKs likely disrupt their structure. However, we suggest that one missense allele, *e2195*, affects a specific FEM-1 binding activity. *e2195* affects a non-conserved residue in the 6th ANK repeat of FEM-1 and encodes a stable protein that exerts a weak dominant negative effect on *fem-1(+)* activity.

fem-1 directly or indirectly inhibits *tra-1* activity in the XO soma. It acts similarly in the germline, but it has an additional role independent of its effect on *tra-1*. This raised the possibility that FEM-1 has tissue specific activities. However, all of the alleles that we have characterized fall into a single allelic series and affect both the soma and the germline.

FEM-1 is unusual amongst the sex-determining proteins in that it has been highly conserved through evolution. Homologues of *fem-1* exist in Drosophila, mouse and humans. Studies of FEM-1 in cultured mammalian cells raised the possibility that *fem-1* has a conserved role in mediating apoptosis that is independent of its role in the sex determination pathway (Chan *et al.,* 1999, Chan *et al.*, 2000). Observations of programmed cell death in four different cell or tissue types in *fem-1* mutants argue strongly against any non-redundant role for FEM-1 in regulating apoptosis in *C. elegans*, except insofar as it indirectly regulates the sex-specific deaths of 6 neurons by regulating TRA-1A activity.

53. Molecular Characterization of the Dosage Compensation Gene, *dpy-21*

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Dosage compensation in Caenorhabditis elegans equalizes X-chromosome gene expression between XO males and XX hermaphrodites. DPY-21 is one of several proteins required for proper dosage compensation. *dpy-21* mutations disrupt X gene expression less severely than other dosage compensation mutations and cause only 20% larval lethality compared to extensive maternal-effect lethality. Further, unlike other dosage compensation *dpy* mutations which affect gene expression only in XX animals, *dpy-21*1 mutations appear to affect X-linked gene expression in XO animals. Assays of different X-linked genes show that *dpy-21* mutations can cause both elevated and reduced gene expression in males.

To better understand the unusual phenotypes of *dpy-21* mutations, we began a molecular characterization of *dpy-21*. The *dpy-21* gene was initially mapped to a region containing two YACs. *dpy-21* was cloned then by injecting single-stranded RNA from candidate ESTs derived from those YACs into *xol-1* mutants and assaying for suppression of male lethality. Two positive ESTs were found and used to assemble the *dpy-21* mRNA sequence in conjunction with 5' and 3' RACE. To verify the identify *dpy-21*, we sequenced seven *dpy-21* alleles and found DNA lesions within the predicted coding sequence: three premature amber STOP codons, three missense mutations, and one in-frame deletion. The 5610 nucleotide *dpy-21* transcript encodes a 1651 amino acid protein. The DPY-21 protein has no sequence similarity to known proteins, but putative homologs, with strong sequence identity within the carboxy-500 amino acids of DPY-21, are present in the *Drosophila melanogaster* and human genomes. To further explore DPY-21's role in dosage compensation, antibodies were made against the amino terminus and a 600 amino acid region in the middle of DPY-21. The anti-DPY-21 antibodies recognize a single 215 kDa band in embryonic extracts that is absent in extracts made from a *dpy-21* mutant containing a premature STOP codon. Immunofluorescence experiments show that DPY-21 localizes to the X chromosome in XX embryos with greater than 30 cells, when the dosage compensation complex assembles. The DPY-21 localization is dependent on SDC and DPY proteins; since in *sdc-2, sdc-3,* and *dpy-27* null mutants, DPY-21 no longer localizes to the X chromosome. Interestingly, other dosage compensation proteins have been shown to localize to the X chromosome in *dpy-21* mutants; therefore, DPY-21 is not required for the stability or X localization of the dosage compensation complex. However, DPY-21 is a part of the dosage compensation complex as evidenced by DPY-21's ability to immunoprecipitate SDC-3. These results lead us to theorize that DPY-21 may modify or regulate the dosage compensation complex activity. Dosage compensation proteins are recruited not only to the X chromosome, but also to the *her-1* locus. The *her-1* gene is necessary for male development and is repressed in the hermaphrodite by SDC proteins. At the X chromosome, dosage compensation proteins effect a two-fold reduction of gene expression, while at the *her-1* locus they effect a twenty-fold reduction in *her-1* expression. DPY-21 does not appear to localize to the *her-1* locus. DPY-21 is the first dosage compensation protein to show a difference in localization between the X chromosome and the *her-1* locus. An important issue is to understand the difference in gene regulation between the X chromosome and the her-1 locus that results in the ten-fold difference in transcription. These findings may indicate that DPY-21 is a protein necessary for the differential regulation of the dosage compensation complex at the X chromosome and at the *her-1* locus. Unexpectedly, DPY-21 is also expressed in the hermaphrodite and male germlines. It is interesting to hypothesize that the germline function may be the conserved role of the putative DPY-21 homologs in *Drosophila melanogaster* and humans. Future experiments will address this issue.

54. Searching for mechanisms of neuronal synchrony in the convulsing worm

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Mutations in a few *C. elegans* genes can result in a convulsive phenotype marked by end-to-end full body contractions followed by relaxation caused by the simultaneous excitation 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. pentylenetetrazole [PTZ] and pilocarpine). Mutants affecting GABA function (*unc-25, unc-46, unc-47* and *unc-49*) have anteriorly restricted convulsions in response to PTZ exposure and full body convulsions on high concentrations of pilocarpine.

To investigate the underlying molecular mechanisms of these convulsions, we are looking in detail at the specific neuronal requirement for UNC-43. Transgenic expression of a wild-type *unc-43* cDNA solely in neurons can alleviate drug-induced convulsions in *unc-43(lf)* animals. Using the *unc-30* promoter which expresses our construct in the inhibitory motor neurons, two independent transgenic lines show partial rescue of *unc-43(lf)* convulsions. Expression in the locomotory interneurons under the *glr-1*promoter fails to rescue. We are using other promoters to further delineate the site of action of *unc-43* in the regulation of worm locomotion.

Additionally, we have conducted screens to discover other genes able to confer convulsion susceptibility in worms. We have screened through 35,000 EMS mutagenized haploid genomes and have identified ten independent mutations that have full body convulsions on PTZ and all are confirmed alleles of *unc-43*. Another twelve alleles, identified by their anterior convulsions, are defective in known GABAergic genes and show full body convulsions when exposed to pilocarpine. (This screen was a collaborative effort between A. McCormick, E. Newton, C. Miller and G. Merrihew.) Next we screened through all available Uncs testing a single representive allele on PTZ and pilocarpine. Of some 130 alleles tested, only a rare dominant allele of *unc-93* showed convulsion sensitivity to these neurostimulants. Interestingly, *unc-93(e1500sd)* has a unique ÒrubberbandÓ reflex when prodded, a phenotype which is only shared by dominant alleles of *unc-110*, *sup-9* and *sup-10*. Dose response curves testing *unc-93(e1500sd)*, *sup-9(n1550sd)* and *sup-10(n983sd)* on pilocarpine show significant convulsion sensitivity.

How the rubberband contraction followed by relaxation response is generated is poorly understood. However, we are interested in any link between this phenomenon and the shrinker response of GABA mutants (which have full body convulsions in response to pilocarpine) and the spontaneous convulsion phenotype of *unc-43(lf)* that can be exasperated by picking, high temperature and neurostimulant exposure. Regardless, these screens demonstrate that full body convulsions can be generated by mutations in only a few genes.

These experiments establish *C. elegans* as a model for mechanisms underlying simultaneous excitation of neuronal networks. Similar types of neuronal synchrony may cause human epilepsies, since CaMKIIa and GAD65 (one of two glutamic acid decarboxylases found in mammals) mouse knockouts are susceptible to spontaneous and drug induced epileptic seizures. Our hope is to characterize key molecular components controlling seizures and perhaps to identify new drug targets for treatment.

55. Constitutive promotion of muscle protein degradation by FGF is prevented by a DAF-2 signaling pathway

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In mammals, insulin and insulin-like growth factor (IGF) promote protein accumulation in muscle as the result of increased protein synthesis and decreased protein degradation. Under conditions of low insulin signaling (as in untreated diabetes) muscle wasting is observed as the result of decreased protein synthesis and increased protein degradation. Despite obvious clinical importance, intramuscular mechanisms by which insulin opposes protein degradation are not well characterized.

To discover and study intracellular signal transduction pathways that regulate protein degradation, we have employed transgenic strains of *C. elegans* expressing a non-myofibrillar, enzymatically active myosin::ß-galactosidase fusion protein produced by *ccIs55 (unc-54::lacZ)* in body-wall and vulval muscle. We have previously reported that this reporter protein is completely stable in fed animals but is degraded in response to starvation, denervation or loss of insulin signaling, conditions that promote muscle wasting in mammals. We have also reported that this reporter protein is degraded by a pre-existing proteolytic system in fed adults when the Raf-MEK-MAPK pathway is activated in response to acute signals from LET-60 (Ras) or EGL-15 (FGFR), and that FGFR signals via Ras. Most recently we reported that mutational deactivation of DAF-2 signaling intramuscularly via AGE-1, PDK-1, and AKT-1 results in reporter protein degradation and that this effect requires signaling via Raf-MAPK. Protein degradation observed in response to loss of insulin signaling or in response to Ras activation appears to be at least partially distinct from protein degradation in response to starvation or denervation, possibly suggesting that the former signals may be relevant to protein degradation required for muscle adaptation, remodeling or de-differentiation.

As protein degradation is observed in animals with a reduction-of-function mutation in *daf-2 ($m41^{ts}$* or *$e1370^{ts}$)*, DAF-2 appears to normally oppose protein degradation in muscle. Thus, another intramuscular pathway must normally signal protein degradation. Here we report that constitutive protein degradation in *C. elegans* muscle is signaled by FGF (EGL-17 and LET-756 acting redundantly). This result implies that the 'default' state of Ras signaling in muscle is 'on' but that detrimental effects such as protein degradation are minimized by a balance of intramuscular DAF-2 (IGFR) signal. Since the decrement of DAF-2 signal required to permit protein degradation is comparable to that which shifts development toward dauer larva formation, it follows that the activity of the DAF-2 pathway can vary within a physiologically significant range to modulate muscle protein degradation. Furthermore, the reporter degradation observed in animals with increased EGL-15 signaling can be prevented by increasing signal via PtdIns-P_3 (*clr-1 ($e1745^{ts}$); daf-18 (e1375)* animals) demonstrating that balance between signals can be maintained outside of physiologic range by increasing the intensity and/or duration of signal via both pathways. Thus it appears that control of protein degradation in *C. elegans* muscle is not a binary decision but is rather controlled by the timing and/or intensity of signaling by EGL-15 and DAF-2 in addition to at least partially independent signaling in response to starvation and/or denervation.

56. A conserved p38 MAP kinase pathway in *C. elegans* innate immunity

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The evolutionary conservation in the mechanisms of innate immunity between *Drosophila melanogaster* and mammals suggests that the study of immune function in diverse species may yield key insights into the origins and molecular mechanisms of the innate immune system. The development of experimental host-pathogen systems utilizing invertebrate hosts greatly facilitates the genetic analysis of immune function of a host organism. We report on a forward genetic analysis of immune function in *C. elegans*. A genetic screen for *C. elegans* mutants with enhanced susceptibility to killing by *Pseudomonas aeruginosa* led to the identification of two genes required for pathogen resistance: *sek-1*, which encodes a mitogen-activated protein kinase kinase (MAP kinase kinase), and *nsy-1*, which encodes a MAP kinase kinase kinase. RNAi and biochemical analysis further established that a p38 MAP kinase ortholog, *pmk-1*, functions as the downstream MAP kinase required for pathogen defense. These data suggest that the p38 MAP kinase pathway represents an ancient, evolutionarily conserved component of the metazoan defense against pathogen attack.

57. Gustatory and olfactory neurons regulate C. elegans longevity

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Caenorhabditis elegans with impaired sensory function live longer than wild type, which suggests that the lifespan of the animal might be regulated by the perception of an environmental signal(s) (1). Apfeld and Kenyon (1) showed that a sensory defect in the amphids, which are the principal chemosensory organs of the worm located at its head, is sufficient to extend the life of the animal. Thus, at least some of the neurons whose activity regulates worm lifespan must be amphid sensory neurons. Each amphid organ contains neurons that function in taste reception, olfaction, mechanosensation, and thermal and pheromone perception (reviewed in ref. 2).

To gain insight into which of the amphid sensory neurons are necessary for proper lifespan control, we ablated subsets of the amphid neurons in wild-type and mutant worms. A subset of gustatory neurons that are implicated in the regulation of dauer formation controls adult lifespan. We find that some of these neurons inhibit longevity, whereas others promote longevity. However, not all sensory neurons implicated in the control of dauer formation affect lifespan and dauer pheromone also does not appear to regulate longevity. Thus, it is possible that these neurons control lifespan by sensing stimuli other than dauer pheromone. These gustatory neurons may regulate lifespan by sensing the presence or absence of specific food products rather than the level of total food in the environment because not every gustatory neuronal ablation affected worm lifespan. In addition, olfactory neurons, which also presumably sense food or its breakdown products, are also required for proper lifespan control. Furthermore, we observed that the gustatory and olfactory neurons regulate lifespan, at least in part, through the insulin/IGF-like DAF-2 pathway. Since several putative DAF-2 ligands are also expressed in gustatory (3) and olfactory neurons, it is possible that an environmental signal(s) sensed by these neurons regulate the production or release of these ligands, and thereby control animal lifespan.

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58. C. elegans - a new model for the human peroxisomal disorders

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We used functional knockouts (by RNAi) and isolated gene knockouts of genes encoding peroxisomal proteins to evaluate the importance of various peroxisomal enzymes and peroxins for the development of *Caenorhabditis elegans* and to compare the roles of these proteins in the nematode to their roles in yeasts and humans. The nematode counterparts of the human ATP-binding cassette half-transporters, the enzymes alkyldihydroxyacetonephosphate synthase and delta^{3,5}-delta^{2,4}-dienoyl-CoA isomerase, the receptors for peroxisomal membrane and matrix proteins (Pex19p and Pex5p), and components of the docking and translocation machineries for matrix proteins (Pex13p and Pex12p) are essential for the development of *C. elegans*. Unexpectedly, RNAi silencing of the acyl-CoA synthetase-mediated activation of fatty acids, the alfa- and beta-oxidation of fatty acids, the intraperoxisomal decomposition of hydrogen peroxide, and the peroxins Pex1p, Pex2p and Pex6p had no apparent effect on *C. elegans* development. Knockouts of genes for peroxisomal catalase ctl-2 (Y54G11A.5b) and for an ABC half-transporter (T02D1.5), an ortholog of human ALDP, have also been isolated. Whereas worms with the second gene knockout did not exhibit any noticeable phenotype, worms harboring the peroxisomal catalase gene knockout had a shortened lifespan. The described analysis provides a basis for the use of *C. elegans* as a valuable model system with which to study the molecular and physiological defects underlying the human peroxisomal disorders.

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59. A model of cholesterol trafficking and NP-C1 disease in *C. elegans*

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Niemann-Pick type C1 disease (NP-C1) is a fatal neurodegenerative disease caused by loss-of-function mutations in the human NPC1 gene. Cytological characterization of cells isolated from NP-C1 patients implicates the gene NPC1 in intracellular trafficking of cholesterol, but its exact function remains unclear. The genome of the nematode *C. elegans* contains two genes homologous to the human NPC1, *ncr-1* and *ncr-2* (formerly *npc-1* and *npc-2* respectively). Worms lacking the function of both *ncr* genes form dauer larvae constitutively (Sym *et. al*., 2000), then recover spontaneously and develop into adults that are thin, infertile, and die prematurely. These phenotypes are significantly ameliorated by increased cholesterol in the growth medium, suggesting a role of the *ncr* genes in cholesterol processing. The unusual dauer phenotype of the *ncr* double mutant is reminiscent of mutants of *daf-9* and the dauer-constitutive mutants of *daf-12*. *daf-12* encodes a nuclear hormone receptor that integrates signals from multiple dauer sensing pathways and whose output is essential for dauer formation (Antebi *et. al.* 2000). *daf-9* encodes a cytochrome P450 enzyme likely involved in biosynthesis of the *daf-12* ligand (Gerisch *et. al.* 2001, Jia *et. al.* 2002). The expression pattern of *ncr-1* overlaps with that of *daf-9*, consistent with a role in cholesterol processing and steroidogenesis. In order to identify additional genes that function together with the *ncr* genes, we screened 59,200 mutagenized genomes for mutants that suppress the dauer phenotype of the *ncr* double mutant. From the screen we isolated 5 dominant and 33 recessive alleles that represent at least 4 complementation groups. We are currently mapping and sequencing these suppressor genes.

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60. 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. To identify DAF-16 dependent transcriptional alterations that occur in a long-lived *C. elegans* strain, we used cDNA microarrays and genomic analysis to identify putative direct and indirect DAF-16 transcriptional target genes. Our analysis suggests that DAF-16 action regulates a wide range of physiological responses by altering the expression of genes involved in metabolism, energy generation, and cellular stress responses. Furthermore, we observe a large overlap between DAF-16 dependent transcription and genes normally expressed in the long-lived dauer larval stage. Finally, we examined the *in vivo* role of 35 of these target genes by RNA-mediated interference and identified one gene encoding a putative protease that is necessary for the *daf-2* Age phenotype.

61. ANC-1 tethers nuclei by connecting the nuclear envelope to the actin cytoskeleton
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Little is know about organelle membrane proteins that mediate the anchorage of nuclei and organelles within cells. We show here that ANC-1 functions to position syncytial nuclei and muscle mitochondria by linking the actin cytoskeleton to the nuclear envelope. Mutations in *anc-1* disrupt the proper positioning of nuclei and mitochondria in *Caenorhabditis elegans*. *anc-1* was shown to encode an 8546 residue protein consisting of mostly coiled regions and a C-terminal nuclear envelope localization domain (KASH domain) conserved with the *Drosophila klarsicht* and *msp-300* and vertebrate *syne-1* and *syne-2* genes. The N-terminal regions of ANC-1, Msp-300, Syne-1, and Syne-2 contain two calponin-like domains; the ANC-1 domain bound to filamentous actin *in vitro*. When overexpessed, the N-terminal region of ANC-1 co-localized with actin filaments, disrupted muscle function, and partially disrupted nuclear anchorage. An actin disrupting mutation in *unc-60*/cofilin also disrupted mitochondrial anchorage. Antibodies against the large repeat region of ANC-1 were localized cytoplasmically, and were enriched at the nuclear periphery. Localization of ANC-1 to the nuclear envelope was dependent on UNC-84, another nuclear envelope-localized protein required for nuclear anchorage. Overexpression of the KASH domain localized to the nuclear envelope and caused a dominant negative nuclear anchorage defect. We propose that ANC-1 connects nuclei to the cytoskeleton by interacting with UNC-84 at the nuclear envelope and directly binding to actin in the cytoplasm. Similarity in the overall structure between ANC-1, Msp-300, and vertebrate Syne proteins suggests that this novel nuclear anchorage mechanism is conserved. Potential similarities between the mechanisms of ANC-1 and Dystrophin will be discussed.

62. Identification of *tph-1* regulators reveals a role of TRP channels in serotonin synthesis

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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 stained with 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 define genes that regulate *tph-1* expression.

Three pieces of evidence indicate that *tph-1* expression in the pair of the ADF chemosensory neurons is regulated by cell-specific mechanisms. First, we found that

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.

Second, 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 in NSM, AIM, RIH, and HSN. Thus, distinct regulators regulate *tph-1* expression in ADF and the UNC-86-expressing serotonergic neurons.

Third, we mutagenized wild-type hermaphrodites carrying an integrated *tph-1*::gfp construct, and have isolated 23 mutants showing reduction/absence GFP in ADF. We name these mutants as *nss* for neuron-specific serotonin deficient mutants. Cloning of *nss*mutant genes revealed that *nss-2(yz6)* is an allele of *osm-9*, a 6-transmembrane domain protein similar to TRP ion channels (Colbert, Smith, and Bargmann, J. Neuroscience, 17, 8259-8269, 1997), and that *nss-3(yz5)* encodes a protein with the predicted sequence similar to *osm-9* and TRP channels (*C elegans* sequencing consortium). Furthermore, expressing wild-type *nss-2/osm-9* in ADF is sufficient to recover *tph-1::gfp*expression in the mutants, suggesting that these factors act in ADF to control the serotonin biosynthesis.

63. Pharyngeal synchrony in *Rhabditida*

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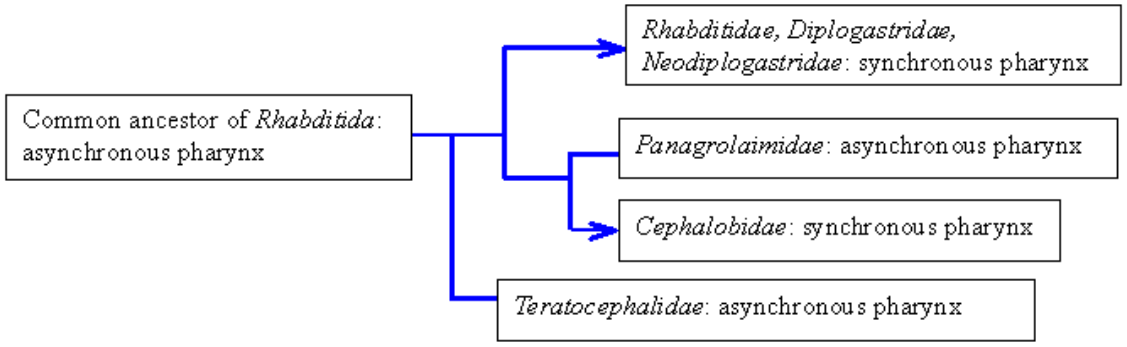
When *C.elegans* feeds, two parts of its pharynx, the corpus and the terminal bulb, contract simultaneously. This synchrony is achieved via gap junctions between the pharyngeal muscle cells, a component of which is encoded by *eat-5*. Using video recordings, we have looked at feeding behavior of *Rhabditida* nematodes from the following families: *Rhabditidae*, *Teratocephalidae*, *Cephalobidae*, *Panagrolaimidae*, *Diplogastridae*and *Neodiplogastridae*. In most of the surveyed *Rhabditida* the two pharyngeal compartments contract synchronously. Deviations from this feeding pattern are found in *Teratocephalidae* and *Panagrolaimidae*.

In a *Teratocephalidae* species, *Teratocephalus lirellus*, the corpus contracts alone most of the time. When the terminal bulb does contract, however, it usually does so synchronously with the corpus. Considering that *Teratocephalidae* is a suggested outgroup for *Rhabditida* based on the rDNA -derived phylogeny, the common *Rhabditida* ancestor may have had a partially or fully asynchronous pharynx.

In *Panagrolaimidae,* the corpus and the terminal bulb are asynchronous to varying degrees. In *Panagrellus redivivus* and *Panagrolaimus PS1159* the corpus and the terminal bulb contract synchronously when the pharynx pumps infrequently. During periods of rapid contractions, the terminal bulb and the corpus often fall out of synchrony, a phenomenon more pronounced in *PS1159*. In both species, corpus-independent terminal bulb contractions are observed.

Arrows show where the synchronous pharynx has evolved:



We recorded action potentials from either of the two pharyngeal compartments with intracellular electrodes while simultaneously taking videos of *P.redivivus* and *Panagrolaimus PS1159* pharynxes. In *P.redivivus* pharynxes, when corpus fires an action potential with an amplitude of 100 mV, a small partial 10 mV depolarization is recorded in the terminal bulb. Therefore, 90% of the corpus action potential amplitude is lost at the gap junctions. This dampening effect of the gap junctions is even more pronounced in the reverse direction - the terminal bulb action potentials were not seen even partially in the recordings from the corpus. In *Panagrolaimus PS1159*, a species with even weaker electrical coupling of pharyngeal contractions, action potentials arising in either of the two compartments could not be detected in the opposite one.

In conclusion, the degree of synchrony between the corpus and terminal bulb contractions in *Rhabditida* correlates with the efficiency of gap junctions. The *Rhabditida* ancestor may have had an asynchronous pharynx. Most of the *Rhabditida* have evolved a synchronous pharynx, in the case of *C.elegans* this is due to gap junctions between the muscle cells. Other, possibly neuronal mechanisms, may have played role in other clades. *Panagrolaimidae* is a *Rhabditida* family that has not evolved efficient gap junctions in the pharynx.

64. Evolution of Nematode Feeding Behavior

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Different nematodes live in different environments and therefore must employ different feeding behaviors in order to be successful. Feeding behavior is controlled by the pharyngeal nervous system, which although structurally similar in a variety of nematodes, directs a variety of behaviors across different species. C. elegans and P. redivivus differ in that the terminal bulb of P. redivivus is capable of pumping independently of the corpus, while in C. elegans these two compartments always pump synchronously. Despite this difference, the nervous systems of these two nematodes are conserved at the structural (by Nomarski optics), chemical (by antibody staining), and functional levels. In all cases to date the function of neurons in P. redivivus can be explained by their connectivity in C. elegans. Laser ablation of MC in P. redivivus and C. elegans results in a decreased rate of corpus pumping. In this case, the function of MC is the same in both species. In C. elegans the motor neuron M2 has synapses on the isthmus but has no detectable function. In P. redivivus and a related species, ablation of M2 causes a growth rate defect, apparently due to a defect in isthmus peristalsis. Thus in this case, the function in P. redivivus is consistent with the connectivity in C. elegans. In C. elegans the motor neuron M4 synapses on the isthmus and the terminal bulb, functioning to control isthmus peristalsis. In P. redivivus, M4 does not appear to play an important role in isthmus peristalsis, but rather is necessary for independent terminal bulb pumping. Again, the function in P. redivivus is consistent with the connectivity in C. elegans.

One might ask why P. redivivus wastes energy by pumping its terminal bulb independently. We would argue that rather than being energy-wasting, this mechanism is energy-saving. We postulate that P. redivivus saves energy, as compared to C. elegans, by not pumping the entire length of the pharynx once food has been passed back to the terminal bulb. This therefore allows P. redivivus to get away with fewer contractions of the corpus. We would expect then that P. redivivus would be able to grow normally when corpus pumping is slow. In fact ablation of MC, while resulting in slower growth in C. elegans, has no effect on growth rate in P. redivivus. We would also expect that in the absence of independent terminal bulb contractions, P. redivivus will not be able to grow normally when corpus pumping is slow. In fact ablation of MC and M4 together result in a decreased rate of growth, while ablation of either neuron alone does not cause a detectable decrease in growth rate. It is plausible then that independent terminal bulb pumping is an energy-saving mechanism for P. redivivus, possibly reflecting the need for greater efficiency with respect to limited food source.

In conclusion, different nematodes under different environmental conditions and with different life cycles employ different feeding behaviors to survive. They employ different behaviors despite largely conserved nervous systems by altering the strength of different neurons and different synaptic connections within those neurons. Major goals of the laboratory are to understand the molecular mechanisms by which nematodes alter the strength of synaptic connections as well as to understand those constraints that resulted in conservation of nervous system structure.

65. *slo-1* modulation of neuronal activity in the pharynx

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In studying the evolution of nematode feeding behaviors, Steciuk et.al. showed that differential activity of homologous (and structurally identical) neurons lead to different feeding behaviors between *C. elegans* and *P. redivivus*. In particular, the neurons M4 and possibly M5 are active to excite the pharyngeal terminal bulb muscle in *P. redivivus*, but not *C. elegans*. A key question is thus: "What are the molecular mechanisms for the differential neuronal activity observed between *P. redivivus* and *C. elegans*?"

Our analysis of *slo-1* mutants in *C. elegans* suggests that one such mechanism may involve the *slo-1* encoded Ca^{++}/voltage gated K^{+} channel. Several pieces of data suggest that loss of *slo-1* function activates M4/M5 neurons and leads to increased terminal bulb excitation in *C. elegans*. First, *slo-1* worms have independent positive spikes that are not present in wild type electropharyngeogram recordings (electrophysiological recordings of pharyngeal activities). These spikes are eliminated by laser ablation of M4 and M5 neurons. Second, to assess terminal bulb excitations in *slo-1*, we used *eat-5* worms where a mutation in the *eat-5* encoded gap junction electrically isolates the terminal bulb from the remainder of the pharynx. s*lo-1;eat-5* worms have increased number of terminal bulb contractions compared to *eat-5* worms, indicating increased terminal bulb excitation due to *slo-1*. Finally, *slo-1;eat-5* worms have improved growth compared to *eat-5*worms, which is consistent with increased terminal bulb pumping.

Thus, *slo-1* mutant *C. elegans* animals gain M4/M5 neuronal activity and exhibit increased terminal bulb excitation, conditions normally present in *P. redivivus* but not in *C. elegans.* This suggests that *slo-1* may be one factor that accounts for the differential activity between homologous neurons in *C. elegans* and *P. redivivus.* We thus hypothesize that *slo-1* acts as an "evolutionary volume knob" to modulate neuronal activity.

66. Long-term memory requires non-NMDA excitatory receptor function and produces decreased GLR-1::GFP expression

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Memory was investigated in the microscopic worm *C. elegans* by examining retention for habituation to a mechanosensory (tap) stimulus at 24 hours post-training. Our lab has previously demonstrated that memory results when training is distributed into training blocks separated by rest periods. Memory is not seen if training is administered in a single massed training block. Further, when protein synthesis is inhibited by administering heat shock after each training block, distributed training no longer produces memory. The distributed training protocol was applied to *glr-1*worms (n2461 and ky176; a non-NMDA excitatory glutamate receptor). *glr-1* worms did not show retention following a distributed training protocol with a tap stimulus. A stronger mechanosensory stimulus should result in a larger amout of glutamate being released from the presynaptic sensory terminal thus enabling a larger response. When distributed training was given with a stronger stimulus (train of taps) *glr-1* worms still do not show memory suggesting that GLR-1 is required for memory and that greater glutamate release had no effect. We also examined the effects of pharmalogical blockade of glutamate receptors with DNQX during training and found that worms trained on DNQX streaked plates showed no retention compared to a vehicle control group.

Previous studies using other animal models show that memory is related to anatomical changes at the synapse. To visualize synaptic changes produced by memory imaging of transgenic green fluorescent protein (GFP) strains was performed using confocal microscopy. One strain ($_{p}$*mec-7*:SNB-1::GFP; provided by M. Nonet) tagged synaptobrevin with a GFP-construct behind a touch-cell promoter (a protein associated with the vesicle membrane; presynaptic) and it was found that no change in amount of GFP expression was noted 24-hours after distributed training. However, we also examined the effects of memory in a GLR-1::GFP transgenic strain (KP1477; provided by J. Kaplan) and found that distributed training produced a significant decrease in the amount of GFP expressed in the ventral cord compared to untrained controls. Interestingly, there was no significant difference between the number of GFP clusters between trained and untrained animals suggesting that the number of places GFP is expressed is not changed by memory, but rather the amount of GFP expressed at each spot is decreased. Current experiments are examining if this decrease in GLR-1::GFP is protein synthesis dependent.

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67. Genetic Analysis of the Neuromodulatory Control of Search Behavior in *C. elegans*
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Immediately following removal from food, *C. elegans* engages in area-restricted search, a behavior found in a wide variety of animals. In *C. elegans*, this behavior is characterized by an initial high frequency of sharp turns that then decays to less frequent turning over time. To understand the neuromodulatory control of search behavior in *C. elegans*, we used a computer assisted path analysis system (DIAS) to quantify the temporal dynamics of worm movement under genetic, neural, and pharmacological manipulations. Our results show that *C. elegans* hermaphrodites perform an area-restricted search after removal from or following brief encounters with food. Glutamate receptor function in the command interneurons, known to control reversal frequency, is required for this behavior. Mutations that modify glutamate-gated currents also modify turning frequency. The dopaminergic neurons in *C. elegans* are presynaptic to the glutamatergic command interneurons, suggesting a possible role for dopamine in the modulation of glutamatergic signaling. Exogenous application of dopamine increases the frequency of high angled turns, whereas dopamine antagonists inhibit this effect. Genetic ablation of the dopaminergic neurons eliminates area-restricted search. We also show that normal glutamate activity is required for dopaminergic modulation of behavior. Taken with previous results from other labs, our work suggests that dopaminergic neurons respond to food by modulating glutamatergic signaling. This increases the frequency of high angled turns and generates an area-restricted search following removal from food.

68. Fluoxetine response genes in *C. elegans*

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Serotonin specific reuptake inhibitors (SSRIs) have been particularly effective in treating major depressive disorder, a significant public health problem. Fluoxetine (Prozac) is the prototypical SSRI and has been widely prescribed. This drug was designed to exert its effect upon the serotonin reuptake transporter (SERT), but its therapeutic action and range of side effects are inadequately explained by SERT inhibition alone. We have undertaken mutational analysis of a SERT-independent fluoxetine-induced behavior in *C. elegans*, nose contraction (Choy & Thomas 1999). *nrf* mutants (nose resistant to fluoxetine) fall broadly into two phenotypic classes. Peg (pale egg) phenotype *nrf* mutants have defects in an inferred pathway that also influences the abundance of yolk in eggs. The molecular identity and functional expression of these genes in the intestine is consistent with a proposed model of hydrophobic molecule transport. Our recent efforts have focused on the non-Peg *nrf* genes, and we present the molecular identification of some of these genes here. *nrf-2* was mapped very close to *vhp-1* and transgenically rescued with the *vhp-1* gene. Sequencing of the single *nrf-2* mutant allele reveals a stop codon in the middle of this 657 aa predicted protein. *vhp-1*encodes a protein with similarity to VHP-1-like dual specificity protein tyrosine phosphatases. *nrf-7* was mapped on the basis of its 27° Daf-c phenotype, and was rescued transgenically by *tax-2*. *tax-2* encodes a cGMP-gated channel involved in chemotaxis, and is thought to form a heterooliogomer with *tax-4*. Alleles of *tax-2*, *tax-4*, and *daf-11* (a guanylyl cyclase) all tested Nrf. The coordinate expression of these proteins in exposed sensory neurons suggests that they may be responding to or transporting fluoxetine through an unidentified pathway in these cells. We are currently exploring how the *nrf-2* phosphatase may link to these genes or to nose muscle contraction. Identification of the remaining *nrf*genes may lead to a mechanistic description of this fluoxetine-induced behavior and lay the groundwork for development of improved antidepressant therapies.

69. Different pathways function at different times to regulate longevity of *C. elegans*

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Recently, several genes and pathways have been identified that control the aging process in the nematode, *C. elegans*. Two pathways, the insulin/IGF-1 signaling pathway and the mitochondrial electron transport (ETC) pathway, dramatically increase the lifespan of *C. elegans* when inactivated. Here we report that these pathways do not function in a passive manner during the animal's life cycle, but rather normally function at discrete times to regulate longevity. Surprisingly, the insulin/IGF-1 pathway functions during early adulthood to regulate aging. The timing of this event is separable from its roles in development and reproduction. In contrast, the ETC regulates the aging process during development, but not during adulthood. Our data suggest that the ETC could play an early regulatory role in establishing the rate of living for the adult animal, while the insulin/IGF-1 pathway functions solely during adulthood to regulate longevity.

70. Characterization of *nmy-1*, a suppressor of *mel-11*

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C. elegans elongation, whereby the embryo is transformed from an egg into a vermiform larva, is driven by actin-mediated lateral epidermal cell (seam cell) shape changes, which leads to a decrease in the circumferential axis with a concomitant fourfold increase in the longitudinal axis[1]. In higher eukaryotes, non-muscle contractile events such as smooth muscle contraction and focal adhesions are regulated by Rho-binding kinase and myosin phosphatase. Myosin phosphatase acts a block to contraction by counteracting the activity of myosin light chain kinase and removing the positive regulatory phosphate from myosin light chain. Rho-binding kinase removes the block to contraction by down-regulating myosin phosphatase activity. We have shown that the *C. elegans* homologues *let-502* (Rho-binding kinase) and *mel-11* (myosin phosphatase) similarly regulate non-muscle contractile events such as elongation, cytokinesis and spermathecal contraction in the worm[2-5].

During embryonic elongation, LET-502 and MEL-11 likely regulate the activity of MLC-4 (myosin light chain)[6]. Loss-of-function *let-502* and *mlc-4* mutants arrest due to failed elongation and *mel-11* mutants die due to hyper-contraction. Genetically, *mlc-4* is downstream of *let-502* and *mel-11*. *nmy-2* encodes the non-muscle heavy chain partner for MLC-4 during cytokinesis[7]. However, a role for NMY-2 during later stages of embryogenesis has not been determined due to early phenotypes. We have found that the other worm *nmy* locus, *nmy-1*, encodes the likely MLC-4 partner during elongation. We have isolated three *nmy-1* alleles as suppressors of *mel-11*, one of which contains a nonsense mutation that behaves as a strong loss-of-function or null. This mutant displays a range of phenotypes such as sterility, distal tip cell migration defects, weak embryonic lethality (~10-20%) and morphogenetic defects. However, since the majority of larvae survive to adulthood and are phenotypically much weaker than *mlc-4* zygotic null mutants, we hypothesize that *nmy-2* may partially compensate for *nmy-1* function during embryonic morphogenesis. In addition, antibodies reveal that NMY-1 and NMY-2 are expressed in similar patterns; however NMY-2 is more predominant in early embryos while NMY-1 has stronger expression later at the onset of elongation.

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71. Nuclear POP-1 asymmetry between A-P sisters is regulated by a 14-3-3 mediated nuclear export mechanism

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We have shown previously that the HMG domain protein POP-1 is asymmetrically localized in the nuclei of all sisters born of A-P divisions using a monoclonal antibody to the POP-1 protein. In most cases examined, the POP-1 asymmetry is required for A-P fate differences between sisters. We and others have shown that the A-P fate difference and POP-1 asymmetry between MS and E sisters require a Wnt/MAPK signaling pathway. How the Wnt/MAPK signaling results in a lower level of nuclear POP-1 and POP-1 activity in the posterior sister is not clear.

Using a functional POP-1-GFP reporter expressed from an exogenous promoter, Maduro and Rothman have shown that the coding region of POP-1 is sufficient to confer the asymmetric pattern observed by POP-1 antibody staining. Moreover, the overall level of POP-1 in A-P sisters is equal and the apparent difference in the nuclear POP-1 is a result of differential nuclear-cytoplasmic distribution. However, the mechanism by which POP-1 is transported between cytoplasm and nuclei as well as how the differential distribution is achieved between A-P sisters remain unclear.

When we added a SV40 nuclear localization sequence to the POP-1-GFP reporter, we observed no change in the nuclear asymmetry between A-P sisters. This result suggests that the difference in nuclear POP-1 level between A-P sisters is likely due to difference in nuclear export but not import. Interestingly, we showed that POP-1 asymmetry is completely abolished in the *par-5* mutant embryos up to the 32-cell stage, with all cells exhibiting a high level of nuclear POP-1-GFP. The *par*-5 gene encodes a 14-3-3 protein that regulates many cellular functions, including mediating nuclear export of a variety of proteins, providing an intriguing model for how POP-1 export might be regulated.

Two lines of results strongly suggest that POP-1 nuclear export is regulated by a 14-3-3-mediated mechanism. First, we show that POP-1 and PAR-5 proteins physically interact in a co-immunoprecipitation assay using *C. elegans* embryo extracts. Second, we can restore the nuclear POP-1 asymmetry in many pairs of A-P sisters, while maintaining the partitioning defects of *par*-5 mutant embryos, when we expressed PAR-5-GFP in 8 cell-stage *par*-5 mutant embryos.

We are in the process of characterizing regions of POP-1 necessary and/or sufficient for its nuclear asymmetry. Most of 14-3-3-mediated nuclear export involves phosphorylation of target proteins. We are currently investigating whether phosphorylation of POP-1 is important for its interaction with PAR-5 and what kinase might be responsible for the differential phosphorylation leading to differential nuclear export between A-P sisters.

72. The Role of *par* Genes in the Regulation of POP-1 Asymmetry

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Cells born in all regions of the *C. elegans* embryo appear to recognize their polarity with respect to that of the embryo. We have shown previously that the HMG domain protein POP-1 is asymmetrically localized in the nuclei of all sisters born of anterior-posterior (A-P) divisions and that *pop-1* activity is required for A-P fate differences between sisters. Recently, this asymmetry was shown by others to be due to the differential distribution of POP-1 between the cytoplasm and the nucleus. Asymmetric levels of POP-1 between the sister blastomeres MS and E have clearly been shown to require a signal from P2, now molecularly defined by a Wnt/MAP kinase signaling pathway. While mutations in any one component of the pathway result in an equal level of POP-1 in the MS and E nuclei, their effect on POP-1 asymmetry in cells other than MS and E varies. Using a *pop-1-gfp* transgene (gift of M. Maduro, J. Rothman), we show that POP-1-GFP quickly reestablishes proper A-P asymmetry in the descendants of MS and E in *mom-2* mutant embryos. However, POP-1-GFP remains equal in all A-P sisters of the MS and E lineage of *lit-1* and *mom-4* mutant embryos. Although the mechanism by which POP-1 asymmetry is initiated and regulated in cells other than MS and E remains unclear, our observation suggests a global mechanism for transmitting information regarding A-P polarity.

The *par* genes have been shown to be critical for several aspects of A-P polarity in the early embryo. Four of the six PAR proteins are themselves asymmetrically localized in the one-cell embryo; PAR-1 and PAR-2 localize to the posterior cortex while PAR-3 and PAR-6 localize to the anterior cortex. Mutations in any individual *par* gene often disrupt the asymmetric localization of other PAR proteins as well as the asymmetric segregation of cell fate determinants. However, all *par*genes do not have the same effect on asymmetric segregation of factors. For example, it has been shown that several proteins with an asymmetric distribution in wild type embryos maintain the wild type pattern in *par-2* mutants, while being aberrantly localized in *par-3* mutants.

To better understand how global POP-1 asymmetry is established, we decided to investigate whether the *par* genes play a role in regulating the asymmetric distribution of POP-1 in each A-P division. In order to facilitate such studies, we have generated an integrated line carrying *gfp::pop-1* under an EMS specific promoter, *med-1.* In all *par* mutants examined, the *med-1* promoter is activated in multiple blastomeres, making it possible to monitor A-P divisions in many lineages, and to follow the dynamic pattern of POP-1-GFP distribution in live embryos.

Surprisingly, despite the apparent early acting role of *par-2* in establishing embryonic polarity in response to the sperm microtubule organizing center (MTOC), POP-1-GFP asymmetry appears to be properly maintained in *par-2(lw32)* mutant embryos. On the contrary, *par-3* and *par-6* appear to play an important role in the establishment of global POP-1-GFP asymmetry as well as in the orientation of POP-1-GFP asymmetry. In *par-3(it71)* and *par-6(zu222)* embryos, 50% of A-P sister pairs have similar levels of POP-1-GFP while 50% of A-P sisters have asymmetric POP-1-GFP levels with a random orientation. The similarity of POP-1-GFP distribution in these two mutant backgrounds is consistent with the finding by others that PAR-3 and PAR-6 appear to form a complex. In *par-5* mutant embryos, POP-1 asymmetry is completely abolished prior to the 32 cell stage, with all cells expressing an equal level of nuclear POP-1-GFP. Although PAR-3 is inappropriately localized in the *par-5* mutant, the abnormal POP-1-GFP pattern in this context is unlikely to be an indirect effect of mislocalized PAR-3. *par-2* mutants also fail to properly localize PAR-3 but do not exhibit the same effect on POP-1 asymmetry.

Our current model suggests that in addition to providing positional information, PAR-5 protein likely plays a direct role in the regulation of POP-1 (see abstract by M. Lo and R. Lin). We are currently performing screens for additional genes functioning in the regulation of global POP-1 asymmetry.

73. LET-99 determines spindle position and is asymmetrically enriched in response to PAR polarity cues in *C. elegans* embryos

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Asymmetric cell division depends on coordinating the position of the mitotic spindle with the axis of cellular polarity. In *C. elegans* embryos, the initial cellular polarity is established through the asymmetric localization of PAR proteins, which subsequently regulates the asymmetric distribution of the cell fate determinants and spindle positioning. However, it is still unclear how spindle positioning is coordinated with the PAR polarity cues. We provide evidence that LET-99 is a link between PAR polarity cues and the downstream machinery that determines spindle positioning in *C. elegans* embryos. In *let-99* 1-cell embryos the nuclear-centrosome complex exhibits a hyperactive oscillation that is dynein-dependent, instead of the normal anteriorly-directed migration and rotation of the nuclear-centrosome complex. Further, at anaphase in *let-99* embryos the spindle poles do not show the characteristic asymmetric movements typical of wild type. LET-99 is a DEP domain protein that is asymmetrically enriched in a band that encircles P lineage cells. The LET-99 localization pattern is dependent on PAR-3 and PAR-2 and correlates with nuclear rotation and anaphase spindle pole movements in wild-type embryos, as well as with changes in these movements in *par* mutant embryos. In particular, LET-99 is uniformly localized in 1-cell *par-3* embryos at the time of nuclear rotation. Rotation fails in spherical *par-3* embryos where the eggshell has been removed, but rotation occurs normally in spherical wild-type embryos. The latter results indicate that nuclear rotation in intact *par-3* embryos is dictated by the geometry of the oblong egg and are consistent with the model that the LET-99 band is important for rotation in wild-type embryos. Together the data indicate that LET-99 acts downstream of PAR-3 and PAR-2 to determine spindle positioning, potentially through the asymmetric regulation of forces on the spindle.

74. New Insights into the Mechanisms Underlying the A-P Polarity Brought by *pod* Genes

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The polarization of cells is necessary for developmental events and the function of cells. We are particularly interested in how a fertilized egg of the *C. elegans* responds to a cue and establishes polarity, giving rise to a complex multicellular organism. Despite our knowledge of several polarity-specific genes, namely *par*genes, little was known about the mechanism by which these PAR proteins become asymmetrically positioned. We developed and carried out a novel genetic screen to identify new components involved in polarity and isolated a previously unidentified polarity gene, *pod-2* for polarity and osmotic defective #2. Discovery and characterization of *pod-2* together with previously reported *pod-1*gene demonstrated that the *pod*genes were a new, untapped class of polarity mutants and they had been missed in other screens in the past. This gave rise to a directed screen for other *pod* genes that led to the discovery of *pod-3* through *8* encoding anaphase promoting complex (APC). The molecular cloning of *pod-2* led to a surprising discovery that a polarity gene, *pod-2*, encodes Acetyl CoA Carboxylase, an enzyme involved in fatty acid synthesis. Recent insights brought by additional *pod* genes in cellular mechanisms by which PAR proteins become resolved into reciprocal asymmetric domains via interaction between the paternal pronulceus and the posterior cortex will be discussed.

75. Mutants that affect gamete function at fertilization

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The main goal of our lab is to understand the molecular mechanisms of fertilization. Initially, we have focused on the characterization of a group *spe* and *fer* genes required by sperm for fertilization. Although sterile, worms with these mutations produce morphologically wild-type sperm with normal motility that can come in contact with oocytes. Therefore mutations in these genes disrupt gamete recognition, adhesion, signaling and/or fusion. We have expanded our studies to include gene products required by the oocyte for fertilization. We are using RNAi to test candidate egg cell surface components for possible functions in fertilization. Additionally, we are conducting a genetic screen to identify conditional "egg sterile" mutants. We will report our progress on the genetic, phenotypic and molecular analysis of these genes. This work will lead to a better understanding of the specialized cell-cell interactions that occur at fertilization.

76. Development and fertility in C. elegans clk-1 mutants depends upon transport of dietary coenzyme Q8 to mitochondria

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The C. elegans clk-1 mutants lack coenzyme Q9 and instead accumulate the biosynthetic intermediate demethoxy-Q9 (DMQ9). clk-1 animals grow to adulthood, albeit slowly, if supplied with Q-containing E. coli. However, depending upon when Q is withdrawn from the diet, these animals either arrest development as larvae or become sterile adults. To understand this stage-dependent response to a Q-less diet, the quinone content of wild-type and clk-1(qm30) worms during development was determined. The quinone content varies in the different developmental stages in wild-type fed Q-replete E. coli. The amounts peak at the second larval stage, which coincides with the stage of arrest of clk-1 larvae fed a Q-less diet from hatching. Levels of the endogenously synthesized DMQ9 are quite high in the clk-1(qm30) arrested larvae and sterile adults derived from dauer larvae that were fed Q-less food. Analysis of quinones from mutant and wild-type animals fed a Q-less diet establishes that the Q8 present is assimilated from the E. coli. Furthermore, this E. coli-specific Q isoform is present in mitochondria isolated from fertile clk-1(qm30) adults fed a Q-replete diet. These results suggest that DMQ cannot functionally replace Q and that the uptake and transport of dietary Q8 to mitochondria prevents the arrest and sterility phenotypes of clk-1 mutants.

77. FRK-1: Roles of a Fer-type non-receptor tyrosine kinase in cadherin and integrin cell adhesion systems and Wnt signaling

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Although the expression and altered function of non-receptor tyrosine kinases (NRTKs) in various tumor types have been described, the normal function of many NRTKs has remained elusive. Members of the mammalian *fes/fps/fer* gene family encode for NRTKs originally identified as viral oncogenes. Although the *fes/fps* genes have been studied extensively, most studies of Fer kinase have been performed *in vitro*, and it's *in vivo* functions have not yet been identified.

We are investigating the function of the *C. elegans* homologue of Fer kinase, *frk-1* (Fer related kinase -1). FRK-1 is most similar to the truncated isoform of the Fer kinase, FerT, which lacks the coiled-coiled domains found in the N-terminus of the longer mammalian isoform. FRK-1 antibodies detect only a single 45kDa protein in *C. elegans* extracts, suggesting that *C. elegans*, unlike mammals, does not express a long isoform. FRK-1 is detectable throughout the life of the worm, including very early embryos, suggesting that it is maternally provided. In early embryos, immunoreactive FRK-1 localizes to the cell boundaries and nuclei; however, it is detectable exclusively at the plasma membrane later in embryogenesis, at the time that most cells have become post-mitotic and just prior to enclosure. Expression is seen in most epithelial tissue types including epidermis, pharynx, intestine, and muscle.

Consistent with a role in epithelial morphogenesis, RNAi of *frk-1* leads to embryonic lethality with severe defects in morphogenesis resulting from a failure in epidermal enclosure. *mDf7*, which deletes *frk-1*, results in a similar morphogenetic phenotype and a failure to express seam cell-specific differentiation markers (Terns et al., 1997). We found that the enclosure defect of *mDf7* is rescued by *frk-1(+)*; rescued animals elongate partially and express markers of seam differentiation.

In cultured mammalian cells, Fer kinase has been shown to interact with β-catenin in the plasma membrane cadherin complex, and associates with the β-integrin complex in the absence of cadherin function. We found that membrane localization of FRK-1 is severely reduced in mutants of *hmp-2* and that it becomes primarily nuclear-localized in epidermal cells. While HMP-2 is a non-nuclear β-catenin that has not been implicated in Wnt signaling, we found that loss of *frk-1* rescues the gutless phenotype caused by the absence of WRM-1 (the nuclear, Wnt-involved β-catenin). This observation suggests that HMP-2 can function in the Wnt signaling pathway, but does not normally do so because FRK-1 retains it at the plasma membrane. Surprisingly, FRK-1 also localizes to the nuclei of epidermal cells in the *pat-3* (β-integrin) mutant, revealing a potential integrating function in both cadherin- and integrin-mediated cell adhesion systems.

We are currently investigating the role of FRK-1 in cellular adhesion and cell rearrangements that occur during morphogenesis by examining how FRK-1 interacts with cadherin and integrin complexes at the cell membrane and whether FRK-1 mediates cross-talk between these two adhesion systems. We are also further examining interactions of FRK-1 with the Wnt signaling pathway.

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78. AIR-2 Regulates the Selective Release of Chromosome Cohesion during Meiosis I

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Accurate chromosome segregation during cell division requires the precise regulated release of chromosome cohesion. During mitosis, sister chromatids are initially linked by cohesin proteins until anaphase when the disruption of cohesin complexes by the protease separase triggers the separation of sister chromatids. However, during meiosis chromosome dynamics are more complicated, as homologous chromosomes separate at anaphase I whereas sister chromatids remain attached until anaphase II. How the selective release of meiotic chromosome cohesion is regulated during meiosis I remains unclear. We report here a mechanism whereby the differential phosphorylation of the meiotic cohesin REC-8 by the aurora kinase AIR-2 may regulate the selective release of chromosome cohesion during meiosis I.

We demonstrate that the aurora/ipl1-related kinase AIR-2 is required for the release of meiotic chromosome cohesion. We performed 4-D imaging of meiotic chromosomes in *air-2(RNAi)* embryos using a reporter strain carrying histone H2B-GFP. When AIR-2 is depleted by RNAi, we observed the 6 bivalents failed to separate during anaphase I. We also showed that AIR-2 localizes to sub-chromosomal regions corresponding to the chromosome arms distal to the chiasmata, regions believed to contain cohesions required to hold homologs together. In *air-2(RNAi)* embryos, we observed that the meiotic cohesin REC-8 is not removed from the bivalents. We showed AIR-2 phosphorylates REC-8 *in vitro* primarily at Thr625, adjacent to a potential separase cleavage site. In yeast, the phosphorylation of Scc1, a mitotic counterpart to Rec8, has been demonstrated to enhance Scc1 cleavage by the protease separase. All of this evidence suggests AIR-2 regulates the selective release of meiotic chromosome cohesion by promoting localized REC-8 degradation via phosphorylation of the REC-8 positioned only in the chromosome arms distal to the chiasmata.

Interestingly, the activity of two PP1 phosphatases, CeGLC-7α and β, is required for the proper localization of AIR-2 to the chromosome arms distal to the chiasmata. When CeGLC-7α/β are depleted by RNAi, we discovered AIR-2 is mis-localized throughout the bivalents in metaphase I. Furthermore, in *Ceglc-7α/β(RNAi)* embryos, we observed chromosomal REC-8 was dramatically reduced throughout the bivalents at metaphase I. Coincidentally, sister chromatids in addition to homologous chromosomes were observed to separate at the onset of anaphase I in *Ceglc-7α/β(RNAi)* embryos. This suggests that the mis-localization of AIR-2 results in the premature removal of REC-8 from between sister chromatids and the precocious separation of sister chromatids during anaphase I. These results further support the conclusion that AIR-2 promotes the release of chromosome cohesion via the phosphorylation of REC-8.

79. HIM-17, a novel protein required for proper initiation of meiotic recombination in *C. elegans*

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During prophase of meiosis I, homologous chromosomes must locate their partners and undergo crossover recombination to ensure correct segregation at the first meiotic division. We have used a combination of classical genetics and functional genomics to identify HIM-17, a novel protein that is required for formation of crossovers. We have obtained five mutant alleles of *him-17*, including one that appears to be a null allele. All five alleles result in defects in meiotic chromosome segregation, as evidenced by production of high frequencies of both males and inviable embryos (indicative of X chromosome and autosomal missegregation, respectively).

We have strong evidence that HIM-17 is involved in the proper formation of the double-strand DNA breaks (DSBs) that initiate meiotic recombination. Ionizing radiation (which artificially generates DSBs) can bypass the defect in recombination seen in *him-17*, producing functional chiasmata. This response to ionizing radiation is identical to that seen in worms lacking SPO-11, a protein that is believed to generate DSBs (1). The most straightforward interpretation of this result is that SPO-11-induced breaks are not being formed in *him-17* mutants. This is consistent with the observation that persistent recombination intermediates capable of triggering a DNA damage checkpoint are not detected in *him-17* mutants. This result also indicates that the recombination machinery downstream of DSB initiation is present and can function correctly in the absence of HIM-17. To date, *him-17* and *spo-11* are the only two mutants we have found that can be rescued by radiation-induced DSBs.

Other aspects of the *him-17* mutant phenotype suggest that HIM-17 has roles beyond meiotic DNA metabolism. For example, while all six pairs of chromosomes require HIM-17 for formation of crossovers, the X chromosomes are much more affected by a partial loss of HIM-17 function. This indicates that the X chromosomes rely much more heavily on HIM-17 function than the autosomes. Because the X chromosomes have different chromatin properties than the autosomes in wild-type meiosis (2), we are drawn to the hypothesis that HIM-17 promotes DSB formation by modifying chromatin to allow SPO-11-generated breaks. Another interesting feature of *him-17* mutants is that they are sterile at 25°: these worms do not produce any embryos, and their germlines show altered progression of meiosis. This phenotype has been seen with all five mutant alleles as well as with RNAi, suggesting that HIM-17 is involved in a process that is temperature sensitive. It could be that this phenotype is a result of altered gene expression in mutant germlines, perhaps due to abnormal chromatin. We are investigating this phenotype in more detail, and are using antibodies that recognize histone modifications to examine chromatin structure in *him-17* meiotic nuclei.

1. Dernburg et al (1998) Cell 94: 387-398.
2. Kelly et al (2002) Development 129: 479-492.

80. Characterization of HCP-6, a C. elegans protein required to prevent chromosome twisting

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Previous studies of mitosis show that capture of single kinetochores by microtubules from both centrosomes (merotelic orientation) is a major cause of aneuploidy. We have characterized *hcp-6*, a temperature sensitive, chromosome segregation mutant in *C. elegans* that exhibits chromosomes attached to both poles via a single sister kinetochore. We demonstrate that the primary defect in this mutant is a failure to fully condense chromosomes during prophase. Although centromere formation and sister centromere resolution remain unaffected in *hcp-6*, the chromosomes lack the rigidity of wild-type chromosomes and twist around the long axis of the chromosome. As such, they are unable to establish a proper orientation at prometaphase, allowing individual kinetochores to be captured by microtubules from both poles. We therefore propose that chromosome rigidity plays an essential role in maintaining chromosome orientation to prevent merotelic capture.

81. TEP-1, a *C. elegans* telomere binding protein, is required for chromosome stability

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We identified by yeast one-hybrid screening a *Caenorhabditis elegans* gene encoding a homeobox-containing protein that specifically binds double-stranded telomeres. We found that TEP-1 localizes to telomeres in vivo. Modeling of the TEP-1 homeodomain revealed that its three-dimensional structure has a strong similarity to that of hTRF1 and Taz1p, human and *Schizosaccharomyces pombe* telomere binding proteins, respectively, although TEP-1 does not share amino acid sequence similarity with these proteins. Like hTRF1, TEP-1 bends DNA by binding telomeric sequences. Distinct functions of the TEP-1 domains were identified: the N-terminal domain and the homeobox domain function in telomere binding, and the C terminal region functions in DNA bending and loop formation. Two dimensional gel electrophoresis and Southern analysis of telomeric DNA showed that, like TRF2, TEP-1 is required for loop formation at telomeric regions in vivo. Overexpression of TEP-1 enhances loop formation at the ends of telomeres, while a C terminal deletion mutant or a dominant negative mutant cannot form loops. We also show that tep-1 genetically interacts with *mrt-2* and *cep-1*, which encode the telomere checkpoint protein and the *C. elegans* p53 homolog, respectively, indicating that tep-1 acts with these genes to maintain chromosome stability. We propose that TEP-1 is a functional homolog of the mammalian TRF proteins and that it acts as a telomere protecting protein in maintaining chromosome stability.

82. An RNAi-based screen for genes involved in the regulation of lifespan by the reproductive system

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Longevity in C. elegans is mediated through an elaborate coordination of environmental cues and endogenous hormonal control. Within the animal, the reproductive system plays a major role in determining the rate of aging. This is evidenced by experiments in which laser ablation of the germline precursor cells in an L1 results in a 60% extension in mean adult lifespan (1). Intriguingly, this extension is sensitive to the amount of germline proliferation in the adult, implying that in a normal adult worm, lifespan is downregulated in accordance with cues from mitotic germ cells (2). However, signals from the somatic gonad counteract this signal, since ablation of the somatic gonad precursor cells rescues the lifespan extension seen in germline-ablated animals(1).

These dynamic cues from the reproductive system are dependent on multiple genes. Lifespan extension in germline-ablated animals is dependent on daf-16, a forkhead transcription factor, daf-12, a nuclear hormone receptor, and daf-9, a cytochrome P450 (1,3). The somatic gonad promotes longevity through modulation of the DAF-2/insluin/IGF-1 pathway, daf-9, and possibly daf-12 (4). We were interested in identifying additional members of the germline and somatic gonad signaling pathways. To this end, we conducted a RNAi-based screen for suppressors and enhancers of germline-mediated lifespan extension. Suppressors will include genes downregulated by signals from the germline and genes that potentiate the somatic gonad longevity signal. Enhancers will identify novel genes that act independently of the reproductive system to shorten lifespan. From a screen of a Chromosome I RNAi library (5), we found 45 RNAi clones, including daf-16(RNAi), that suppress longevity. Further analysis has identified a group of 11 genes that suppress germline-mediated lifespan extension, but have little or no effect on wild-type longevity. Epistasis anaylsis and studying wild-type gene function will elucidate the role of the genes that act specifically in the reproductive pathway to regulate aging.

1. Hsin and Kenyon, Nature 1999 2. Arantes-Oliveira et al, Science 2002 3. Gerisch et al, Dev Cell 2001 4. JRB and CK, unpublished; Adam Antebi, personal communication 5. Courtesy of J. Ahringer

83. Measuring *C elegans* Sensory Functions Across Lifespan

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Senescence in *C. elegans*is accompanied by intestinal and cuticular changes as well as decreased pumping rate. In addition, defecation rate and movement decline across lifespan(1). We have been investigating whether neuronal function also changes over lifespan by using a variety of behavioral assays to study sensory functions in aged worms. We observed a mid-life decline in chemotactic behavior. However, at mid-life animals can still sense volatile repellants at close range, respond to light touch, and move. This suggests that the changes in chemotactic behavior might be divorced from sensory function, possibly due to muscular deterioration or altered behavioral responses. We are currently conducting experiments to distinguish between these possibilities.

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84. Gene expression changes in *clk-1* and caloric-restricted worms.

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Caloric restriction has been shown to extend lifespan in model organisms including the rat, mouse, fly, and worm. Caloric restriction is thought to act by reducing the metabolic rate of the animal. Mutations in *clk-1* extend lifespan and alter the timing of several developmental and behavioral processes. *clk-1* and caloric restriction extend lifespan through common pathways. *clk-1* encodes a enzyme required for synthesis of ubiquinone, a component of the mitochondrial electron transport chain, and thus may directly alter metabolism. Spotted DNA microarrays containing most worm genes are being used to profile gene expression changes caused by a *clk-1* mutation or by caloric restriction in adult worms. These gene expression changes will be compared with the gene expression profiles of worms in the dauer state and the changes that occur during normal aging.

85. Protein Repair Deficient Worms Reproduce and Age Normally

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Proteins are susceptible to several types of spontaneous damage, including the isomerization of normal asparagine and aspartic acid residues to L-isoaspartyl residues. This reaction causes a kink in the protein chain by rerouting the backbone through the side-chain of the asparagine or aspartic acid residue. This damage can be corrected by the protein L-isoaspartyl-O-methyltransferase that is found in nearly all organisms examined to date. The gene coding for this protein in C. elegans is pcm-1. A recent RNAi screen by Maeda et al. (Curr Biol. 2001, 11, 171-6) found an embryonic lethal phenotype in 14% of the eggs of pcm-1 RNAi treated worms. This gene has previously been knocked out and analyzed in a him-8 mutant background. The brood size of the him-8; pcm-1 worms was not less than that of the him-8 worms, suggesting no embryonic lethality (Kagan et. al., Arch Biochem Biophys. 1997, 348, 320-8). However, the brood size of both these strains was about half that of wild type. We were concerned that phenotypes of the methyltransferase knockout worms were masked by the him-8 mutation. We backcrossed pcm-1(qa201) five times to N2. The analysis of pcm-1 in a wild-type background demonstrated that brood size and embryonic lethality are indistinguishable from wild type. We do not find the 14% embryonic lethality that was noted in the RNAi experiment. We also analyzed the adult life span at 25oC of the pcm-1 delete worms and found that it was nearly identical to that of wild-type worms. We then hypothesized that if this mutation were in a long-lived background such as daf-2(m41), then there may be a shortening of life span due to the accumulation of damaged proteins over a longer period. This however was not the case. The daf-2; pcm-1 double mutant worms lived as long as daf-2 single mutants. We are currently analyzing the two phenotypes that were noted in the him-8; pcm-1 worms, decreased dauer life span, and a failure to thrive in a mixed population of wild-type and pcm-1 worms. We are also planning to investigate several conditions that may affect the methyltransferase knockouts more severely than the wildtype worms, such as oxidative stress, pH, and temperature.

86. Showing sensitivity to *Bacillus thuringiensis* toxins

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Our lab is studying the mechanisms of action and resistance to *Bt* crystal proteins using the nematode *C. elegans*. These invertebrate-specific bacterial toxins play an important role in controlling insect pests around the world. To date, we have characterized in detail how toxin resistance develops. A different approach to studying this host-pathogen interaction is to look for hypersensitive mutants that are susceptible to lower levels of toxins than wild type. Such mutants can teach us about pathways involved in response to toxin and in defense of an organism to a pore forming toxin, since removal of a defense mechanism is predicted to lead to hypersensitivity. We set to see if hypersensitive mutants 1) can be isolated; 2) can be recovered; and 3) can be worked with. Results of our initial genetic screening will be presented.

Another way we are "sensitive" to toxin is to try to develop ways to quantitate response of nematodes to toxin. There has been little work in the *C. elegans* field to develop reliable, dose-dependent quantitative assays. The assay that I am currently working on involved looking at the effects of toxin on hermaphrodite growth rate. I will present our current status on how growth rate can be measured, semiautomated, and the response of growth rate in wild-type and mutants in various doses of toxin.

Overall, the lab is interested in the study of *Bt* toxins and how it works. Since Bt toxins are at the center of the world debate on transgenic crops (Genetically Modified Organisms), we feel it is important to educate people about the risks and benefits of this technology so that intelligent decisions can be reached. Our lab is in a good position in informing the general public about *Bt*. One way we are delivering this information is through the use of animation. An important goal is to develop a web site understandable by general populace to help people understand *Bt*, how it is used, the benefits of this technology, and the concerns regarding its use. I will present the current status of this developing tool.

87. *C. elegans as a model host for the Bacillus thuringiensis toxin Cry5B*

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Bacillus thuringiensis(*Bt*) is a sporulating bacterium that produces crystallized toxins (Cry proteins) with insecticidal activities. The use of these toxins has become widespread, both in genetically engineered crops and as sprays to control insects that carry human diseases. However, their effectiveness at combating pest insects is threatened by the possibility of selection for resistance among the insect population. Understanding the molecular mechanisms of toxicity and resistance to Bt-toxins is a primary goal in our lab.

In vitro studies in insects have demonstrated that, following ingestion by a susceptible host insect, the toxin is solubilized and proteolytically activated. The toxin can then bind to receptors on the apical surface of the gut and insert into the membrane, forming pores and causing lysis of these cells.

Certain *Bt*-toxins have nematicidal properties, including the toxin Cry5B. When *C. elegans* is fed *E. coli* engineered to express the Cry5B toxin gene, the animals suffer intestinal damage similar to that described for insect larvae feeding on insecticidal *Bt*-toxins. To help elucidate the molecular mechanisms of toxicity, a genetic screen was performed to isolate *C. elegans* mutants resistant to Cry5B. Five complementation groups were identified and named *bre* for Bacillus resistant.

Previous work has shown that BRE-5 and BRE-3 are putative glycosyltransferases. These are the enzymes responsible for the synthesis of the oligosaccharide chains commonly found attached to proteins and lipids at the cell surface. The model that has been developed is that BRE-5 and BRE-3 participate in building a carbohydrate structure that serves as a binding site for the toxin. Failure to build this structure in the mutants results in the inability of the toxin to bind the gut cells. Subsequently, no pore is formed and the animals are resistant to the toxin.

I have recently determined the molecular identity of another *bre* gene, *bre-4.* Using single nucleotide polymorphisms to map this mutation, I was able to narrow the *bre-4* region to an interval containing twenty predicted open reading frames. One of these twenty genes, a predicted galactosyltransferase, is mutated in three independent *bre-4* mutants, indicating that *bre-4* is likely to be this gene. Our BRE-4 data fits well with the –BRE-5/BRE-3 model of toxicity and resistance as another player in building this toxin-binding oligosaccharide and reinforces the prominence of carbohydrates in*Bt*-toxin binding and resistance.

More recently, I have begun a separate project aimed at more globally identifying genes important for Bt toxin action and resistance in *C. elegans.* Using full genome microarray chips in collaboration with the Kim Lab at Stanford, I plan to compare the expression profiles of *C. elegans* fed Cry5B versus the closely related but non-toxic Cry5A. The goal is to understand the primary response in the gut of the animal when exposed to toxin. This approach should help uncover both the killing strategies of the toxin and the defense mechanisms of the host.

88. Functional characterization of a structural motif in UNC-11 that has been implicated in phosphoinositide binding.

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In *C. elegans*, the *unc-11* gene encodes a family of proteins with structural and functional homology to the mammalian monomeric clathrin adaptor protein AP180 (Nonet et al., 1999). In the nematode these proteins have a role in the retrieval and sorting of the integral synaptic vesicle protein synaptobrevin from the plasma membrane and to synaptic vesicles (Nonet et al., 1999).

AP180 family members, contain a conserved inositide-binding motif (Ford et al., 2001). Functional studies with the mammalian proteins have revealed that this motif is essential for the interaction of these proteins with inositides and its phosphorylated lipid derivatives, phosphoinositides (Gaidarov and Keen, 1999; Hao et al., 1997). Moreover, it has been suggested that binding to $PI(4,5)P_2$ is necessary for the recruitment of AP180 to membranes enriched in this phosphoinositide and the targeting of clathrin coats to such sites (Takei et al., 1998).

The residues directly involved in PIP_2 binding, deduced from the analysis of the crystal structure of the N-terminal half of the ubiquitously expressed family member (CALM), are conserved in the UNC-11 proteins (Ford et al., 2001). All potential UNC-11 isoforms share a phosphoinositide-binding motif centered on three lysine residues at amino acid positions 53-55 of all isoforms. This PIP_n binding site is missing entirely from the mutant allele *unc-11 (q358)*.

To determine the functional significance of this motif *in vitro* we analyzed the inositide-binding properties of recombinant wild type (UNC-11A) and mutant (Q358-2) proteins in two established biochemical assays (Ye and Lafer, 1995; Hao et al., 1997). We found that the mutant protein was as competent as the wild-type protein in promoting the assembly of clathrin *in vitro*. In contrast to the wild type protein, cages made by the mutant protein could not bind IP_6. This finding suggested that the motif missing in *unc-11(q358)* was required for inositide-binding but was not necessary for promoting clathrin assembly.

To determine whether *in vivo* binding to phosphoinositides is necessary for function, and physiologically relevant, we introduced the *q358* mutant gene into nematodes lacking endogenous *unc-11* function and compared the expression pattern of the resultant mutant proteins to that of the proteins derived from a wild type gene. Surprisingly the mutant protein partially rescued the loss of function phenotypes. Since only a small percentage of the mutant protein, when compared to the wild type protein, made it to the synaptic terminal this observation suggested that once at the synaptic terminal the mutant protein was functional. Moreover, the fact that most of the mutant protein was retained in the neuronal cell bodies, a subcellular distribution pattern distinct from that of the wild type protein, suggested that binding to phosphoinositides was important for either the transport or the targeting/retention of UNC-11 at the terminal.

We are pursuing this observation to determine if the phosphoinositide binding motif is sufficient for synaptic localization of UNC-11 proteins. Moreover, we have generated another mutant gene in which three lysine residues involved in PIP binding have been mutate to alanines. Preliminary analysis indicates that this mutant protein is also retained in the neuronal cell bodies.

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89. *cen-1* is required for centrosome maturation in the early *Caenorhabditis elegans* embryo.

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We have isolated a maternal effect lethal mutant we call *cen-1(or430ts)*, for **cen**trosomal defective, in a screen for temperature-sensitive embryonic-lethal cell division mutants. At the non-permissive temperature pronuclear migration, rotation and movement of the centrosomal/nuclear complex and spindle formation are all severely, although variably, affected in single-celled *cen-1* mutant embryos. Indirect immunofluorescence analysis of fixed embryos and time-lapse spinning disk confocal microscopy using a beta-tubulin::GFP reporter reveal that astral microtubules associated with the sperm pronucleus are far less robust than in wild-type, although free cytoplasmic microtubules are present in early pronuclear stage embryos. This suggests that the primary defect is not in microtubule assembly. Instead, these defects in microtubule accumulation probably result from abnormal centrosomes: SPD-5, AIR-1, ZYG-9 and gamma-tubulin, all known components of centrosomes (D. Hamil, personal communication, 1,2,3,4), fail to accumulate to normal levels in *cen-1* mutant embryos. *cen-1* therefore appears to be an important factor required for centrosomal maturation. Consistent with this conclusion, *cen-1* mutant embryos share other phenotypes caused by depletion of centrosomal proteins. These include collapse of centrosomes towards one another upon nuclear envelope breakdown, as reported for *air-1* depleted embryos (1), and occasional separation of centrosomes from the male pronucleus, as seen in *tbg-1* depleted embryos (4). We are currently positionally cloning *cen-1*, which maps to the right arm of LGIV, at approximately 5.57 map units.

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90. Identification of Genes Involved in Sterol Metabolism in *C. elegans*

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Cholesterol is required for normal growth and reproduction in *C. elegans*. To investigate whether sterols might regulate worm physiology, we tested the effects of mammalian sterol biosynthesis inhibitors on worm growth, reproduction, and lifespan. Among those studied, a class of imidazole based inhibitors: ketoconazole, miconazole, sulconazole, and clotrimazole, each of which inhibits a broad yet unique spectrum of P450 enzymes, significantly affected *C. elegans* growth and reproduction. At low concentrations (100μM ketoconazole), the inhibitors reduced growth rates and brood size, similar phenotypes to those observed in cholesterol-deprived media. At intermediate concentrations (200 μM ketoconazole), worms displayed a severe growth defect, remaining at L1 or L2 for at least seven days. At high concentrations (300 μM ketoconazole), worms died at L1 or L2. In addition, the imidazole based inhibitors significantly reduced adult lifespan. Based on these phenotypes, we have screened for mutants that are resistant to the imidazole compounds. We have isolated several such mutants and are currently mapping and characterizing them.

91. Intermediate Filament IFB-1 Functions in Epidermal Morphogenesis and is a component of trans-epidermal attachments

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In screens for epidermal morphogenesis mutants, we identified a mutation, *ju71*, that affects one of the *C. elegans* intermediate filament (IF) genes, *ifb-1* (Woo and Chisholm. 2001 Intl. worm meeting).*ifb-1(ju71)* mutants display abnormal head epidermal morphology and 50% embryonic and larval lethality. The mutant embryos arrest at the three-fold stage of embryonic elongation and eventually rupture at the head epidermis.

ifb-1 encodes two isoforms, differing at the N-termini due to alternative first exons. The lesion in *ju71*is a 617 bp deletion whose 3 breakpoint lies 250 bp upstream from the predicted IFB-1S (short isoform) initiator ATG. A genomic clone encoding only IFB-1S fully rescues *ifb-1(ju71)* mutants, suggesting that the *ju71* mutation specifically affects the short isoform. Consistent with these results, RNAi of both *ifb-1* isoforms resulted in 100% lethality (also reported by Karabinos, et. al. *Proc. Natl. Acad. Sci. USA 98*: 7863-8). The *ifb-1(RNAi)* animals either arrested at the two-fold stage during embryonic elongation with lumpy epidermal morphology, or as paralyzed larvae. We are currently examining the individual functions of the two *ifb-1* isoforms using isoform-specific RNAi and transgene analysis. We are also performing Northern and Western blot analysis to determine the effect of the *ju71*mutation on transcript and protein levels.

A functional IFB-1S::GFP localizes to trans-epidermal attachments (TEAs, *a.k.a.* fibrous organelles) in embryos and larvae. To characterize the role of IFB-1 in TEAs, we are examining the expression patterns of other components of TEAs in *ifb-1(ju71)* mutants, and also the localization of IFB-1S::GFP in other embryonic elongation mutants. Since *mup-4* mutants display defects in embryonic morphogenesis overlapping with those of *ifb-1,*and the intracellular domain of MUP-4 is homologous to an IF-associated protein (Hong et. al. *J. Cell. Biol. 154*: 403-414), we are testing whether *ifb-1* and *mup-4*function in the same pathway in embryonic morphogenesis.

The lumpy two-fold arrested phenotype of *ifb-1* RNAi embryos also resembles that of other mutants with defects in embryonic trans-epidermal attachments such *vab10*, which encodes the conserved hemidesmosome component Plectin (Bosher et al., 2001 Intl. Worm Meeting) and *vab19*, which encodes a novel conserved protein (Ding and Chisholm, 2001 Intl. worm meeting). To identify new genes functioning in IF-mediated epidermal morphogenesis we performed a semi-clonal screen for new elongation-arrested lumpy mutants (see abstract of Ding et. al., this meeting). We are currently characterizing these mutants.

92. *let-711*: A GENE INVOLVED IN SPINDLE POSITIONING

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The timing and orientation of cell division is critical for establishing cell contacts and partitioning asymmetrically localized cytoplasmic factors required for proper development. Following fertilization in *C. elegans* embryos, the two parental pronuclei meet in the posterior of the cell, move to the center (centration), and the nuclear-centrosome complex rotates 90^{o}. This movement of the nuclear-centrosome complex places the centrosomes on the anterior-posterior axis, enabling the spindle to set up on this axis. Centration and rotation are dependent on astral microtubules, and the microtubule motor dynein and its associated dynactin complex.

We are studying a gene, *let-711*, which functions in spindle positioning in many cells of the early embryo. Worms homozygous or heterozygous for different *let-711* alleles show phenotypes ranging from larval lethality to sterility to maternal effect defects in spindle orientation. To characterize the spindle positioning defects, we have used time-lapse video microscopy to examine embryos from worms homozygous for a maternal allele, *it150ts* (previously known as *spn-3*), as well as those from worms heteroallelic for *it150ts* and a stronger allele (*s2587*). Embryos from *it150ts* homozygotes had spindle positioning defects during the second and third cleavages. Analysis of embryos from the stronger allelic combination revealed a role for *let-711* in spindle positioning during the first cleavage. During this division mutant embryos often failed to position their spindle correctly due to a failure in centration, nuclear-centrosome rotation, or both. However, polarity markers in *it150ts*/*s2587* and *it150ts*/deletion embryos were normal, suggesting *let-711* functions downstream of polarity establishment or in a parallel pathway. Immunolocalization of microtubules and a centrosomal marker showed the centrosomes to be larger in *it150ts*/*s2587* embryos at all stages of the first mitosis. This enlargement could be due to a primary defect in centrosome structure that then results in centration and rotation defects. Alternatively, there could be a more direct effect on microtubule function that both alters the structure of the centrosome and affects nuclear-centrosome movements. To distinguish between these two possibilities we are examining the localization of different centrosomal markers in *it150ts*/*s2587* embryos. As well, we are analyzing embryos doubly mutant for *let-711* and microtubule associated proteins. *let-711* has been mapped to a region spanned by 7 cosmids and candidate genes from this region are currently being sequenced. Through the identification of *let-711* and further characterization we hope to better understand how this gene is involved in the movements of the nuclear-centrosome complex in the early embryo.

93. A protein phosphatase 4 homologue, PPH-4.1, is essential for centrosome maturation in mitosis and sperm meiosis in C. elegans

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The centrosome is the primary microtubule organizing center in eukaryotic cells. Centrosomes mature during the G2/M transition, accumulating the pericentriolar materials (PCM) that direct the assembly of microtubules. The regulation of this dynamic change of centrosomes remains largely unknown. Protein phosphatase 4 is known to localize to mitotic centrosomes in mammals and Drosophila, but its in vivo functions are not well understood.

Here, we report that one of the two C. elegans protein phosphatase 4 homologues, PPH-4.1, is required for centrosome maturation. RNAi analysis revealed that PPH-4.1 was required for the formation of spindles in mitosis and sperm meiosis, while it was dispensable for female meiotic divisions, which do not depend on centrosomes. In mitotic pph-4.1(RNAi) embryos, localization of gamma-tubulin and a Polo-like kinase homologue to the centrosome was severely disrupted. Immunofluorescence staining revealed that PPH-4.1 was present at centrosomes from prophase to telophase, but not during interphase. Depolymerization of astral microtubules by nocodazole or cold treatment did not affect the centrosomal localization of PPH-4.1, suggesting that localization of PPH-4.1 to centrosomes is not dependent on microtubules. These results indicate that PPH-4.1 is involved in the recruitment of PCM components to the centrosome, and is essential for the activation of microtubule nucleation potential of the centrosome. We are currently examining the mechanisms of cell cycle-dependent localization of PPH-4.1 on centrosomes. Besides the centrosomal phenotypes, absence of chiasmata between homologous chromosomes was often observed in oocytes of pph-4.1(RNAi) animals. Thus, in addition to spindle formation, PPH-4.1 appears to play a role in either the establishment or the maintenance of chiasmata during meiotic prophase I.

94. Mutations affecting mitotic spindle position in the *C. elegans* embryo.

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We have been screening EMS-mutagenized worms for temperature-sensitive mutations affecting cell division mechanics. Six new mutant strains have been isolated in which mitotic spindles are abnormally positioned during the embryo's first two divisions. We call these mutants or470, or473, or578, or579, or580, and or581. We will describe these embryonic-lethal phenotypes in detail, and report on our progress in mapping and genetically characterizing these mutations.

95. Analysis of the Intraciliary Transport Motor, OSM-3

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Chemosensation in *C. elegans* is mediated by chemosensory neurons that terminate in sensory cilia, which detect chemical cues in the external environment. The *osm-3* gene encodes a kinesin-related microtubule-based motor protein (Signor et al., 1999a). OSM-3 is thought to contribute to chemosensory neuronal function by transporting ciliary precursors from the neuronal cell body to the tips of the cilia, because *osm-3* mutants display defects in chemosensory cilia structure and accumulation of vesicles in the sheath cell (Perkins et. al, 1986). Consequently, these *osm-3* mutants are unable to discriminate between high and low osmolarities or form dauer larvae (Tabish et al, 1995). To directly test the role of OSM-3 in microtubule-based transport and ciliogenesis, and its relation to the heteromeric kinesin-II intraciliary transport motor (Signor et al, 1999a, 1999b), we used an in vivo motility assay (Orozco et. al, 1999; Zhou et. al, 2001). Here, we created an *osm-3*::GFP transgene which rescued defects in ciliary structure as assayed by dye-filling and dauer larva formation, when introduced to the homozygous loss-of-function (*p802*) mutant allele as an extrachromosomal array. OSM-3::GFP moves along chemosensory cilia with an average velocity of 0.93+/-0.431 microns/s (n=441) [average anterograde velocity = 0.93+/-0.417 microns/s (n=286); average retrograde velocity = 0.96+/-0.457 microns/s (n=155)]. To identify domains that are important in cargo-binding and transport, we have sequenced multiple alleles of *osm-3* and are assessing the effects of their lesions on intraciliary transport and ciliary structure. Finally, we have begun to assess intraciliary transport of putative OSM-3 cargoes in *osm-3* mutant backgrounds. We find that transport of the IFT-raft subunit, OSM-6::GFP, persists in *osm-3* mutant backgrounds suggesting that OSM-3 drives an intraciliary transport pathway that is distinct from that driven by kinesin-II (Signor et. al, 1999).

96. A Genetic Analysis of Neuronal Polarity in C. elegans

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Neurons are highly specialized cells designed to receive, integrate, and transmit information. To accomplish these tasks, the neuron is functionally and structurally divided into different compartments. The somatodendritic compartment, consisting of the cell body and the dendritic processes, is specialized for the receipt and integration of information. Within dendrites, specialized areas like sensory cilia and dendritic spines are local signaling centers. The axonal compartment, consisting of a longer axonal process, is designed to transmit information quickly to other neurons and to muscle. How are these compartments established and maintained?

To help answer this question, I am using the AWB amphid neuron as a model for neuronal polarity. AWB is a bipolar neuron with one well-defined dendrite that ends in a sensory cilium at the tip of the nose, and one axonal process that projects to the nerve ring. I am using GFP-tagged ODR-10, driven by the AWB-specific str-1 promoter, as a marker for ciliary localization. ODR-10 is a G protein-coupled odorant receptor that is highly enriched in the cilia of wild-type animals. To label axons, I am using GFP-tagged versions of the PDZ domain protein LIN-10 and the synaptic vesicle protein SNB-1. These proteins are present in axons and excluded from dendrites of amphid sensory neurons in wild-type animals. I mutagenized str-1::odr-10::GFP and conducted a clonal screen to isolate mutants with defects in ODR-10 localization to cilia. The clonal screen allowed me to isolate sick and slow growing mutants that would have been missed using more traditional methods. I found alleles of odr-4 and odr-8, the two genes previously known to affect ODR-10 localization to cilia, as well as alleles of two additional genes. Preliminary mapping and characterization of these mutants will be presented.

97. *glo-1* is necessary for lysosome-related organelle biogenesis in *C. elegans*

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Lysosome-related organelles represent a diverse group of specialized, cell type specific organelles that share a significant number of characteristics with conventional lysosomes. The *C. elegans* intestinal-specific gut granule is a member of this group of organelles. Gut granules are acidified, contain lysosomal proteins, and are the terminal endocytic compartment. However, unlike other lysosomes in *C. elegans*, gut granule contents are birefringent and autofluorescent, and they are likely to have a specialized role in the digestive physiology of the animal.

To identify the pathways involved in the biogenesis of lysosome-related organelles, we screened for mutants with defects in gut granule assembly. From our screens we identified three *glo* (for Gut granule LOss) genes that are necessary for gut granule biogenesis. Here we present our analysis of *glo-1*. *glo-1* mutants mislocalize gut granule contents to the intestinal lumen during embryogenesis. In addition, *glo-1* larvae and adults lack birefringent and autofluorescent gut granules and do not contain acidified and terminal endocytic compartments in the intestine. Surprisingly, despite the loss of functional lysosomes in the intestine, *glo-1* animals are viable and fertile.

In order to understand how *glo-1* functions in gut granule biogenesis we cloned the wild-type *glo-1* gene. *glo-1* is predicted to encode a GTPase with significant similarity to Rab GTPases. Rabs play important roles regulating targeting, trafficking, and transport in the secretory and endocytic pathways. The phenotype of *glo-1* mutants supports a similar function for GLO-1 in the biogenesis of gut granules.

98. Synaptic vesicle kinesin and synapse development

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Kinesins are a large family of motor proteins responsible for fast transport of a variety of cellular organelles over long distances. The *C. elegans* genome encodes at least 20 different kinesin-like molecules and as in other organisms - each kinesin is believed to transport a specific and unique cargo. UNC-104 is a *C. elegans* kinesin involved in anterograde axonal transport. We would like to determine the variety of cargo transported by UNC-104, the developmental role such cargo may elicit, and finally the mechanism of cargo recognition.

UNC-104 transport is essential for the formation of neuromuscular junctions in the worm. In wild-type GABA neurons, synaptobrevin (a synaptic vesicle protein) is always found juxtaposed to GABA receptor clusters. The neurotransmitter GABA is not required for this clustering of GABA receptors, since clusters still form in mutants that do not make GABA (*unc-25*). By contrast, in *unc-104* synaptic vesicle clusters are not transported to the dorsal nerve cord and GABA receptors do not cluster at neuromuscular junctions. These data suggest that a cargo vesicle transported by UNC-104 is required for clustering of postsynaptic receptors.

We are also studying the molecular mechanism that underlies cargo recognition. It is likely that UNC-104 identifies its cargo by binding a lipid component of the vesicle membrane via its pleckstrin homology (PH) domain. PH domains are motifs known for their lipid binding activity and we have demonstrated that the recombinant UNC-104 PH domain specifically binds phosphoinositides. We are currently testing the necessity of lipid-binding activity for UNC-104 mediated vesicle transport.

99. *slr-2* and *slr-8*, two genetic modifiers of *lin-35/Rb* in *C. elegans*

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Due to the eventual saturation of the *C. elegans* genome with mutations that produce easily detectable autonomous phenotypes, it will be important to conduct screens for mutations that display synthetic interactions genetically. *lin-35*, the *C. elegans* ortholog of the human tumor suppressor gene Rb, displays only a subtle null phenotype but shows functional redundancy with a number of *C. elegans* genes (Lu et al., 1998; Boxem and 2001; Fay et al., 2002). By using a nonbiased synthetic mutant screen, we have identified genes that assist *lin-35* in the control of *C. elegans* development and cell cycle regulation. This screen consists of mutagenizing *lin-35 (-/-)* animals that harbor an extrachromosomal array *(kuEx119)* containing rescuing copies of the wildtype *lin-35* gene along with a ubiquitously expressed GFP reporter (Fay et al. 2002). This screen has resulted in the isolation of 11 mutants (termed *slr* for *synthetic* with *lin-35/Rb*) that display a spectrum of lethal phenotypes in conjunction with *lin-35* mutations. We report here progress of two mutants, *slr-2* and *slr-8*, which both show strong synthetic interactions with *lin-35*.

slr-2(ku294) single mutants appear identical to wild type and exhibit only a slight reduction in fertility compared to N2. In contrast, *lin-35; slr-2* double mutants arrest as L1 or L2 larvae and exhibit a moderate Dpy phenotype. When placed on *lin-35* RNAi feeding plates, *slr-2* animals often progress to become sterile Dpy adults, in some cases exhibiting a variety of germline defects. Using two and three-point mapping techniques, we have placed *slr-2* on the right arm of LGV between *rol-9* and *unc-76*. We are currently using SNP mapping to narrow the region and identify the affected locus.

lin-35; slr-8(fd2) double mutants either arrest as Unc larvae or progress to become thin sterile adults. These adults typically display defects in gonad development wherein the gonad arms fail to form and/or migrate. Embryos produced by *lin-35; slr-8* animals occasionally remain internal and fail to hatch. In contrast, *lin-35; slr-8* animals containing the array *kuEx119* appear indistinguishable from wild type. *slr-8* has been mapped to a region of chromosome I near dpy-5. We are presently using SNP mapping to identify the *slr-8* locus.

Lu and Horvitz (1998). *Cell* **95**: 981-991.
Boxem and van den Heuvel (2001). *Development* **128**:4349-4359.
Fay et al. (2002). *Genes and Development* **16**: 503-517.

100. Germline precursor cells arrest at G2/prophase during embryogenesis and dauer diapause

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We and others previously reported that the *cki-1* gene, which encodes a CIP/KIP cyclin-dependent kinase inhibitor (CKI), is required for many somatic cells to exit from the embryonic cell cycle at the proper time. In contrast, we found that the two germline precursors (Z2/Z3 cells) undergo cell cycle arrest by a *cki-1*-independent mechanism during embryogenesis. Since CIP/KIP CKIs have been shown to be essential for developmental G1 arrest in many animals, we tested the hypothesis that the Z2/Z3 cells become mitotically quiescent at a non-G1 phase.

We found that arrested Z2/Z3 cells start displaying condensed chromosomes before the bean stage. The fluorescence intensity of propidium iodide-stained Z2/Z3 cells in starved L1 larvae is approximately twice that of the adjacent Z1/Z4 somatic gonad cells, suggesting that arresting Z2/Z3 cells have a 4N DNA content. Moreover, we found that the Z2/Z3 cells in previously starved L1 larvae in which cell-cycle arrest was relieved by feeding can divide even in the presence of hydroxyurea, an S-phase inhibitor, suggesting that germline progenitor cells are arrested after S phase in starved L1 larvae. These findings suggest that embryonic Z2/Z3 cells arrest at G2/prophase.

Phosphorylation of Ser10 on histone H3 is generally correlated with mitosis and chromosome condensation. We found that the Z2/Z3 cells become immunoreactive with anti-phospho H3 (ser10) antibody at around the stage when chromosomes start condensing and continue to express this epitope throughout diapause. Interestingly, our preliminary data indicate that this immunoreactivity is weaker than that in cells undergoing mitosis; we also see phospho-H3 immunoreactivity in apparently all arrested germ cells in both starved L1 as well as dauer larvae, implying that there may exist two distinct states of histone H3 phosphorylation. We are currently attempting to determine whether centrosomes have been duplicated in arrested Z2/Z3 cells, at which phase of the cell cycle germ cells in dauer larvae are arrested, how the phosphorylation state of histone H3 changes in developing germ precursors, and the identity of the molecular cues that cause this germline arrest.

Our observations demonstrate that developmental diapause at both the L1 and probably dauer stages promotes a post-S phase arrest in the germline. This system may serve as a mechanism to ensure the integrity of the genome in the immortal germline cells.

101. Developmental control of cell cycle in *C. elegans*

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The decision to enter a new division cycle in response to extracellular signals occurs during the G1 phase of the cell cycle. How the basic cell-cycle machinery is regulated by these signals is poorly understood. We use the development of the *C. elegans* vulva as a model to study the regulation of cell cycle entry during development, since the vulva precursor cells (VPCs) display a developmentally regulated period of cell cycle arrest.

We have previously described a screen to identify negative regulators of VPC divisions. Briefly, mutants are identified in the *lin-12(gf)* background as animals with greater than six pseudovulvae. By observing the vulval lineages during the L2 and L3 stages, the origin of these extra pseudovulvae can be verified to result from ectopic divisions of the VPCs. To date, we have identified at least four *elm* (enhancer of *lin-12(gf)* multivulvae) loci that result in extra cell divisions.

Two mutants, *elm-1(he118)* and *elm-4(he135)*, exhibit extra cell divisions within the VPC lineage with high penetrance. The extra division phenotype of *elm-1(he118)* appears to be restricted to the VPCs while *elm-4(he135)* mutants are defective in multiple cell lineages. Interestingly, the Elm phenotypes of both mutants are enhanced by loss of *lin-35* Rb activity. Since *lin-35* acts in parallel to the cyclin dependent kinase inhibitor, *cki-1*, to inhibit cell cycle progression (Boxem and van den Heuvel, 2001), these data suggest that *elm-1* and *elm-4* act within the *cki-1*-mediated pathway. In addition, the extra VPC phenotype of *elm-1(he118)* is dependent upon the expression of *cye-1*, which encodes the target of *cki-1* inhibitory activity. These loci are currently being identified and progress in cloning will be presented.

In addition, we identified three mutations, *lin-31(he136)*, *lin-1(he117)* and *lin-1(he119)*, which confer a low penetrance extra VPC division phenotype. *lin-1* and *lin-31* encode transcription factors of the Ets and Forkhead families, respectively. A role for a LIN-1/LIN-31 complex in regulating vulval cell fate determination during L3 has been described (Tan *et al*., 1998). Although ectopic VPC divisions have been observed previously in *lin-31(lf)* mutants (Miller *et al.,* 1993), the finding that *lin-1* mutants are also Elm suggest that the LIN-1/LIN-31 complex also plays an earlier role in VPC development to mediate a temporary withdrawal from the cell cycle. *cki-1* is a candidate target of LIN-1/LIN-31 transcriptional regulation since its promoter contains consensus binding sites for Ets and Forkhead transcription factors, *cki-1* reporter expression correlates with *lin-31* promoter activity and *cki-1* activity is required for the VPC developmental arrest. Using a *cki-1::GFP* transgene (a kind gift from V. Ambros) we determined that expression of the *cki-1* reporter 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 result from decreased expression of the cell cycle inhibitor *cki-1*. Moreover, the extra cell phenotype of *lin-31(he136)* is enhanced by *lin-35(RNAi)*, again supporting a role within the *cki-1* pathway. We propose that cell-type specific transcriptional regulation of *cki-1* by *lin-1* and *lin-31* defines part of a network that mediates the temporal withdrawal of the VPCs from the cell cycle during development.

102. *cep-1*/p53-Independent Activation of Apoptosis in the *C. elegans* Germline

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We have isolated a deletion mutant in the *C. elegans* p53 gene ortholog*, cep-1*. In agreement with the phenotypic analysis of *cep-1* RNAi (Derry et al., 2001; Schumacher et. al., 2001) and a dominant negative *cep-1* mutant (Derry et al., 2001), we find that the *cep-1* deletion blocks radiation-induced apoptosis in the meiotic germline, but does not alter physiological germ cell death. In addition, the *cep-1* deletion mutant displays hypersensitivity to radiation-induced germline proliferation defects, although this phenotype is less penetrant than that of the checkpoint mutants *hus-1* and *rad-5*. Some experiments suggest that the *cep-1* deletion may also partially block cell cycle arrest of the mitotic germline in response to DNA damage.

We are carrying out an RNAi screen for increased germline apoptosis in the *cep-1* deletion mutant, with the goal of identifying candidate drug targets for inducing apoptosis of p53 mutant tumor cells. Inactivation of *cpl-1*, an ortholog of the protease cathepsin L, was found to induce germline apoptosis in a manner independent of *cep-1* gene activity, but dependent on *ced-3*/caspase and *ced-4*/Apaf-1 activities. We will discuss models by which the function of a lysosomal protease might normally prevent apoptosis.

103. Identification of candidate genes that function in CED-3-independent programmed cell death

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Among the known cell death regulators in C. elegans, the caspase CED-3 (Ellis et al., 1986) is a key member of the pathway, both as the terminal component in the core pathway and as the trigger for chromatin condensation, cytoskeletal degradation, and intracellular vacuolization accompanying apoptotic death. In the absence of functional CED-3, cells that would normally undergo programmed cell death instead develop, differentiate, and integrate into the animal's tissues. Despite the knowledge provided by the discovery of the core cell death machinery, the molecular processes that mediate the biochemical and cellular events downstream of CED-3 remain poorly understood.

Based on the recent finding in our laboratory that an apparent cell death suppressor, icd-1, leads to CED-3-independent cell death when debilitated (Bloss et al., 2002), we are analyzing candidate genes that may protect cells from dying and that function downstream, or independently, of ced-3. To this end, we are studying previously identified zygotic embryonic lethal mutations from the laboratory collection (Rothman, 1991) that lead to widespread cell death in the absence of ced-3 function. We are also using an RNAi library of bacterially expressed dsRNAs (Kamath et al., 2001) to identify genes whose loss of function results in the occurrence of apoptotic cell death in ced-1;ced-3 worms. Candidate genes that show RNAi-induced apoptosis in ced-3 mutant worms will be further characterized for their role in programmed cell death.

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104. ceh-16: the C. elegans ortholog of the evolutionary conserved engrailed gene

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Engrailed is an evolutionary conserved transcription factor. It contains a homeodomain as a DNA binding moiety. In the fly, engrailed is essential for segment polarity. There, secreted Wnt molecules from anterior cells are required for correct engrailed expression of posterior neighbouring cells. Engrailed in turn drives the expression of Hedgehog, a secreted molecule, resulting in a positive feedback loop for the Wnt expression of the anterior cells. In C. elegans the open reading frame C13G5.1 (LG III) encodes a predicted gene product with high identity to the Drosophila protein Engrailed. Since no cDNAs were available from the C. elegans community, we cloned the cDNA encoded by C13G5.1 using RTPCR. The genefinder prediction was largely confirmed. The gene contains a homeodomain encoding hoemeobox. The degree of amino acid identity within engrailed homeodomains of different species is very high.

Furthermore, we analyzed the expression pattern of C13G5.1::gfp in transgenic animals (the construct consists of the promoter region, the entire ORF and a C-teminal gfp fusion). C13G5.1::gfp is transiently expressed in the seam cells of embryos. Expression starts from prior to morphogenesis to the three-fold stage. Moreover, the construct is expressed in one pair of sensory neurons in the head and two pairs of neurons in the tail (the identity of all neurons remains to be determined). These expression data were confirmed by antibody staining using specific monoclonal antibody directed against a portion of the Engrailed homeodomain (the antibody is a kind gift of Dr. Nipam Patel, University of Chicago).

To elucidate the in vivo function of engrailed in the worm we are studying a mutant with a deletion in the gene generated by EMS mutagenesis. The deletion spans the majority of the ORF, deleting the promoter region as well as the conserved sequence region. Thus the mutant constitutes most likely a null allele. Preliminary analysis of the mutant revealed severe hypodermal defects. Homozygous animals die as embryos with this phenotype being fully penetrant. Finally, the mutants are rescued by the C13G5.1::gfp construct. A more detailed analysis will be presented at the meeting.

105. A New Mutation that Affects Distal Tip Cell Migration

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During larval development of *C. elegans* hermaphrodites a pair of distal tip cells (DTCs) lead the extension of two gonadal arms through three sequential phases of migration. DTCs, which are located on the anterior and posterior ends of the gonad primordium at L1, first move centrifugally, then make a dorsal turn and finally move centripetally. We have performed a genetic screen to look for mutations that affect DTC migration and isolated the mutation *tp3*.

We found that 90 % of *tp3* mutants are defective in DTC migration as inferred from the gonadal shapes. Approximately 80 % of these DTCs have a shorter 1st phase migration path than those in wild type. To further analyze this defect, we followed DTC migration in *tp3* and wild-type worms using Nomarski microscopy. We found that DTCs in *tp3* mutants not only migrated slowly but also underwent a precocious dorsal turn as compared to those in wild type. Previous studies showed that cell-autonomous expression of *unc-5* in DTCs is necessary and sufficient to drive the dorsal turn of DTCs (Su, M. et al., 2000). We therefore examined the timing of *unc-5* expression in *tp3* mutants using the *unc-5*::GFP transgene. *unc-5* expression was observed at the time of the precocious dorsal turn in *tp3* mutants. These results suggest that the early dorsal turn observed in DTCs of *tp3* mutants may result from precocious *unc-5* expression in these cells. We are currently doing experiments to confirm this.

In addition to the precocious dorsal turn and slow 1st phase migration, nearly 50 % of anterior DTCs and 10% of posterior DTCs of *tp3* mutants migrate in the opposite directions at the third migration phase: they migrate centrifugally but not centripetally. Therefore, the *tp3* mutation appears to affect the migration direction during the 3rd phase migration. To understand how *tp3* affects DTC migration, we have been taking the SNP mapping strategy to map *tp3* on the genetic map.

Reference:

Su, M., Merz, D. C., Killeen, M. T., Zhou, Y., Zheng, H., Kramer, J. M., Hedgecock, E. M., and Culotti, J. G. (2000). Regulation of the UNC-5 netrin receptor initiates the first reorientation of migrating distal tip cells in *Caenorhabditis elegans*. Development 127, 585-94.

106. *mig-29* encodes a SEC34 vesicle trafficking protein required for cell migration in *C. elegans* .

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The distal tip cells (DTCs) migrate on the body wall basement membrane in a U-shaped pattern during development of the gonad. We have genetically identified genes that can mutate to show meandering DTC phenotypes. In these mutants, the DTCs can move, but they cannot migrate along the normal pathways. One of these genes *mig-17* encodes a secreted ADAM (**A D**isintegrin **A**nd **M**etalloprotease) protein. MIG-17 is secreted from the body wall muscle cells and subsequently localized on the surface of the gonad, suggesting that the proteolytic remodeling of basement membranes are important for correct migration of DTCs.

To understand the MIG-17 mediated mechanism controlling the DTC migration, we investigated the *mig-29(k181)* mutation having *mig-17*-like meandering DTC phenotypes. The *mig-29 (k181)* mutation was isolated from a mutator strain that often results in trasposon insertion mutations. The mutation was mapped between *lin-17* and *unc-11* on linkage group *I*. SNP mapping located *mig-29* between two genomic clones W09D8 and Y54E10BL. A newly inserted transposon *Tc4* was found in the predicted gene Y71F9AM.4 - Y71F9AM.3. This transposon was jumped out in a spontaneous wild-type revertant. An about 20-kb genomic fragment containing about 2.5-kb 5'-upstream and 1.5-kb 3'-downstream regions rescued the DTC migration defects of *mig-29*. Sequence analysis of *mig-29* cDNAs revealed that *mig-29* has 14 exons encoding a 794 amino acid protein. The MIG-29 protein is highly homologous to human and yeast SEC34 proteins that are required for vesicle trafficking from ER to Golgi or within the Golgi. We found that the gonadal localization of MIG-17::GFP is weaker in *mig-29* than in the wild-type background, suggesting that MIG-29 affects the secretion of MIG-17 from the muscle cells.

107. Extending the *unc-53* pathway - Part 1: Yeast two hybrid reveals interactors

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Our approach to understanding the processes that direct cell migration and outgrowth has focussed on identifying the molecules that act as initiators or respondents to instructional signaling cues.

The *unc-53* gene is required for the directional guidance and extension of a subset of cells along the longitudinal axis of the worm, including the mechanosensory neurons, the sex muscles and the excretory canals. The *unc-53* locus is large, containing 23 exons spanning 31kb of genomic DNA. In addition to the presence of multiple promoters, *unc-53* transcripts are subject to alternative splicing, suggesting the existence of multiple protein isoforms *in vivo*. UNC-53 binds F-actin and SEM-5/GRB2 *in vitro*, implicating UNC-53 in both signal transduction and actin cytoskeleton dynamics (Stringham et al., 2002, *Development* 129: 3367-3379).

In order to understand the role of UNC-53 in cell migration and outgrowth, we are utilizing a yeast two-hybrid screen to identify potential interactors. A screen of a *C. elegans* cDNA library with the N-terminal portion of the UNC-53 gene product yielded 6 putative candidates. These candidates were identified based on their ability to grow on triple DO media containing a limiting amount of 3-aminotriazole and their ability to activate the beta-galactosidase reporter gene. We are in the process of determining the molecular identity of these candidates.

We are also performing immunohistochemistry on wild type and various *unc-53* alleles using antibodies raised against the N- & C- terminal portions respectively of the UNC-53 gene product. Our results should shed light on the tissue specificity of UNC-53 isoforms as well as their role at specific stages of development.

A second approach to isolate molecules involved in the *unc-53* pathway employs the use of genetic screens to identify suppressors of *unc-53* mutant phenotypes (see Abstract by Stauffer et al, this meeting).

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108. Extending the *unc-53* pathway- Part II: Isolation of genetic suppressors.

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Strong hypomorphic alleles of *unc-53* display reduced extension and guidance defects in the longitudinal outgrowth of a subset of cells including the ALN and PLN axons, the excretory canals, and the sex muscles, the latter resulting in an egg laying defective phenotype in hermaphrodites. In contrast, overexpression of UNC-53 in muscle cells results in exaggerated outgrowth during embryogenesis. UNC-53 encodes a 1583 amino acid protein that *in vitro* binds F-actin and SEM-5/GRB2 (Stringham et al., 2002, *Development* 129: 3367-3379.) suggesting UNC-53 plays a role in both signal transduction and modelling of the actin cytoskeleton. We are interested in identifying additional molecules which operate in the UNC-53 pathway to control longitudinal pathfinding.

We screened for genetic suppressors of the strong egg laying defective phenotype of the *unc-53* allele, *n166*. In a screen of 6182 haploid genomes two intragenic and two extragenic suppressors of *unc-53 (n166)* were isolated. One of these alleles *pm25*, was selected for further analysis. In *n166 pm25* double mutants, both egg-laying and excretory canal outgrowth is partially restored; in contrast, the uncoordinated phenotype is enhanced. *Pm25* animals are coilers, and preliminary analysis using an *unc54::GFP* reporter reveals that while the phenotype of the sex muscles is essentially wild type, there is some ectopic branching of body muscles in *pm25* mutants. *Pm25* was mapped to LG I and found not to complement *unc-75 (e950)*, a gene implicated in synaptic transmission. *Unc-75* mutants are constitutive egg layers and are hypersensitive to serotonin (Schafer et al., 1996, *Genetics* 143:1219-1230). Loria and Hobert have described sprouting defects in the DVB motorneuron of *unc-75* mutants (2001 IWM Abstract #51). We have observed that the excretory canals of both *pm25* and *unc-75 (e950)* mutants terminate somewhat prematurely, often ending in a swirly "cauliflower" like structure. Currently, we are characterizing the neuronal phenotype of *pm25n166* and *pm25* mutants.

Interestingly, genes involved in neuronal activity have been shown to suppress the axon guidance defects in animals expressing the N-terminal fragment of UNC-6/netrin. (Wang and Wadsworth, 2002, *J. Neurosci.* 22:2274-2282). We now wish to investigate whether other genes involved in synaptic transmission will suppress *unc-53* alleles.

To identify additional molecules involved in the *unc-53* pathway, our lab is using the yeast two hybrid system to isolate potential molecular partners. (See Abstract by Adeleye et al., this meeting)

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109. A Wnt gradient may guide HSN migration.

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The migration of neuronal cell bodies is a critical feature of neural development in all animal phyla. To investigate the molecular mechanisms of this process, we study the migrations of the hermaphrodite specific neurons (HSNs) of *Caenorhabditis elegans.* The HSNs are born in the tail of the comma-stage embryo and migrate anteriorly to the midbody. Later, during postembryonic development, the HSNs extend axons and synapse onto vulval muscles to control egg laying.

Migrations of both the HSNs (Desai *et al.* 1988) and the Q neuroblasts (Harris *et al.* 1996) require the function of the *egl-20* gene, which encodes a Wnt homolog. Wnts are secreted glycoproteins with diverse functions in many developmental processes. Desai *et al.* (1988) demonstrated that loss of *egl-20* leads to posterior displacement (undermigration) of the HSNs, and we have observed that EGL-20 overexpression leads to anterior displacement (overmigration). Endogenous EGL-20 is expressed in the embryonic tail during the stage when the HSNs are migrating, raising the possibility that an EGL-20 gradient guides HSN migration. In contrast, EGL-20 plays a permissive role in Q cell migration by controlling *mab-5* expression (Maloof *et al.* 1999). Supporting the possibility that an EGL-20 gradient is essential for HSN migration, global overexpression of EGL-20 from a heat-shock promoter leads to both overmigration and undermigration of the HSNs.

We are pursuing experiments to further address this hypothesis. We will misexpress *egl-20* from region-specific promoters. Undermigration in response to *egl-20* expression along the HSN migration path would be consistent with an EGL-20 gradient guiding the HSNs, while overmigration would suggest a permissive function for EGL-20.

We are also investigating candidate genes that act downstream of EGL-20. Diverse Wnt signaling cascades work through homologs of *frizzled* (*fz*, a transmembrane receptor) and *dishevelled* (*dsh*, a cytosolic phosphoprotein). We aim to determine which of the four *fz* homologs (*mig-1*, *mom-5*, *lin-17*, and *cfz-2*/F27E11.3) and three *dsh* homologs (*dsh-1*, *dsh-2*, and *mig-5*) function to transduce the EGL-20 signal, and whether their function is required in the HSNs. We have observed that a high copy transgene array of *dsh-1* results in HSN migration defects and a GFP reporter driven by the *dsh-1* promoter is expressed in HSNs, consistent with *dsh-1* acting downstream of *egl-20*. We are further examining the role of *dsh-1* and of other candidate genes as potential effectors of EGL-20-mediated HSN migration.

110. Identification of proteins interacting with MIG-13 in Q cell migration

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We are studying cell migration in the context of anteroposterior migrations of the Q neuroblasts and their descendants. QR and QL are born in right-left symmetrical positions in the animal. QR and its descendants migrate towards the anterior of the body, while QL and its descendants migrate towards the posterior. Proper positioning of QR and its descendants in the anterior requires *mig-13*[1]. Interestingly, *mig-13* functions non cell-autonomously to specify the stopping point of migrating cells but is not associated with any specific landmark[1]. *mig-13* encodes a novel transmembrane protein[1]. We currently know very little about the mechanism by which MIG-13 functions. In order to learn more about how migrating cells determine a final stopping point, we are performing biochemical experiments to isolate proteins that bind to MIG-13. Immunoprecipitations were performed using extracts from worms expressing a rescuing GFP-MIG-13 fusion, or as a control, from worms expressing GFP alone. A commercial anti-GFP antibody was used to isolate the fusion protein (or GFP alone in the control) and any associated proteins. Immunoprecipitated samples were analyzed by mass spectrometry to identify proteins present in each sample. We will present preliminary data from this mass spectrometry analysis.

Identification of proteins interacting with MIG-13 will enable us to perform further biochemical experiments to characterize these interactions; in addition, we will be able to test the functional significance of these protein-protein interactions using RNAi or other genetic approaches.

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111. Mechanisms of odor discrimination

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We are using the chemotactic response to volatile odors by *C. elegans* to ask how animals discriminate between multiple inputs to produce the appropriate behavior. The AWC amphid neurons of *C. elegans* sense the attractive odors benzaldehyde (bz), butanone (bu), isoamyl alcohol (iaa), pentanedione (pd), and trimethylthiazole (tmt). The AWC pair alone can discriminate between bu, pd, tmt, and bz/ia - that is, in a uniform background of one odor, animals can still chemotax towards a point source of a different odor. The mechanisms for odor discrimination are not well understood.

Wes and Bargmann (2001) isolated a mutant, *nsy-1*, that was defective in discriminating a point source of bz from a background of bu. They also found that *nsy-1* animals failed to sense pd. *nsy-1* acts during development to direct alternative cell fates of the left and right AWC neurons. Laser ablation studies in wild-type animals showed that ablating one of the two AWC neurons can phenocopy the *nsy-1* defects in chemotaxis and discrimination. Thus the loss of developmental asymmetry in AWC neurons explains the odor discrimination defect in *nsy-1* mutants.

nsy-1 also fails to discriminate where animals are asked to find a point source of bu in a background of bz. It is not known if this defect is also due to the loss of neuronal diversity. I plan to do laser ablation studies to determine the requirement for neuronal diversity, and to use heat-shock expression of *nsy-1* to determine when *nsy-1* is required for proper discrimination.

nsy-1 mutants are still able to discriminate between some odor pairs sensed by the AWC neurons. This observation suggests that there are other *nsy-1*-independent mechanisms of discrimination. I plan to screen for discrimination mutants to identify genes that are required for these mechanisms.

Reference

Wes and Bargmann. (2001) *C. elegans* odour discrimination requires asymmetric diversity in olfactory neurons. *Nature*. 410:698.

112. Imaging neuronal activity in AWC chemosensory neurons

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Particular odorants reliably elicit chemotactic behaviors from C. elegans, but the response can depend on odorant concentration, odorant type and the initial state of the animal. The detection of several volatile attractive odorants is mediated by the AWC chemosensory neuron pair; analyses of animals mutant in their ability to recognize these odorants have revealed many gene products involved in AWC olfactory signaling. However, it has been difficult to study the effect of olfactory stimulation on the activity of chemosensory neurons themselves. Recently, the Schafer lab has successfully measured neuronal activity by using the genetically encodable calcium indicator cameleon. Our goal is to optimize calcium imaging of AWC chemosensory neurons in live animals presented with attractive volatile odorants, including benzaldehyde, butanone and isoamyl alcohol. We will probe the variation of calcium transients that can be elicited by odorant type, concentration, and stimulations at different durations and time intervals. We will evaluate how molecular components of olfactory transduction pathways influence calcium signaling and behavior by measuring calcium transients in chemotaxis mutants.

113. Modulation of Olfactory Adaptation in *C. Elegans*

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In a world filled with numerous stimuli, organisms must learn to ignore (adapt to) persistent stimuli in order to recognize a novel stimulus that could indicate a new food source or oncoming danger. *C. elegans* is able to sense and adapt to a wide variety of odors in its environment. Olfactory adaptation in *C. elegans* is not only odor-specific but also modulated by experience. When a starved animal is exposed to an odor for a prolonged period, it adapts to that odor much more strongly than an unstarved animal (termed hyperadaptation) (Colbert and Bargmann, 1995). Our question is, how does the stress of starvation effect hyperadaptation?

Together, the bilaterally symmetric AWC neurons in *C.elegans* sense at least five odorants: benzaldehyde, butanone, isoamyl alcohol, 2,3-pentanedione, and 2,4,5-trimethylthiazole. In our model for olfactory signal transduction in AWC, odor binds a seven transmembrane G-protein coupled receptor, stimulating a G-alpha which leads to the opening of the cGMP-gated channel comprised of the subunits TAX-2 and TAX-4. The guanylyl cyclases ODR-1 and DAF-11 are proposed to provide the cGMP needed to open TAX-2/4. New evidence shows that cGMP also stimulates the cGMP-dependent protein kinase (PKG), EGL-4, which regulates adaptation. EGL-4 acts cell autonomously within AWC to promote adaptation. Using an inducible promoter, EGL-4 was shown to be required only shortly before adaptation and not throughout development (Eastman et al., WCWM 2002).

Knowing that EGL-4 is required for adaptation, we looked at the effect of starvation on adaptation in *egl-4* mutants. Surprisingly, when *egl-4(n479*) (null) mutants were starved, they adapted to the levels of unstarved wild-type worms. Mutants that carried the kinase-defective (KD) allele of *egl-4 (n478)* however, failed to adapt when starved. Loss of the TGF-beta dauer formation pathway suppresses the *egl-4(n478)* mutant animal's adaptation defects, such that *egl-4(n478);daf-3(e1376)* adapts. These results suggest that EGL-4 is not the only component involved in regulating adaptation, and there certainly appears to be an alternate pathway involved. Of particular interest is the fact that the *egl-4* null mutant can adapt when starved, but the KD mutant cannot, suggesting that the null mutant may trigger the alternate pathway. The fact that (KD) *egl-4(n478)* mutant worms failed to adapt when starved may be interpreted to indicate that (KD)EGL-4 is actively blocking starvation-mediated adaptation. We are using cell-specific promoters to determine which cells require EGL-4(KD) expression to inhibit starvation-enhanced adaptation. In this way, we may identify the neurons involved in the starvation-mediated enhancement of adaptation. In future experiments, we hope to clarify the functional differences between the *egl-4* alleles and to perform a screen to identify members of the EGL-4-independent alternate adaptation pathway in AWC.

Colbert, H.A. and Bargmann, C.I. (1995). Neuron *14,* 803-812.

114. Visualizing OSM-9::GFP in AWC during odor exposure

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In order for *C.elegans*to flourish in the complex environment of soil, it must exhibit a dynamic and broad-ranged ability to sense attractive and repugnant odors. Members of the sensory ganglion the bilaterally symmetric AWCs, AWAs and AWBs, ADLs, ASHs facilitate the worm's ability to sense attractants and repellents, respectively. Within the pair of AWC neurons, chemoattractants such as benzaldehyde, and isoanylalcohol stimulate the olfactory signaling pathway and allow the worm to move toward the alluring odors. The basic framework of olfactory signaling within AWC has been sketched: a G-protein coupled receptor initiates a cyclic GMP-based signaling cascade which results in the opening of a cGMP gated cation channel, comprised of TAX-2 and TAX-4 subunits. Channel opening would depolarize the neuron and send a signal via interneurons to the head motor neurons which direct the worm towards the odor's source. Extensive odor-saturation of the olfactory neuron leads to its long-term adaptation.

The mechanisms that facilitate adaptation of the AWC response remain a mystery, however, one gene, *osm-9,* is known to be required (Colbert and Bargmann, 1995). OSM-9 is a TRP-like (TRPL) channel (Colbert et al., 1997) required for adaptation of AWC. Recent reports of a decrease in the concentration of TRPL within the rhabdomeres of fly retinal cells in response to light (Bahner et al., 2002) prompted us to examine the possibility that the concentration of OSM-9 within the AWC sensory cilia is altered in response to odor. We will determine whether the fluorescence pattern of a GFP-tagged form of OSM-9 is altered subsequent to odor adaptation. Observed changes in the sub-cellular localization or intensity of OSM-9::GFP, will be examined and presented.

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115. Helping Chromosomes Pack Up and Split: The Role of Condensin in Mitotic and Meiotic Chromosome Segregation

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As a cell divides, a complete copy of the genome must be faithfully segregated to each new daughter cell. Dramatic changes in chromosome structure are necessary to ensure accurate chromosome separation. We are studying how a conserved protein complex called condensin promotes chromosome condensation and segregation. Condensin contains proteins of the SMC (structural maintenance of chromosomes) family, conserved chromosomal ATPases required for various aspects of chromosome dynamics. C. elegans contains two condensin-like complexes, one with a conserved function in chromosome segregation, and another without mitotic function that regulates X-linked gene expression during dosage compensation. Both complexes share the protein MIX-1, which performs its dual roles in combination with different proteins partners. The mitotic C. elegans condensin, like condensin in other organisms, contains a pair of conserved SMC proteins (MIX-1 and SMC-4), positively supercoils DNA in vitro, and promotes mitotic chromosome structure and segregation in vivo. Time-lapse analysis of MIX-1 or SMC-4 depleted embryos carrying histone-GFP revealed defects in chromosome condensation at prometaphase, yet a surprising degree of compaction by metaphase. SMC-4 or MIX-1 RNAi caused failed sister chromatid separation in both mitosis and meiosis II, although defects in homolog separation at meiosis I were not observed. We are currently investigating the meiotic localization and function of this complex in more detail in both sperm and oocytes. Interestingly, SMC-4 and MIX-1 localized to the outer faces of condensed mitotic chromosomes in a pattern that resembled that of centromere proteins, and required the AIR-2/Aurora-B kinase for this localization. RNAi of SMC-4 or MIX-1 did not prevent the mitotic association of centromere proteins such as HCP-3/CENP-A, but did disrupt their restricted localization towards the spindle poles. Thus, condensin may help build and position the centromere as it organizes mitotic chromosome structure. We are performing mass spectrometry to identify proteins immunoprecipitated with SMC-4 or centromere component antibodies, to determine whether condensin physically interacts with centromere proteins and to identify other members of the SMC-4/MIX-1 complex.

116. Cohesin proteins regulate the reorganization of the holocentric chromosome during mitosis in the C. elegans embryo

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During mitosis, chromosomes orient such that sister kinetochores face opposite poles of the mitotic spindle. This bipolar orientation is aided by the positioning of centromere proteins to opposite surfaces of the mitotic chromosome. In the nematode Caenorhabditis elegans, centromere proteins required for kinetochore assembly undergo a reorganization to face in opposite directions. This process of sister centromere resolution is dependent on the CENP-C-like protein, HCP-4. Because chromosome proteins involved in biorientation may be involved in sister centromere resolution, we examined the function of Cohesin-Homolog-(COH)-2, COH-4, and CeSMC1, C. elegans orthologs of the yeast cohesin proteins SCC1, SCC3, and SMC1 respectively. Loss of cohesin protein function did not alter sister chromatid cohesion but did suppress a block in centromere resolution resulting from a loss of HCP-4. This result indicates that cohesins negatively regulate sister centromere resolution and that one function of the HCP-4 protein is to relieve this inhibition. Consistent with cohesin proteins negatively regulating sister centromere resolution, cohesin protein expression decreased during mitosis. These results suggest that cohesins removal from different chromosomal domains, including the centromere, in an ordered pathway plays a critical role in organizing the mitotic chromosome by regulating the resolution of sister centromeres and that this process is regulated by specific proteins such as HCP-4. To further investigate this model we have conducted both a functional genomic screen and genetic screen to identify proteins involved in regulating sister centromere resolution and biorientation of mitotic chromosomes.

117. Proteomic approach identifies a single-stranded telomere binding protein in the nematode Caenorhabditis elegans

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The integrity and proper functions of telomeres rely upon associations between the telomeric repeats and specific binding proteins. Several proteins that are essential for chromosome capping and telomerase regulation interact with the single-stranded 3' terminal extensions. However, telomere binding proteins have not been identified in C. elegans. Our previous study showed that the C. elegans nuclear extract contained at least one protein that specifically binds single-stranded telomere DNA sequences. In this study, we aimed at identifying single stranded telomere binding proteins in C. elegans. In order to identify the telomere binding proteins, we performed affinity purification experiments follwed by MALDI-TOF analysis. We selected from the MALDI-TOF data several candidate proteins, many of which contained RNA binding motifs. While most candidate proteins were able to bind telomeric sequences, their binding was not specific. One of the candidate proteins, a C. elegans hnRNP protein, was able to specifically bind the nematode telomeric sequecnes. Although the major function of hnRNP may be in the splicing events, hnRNPs are also involved in telorme functions. There were reports that human hnRNP proteins directly bind telomeric sequences. We confirmed the specific binding of the hnRNP protein identified in our experiment to telmeres by competition with clod telomere competitors. We found that the minimal number of the telomere repeats for binding was 2 repeats of TTAGGC. Single nucleotide substitution at the 2nd T nucleotide in (TTAGGC)2 almost abolished the binding capacity, indicating that this nucleotide is involved in direct contact with the hnRNP protein. Interestingly, while the binidng activity of hnRNP was not strong when binding two copies of the telomeric sequences, it showed much stronger binding activity when binding to telomre sequences longer than 3 copies of TTAGGC. Because the telomeres of the nematode chromosomes contain the 3¡¯ single stranded overhang longer than three copies of TTAGGC, we propose that hnRNP must bind strongly to the teomeres in vivo. Single substitution at the 2nd T nucleotide of the first telomere repeat in the oligomer of (TTAGGC)3 essentially abolished binding capacity, supporting the idea that the dimer of hnRNP binds two antiparallel strands of single stranded telomeres. hnRNP was expressed in the nuclei of all cells from embryogenesis throughout the adulthood. hnRNP was distributed in puncated forms, as a large number of very small spots, or as a small number of large spots. The latter may be telomeres, and the former may be spliceosomes. We are now examining the physilogical roles of hnRNP by RNAi and overexpression. We will present updated results.

118. Posterior body contraction - novel fast signaling

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The C. elegans defecation cycle is characterized by the sequential contraction of the posterior body wall muscles, anterior body wall muscles and enteric muscles. This motor program occurs every fifty seconds and requires the coordinated activity of three distinct tissues - the intestine, the muscles and neurons. Laser ablation of candidate motor neurons and analysis of synaptic transmission mutants suggest that the periodicity and execution of the first step of the defecation motor program, the posterior body contraction, is controlled by a non neuronal cell type. In vivo calcium imaging studies revealed a correlation of intestinal calcium spiking and the initiation of the posterior body contraction (Dal Santo et al., 1999). Because alteration of intestinal inositol 1,4,5-trisphosphate receptor (IP3R) function by mutation or overexpression can stop, slow, or speed the clock's rhythm, the IP3R intracellular calcium release channel is likely to directly regulate the intestinal clock through cyclic releases of calcium (Dal Santo et al., 1999).

To determine how cyclic calcium spiking within the intestine leads to posterior body wall contraction, mutants that specifically eliminate the posterior body wall contraction (pbos) were identified. Two of these mutants, pbo-4 and pbo-5 have been cloned and characterized. pbo-4 encodes a protein homologous to Na+/H+ exchangers (NHEs) and is expressed only in the posterior intestine. Activation of mammalian NHEs can be regulated by calcium. The identification of a potential calmodulin binding site as well as consensus calmodulin dependent kinase II phosphorylation sites in pbo-4 further suggests calcium levels may modulate PBO-4's activity. pbo-5 encodes a novel, ligand-gated ion channel that is distantly related to acetylcholine receptors. PBO-5 is expressed in the posterior body wall muscles, adjacent to the PBO-4 expressing cells. One possible model is that PBO-4 releases protons from the intestine in response to calcium spikes. PBO-5 could recognize this signal and depolarize the muscles to initiate the posterior body contraction. We are testing this model by characterizing the electrophysiological properties of PBO-5 in Xenopus oocytes. In addition, we recently identified a closely related acetylcholine receptor, F11C7.1, that is also expressed in the posterior body wall muscles. Therefore, the electrophysiological properties of an F11C7.1 and PBO-5 oligomer are being analyzed. Finally, two additional pbo alleles are being mapped. One of these alleles, ox10, maps to the right arm of V, between unc-51 and rol-9.

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119. Wormatlas: A web-based behavioral and structural atlas of C. elegans

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We have recently launched the prototype of Wormatlas (www.wormatlas.org). This atlas is designed to serve the scientific community with the main goal of bringing all the anatomical information pertinent to C. elegans within one readily accessible and easy to use web site. By creating extensive links to the WormBase as well as the C. elegans WWW server, we are aiming to provide users with seamless links between these databases. We hope to create the most comprehensive and complete online anatomy atlas for any genetic model organism.

Wormatlas is designed to have two main sections, Index and Guides, with multiple chapters within each section. The Index section will contain the Handbook, Slidable Worm, Literature Archive, Cell Identifications, Neuron Data, Glossary, and Methods. The main goal of the Handbook is to provide a relatively simplified, image-supported and curated information about the general and specific anatomy of C. elegans. The images included in the Handbook will be annotated scanning and transmission electron (TEM) micrographs, computer-drawn images as well as DIC and fluorescent micrographs. The Slidable Worm is designed to provide 600-1200 annotated and nonannotated versions of TEM cross-sections of the animal available for viewing by the users with the help of a newly designed JAVA applet interface. The images will come from the original images from the MRC/LMB archive, from our Caltech/AECOM archives, and possibly others. The Literature Archive will provide on-line copies of landmark articles and treatises about the anatomy of the nematode. These HTML format articles include multiple web links to other sites in Wormatlas and WormBase to strengthen their interactivity for the structures mentioned. The Glossary aims to provide a comprehensive list of all nomenclature used to describe any cell structure in the nematode. Cell Identification and Neuron Data are planned to provide enough detail on features of single cells, esp. neurons, to aid researchers in recognizing and studying individual cells by their 3D shapes and positions, comparing TEM, DIC and GFP information, and by providing links to curated data on their gene expression patterns. Finally, Anatomical Methods will provide an up to date summary of the different modalities that are currently used in cell identification and tissue pathology studies. This website is designed to accommodate the vast amount of structural, behavioral and gene expression data that has appeared since publication of "The Mind of a Worm" in a dynamic and easily updatable medium. This curated information can be viewed in individual neuron pages as well as neuron data appendices. In the future, we want to develop interactive user interfaces to visualize behavioral circuitries and perhaps neurophysiology information as they become available.

The second section of Wormatlas provides guides for optimal usage of the information included in the first section. It offers general information relevant to C. elegans as well as specific usage directions for Wormatlas. For instance, we have created a color coding system in which the main structural elements of the animal have each been assigned a specific color from the web-safe color palette. The uniform color code will help viewers to perceive anatomical relationships and tissue symmetries even without any symbolic annotation.

Our close collaboration with WormBase researchers has helped to create a common display language, in data sharing, and in development of a shared Gene Ontology vocabulary. Wormatlas is being created to serve the scientific community and as such, we greatly appreciate your input, data sharing, suggestions and criticisms that help improve the web site. We are actively seeking peer review as each new chapter is readied for release.

120. The Incyte Genomics BioKnowledge Library™: A comprehensive, integrated resource for the analysis of functional conservation between model organismal and mammalian proteins

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The BioKnowledge Library ™ (http://www.incyte.com) is a collection of literature-based databases that integrates genomic and proteomic data to provide information about biological function. The complete database volume set, now available through subscription, includes WormPD ™, YPD ™ (*Saccharomyces cerevisiae*), PombePD ™ (*Schizosaccharomyces pombe*), and for the first time to academic users, MycoPathPD ™, HumanPSD ™, and GPCR-PD ™. MycoPathPD is an annotated database of 17 human fungal pathogens. HumanPSD is a survey database of over 29,000 human, mouse, and rat proteins. Also a mammalian protein database, GPCR-PD provides literature-based annotations for G protein-coupled receptors, their ligands, and downstream signaling proteins. All the databases provide Gene Ontology (GO) terms, are fully interconnected and searchable, and are accessible in a Web-based format.

In addition to the inclusion of the mammalian and fungal pathogen databases, the subscription-based format of WormPD provides several enhancements over the original version. These include the addition of almost 34,000 GO terms, a controlled vocabulary list that describes biological function, molecular function, and cellular component data, and modifications to transcription profile information that allow searching for similar expression patterns. Furthermore, over 800 uncloned genetic loci pages are now included in the database. Finally, since the mammalian databases are accessible to academic users, predictions of functional conservation between worm and mammalian proteins are facilitated by the integration of information, which includes disease involvement and mouse mutant phenotype data. To highlight the increased utility of the BioKnowledge Library to academic users, comparisons of worm and mammalian members of the olfactory receptor and DEG/ENaC protein families will be provided.

121. DPR-1, a membrane-associated nuclear hormone receptor, prevents dauer recovery
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DPR-1 (dauer pheromone responsive-1, previously END-2) is expressed throughout the life of the worm in the endoderm lineage. DPR-1 is a nuclear hormone receptor (NHR) and as such is expected to reside primarily in the cytoplasm or nucleus. While at different stages in the development of the worm DPR-1 is detected in the cytoplasm or nucleus, it moves to the membrane in response to crowded growth conditions. This membrane localization is extremely unusual for an NHR. The redistribution of DPR-1 to the membrane is detected both by immunocytochemistry and in western blots of worm extracts. We found that dauer pheromone also causes DPR-1 to become membrane-localized. The membrane targeting of DPR-1 may be a direct response to dauer pheromone, as the change in localization can occur very early in larval development, at the beginning of the L1 stage. Dauer pheromone is a small lipophilic molecule with characteristics of a bile acid, suggesting that it might act through an NHR. We are currently investigating whether DPR-1 might be such a dauer pheromone receptor.

Observations of worms overexpressing DPR-1 implicate a role for it in dauer regulation. We observed dauer larvae on sparsely populated plates of transgenic worms carrying a myc-tagged version of DPR-1. While these worms appear to enter the dauer state normally, we found that they are slow to recover from dauer and some never recover. The effect of myc-tagged DPR-1 may be due to the overexpression of DPR-1 or to a change in the protein caused by the addition of the myc tag. These results suggest that DPR-1 is a previously unidentified regulator of dauer development.

We found that GFP-tagged DPR-1 is not expressed in dauer larvae. However, western blot analysis of endogenous DPR-1 suggests that it is present, but in a different (smaller) form than in non-dauers. This smaller form ("DPR-d") is membrane-associated, and behaves like an integral membrane protein, while the membrane-bound form of DPR-1 in normal developing larvae behaves like a peripheral membrane protein. Using further biochemical analyses, we are trying to determine how the different forms of DPR-1 interact with the plasma membrane and are examining the structural basis for the differences in size and membrane association.

122. *bug-3*, a new regulator of asymmetric cell division in *C. elegans*

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We are interested in studying the mechanisms regulating asymmetric cell division in the *C. elegans* nervous system. Previously, our lab identified *ham-1* as a regulator of the HSN/PHB neuroblast's asymmetric division (1). In wild-type animals, each of the two bilaterally symmetric HSN/PHB neuroblasts divide to produce an anterior daughter that dies and a posterior daughter that we call the HSN/PHB precursor. The precursor then divides to produce the HSN and PHB neurons. In *ham-1* mutants, the cell normally fated to die is often transformed into an HSN/PHB precursor resulting in extra HSNs and PHBs (~35% of sides in the *gm279* null allele). *ham-1* mutants also have missing HSNs and PHBs (~20% of sides in *gm279*). HAM-1 encodes a novel cytoplasmic protein that is asymmetrically distributed to the posterior of the HSN/PHB neuroblast and segregated into the HSN/PHB precursor, the daughter cell that normally lives. These observations have led us to propose that HAM-1 acts as a tether for determinants that control precursor cell fate. In *ham-1* mutants, we propose that these determinants are segregated to both daughter cells, resulting in either transformation of the anterior daughter into a precursor (resulting in extra HSNs and PHBs) or failure of the posterior daughter to correctly differentiate (resulting in loss of HSNs and PHBs)

Subsequent screens for mutants with the Ham-1 phenotype identified three recessive maternal-effect mutations in the *bug-3* (*b*ack-end *ugly*) gene. *bug-3* mutants have a low penetrance of extra HSNs and PHBs (~8% of sides in *gm301* and ~2% of sides in *gm280*) but do not have missing HSNs or PHBs. The penetrance of these phenotypes is similar to what is observed in animals carrying a strong loss-of-function mutation in the programmed cell death killer genes *ced-3* or *ced-4* (~10% extra HSNs or PHBs /side) . To determine whether the extra HSNs and PHBs observed in *bug-3* mutants result merely from defective programmed cell death, we determined the number of HSNs and PHBs in *bug-3 ced-3* double mutants. We found that the penetrance of extra HSNs and PHBs is greatly enhanced in *bug-3 ced-3* mutants (~90-95% of sides have extra HSNs and PHBs). A similar genetic interaction is observed between mutations in *ham-1* and *ced-3*. These results suggest that *bug-3* mutations do not merely disrupt programmed cell death but also affect cell fate. We hypothesize that mutations in *bug-3*, like mutations in *ham-1,* disrupt the asymmetric division of the HSN/PHB neuroblast resulting in transformation of its anterior daughter into an HSN/PHB precursor.

To determine whether *ham-1* and *bug-3* act in the same pathway, we constructed *bug-3 ham-1* double mutants. We found that mutations in *bug-3* are epistatic to mutations in *ham-1*. Specifically, the double mutants never have missing HSNs or PHBs and the penetrance of extra HSNs and PHBs is very low, similar to the penetrance observed in *bug-3* single mutants. Based on these observations, we hypothesize that in *bug-3* mutants, *ham-1* may no longer be required to regulate asymmetric cell division in this lineage. Alternatively, *ham-1* could negatively regulate *bug-3*.

I have mapped *bug-3* to the left arm of LG IV. Current experiments are underway to clone *bug-3* and to determine the origin of the extra HSNs and PHBs in *bug-3* mutants by lineage analysis.

1. Guenther and Garriga (1996). Development **122**: 3509-18.

123. Genetic analysis and molecular cloning of *nsy-2*

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The*C. elegans* AWC olfactory neurons are a bilaterally symmetric pair of similar neurons. However, the promoter of the candidate odorant receptor *str-2* drives asymmetric GFP expression in only one of the two AWC neurons. In wild-type animals, *str-2* is randomly expressed in either the left or the right AWC neuron, but never in both. Axon contact and cell communication between the AWC neurons are required to establish the asymmetric pattern of *str-2* expression. AWC cell communication acts through the voltage-gated calcium channel (*unc-2, unc-36)* and CaMKII *(unc-43),* which in turn activate a JNK/p38 MAP kinase pathway. We are interested in the signaling pathways that define the two distinct AWC fates.

A screen for mutant worms that express *str-2*::GFP in both AWC cells (2 AWC^{ON}) yielded three *n*euronal *sy*mmetry (*nsy*) genes that map to novel loci. *nsy-2* is defined by a single allele, *ky388.* Genetic epistasis analysis suggests that *nsy-2* acts downstream of the CaMKII *unc-43* and upstream of the JNK/p38 MAPKKK *nsy-1*.

The *nsy-2*(*ky388*) allele is temperature-sensitive for its 2 AWC^{ON} phenotype. The proportion of *nsy-2* (*ky388*) animals expressing *str-2*::GFP in both AWC cells ranges from 25% at 15^0C to 81% at 25^0C. To determine the time of *nsy-2* gene action, animals were shifted between 20^0C and 15^0C at different developmental stages. The results suggest that the onset of the requirement for *nsy-2* activity in *str-2* asymmetry occurs in late embryogenesis, a few hours after the initiation of axon outgrowth. This result is consistent with a role for *nsy-2* in the initial communication between the AWC neurons.

In addition to the 2 AWC^{ON} phenotype, *nsy-2* (*ky388*) animals also display late-onset sterility phenotypes that appear after a few generations. Since there is only one allele, we are not certain whether this represents a second phenotype of *nsy-2* or a closely linked mutation.

nsy-2 was mapped to chromosome III by using Tc1 transposable elements and deficiency strains. I now have rescue with one cosmid. Sequencing of candidate genes within the cosmid is underway.

124. Specification of Individual Olfactory Neuron Function

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Specific transcription factor cascades determine the sensory response profiles of individual olfactory neuron types. The orphan nuclear receptor ODR-7 is required for all known sensory functions of the AWA olfactory neurons. 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*. ODR-7 is also required to maintain its expression by autoregulation. In addition, the candidate olfactory receptor *str-2*, which is normally expressed stochastically in one AWC olfactory neuron, is expressed in one or both AWA neurons in *odr-7* mutants. To understand how ODR-7 functions, we have carried out an analysis of the ODR-7 protein and have defined putative ODR-7 regulated DNA sequences. We have defined an element in the *odr-10 and osm-9* promoters that is required for proper AWA-specific expression. This element is also found in the promoters of *odr-7* and *gpa-5*. We are also using a yeast-one hybrid library (1) to screen for response elements.

ODR-7 contains a DNA binding domain (DBD) that is similar to that of other nuclear hormone receptors, but does not contain a well-defined ligand binding domain (LBD). To further investigate ODR-7 function, we have performed a mutational analysis of the putative DBD of ODR-7 and deletion analysis of the LBD of ODR-7. The function of ODR-7 is not affected by many mutations in conserved residues of the DBD. However, we have been able to separate the ability of ODR-7 to regulate the expression of *odr-10* and genes required for pyrazine chemotaxis from its ability to repress *str-2* and to autoregulation. In addition, the LBD contains a region required for the expression of *odr-10*, but not for autoregulation, *str-2* repression, or pyrazine chemotaxis.

Expression of ODR-7 in either the AWB or AWC neurons is not sufficient for expression of *AWA*-specific genes. Surprisingly, ODR-7 expression in AWC represses *str-2* expression and these animals are also defective in chemotaxis to the odorant butanone, but not to other AWC sensed chemoattractants.*nsy-1* encodes a MAPKKK that is required for the asymmetric expression of *str-2* in a single AWC neuron (2). In *nsy-1* mutants, str-2 is expressed in both AWC neurons. ODR-7 is able to repress *str-2* in both AWC neurons in a *nsy-1* background, indicating that it acts either downstream or parallel to *nsy-1* in AWC. Interestingly, expression of the ODR-7 DBD results in the expression of *str-2* in both the AWC neurons.

We are interested in identifying additional genes required for the sensory functions of individual olfactory neurons such as AWA and AWB. We are investigating several different methods by which large number of genes expressed in single olfactory neurons can be identified. Previously, in collaboration with Miriam Goodman, we amplified RNA isolated from single AWA and AWC olfactory neurons. However, we found this method to be laborious and impractical for large scale screening. We are now taking advantage of the recent cell culture system developed at Vanderbilt (3) to isolate RNA from single identified olfactory neurons. Cell specific markers for the AWA and AWB neurons are being tested to determine whether they can be used to isolated these neurons by FACS from dissociated embryos in culture. We are also taking advantage of the nematode-specific trans-splicing system to identify neuron-specific transcripts.

(1) Van Gilst and Yamamoto. IWM 2001 abstract 191
(2) Sagasti *et al.* (2001) Cell 105: 221
(3) Christensen *et al.* (2002) Neuron 33:503

125. Sensory Neuron Cell Fate Determination

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We are interested in understanding signaling pathways that control cell fate in the nervous system. *C. elegans* senses volatile odors through three bilaterally symmetric pairs of neurons, AWA, AWB, and AWC. Some odorant receptors are expressed symmetrically in both neurons of a pair. However, the *str-2* receptor is expressed asymmetrically in only one of the two AWC neurons. Expression of *str-2* is stochastic: fifty percent of animals express *str-2* on the left neuron, and fifty percent express it on the right. Laser ablation experiments and analysis of axon guidance mutants indicate that wild-type axon guidance and probably direct contact between the two AWC neurons are required for normal *str-2* expression (Troemel et al., 1999).

To define the pathway responsible for asymmetric expression of *str-2*, screens have been performed for neuronal symmetry *(nsy)* mutants. A screen for mutants in which both AWC neurons express GFP (two AWC^{ON} cells) under the control of the *str-2* promoter (*str-2::GFP)* revealed that a calcium-activated MAP kinase cascade represses *str-2* expression in AWC (Troemel et al., 1999). Genetic epistasis indicates that a two-AWC^{ON} mutant, *nsy-3,* is upstream of this pathway. *nsy-3* mutations are semi-dominant alleles of *slo-1*, a calcium-activated potassium channel (Wang et al., 2001). SLO-1 is located presynaptically in many types of neurons and can colocalize with voltage-gated calcium channels, making it an interesting candidate for direct participation in this pathway. Further characterization is underway.

To identify additional genes in the pathway, a second screen was performed for mutants in which neither AWC neuron expresses *str-2::GFP* (two AWC^{OFF} cells). These mutants fell into eight complementation groups. The mutants affect different aspects of AWC development. One gene appears to be required for all AWC cell fates. At least three genes, including the guanylyl cyclases *daf-11* and *odr-1*, are required to maintain str-2::GFP expression in AWC^{ON}, but are not required during the interaction that initiates the asymmetric AWC cell fates. Two genes appear to act upstream of the calcium-activated MAP kinase pathway based on genetic epistasis experiments, and are candidates to be involved in cell-cell communication. Both of these genes are currently being further mapped and characterized.

Troemel, E., Sagasti, A., and Bargmann, C. (1999). Lateral Signaling Mediated by Axon Contact and Calcium Entry Regulates Asymmetric Odorant Receptor Expression in *C. elegans*. Cell *99,* 387-398.

Wang, Z., Saifee, O., Nonet, M., and Salkoff, L. (2001). SLO-1 Potassium Channels Control Quantal Content of Neurotransmitter Release at the *C. elegans* Neuromuscular Junction. Neuron *32*, 867-881.

126. Dissecting the *C. elegans* ray developmental pathway with DNA microarrays

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Nematode development provides an excellent opportunity to understand how spatial, temporal and sex-specific information is integrated to produce the variety of neuronal subtypes that compose the nervous system. In the *C. elegans* male tail, each of the eighteen sensory rays is composed of three cells (A- and B-type sensory neurons and an associated structural cell), all of which descend clonally from a single ray precursor cell. We have previously found that multiple steps of ray development require the functions of the bHLH transcription factors LIN-32 (the worm *atonal* ortholog) and HLH-2 (E/*daughterless* ortholog). To obtain a more complete view of the mechanisms that generate the three distinct cell types of each ray, we have undertaken a microarray-based approach to identify genes expressed in mature rays.

We sought genes in two classes: (1) terminal differentiation genes that are informative about ray function and are useful as markers of cell fate, and (2) regulatory factors that may have roles in ray development. In collaboration with Stuart Kim, we have performed seven array hybridizations comparing mRNAs from adult males that completely lack rays (*hlh-2; lin-32*) to those that have eighteen wild-type rays as well as ectopic rays along the body (*lin-22*). Based on these data, we chose a number of genes with reproducibly high expression ratios and generated reporters to determine preliminary expression patterns. Of the 37 reporters we constructed, fifteen are expressed in some or all of the rays. Among the remaining non-ray genes, eleven reporters are expressed elsewhere in the nervous system; these may represent targets of *lin-32* regulation in other neuronal lineages. Three additional reporters were expressed outside the nervous system, and the remaining seven showed no detectable expression. We conclude that our microarray-based approach successfully generated a dataset highly enriched for ray-expressed genes.

Among the ray terminal differentiation genes we have identified, eight show strong reporter expression in most or all of the rays as well as a limited number of other cells. These genes include a beta-tubulin isoform expressed in all ray neurons, a TWIK-family potassium channel expressed in all A-type ray neurons, and a G-protein-coupled receptor expressed in most B-type ray neurons. We have also identified four genes encoding novel putative secreted proteins whose expression patterns are identical to those of the *C. elegans*polycystin genes *lov-1* and *pkd-2*, which have been shown by Barr and Sternberg to have important roles in male mating behavior. These four genes, expressed in the four CEMs, the hook neuron HOB and all B-type ray neurons except R6B, may also function in this process.

In addition, we have identified a number of putative transcription factors that may be important for ray development. Reporters for *hlh-10*, encoding an eHAND-like bHLH protein, and *ces-2*, encoding a bZIP-domain protein, are expressed in several ray neurons (and other neurons as well) and may play roles in ray patterning. In addition, a reporter for the Zn-finger gene *egl-46* is expressed in one neuron of each ray, and the LIM-homeodomain gene *lim-7* appears to be expressed during the ray sublineage.

127. Genetic analysis of the C. elegans Pax-6 locus and characterization of its functions in epidermal morphogenesis

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Pax-6 genes encode proteins with paired domains (PD) and homeodomains (HD), and are involved in eye and brain development in many animals. In *C. elegans*, disruption of Pax-6 function results in variable defects in head epidermal morphogenesis, distal tip cell (DTC) migration, fate transformations in lateral epidermis and male tail, and cell fate transformations in male tail rays. The *C. elegans* Pax-6 locus expresses at least two groups of transcripts. One class of transcripts encodes both PD and HD; other transcripts do not encode the PD and arise from an internal promoter (1,2). *Pax-6* mutant alleles can be grouped into three classes. Class I alleles have molecular lesions in PD-encoding exons and cause *vab-3*phenotypes. The mutation *mab-18(bx23)* affects only non-PD encoding transcripts and results in ray fate transformations (2). Class III alleles affect exons common to both *vab-3* and *mab-18* and cause distinctive phenotypes (Chisholm, 1996 West Coast Worm Meeting). Since none of these mutations appear to abolish Pax-6 function completely, we are performing non-complementation screens to obtain a null allele. We are also directly screening for deletions in Pax-6.

Pax-6 genes are haplo-insufficient in mice and humans. In mice, *Pax6* homozygotes are embryonic-lethal with lack of eyes and forebrain structures, heterozygotes are viable with eye and brain defects. To test Pax-6 haplosufficiency in *C. elegans*, we analyzed *vab-3(e648)/+* animals for head morphogenesis and DTC migration phenotypes and found that *C. elegans*Pax-6 is haplosufficient for these functions in Class I alleles. We are in the process of testing Class III alleles.

vab-3::GFP is widely expressed in head epidermal cells and neurons, beginning at the 100-cell stage (1). Further analysis of embryonic head lineages and cell movements in *vab-3* mutants is necessary to identify affected cells, structures and events during head morphogenesis. For this purpose we are using GFP markers of specific cell fates and subcellular structures, and analyzing the behavior of these markers in *vab-3* mutants.

Another focus of our studies is to identify targets of the Pax-6 gene. We are planning microarray experiments to define the Pax-6 transcriptional program in different developmental stages.

1 Chisholm,A.D.,and Horvitz, H.R. (1995) Nature 377, 52-55
2 Zhang,Y., and Emmons, S.W. (1995) Nature 377, 55-59

128. The roles of EFN-4 in embryonic morphogenesis and oogenesis

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Loss of function in the ephrin EFN-4 causes defects in neuroblast migrations during closure of the ventral gastrulation cleft and in subsequent epidermal morphogenesis (Chin-Sang + Chisholm, 2001 Intl Worm Meeting). Synergistic interactions between *efn-4* mutations and mutations in the Eph receptor VAB1 suggest that EFN-4 has functions independent of the Eph receptor. We have investigated the relative contributions of the four ephrin ligands to VAB-1 signaling during morphogenesis. First, if we reduce *vab-1* expression by RNAi in an *efn-1 efn-2 efn-3*triple null mutant background we do not observe enhancement of the triple mutant phenotype (as determined by 4D microscopy). This is consistent with previous data suggesting that most of the VAB-1 signaling in morphogenesis is provided by EFN-1, EFN-2 and EFN-3. Second, we generated quadruple ephrin mutant animals using *efn-1* RNAi in an *efn-2 efn-3 efn-4*triple null mutant. Quadruply mutant embryos laid by these animals display severe defects in gastrulation cleft closure and a fully penetrant embryonic arrest. Thus, *efn-4(RNAi)* enhances the phenotypes of the *efn-1,-2-,3* triple mutant. Taken together, our data suggest that EFN-4 functions independently of EFNs 1, 2 and 3 and the VAB-1 receptor during morphogenesis. In addition to signaling through Eph receptors, ephrins can also transduce signals to cells expressing them, a process known as reverse signaling. An interesting possibility is that EFN-4 may be a constitutively active reverse signaling ephrin.

Our analysis of the quadruple ephrin mutants may have also revealed an intriguing new role for ephrin signaling in oogenesis. *efn-2,3,4* triple mutants injected with *efn-1* dsRNA develop enlarged oocytes that are fertilized to yield eggs up to twice normal length. A functional EFN-4::GFP transgene is expressed in the sheath cells of the somatic gonad. Large oocytes are not seen in other *efn*mutants nor in *vab-1*mutants suggesting that EFN-4 functions redundantly with other ephrins or with VAB-1 in oogenesis, as in morphogenesis. Enlarged oocytes have also been observed in mutants lacking the SHP phosphatase PTP-2 (Gutch et al., 1998, Genes Dev. 12: 571) and the Rho-binding kinase LET-502 (Piekny and Mains, 2002, J. Cell Sci. 115: 2271). We are planning to determine if these genes might function in a common pathway regulating oocyte size control.

VAB-1 also functions to repress oocyte maturation. Maturation of oocytes is in part triggered by binding of major sperm proteins to oocyte-expressed VAB-1 (Miller et al., 2002 East Coast Worm Meeting abstract 50). However, defects in this process do not result in enlarged oocytes. It is thus possible that EFN/VAB-1 signaling may have multiple roles in oogenesis.

129. The temperature sensitive lethal mutation or566 may define a new gene that functions in early morphogenesis

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Mutations in the EPH receptor tyrosine kinase VAB1 and in its ephrin ligands cause incompletely penetrant defects in gastrulation cleft closure and epidermal enclosure. Fully penetrant defects in cleft closure are seen when multiple pathways are blocked (e.g. *vab-1* and *ptp-3* (Harrington et al., 2002, Development 129: 2141). To find genes that may have essential functions in gastrulation cleft closure we have begun to examine conditional lethal mutants for possible defects in this process. Bruce Bowermans lab identified several new temperature-sensitive (ts)-lethal mutations affecting morphogenesis in their large scale ts-lethal screens, and very kindly provided some of them to us.

We have characterized the phenotype of one ts lethal mutant, *or566*, in most detail. Homozygous *or566* mutants are viable at 15°C. When parents are shifted to 25°C, their progeny display fully penetrant embryonic lethality. We examined the development of *or566* embryos at 25° using 4-D Nomarski microscopy. *or566* mutants display severe defects in ventral gastrulation cleft closure. In normal development, the gastrulation cleft usually closes between 230 and 290 minutes after first cleavage (times at 20°C). In *or566* mutants the cleft is very large throughout gastrulation and does not close. Loose cells are often seen within the egg. Epidermal enclosure begins with approximately normal timing but is abortive due to the enlarged cleft. Preliminary temperature shift experiments are consistent with a temperature sensitive period for *or566* function in early embryogenesis.

The defects seen in *or566*mutants resemble those resulting from defects in Eph signaling or in redundant pathways such as PTP-3. We are currently constructing double mutants between *or566* and other Eph signaling mutants to address whether *or566*affects one of these pathways or a parallel pathway.

We mapped *or566* using snip-SNPs to the right arm of chromosome I. Since no genes involved in gastrulation cleft closure are known to map to this region, *or566*may define a new gene. We will report on our progress in mapping and characterizing this locus.

130. Identification of the downstream genes of *lon-1*

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DBL-1 regulates the body length of *C. elegans* dose dependent manner [1)]. DBL-1 negatively regulates the expression level of LON-1 [2)]. Overexpression of DBL-1 causes Lon phenotype like *lon-1* , and overexpression of LON-1 causes Sma phenotype like *dbl-1* . LON-1 has evolutionary conserved protein domain from yeast, plant to human, but its biological function is not clear. To understand the mechanism of LON-1, we try to get the genes downstream of *lon-1. lon-1 overexpressed allele nkIs10 shows Sma phenotype like dbl-1. We screened the suppressor of Sma phenotype by EMS mutagenesis. Over the 50, 000 genomes, we identified 5 Lon and 10 wild type body length suppressores. We are currently mapping them.*

1)K. Morita, et al (1999) Development 2)K. Morita, et al (2002) EMBO. J

131. Identifying genetic loci that control early pharynx morphogenesis

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During *C. elegans* embryogenesis, the pharyngeal primordium develops from a ball of cells into a linear tube connected to the buccal cavity anteriorly and to the midgut posteriorly. We have shown that the connection of the pharynx to the buccal cavity occurs in three stages[1]: Stage I) lengthening of the nascent pharyngeal lumen by reorientation of apicobasal polarity of anterior pharyngeal cells (Reorientation), Stage II) the arcade cells epithelialize, mechanically coupling the buccal cavity to the pharynx and anterior epidermis (Epithelialization), and Stage III) a concomitant movement of the pharynx anteriorly and the epidermis of the mouth posteriorly to bring the pharynx, arcade cells and mouth into close apposition (Contraction). We have termed this process "pharyngeal extension."

I performed a mutagenesis screen to identify mutants that are defective in pharyngeal extension. Fourteen candidate mutant alleles were discovered by screening 3000 haploid genomes for a Pun (pharynx unattached) phenotype at the L1 larval stage. I watched live embryos during embryogenesis and showed that 13 of the 14 alleles are defective in pharyngeal extension, establishing pharyngeal extension as a genetically tractable morphogenic event. The fourteenth allele displayed a "snap-back" phenotype consistent with weak attachment of the pharynx to the buccal cavity. Five alleles are defective in stage I of pharyngeal extension: the anterior pharyngeal cells do not reorient their apical/basal polarity and the arcade cells fail to connect to the pharynx, resulting in a Pun phenotype. Eight alleles are defective in stage II: reorientation of the pharyngeal cells occurs, but the arcade cells fail to connect to the pharyngeal cells, again, leading to a Pun phenotype. I have assigned linkage groups to nine of the thirteen alleles by snip-SNP mapping and have defined at least six loci.

All mutants have a differentiated pharynx, suggesting that the defects are specific to pharyngeal extension and are not due to a broad pharyngeal developmental defect. The pharynx morphology in these mutants differs from the wildtype in that the long isthmus between the procorpus (anterior bulb) and metacorpus (posterior bulb), predominantly pharyngeal muscle m5, does not elongate, possibly suggesting that growth of m5 requires tension.

We are now focused on characterizing one of the stage II mutants, *px47*. Timelapse microscopy shows that the pharyngeal epithelial cells undergo proper reorientation, but they fail to epithelialize to the arcade cells and thus they produce a Pun phenotype. Sixty percent of homozygous *px47* animals arrest as L1 larvae, presumably because they cannot feed. The remaining 40% of homozygous mutants arrest during mid to late embryogenesis, and these animals have defects in other epithelial tissues (in addition to the Pun phenotype). Midgut cells appear rounded, giving the impression that they are not properly epithelialized, ventral and anterior closure are defective in mid-stage embryos, and tail morphogenesis defects are seen in late-stage embryos. *px47* maps to LGIV, and I am currently using snip-SNP and three-factor mapping to refine the map position. The gene mutated in *px47* alleles may provide us with a powerful tool to study both pharyngeal extension and general epithelialization in *C. elegans*.

[1] Portereiko, MF and SE Mango. (2001)
Early morphogenesis of the *Caenorhabditis elegans* pharynx.
Dev Biol. 233(2):482-94

132. PAR proteins and cell polarity after the one-cell stage

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The directed movement of cells depends on the polar localization of proteins to specific membrane regions. The first cell migrations within the *C. elegans* embryo occur during gastrulation, when those cells destined to form internal tissues such as intestine or muscle move from the embryo's surface to its interior. We are interested in learning how these cells are polarized to allow such directed processes to occur.

There is a well-described requirement for PAR proteins in the anterior-posterior polarity of the one-cell *C. elegans* embryo. However, PAR proteins that localize to the anterior cortex of the one-cell embryo (such as PAR-3 and PAR-6) subsequently adopt an outer-inner asymmetry; by the late 4-cell stage, PAR-3 and PAR-6 are absent from cortical regions where cells contact, and are instead restricted to contact-free, or 'outer', cortical regions. By combining embryos in culture, we previously demonstrated that the cortical localization of PAR-3 and PAR-6 is inhibited by cell-cell contact. Together, these observations suggest that cell-cell contact polarizes the cortex, and that the PAR proteins may play a role in this outer-inner polarization.

As an approach to studying the role of PAR-3 and PAR-6 in cell polarity after the one-cell stage, we are expressing GFP-tagged proteins containing PAR-3 or PAR-6 fused to the ZF1 domain of PIE-1. The ZF1 domain can promote the degradation of a GFP reporter in somatic cells of the early embryo, beginning at the two-cell stage [1]. We find that GFP::PAR::ZF1 proteins rescue the anterior-posterior defects of *par* mutant embryos, and are then degraded in somatic cells. We will report our phenotypic characterization of these PAR-depleted embryos.

1. Reese, K.J., et al. (2000). Asymmetric segregation of PIE-1 in *C. elegans* is mediated by two complementary mechanisms that act through separate PIE-1 protein domains. Molecular Cell 6: 445-455.

133. Studies of mutations involved in vulval development in *C. elegans*

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Our laboratory has been screening and analyzing sterile mutants with abnormal vulval morphology in the past few years. Our studies have also included the characterization of some *evl* genes identified previously by I. Greenwald's lab (1). *evl-14* gene is a homologue of yeast *Pds5*(*Prococious Dissociation of Sisters*), which encodes a sister-chromatid cohesion associated protein. Animals homozygous for loss-of-function mutations in *evl-14* exhibit both mitosis and meiosis defects. *evl-14* RNAi both by feeding and injection reduces embryonic viability, increases incidence of males in the F1 generations, and causes protruding vulvae (Pvl) and sterile phenotype in adult hermaphrodites. Further analysis of this gene is in progress.

The *sur-4* gene is identified by a single gain-of-function mutation (*ku23*) that suppresses the Multivulva phenotype of a *let-60*(gf) mutation. Our genetic analysis of this mutation suggests that this gene acts as a negative regulator in the Ras pathway, and that the activity of theRas pathway is sensitive to the dosage of this gene. Hyperactivation of this gene causes an egg-laying defective phenotype. We have mapped this gene to a small region on LGIII. SNP mapping and RNAi experiments are being carried out to clone this gene.

(1)Seydoux, G, Savage, C., and Greenwald I. (1993). Developmental Biology 157, 423-436.

134. Identifying genetic loci that control early pharynx morphogenesis

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During *C. elegans* embryogenesis, the pharyngeal primordium develops from a ball of cells into a linear tube connected to the buccal cavity anteriorly and the midgut posteriorly. We call this process pharyngeal extension. We have shown that the connection of the pharynx to the buccal cavity occurs in three stages[1]. Prior to extension, the pharyngeal cells are shaped like a wedge, with the tip of the wedge, which faces the interior of the primordium, defining the apical surface. I) During the first stage (Reorientation), the anterior pharyngeal cells reorient their apicobasal polarity to align it along the dorsoventral axis of the embryo. This behavior lengthens the nascent pharyngeal lumen towards the anterior of the embryo. II) In the second stage (Epithelialization), the arcade cells form an epithelium that is continuous with the pharynx posteriorly and the hypodermis anteriorly. Epithelialization mechanically couples the digestive tract to the surrounding hypodermis. III) During the third stage (Contraction), the pharynx moves anteriorly and the epidermis of the mouth posteriorly. These movements suggest that cells of the epidermis, buccal cavity and pharynx contract their apical surfaces, similar to a purse-string.

To identify the molecules that regulate pharyngeal extension, we have performed a genetic screen for mutants that arrest with a pharynx unattached (Pun) phenotype at the L1 stage. We recovered 14 mutants from an EMS mutagenesis of 3000 haploid genomes. Pun animals could reflect either a failure to connect the pharynx with the buccal cavity or a 'snap-back' in which the attachment between the pharynx and the buccal cavity was not strong enough to withstand the forces of embryonic elongation. We used time-lapse videomicroscopy to distinguish between these possibilities and found that only 1/14 mutants belonged to the snap-back category. Of the remaining alleles, four are defective for Stage I Reorientation. The anterior cells appear to be positioned correctly within the pharyngeal primordium but fail to reorient their apicobasal polarity. Eight alleles are defective for Stage II. The anterior pharyngeal cells reorient their polarity but fail to form an epithelium with the arcade cells. For one allele, cells within the primordium appear disorganized, suggesting the extension defects may be a secondary effect. Importantly, the pharynx is well differentiated in all of the mutants, suggesting that the Pun phenotype does not reflect a general defect in pharyngeal development. We have assigned 9/13 alleles to linkage groups by snip-SNP mapping and have defined at least six genes.

Our current focus is one of the Stage II mutants, *px47*. Timelapse videomicroscopy revealed that anterior pharyngeal cells undergo proper reorientation, but that the arcade cells fail to form an epithelium, producing a Pun phenotype. Sixty percent of *px47* homozygotes arrest as L1 larvae, presumably because they cannot feed. The remaining 40% arrest during mid to late embryogenesis with defects that affect multiple epithelia. For example, midgut cells appear rounded, ventral and anterior hypodermal cells fail to enclose the embryo, and the tail is frequently misshapen. *px47* maps to LGIV, and we are currently using snip-SNP and three-factor mapping to refine the map position and clone the gene.

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Early morphogenesis of the *Caenorhabditis elegans* pharynx.
Dev Biol. 233(2):482-94

135. Ephrin/Eph Receptor Kinase Signaling in Caenorhabditis elegans

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The vertebrate Ephrin ligands and their receptors, the Eph receptor tyrosine kinases (RTKs), act as cell surface signaling molecules that have many roles during development. C. elegans has 4 Ephrins (EFN-1 to 4) and 1 Eph RTK (VAB-1). Mutations that affect the EFN-1 ligand or the VAB-1 receptor result in similar embryonic defects in neuroblast organization and epidermal morphogenesis. These phenotypes are variable and not completely penetrant suggesting that there is redundancy during these developmental events. To identify genes that may function with vab-1 we screened for genes that when mutated result in a synthetic lethal phenotype with vab-1. We have screened 1800 mutagenized F1 animals and isolated 3 synthetic lethal genes. We will present results from the synthetic lethal screens, and our initial characterization of these mutants. We will also describe some biochemical experiments to complement the genetic approaches to understand C. elegans Ephrin/Eph RTK signaling.

136. slr-5 and slr-9 function with lin-35/Rb to control pharyngeal morphogenesis in C. elegans.

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In screens for novel lin-35-synthetic-lethal mutants (Fay et al., 2002), we have identified two genes, each defined by a single allele, that collaborate with lin-35/Rb to control pharyngeal development. lin-35; slr-5(ku354) double mutants typically arrest as embryos or starved Unc L1 larvae. Further examination of late-stage embryos and larvae revealed severe defects in pharyngeal development; the majority (>90%) of lin-35; slr-5(ku354) animals fail to undergo pharyngeal elongation, thereby preventing these animals from feeding. We are currently examining the cause for this phenotype, which could result from either cell fate transformations or defects in pharyngeal morphogenesis and attachment. To this end we have analyzed pharyngeal development in lin-35, slr-5 double mutants using a variety of relevant GFP markers. We conclude that the most or all pharyngeal cell-types are present and in their approximate correct numbers, but that morphogenesis is unsuccessful due to a failure of anterior pharyngeal cells to migrate anteriorly and integrate with arcade cells of the developing buccal cavity. In contrast to double mutants, slr-5 single mutants appear completely normal, but show an ~50% reduction in brood size. We have also observed a spectrum of germline/gonad abnormalities in some lin-35; slr-5 double mutants that reach adulthood, suggesting a possible role in germ cell differentiation, proliferation, or fertility. Unlike fzr-1/slr-1, slr-5 single mutants do not appear sensitive to RNAi treatment of lin-36 or efl-1, indicating differences among the slr mutants with respect to their specific genetic interactions.

Approximately 40% of lin-35; slr-9(fd1) double mutants also exhibit severe pharyngeal defects that appear identical to those displayed by lin-35; slr-5 double mutants. Both slr-5 and slr-9 map to LGIII, though extensive mapping and complementation tests indicate that they are not allelic. Mapping data has placed slr-5 close to sma-3 while slr-9 is right and closely linked to vab-7. We are currently using SNP mapping to facilitate the identification of both slr-5 and slr-9 gene products. In addition, we are carrying out cosmid rescue experiments for slr-5, which we have placed between two closely spaced SNPs. Progress on the cloning and phenotypic characterization of these mutants will be reported.

Fay et al. (2002). Genes and Development 16: 503-517

137. Regulation of the FoxA homologue *pha-4* in organ development

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PHA-4 is a critical regulator of pharyngeal development that endows cells with pharyngeal organ identity. Worms homozygous for mutations in *pha-4* completely lack a pharynx and die as embryos or first stage larvae.[1] Conversely, ectopic expression of *pha-4* is sufficient to produce ectopic pharyngeal cells.[2,3] *pha-4* encodes the *Caenorhabditis elegans* homolog of the forkhead box A (FoxA) protein, a transcription factor whose orthlogs are implicated in gut development of organisms ranging from hydra and flies to vertebrates. Despite the importance of *pha-4* in organ development, components that regulate FoxA activity are unknown in any organism.

To identify genes that encode regulators or co-factors of PHA-4, we have undertaken a screen for mutants that suppress a partial loss of *pha-4* function. First, we designed a temperature sensitive configuration of *pha-4* by combining *pha-4* nonsense alleles with a temperature sensitive *smg-1* mutation *(cc546).*[4] *smg-1(cc546); pha-4(nonsense)* survive at 24° when *smg-1* is inactive, but die at 15° when *smg-1* activity is restored.[5] At an intermediate temperature of 20° , homozygous animals arrest with a weak Pha-4 phenotype. We speculate that the weak phenotype reflects intermediate levels of *pha-4* mRNA, protein and activity

To identify *pha-4* suppressors, we mutagenized *smg-1(cc546); pha-4(nonsense)* worms at 24° and shifted their grandchildren to 20° . Most animals die at this temperature and we searched for mutants that restored viability. 27 suppressors from 15,000 haploid genomes were obtained using EMS as a mutagen and 29 more suppressors were found using the Mos1 transposon[6]. Surprisingly, none of our transposon-induced alleles carried a Mos1 insertion. Secondary tests have shown that 16/27 EMS- and 26/29 Mos1-induced alleles are in the *smg* pathway, leaving 14 non-*smg* suppressors that are most likely *pha-4* interacting proteins

We are beginning to characterize the *pha-4* suppressors in detail. We have identified both dominant and recessive suppressors and some of these are associated with a temperature-sensitive Egl phenotype at 20° . We are continuing to map and analyze the *pha-4* suppressors genetically.

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138. Characterizing ELT-7, an apparent redundant partner with ELT-2, in intestinal organogenesis

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The E founder cell is the sole progenitor of the endoderm in the *C. elegans* embryo, giving rise to the 20 cells of the intestine. While many maternal factors (e.g. Wnt/MAPK, SKN-1) and their zygotic gene targets (e.g. two, sequentially-expressed, pairs of redundant GATA factors, MED-1, -2 and END-1, -3) have been shown to specify the E cell, and many other genes are known to be expressed 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.

An *elt-7::gfp* construct shows gut expression beginning at the 2E cell stage continuing through the life of the animal, identical to that of the GATA factor *elt-2* (Fukushige, et al, 1998). Ectopic expression of either *elt-2* or *elt-7* can cause non-gut cells to express terminal gut differentiation markers. 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). Though, *elt-2* is not essential for *ges-1* expression, *elt-7(RNAi);elt-2(0)* animals do not appear to express a *ges-1::gfp* reporter construct. We are currently collaborating with Jim McGhee to confirm this result by esterase staining *elt-7(RNAi);elt-2(0)* embryos, a more sensitive assay for the presence of GES-1. Elimination of ELT-2 results in L1 animals that die with a degraded gut (Fukushige, et al, 1998). While *elt-7(RNAi)* produces animals that appear morphologically normal, *elt-7(RNAi);elt-2(0)* appears to enhance the *elt-2(0)* phenotype, producing L1 animals that do not seem to have a gut lumen under Nomarski optics. We are currently analyzing this phenotype more closely by examining *elt-7(RNAi), elt-2(0),* and *elt-7(RNAi);elt-2(0)* animals with a panel of antibodies, various gfp reporter constructs that are expressed in the gut and electron microscopy.

These observations make *elt-2* and *elt-7* likely redundant partners in intestinal organogenesis, though the redundancy does not appear complete, since animals without ELT-2 arrest and die as L1 larva. This implies that there are targets for gut differentiation that require ELT-2 and not ELT-7, since *elt-7(RNAi)* can enhance the *elt-2(0)* phenotype while not appearing to cause lethality itself. Redundancy among GATA factors appears to be a common theme in development, having already been observed with MED-1, and -2, (Maduro, et. al., 2001) in mesendoderm specification, with END-1, and -3 in gut specification as well as with EGL-18 and ELT-6 (Koh and Rothman, 2001) in hypodermal development.

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Hawkins, M. G., McGhee, J. D. (1995) elt-2, a Second GATA Factor from the Nematode *Caenorhabditis elegans*. J. Biol. Chem. **270**, 14666-14671.

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139. A system for intestine-specific RNA interference

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To help identify genes important in intestinal morphogenesis we are attempting to construct an intestine-specific RNAi system. We are expressing rde-1(+)under control of an intestinal promoter, *end-1*, in *rde-1* mutant embryos (referred to as *end-1*::RDE-1 embryos). We have tested our system by performing RNAi against *hmr-1*, a classical cadherin expressed in all epithelia in *C. elegans* (Costa et al., 1999). Based on expression of *end-1*::GFP reporter constructs, we would have expected penetrant RNAi effects at early stages of embryogenesis; however, we only see significant loss of HMR-1 staining in the intestine of late embryos, approximately two-fold and pretzels. *hmr-1*(RNAi) in *end-1*::RDE-1 embryos, either by injection or bacterially-mediated delivery, produces no lethality in embryos, in contrast to N2 embryos, which die with major defects in body morphogenesis and enclosure. In some of the treated *end-1*::RDE-1 embryos, we have observed partial loss of HMR-1 staining in hypodermal cells with concomitant hypodermal morphogenesis defects; this may be due to the fact that the *rde-1* allele we used, *ne219*, is not a null. We have also attempted to inhibit the function of two other genes reported to be required for intestinal morphogenesis, *die-1* (Heid et al., 2001) and *lin-12*(Hermann et al., 2000) using our system, and failed to observe the expected phenotypic defects. *Hmr-1*, *die-1*, and *lin-12* are expressed both maternally and zygotically, so it is possible that perdurance of maternal product may interfere with our ability to inhibit gene function.

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Heid et al. (2001) Dev. Biol. 236: 165-180.

Hermann et al. (2000). Development 127: 3429-3440.

140. Analysis of *pel-2*, a gene required for embryonic pharyngeal differentiation and body morphogenesis

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Organogenesis is a complex process requiring the coordinated specification, morphogenesis, and differentiation of disparate cell types. To better understand how metazoan organ development is achieved, we are investigating a gene required for embryonic pharynx development, *pel-2* (pharynx and elongation defective). The *pel-2* mutant was isolated in a screen designed to identify zygotic genes essential for embryonic development.

During development of the pharynx, the PHA-4/FoxA transcription factor imparts pharynx organ identity on diverse cell types and functions continuously to maintain pharynx differentiation throughout the lifespan of the worm. Pharynx precursor cells become specified and undergo additional morphogenesis and differentiation by the activity of at least four factors, CEH-22/NK-2 homoedomain, PHA-1/bZip, PEB-1(a novel DNA-binding protein), and PHA-4 which act in concert at the *myo-2* enhancer to induce full expression of the pharynx-specific myosin gene, *myo-2*.

pel-2 embryos arrest late in embryonic development with a pharynx primordium surrounded by a basement membrane. Differentiation and morphogenesis of the pharynx are incomplete in arrested embryos. While *pel-2* mutants express the MAb 3NB12-reactive early pharyngeal muscle marker, these embryos generally fail to express later markers of pharynx-specific myosin and pharynx gland cells, confirming the block in differentiation. *pel-2* is also required for full expression of PHA-1, placing *pel-2* upstream of *pha-1* in the pharynx muscle development pathway. These studies suggest that *pel-2* is involved in the differentiation of pharyngeal tissues and its function may be integrated at the transcriptional level through the activation of the pharynx differentiation-promoting PHA-1 transcription factor. The later block in pharynx differentiation suggests that *pel-2* acts downstream of *pha-4*, which is required for generation of all pharynx cells. However, we found that *pel-2* is required to maintain high levels of *pha-4* expression throughout embryogenesis and may be required to initiate *pha-4* expression, possibly in conjunction with another factor.

As the wild-type embryo progresses through development, the pharynx primordium extends toward the anterior, leading to the attachment of the pharynx to the buccal cavity. This movement is dependent in part on the epithelialization of arcade cells. In the *pel-2* mutant, the pharyngeal primordium fails to extend; however, we found that arcade cells are made in *pel-2* embryos and the epithelium that links the pharynx to the buccal cavity is usually intact. Thus, the inability of the pharynx primordium to extend to the anterior appears to be independent of pharyngeal extension driven by the arcade cells.

Using a multitude of mapping strategies we have narrowed the position of the *pel-2* locus on the right end of chromosome IV, near YAC Y73F8A in an effort to determine the molecular identity of the *pel-2* locus.

141. Dimers of GLD-1 bind to a single TGE repeat of the *tra-2* mRNA 3'-untranslated region

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The developmentally timed switch from spermatogenesis to oogenesis in *C. elegans* hermaphrodites is controlled by the differential expression of two key factors, *tra-2* and *fem-3*. Regulation of both factors occurs at the RNA level via distinct multi-protein complexes. In either case, the repression machinery is targeted to specific sequences present in the 3'- untranslated regions (UTR) of their mRNA transcripts. In the L4 larval stage, *tra-2* mRNA is repressed via the assembly of a protein complex onto tandem TGE repeats in its 3'-UTR. The primary determinant of this complex is GLD-1, a member of the STAR family of RNA-binding proteins.

We have expressed and purified recombinant GLD-1 and have developed a gel mobility shift assay to monitor binding of this protein to its RNA target. Using this approach, we show that one TGE repeat of the *tra-2* 3'-UTR is sufficient for high affinity binding. Preliminary mutagenesis of the pyrimidine-rich TGE reveals a number of adenosine residues that contribute to the specificity of the interaction. Surprisingly, stoichiometric-binding experiments show that two molecules of GLD-1 bind to one copy of the TGE. Lastly, we show that GLD-1 forms dimers in the absence of RNA. Together, these data suggest that the intact 3'-UTR of *tra-2* has several binding sites for GLD-1 that cooperate in order to form a higher order structure necessary for translational repression of *tra-2*.

142. Knockout analysis of putative germline-related genes in *C. elegans*.

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Genes relevant to gametogenesis can be easily recognized in *C. elegans* by phenotypic analysis of their loss-of-function. We have previously screened and identified a set of genes that apparently have germline-related functions, through either RNAi or gene knockout assay. Here we report preliminary characterization of the mutant worms deficient in some of these genes, including F30A10.10, K09A9.4, and *rnp-1*.

The F30A10.10 and K09A9.4 genes both encode ubiquitin C-terminal hydrolase. Disruption of the F30A10.10 gene resulted in formation of an abnormal gonad, which contained neither sperm nor oocytes but showed vacuolous germ cells in the proximal region. In contrast, disruption of K09A9.4 showed less obvious phenotypes. The knockout worm was apparently normal at 20°C, but some population of progeny became sterile after proliferation at 25°C.

The *rnp-1* gene encodes a possible homolog of the *Drosophila* RNA-binding protein LARK. The gonad of *rnp-1(RNAi)* worms contained a reduced number of germ cells, with abnormal oocytes. The *rnp-1* deletion mutant we isolated was more severerly sterile, in which gonadal arms were distorted and oocytes were missing.

In addition, we currently analyze five more genes, some of which are found so far only in *C. elegans*. RNAi of these genes have given phenotypes such as (1) a defect in fertilization, (2) reduction of the number of germ cells and abnormal oocytes, (3) a defect in germ cell proliferation, (4) abnormal oogenesis, and (5) reduction of the number of germ cells, respectively. Isolation of deletion mutants for these genes are in progress.

143. Vulval development and glycosaminoglycan biosynthesis

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Vulval development in *C. elegans* is a dynamic process involving migration and invagination of epithelial cells. A screen for mutants defective in vulval invagination identified eight complementation groups displaying a squashed vulva (*sqv*) phenotype. Initial characterization of the *sqv* genes showed that they define parts of a glycosylation pathway. *sqv-7* encodes a nucleotide sugar multi-transporter and *sqv-4* encodes UDP-glucose dehydrogenase, which catalyzes formation of UDP-glucuronic acid. Studies characterizing *sqv-3* and *sqv-8* identified them as galactosyltransferase-I and glucuronyltransferase-I, respectively, which are required for the assembly of glycosaminoglycans. These long linear polysaccharides are attached to protein cores of proteoglycans, indicating an important role for these matrix molecules in vulval morphogenesis. Characterization of additional *sqv* genes has demonstrated that the pathway of glycosaminoglycan biosynthesis in *C. elegans* is virtually identical to the pathway in vertebrates. Furthermore, all of the relevant steps can be assayed in vitro using the vertebrate system as a guide. These studies further refine our understanding of the glycosaminoglycans required for vulval development.

144. A role for ephrin signaling in vulval development?

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In *C. elegans*, mutations in ephrin/ ephrin receptor signaling lead to a broad spectrum of phenotypes, including defects in ventral cleft closure during gastrulation, embryonic and larval lethality and variable defects in head and tail morphology (1, 2). The variability of mutant phenotypes in worm ephrin signaling makes it challenging to screen directly for suppressors or enhancers (3). With this in mind, we have set about characterizing additional phenotypes in ephrin signaling mutants that could form a basis for future modifier screens.

EFN-4 is a divergent member of the ephrin family and appears to function independent of the VAB-1 Eph receptor in morphogenesis (4). Our preliminary analysis of *efn-4* null mutants has identified low penetrance egg-laying defects, protruding vulvae and gonad eversion through the vulval opening. In addition, a functional EFN-4::GFP reporter construct is expressed in the descendants of the vulval primary cell lineage (P6.p grand-daughters) during the L4 stage. To confirm this, we crossed EFN-4::GFP into multi-vulva (Muv) and vulvaless (Vul) mutants. In *let-60(n1046dm)* muv and class A syn-Muv animals, EFN-4::GFP was seen in ectopic pseudovulvae in addition to the true vulva. In *let-23(sy97)* Vul animals lacking the 1° vulval fate EFN-4::GFP is not expressed in ventral epidermis. We are currently corroborating these results with anti-EFN-4 antibody staining. We are also using double mutant analysis to place ephrin signaling in context with previously characterized genes involved in vulval development. Our hope is to use the power of genetic screens based on vulval development to identify other components of ephrin signaling pathways.

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145. SITE-DIRECTED MUTANTS OF THE *C. elegans* TRANSCRIPTION FACTOR LIN-31 REVEAL DISTINCT FUNCTIONS.

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In order to better understand cell fate determination in *Caenorhabditis elegans*, we are conducting a functional analysis of LIN-31, a winged-helix transcription factor (WH TF) that acts as a tissue-specific effector of the conserved Ras/MAP kinase signaling pathway to promote or suppress vulval cell fates in the development of the hermaphrodite vulva (Miller *et al.*, Genes and Dev., 7:933, 1993). In addition to a DNA-binding domain (DBD), the LIN-31 protein contains several regions of interest: a small acidic-rich region, four MAP kinase consensus phosphorylation sites, and a small region at the C-terminus that displays homology with a subset of WH proteins. These regions could play a number of roles, from transcriptional activation to an interaction domain for LIN-1, which is known to heterodimerize with LIN-31 (Tan *et al.*, Cell, 93:569,1998). Using site-directed mutagenesis techniques, specific mutations were introduced into the gene at these regions of interest. Stable transgenic lines were created through germline microinjection of mutant plasmids into animals with no functional LIN-31 protein. Through phenotypic analysis of multiple transgenic lines, we are beginning to better understand the functional significance and contribution of each of these different sites to LIN-31 function. Our results thus far support the current model (Miller *et al.*, 1993; Tan *et al.*, 1998; Miller *et al.*, Genetics, 156:1595, 2000), 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.

In addition, we are initiating a functional analysis of LIN-31 protein using two assays: ability to bind a putative DNA target sequence and ability to heterodimerize with LIN-1. We used a bacterial expression system to produce GST::LIN-31 fusion protein. Using gel-shift assays, we confirmed function of wild-type protein by demonstrating its ability to bind the transthyretin (TTR) promoter, a consensus sequence recognized by HNF-3, another WH TF sharing DBD sequence homology (Costa *et al.*, Mol. Cell. Biol., 9:1415, 1989). We are now in the process of creating, expressing, and purifying GST::LIN-31 fusion proteins carrying specific mutations, including two point mutations in the DBD believed to disrupt interaction of the LIN-31 with its target DNA (Miller *et al.*, 2000). These mutant proteins will allow us to test *in vitro* their ability to bind the TTR promoter and to heterodimerize with LIN-1.

146. Genome-wide search for LAG-1 target genes that function in the AC/VU decision and VPC specification

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Andrew Yoo and Carlos Bais contributed equally to this work

LIN-12/Notch signaling determines many different cell fate decisions in C. elegans. The released intracellular domain of LIN-12 forms an activator complex with LAG-1, a transcription factor homologous to Suppressor of Hairless (Su(H)) in Drosophila. LAG-1 binds to specific sequences in cis-regulatory regions and activates its target genes. Cis-regulatory domains are characterized by the presence of multiple binding sites for transcription factors. As an initial attempt to identify putative target genes of LIN-12 signaling, we examined the genome database for clusters of LAG-1 binding sites (LBS). Sequences that had been experimentally shown to bind LAG-1 and Su(H) were collected, and a matrix for these sequences was built by multiple alignment. From this matrix, a consensus sequence for the LBS was deduced. We devised a computer program, Cluster Analyzer, to analyze the genome database for clusters of LBS sequences. We set the criteria for an LBS cluster to be 4 or more binding sites within a span of 1000 bp, and we found approximately 260 genes that contain the clusters of LBS in their non-coding regions. This computational approach detected a number of genes already known to be regulated by LIN-12 signaling, including lin-12 itself and lip-1. The putative target genes were further categorized with respect to their predicted functional domains, and a summary of these findings will be presented.

The next step is to assess the expression pattern of selected candidate genes using transcriptional reporter genes. We are particularly interested in potential target genes that are expressed during the anchor cell/ventral uterine precursor cell (AC/VU) fate decision and vulval precursor cell (VPC) fate specification. In the VPCs, there is also the interesting problem of how signals from LIN-12 are integrated with signals from the Ras/MAP Kinase (MAPK) pathway. We have identified a gene that we think is likely to be involved in integrating these signals. A reporter construct containing the LBS cluster is expressed in a dynamic pattern during VPC specification, and appears to be influenced by both the Ras and LIN-12 pathways. We are currently analyzing this gene in detail, and hope to have information about its role in VPC specification to present at the meeting.

147. Genetic analysis of the vulval mutants in *C. briggsae*

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To understand the evolution of developmental mechanisms, we are doing a comparative analysis of vulval patterning in *C. elegans* and *C. briggsae*. *C. briggsae* is closely related to *C. elegans* and has identical looking vulval morphology. However, recent studies have indicated subtle differences in the underlying mechanisms of development. The recent completion of *C. briggsae* genome sequence by the *C. elegans* Sequencing Consortium is extremely valuable in identifying the conserved genes between *C. elegans* and *C. briggsae*.

To identify the genes involved in vulval development in *C. briggsae*, we have taken a genetic approach and are conducting large scale EMS screens. So far, we have identified more than 30 vulval mutants that are vulvaless (vul), protruding vulva (Pvul) or multivulva (Muv). Current experiments involve mapping and phenotypic characterization. Preliminary analysis indicates that some of these have phenotypes distinct from the known *C. elegans* vulval mutants. The genetic mapping of mutants has been facilitated by the availability of markers and their linkage maps (kindly provided by Dr. Baillie's lab, Simon Fraser University). Our analysis of the vulvaless mutants has revealed an ortholog of *C. elegans lin-11*. This has been confirmed by transgene rescue and allele sequencing experiments. *lin-11* encodes a LIM homeodomain protein and functions to specify distinct vulval cell fates. We have determined the regulatory region of *C. briggsae lin-11* that directs gene expression in vulval cells. The temporal pattern of expression closely resembles to that of *C. elegans lin-11*. A comparison of the regulatory sequences between the two nematode species has revealed conserved elements that are likely to be recognized by vulval-specific transcription factors. We are analyzing such elements to identify the gene network that is conserved during evultion and regulates the expression of *lin-11* in vulval cells.

148. Deficiencies in C20 polyunsaturated fatty acids cause behavioral and developmental defects in *C. elegans fat-3 mutants*

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Arachidonic acid and other long-chain polyunsaturated fatty acids (PUFAs) are important structural components of membranes and are implicated in diverse signaling pathways. The Δ6 desaturation of linoleic and linolenic acids is the rate limiting step in the synthesis of these molecules. *C. elegans fat-3* mutants lack Δ6 desaturase activity and fail to produce C20 PUFAs. We examined these mutants and found that development and behavior were affected as a consequence of C20 PUFA deficiency. While *fat-3* mutants are viable, they grow slowly, display considerably less spontaneous movement, have an altered body shape, and produce fewer progeny than wild-type. In addition, the timing of an ultradian rhythm, the defecation cycle, is lengthened compared to wild-type. Since all these defects can be ameliorated by supplementing the nematode diet with gamma-linolenic acid or C20 PUFAs of either the n6 or n3 series, we can establish a causal link between fatty acid deficiency and phenotype.

149. Analysis of learning, short-term and long-term memory in the presenilin mutant strains *sel-12* and *hop-1*

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Alzheimer's disease is a prevalent neurodegenerative disorder with symptoms that include decreased memory ability and cognitive decline eventually leading to dementia and finally death. Presenilin 1 and Presenilin 2 are human genes associated with the occurrence of Alzheimer's disease. Presenilin genes have also been discovered in the nematode *Caenorhabditis elegans*: *sel-12* and *hop-1.* In the worm, these presenilins are associated with the LIN-12/Notch signaling pathway through the Notch receptors LIN-12 and GLP-1. *sel-12* (Levitan and Greenwald, 1995) is ubiquitously expressed and results in an egl phenotype which can be rescued by human PS1 and PS2 constructs (Levitan et al., 1996). *hop-1* (homolog of presenilin) was discovered using reverse genetics techniques by searching the *C. elegans* genome sequence database and looking for genes with a similar amino acid sequence to *sel-12* (Li and Greenwald, 1997). *hop-1* constructs rescue the egl phenotype of the *sel-12* mutations providing evidence that *hop-1* is a presenilin gene. The *hop-1* mutant strain is a deletion mutation and does not show the egl phenotype noted with *sel-12* gene mutation suggesting that *hop-1* may be functionally redundant to *sel-12*. Only in the *hop-1*;*sel-12* double mutant is a novel phenotype noted, e.g., a distorted morphology phenotype. At this time, behavioral analysis of the presenilin mutant worms has focused on thermotaxis: worms with *sel-12* ;*hop-1* mutations are unable to migrate to their "home temperature" when placed on a temperature gradient (Wittenburg et al., 2000). This deficit in thermotaxis was rescued by insertion of a *sel-12* transgene.

In our laboratory, we have been able to show that wild-type *C. elegans* will reverse in response to a mechanical tap to the side of their petri dish, however, with repeated taps the worms habituate to the tap and as a result will show smaller reversals or no response at all to a tap stimulus (short-term memory; Chiba et al., 1990). If habituation training is divided in to distinct training blocks separated by rest periods, worms can remember this habituation training for at least 24 hours (Beck and Rankin, 1997) and up to 72 hours (Rankin, unpublished results). As both the *sel-12*(ar131) and the *hop-1*;*sel-12* mutant worms retain mechanosensory and locomotor ability, our lab is able to use habituation of the tap-withdrawal response to examine both the short-term and long-term memory ability of these presenilin strains. We have found that the *sel-12*(ar131) shows short-term memory that is similar to wild-type when taps are presented at a short interstimulus interval (ISI; 10s), however, *sel-12*(ar131) show more rapid habituation when taps are given at a long ISI (60s). Interestingly, the *hop-1*;*sel-12* double mutant strain appears to show normal short-term memory when taps are given at either a short or a long ISI. Studies examining the long-term memory ability of these presenilin strains are currently being performed.

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150. Toward the identification of novel regulators of b-catenin signaling in C. elegans
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The unbridled transcriptional activation of Wnt-responsive genes is a frequent and early event in a number of human tumor types (Polakis, 2000). In an effort to identify novel regulators of Wnt-mediated signaling, we have initiated modifier screens in C. elegans around two key Wnt components, b-catenin (bar-1, Eisenman et al, 1998) and its negative regulator, axin (pry-1, Korswagen et al., 2002).

We have constructed putative degradation-resistant versions of BAR by removing possible GSK3b phosphorylation sites (8 sites at the N-terminus) and expressed these constructs from a heat shock and lin-31 promotor (gracious gift from P. Tam and S. Kim). Removal of 5 of these 8 sites (bar-1 (D2)), as well as removal of all 8 sites (bar-1 (D3), resulted in robust phenotypes in both expression systems. Early larval expression (via heat shock) results in weakly penetrant larval arrest and highly penetrant vulval rupture and sterility phenotypes. Inducing expression later in larval development (via heat shock or lin-31-driven) results in a highly penetrant Muv phenotype (~90%, similar results obtained by Gleason et al., 2002) that is dependent on pop-1/TCF.

pry-1 encodes the worm axin ortholog (Korswagen et al, 2002). Axin is a scaffolding protein required for the efficient phosphorylation, and subsequent degradation of b-catenin. Loss of pry-1 activity results in temperature-sensitive lethality (ruptured vulva at 15oC) and a weakly penetrant Muv phenotype (~30%) at the permissive temperature (25oC, Gleeson et al., 2002). These phenotypes are dependent on wild-type bar-1 (Maloof et al, 1999) and pop-1 activity, supporting the notion that PRY-1 negatively regulates BAR-1 and POP-1.

In an effort to identify genes that positively influence Wnt signaling, we performed large-scale F2 selection screens for mutations that suppress the phenotypes resulting from early larval heat shock of bar-1 (D2) or the temperature-sensitive lethality of pry-1. All of the strong suppressors (>20) encode alleles of either bar-1 or pop-1. In the hope of identifying modifiers that may have been missed in the selection screens, we are performing large-scale RNAi screens and will report our progress on suppressor identification and the behavior modifiers in various secondary assays.

151. *sbp-1*, a homolog of SREBP, controls lipid metabolism in the worm

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The "Metabolic Syndrome" is characterized by a cluster of metabolic defects, including obesity, insulin resistance, and elevated plasma lipid levels. This syndrome predisposes individuals to develop Type 2 diabetes and cardiovascular disease. Although the relationship among these metabolic defects is not well understood, it has been shown that treatments which lower plasma lipid levels are beneficial for these patients. Our goal is to use enhancer and suppressor genetics to identify potential new therapeutic targets for regulation of lipid metabolism.

The Sterol Regulatory Element Binding Proteins (SREBPs) are a family of bHLH transcription factors that control the expression of genes involved in lipid metabolism in mammals, including those encoding the enzymes of cholesterol and fatty acid biosynthesis and transport as well as adipocyte differentiation. The activity of these transcription factors is post-translationally regulated: the inactive form is a transmembrane protein that is localized to the ER and nuclear envelope and is activated by two proteolytic cleavages that release the amino-terminal bHLH domain to allow entry into the nucleus. This proteolysis is regulated by nutritional signals, including cellular sterol and unsaturated fatty acid levels. The transcript level of SREBP is also regulated by nutritional signals including insulin signaling. In C. elegans, there is a single SREBP homolog, sbp-1 (Sterol Regulatory Element Binding Protein-1), encoded by Y47D3B.7. We generated a deletion in the gene by excision of a Tc1 element, resulting in removal of the C-terminal regulatory regions of the gene while leaving the amino-terminal bHLH transcription factor domain intact. This mutation is a temperature-sensitive loss-of-function, and results in embryonic or larval arrest and pale intestine (Pin) phenotypes, similar to the RNAi phenotype. A second, weaker allele results in a viable Pin phenotype. The Pin phenotype results from defects in lipid synthesis and storage: well-fed sbp-1 mutants have a paucity of lipid droplets in the intestine, resembling the phenotype of starved worms. The intestines of these animals stain poorly with lipid dye Sudan black as compared to the wildtype, and lipid profiles of sbp-1 (ts) mutants indicate defects in fatty acid and triacylglycerol synthesis. Furthermore, although C. elegans is auxotrophic for cholesterol, we have shown that sbp-1 mutants are hypersensitive to cholesterol deprivation, perhaps due to the overall lipid deficiencies in these animals. Examination of an sbp-1p::GFP reporter construct indicates that the sbp-1 gene is expressed in the intestine from early embryogenesis through adulthood, consistent with the intestinal phenotype. Two of the proteins involved in regulated cleavage of SREBP in mammals, Site 2 Protease (S2P) and SREBP Cleavage Activating Protein (SCAP) also are conserved in worms. RNAi experiments with these genes indicate that they interact with each other to give strong Pin phenotypes, and also that RNAi of S2P can enhance the weak sbp-1 allele to give a strong Pin phenotype. Therefore, we believe that aspects of regulation of SREBP cleavage are conserved in worms as well. Because dauer formation has an (opposite) effect on lipid accumulation in the intestine, genetic interactions were examined between sbp-1 and dauer-constitutive mutations in the daf-2 (insulin receptor) and daf-7 (TGF-b) pathways. sbp-1 mutants or RNAi are weakly suppressed for the Pin phenotype by mutations in both pathways.

152. A Genetic Selection for Amphetamine Resistance

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We are using amphetamine (AMPH) as a pharmacological agent to study monoaminergic function in *C. elegans*. Previous studies in vertebrates have shown that AMPH can bind and inhibit the plasma membrane reuptake transporters for dopamine, serotonin and norepinephrine, resulting in a prolonged increase in amine transmitters in the synaptic cleft. Also, AMPH has been shown to block the uptake of dopamine by the *C. elegans* plasma membrane transporter (ceDAT) in vitro (Jayanthi, L.D. et al., Mol Pharm 54:601-9). In worms, AMPH inhibits both pharyngeal pumping and egg-laying, which correlates well with the behavioral effects of exogenous dopamine, but not serotonin. Chronic exposure to 5mM AMPH from early larval stages results in a dramatic slowing of the rate of development. We have used EMS mutagenesis and a genetic selection for resistance to chronic AMPH in order to identify mutants which may be altered in monoaminergic function. From an initial screen of 4500 genomes, 12 mutants were isolated which show faster growth on 5mM AMPH relative to N2. Isolated mutants demonstrate varying degrees of resistance to developmental delay, with some mutants growing at nearly wild-type rates on 5mM AMPH. Also, some of the mutants isolated in the chronic AMPH screen show resistance to the acute effects of AMPH on both egg-laying and pharyngeal pumping. We have also begun to analyze the responsiveness of these mutants to exogenous dopamine and serotonin and are attempting to genetically map those mutations which result in altered post-synaptic function.

153. A Genetic Study of Muscular Dystrophy in *C. elegans*

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Muscular dystrophy (MD) is a common genetic disorder characterized by progressive muscle weakness and wasting. Mutations in dystrophin were identified as one cause of this disease in humans. Mutations in the C. elegans homologue of the dystrophin gene, dys-1, cause exaggerated bending of the anterior body during locomotion and muscle degeneration in a sensitized genetic background (1, 2).

We have previously reported that in a genetic screen for mutants showing ethanol-resistant or -inducible egg-laying behavior, twelve mutants exhibit a dys-1-like phenotype (2000 wcm abstract 170 and 2001 iwm abstract 893). The dys-1-like mutants represent one of four different classes of mutants identified in this screen. The dys-1-like mutants exhibit a resistant egg-laying behavior at a high ethanol concentration that inhibits more than 90% of N2 egg-laying. Furthermore, egg-laying of these mutants is induced at relatively low ethanol concentrations that moderately suppress egg laying of N2 animals. More eggs are laid in the presence than in the absence of ethanol in these mutants. This behavior is also observed in slo-1 mutants. Although the underlying mechanism of the altered egg-laying behavior is not clear, it is intriguing that this screen led to muscular dystrophy mutants. Genetic mapping and complementation testing of the isolated dys-1-like mutants indicate three different known genes and one additional gene. Specifically these include two alleles of dys-1, two alleles of dyb-1, three alleles of dyc-1 and five alleles (eg28, eg114, eg115, eg121, eg137) of an additional dys-1-like gene located on the left arm of chromosome III.

As a step toward understanding the possible role of the novel dys-1-like gene in muscle degeneration, we constructed several double mutants of eg28 with other known mutants. The locomotory defect of eg28;dys-1 is indistinguishable from that of the eg28 and dys-1 single mutants. Locomotion of the double mutant, eg28; hlh-1(cc561ts), progressively deteriorates and resembles the defect observed in dys-1;hlh-1 (2). Together these results indicate that eg28 may act in the same pathway as dys-1. Unlike other known aldicarb-hypersensitive MD mutants, however, eg28 is somewhat resistant to aldicarb, suggesting that this gene may have another function. We are currently attempting to map and molecularly characterize this gene. We have mapped eg28 to a 50 kb region on chromosome III. In an attempt to rescue the locomotory phenotype, injection of genomic PCR fragments covering this region has been performed. Molecular characterization of eg28 may enhance our understanding of muscle degeneration in C. elegans and in human muscular dystrophies.

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154. Mapping chromosomal targets of the dosage compensation complex

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The worm *C. elegans* compensates for the difference in X-linked gene dosage between XX hermaphrodites and XO males by reducing gene expression two-fold in XX animals. Downregulation of gene expression is achieved by the dosage compensation complex (DCC), a large multiprotein complex that binds the two X chromosomes in hermaphrodites. However, the *cis*-acting chromosomal elements that recruit and bind the complex are not known. Using free and attached X chromosomal duplications we are attempting to identify regions of the X chromosome that are sufficient to recruit the complex. By analyzing the volume and number of DCC-bound chromosomal bodies, a large duplication of the right 30% of X, mnDp10, was shown earlier to be competent to bind the DCC (1). Using combined fluorescent in situ hybridization (to mark X chromosomal territories) and immunofluorescence (to mark chromosomes that bind the dosage compensation complex) we confirmed these results. By analyzing smaller duplications that span the entire chromosome we hope to map chromosomal targets of the DCC.

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155. An XO-lethal mutation identifies a gene with interesting friends.

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Sex-specific lethality is a hallmark of mutations that affect X-chromosome dosage compensation, the process of globally reducing gene expression across both X chromosomes of hermaphrodites to equalize expression with the single X of males. For instance, mutations in *sdc* (Sex and Dosage Compensation) genes, which activate dosage compensation and repress transcription of the male sex determination gene *her-1,* cause XX-specific lethality and transformation toward the male fate. Conversely, a mutation in *xol-1* causes XO-specific lethality and transformation toward the hermaphrodite fate. XOL-1 is required in males to repress the SDC proteins, thus preventing dosage compensation and allowing expression of *her-1*.

We identified a maternally rescued, XO lethal we temporarily call *nox-1* (Not *xol-1*) in a screen for sex-specific phenotypes. *nox-1* XX hermaphrodites are viable, but have phenotypes that are consistent with perturbed gene expression. Using single nucleotide polymorphisms, we mapped *nox-1* to a single cosmid; subsequent transgenic rescue experiments determined that a gene able to rescue *nox-1* XO males encodes a WD-repeat protein. Sequencing of two *nox-1* alleles, *y2* and *y403*, indicated that both contained early stop codons in this gene. Taken with the phenotype of *nox-1(y2) in trans* to a deficiency, these data suggest that *y2* and *y403* are null alleles.

Is the XO lethality of *nox-1* due to incorrect activation of dosage compensation? Overexpression of *xol-1* rescues the XO lethality of *nox-1,* suggesting that *nox-1* affects the specification of dosage compensation. However, *nox-1* does not seem to be acting simply upstream of *xol-1,* since rescued animals are transformed into hermaphrodites, a phenotype not seen with *xol-1* overexpression alone. Furthermore, proteins that carry out dosage compensation do not localize inappropriately to X in dying *nox-1* XO embryos, as they do in dying *xol-1* XO embryos.

Coincidentally, the WD-repeat protein was concurrently being characterized in our lab as part of a protein complex containing ASH-2 (a trithorax-related protein) and DPY-30 (an XX-specific lethal shown to affect dosage compensation). We have shown that these three proteins co-immunoprecipitate from embryonic extracts. DPY-30 was found to co-immunoprecipitate with two distinct protein complexes: the SDC complex, implying that *dpy-30* influences sex and dosage compensation by acting with the SDC proteins; and the complex containing NOX-1 and ASH-2. Furthermore, recombinant DPY-30 and ASH-2 interact *in vitro*, and we are currently investigating how all three proteins physically interact. *nox-1, ash-2*, and *dpy-30* are homologous to components of a yeast complex containing Set1p, which has been implicated in the methylation of the N-terminal tail of histone H3 on lysine 4. This modification is noticably reduced in *dpy-30* mutant embryos, implying that histone methylation may also be affected by mutations in *nox-1* and *ash-2*.

In another coincidence, a screen performed in our lab for suppressors of a mutant strain that inappropriately allows *xol-1* expression in XX animals has yielded a likely loss-of-function *ash-2* allele. Therefore, like wild-type *nox-1, ash-2* may promote male viability.

Mutations that compromise dosage compensation often show sexual transformation phenotypes in sensitized backgrounds, reflecting a complex relationship between X expression and regulation of *her-1*. *nox-1, ash-2,* and *dpy-30* affect sexual fate in such assays. For example, like mutations in dosage compensation genes, partial reduction of function by *nox-1*, *ash-2*, or *dpy-30* RNAi suppresses the transformation to male fate associated with gain-of-function *her-1* XX animals.

We are currently using genetic and molecular tools to investigate the relationship between *nox-1*, *ash-2*, and *dpy-30*, and to determine whether *nox-1* and *ash-2* function directly in X expression or sex determination. Study of *nox-1, ash-2,* and *dpy-30* could reveal a novel and intriguing perspective on how genes are regulated in a developmental context.

156. *dsh-2* is required to polarize asymmetrically dividing cells.

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To investigate the mechanisms underlying asymmetric cell division, we have focused on the lineage that generates the HSN and PHB neurons. During embryogenesis, the HSN/PHB neuroblast divides asymmetrically to generate an anterior daughter that undergoes programmed cell death and a posterior daughter, the HSN/PHB precursor. This precursor then divides to produce the HSN neuron and the phasmid neuron PHB, a sensory neuron located in the tail. In *ham-1* mutants, the neuroblast often divides symmetrically producing two HSN/PHB precursors. As a result, we observe HSN and PHB neuron duplications. The PHB neuron duplication can be visualized using an integrated *srb-6::GFP* reporter, *gmIs12*, that expresses in PHB, as well as PHA, a second phasmid neuron in the tail unrelated lineally to PHB. Using *gmIs12* we discovered that mutations in a *C. elegans dishevelled* homolog, *dsh-2*, also produced phasmid neuron duplications, a Ham-1-like phenotype. We raised antibodies to DSH-2 and showed that DSH-2 and HAM-1 co-localize during embryogenesis. These results suggested that HAM-1 and DSH-2 may function together to polarize the HSN/PHB neuroblast. However, direct lineage analysis of *dsh-2* mutant embryos did not reveal any defects in the division of the HSN/PHB neuroblast. Instead, we observed that the cell deaths ABpl/rpppapp were often absent or delayed producing large persistent corpses. We also observed that the absence of these cell deaths correlated with the production of extra phasmid neurons. These cell deaths are closely related lineally to PHA. Using both PHA and PHB specific GFP markers, we determined that the majority of extra phasmid neurons in *dsh-2* mutants were due to PHA duplications. In *dsh-2* mutant embyos, we also observed duplications of PVC and loss of a phasmid sheath cell and hyp8/9 cell. These results are consistent with a defect in the asymmetric cell division of ABpl/rpppa. We propose that in *dsh-2* mutants ABpl/rpppa often divides symmetrically resulting in the transformation of the posterior daughter cell, which normally gives rise to the phasmid sheath cell, hyp8/9 and the ABpl/rpppapp cell death, into a second anterior-like daughter, which normally gives rise to PHA and PVC.

157. Identification of Embryonic Mesoderm Genes Using Microarrays

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At the 8-cell stage of *C. elegans* embryogenesis, the MS and E blastomeres are born. E will give rise to the entire intestine, while MS makes many mesodermal cell types, including body wall muscle and the posterior half of the pharynx. The fates of MS and E are specified by the MED-1,2 GATA transcription factors. While many of the regulators that function in E lineage development have been identified, there is comparatively little known about how MS development proceeds downstream of the MEDs. As a first approximation toward identifying MED target genes in MS, and identifying regulators that might function in the elaboration of the MS lineage, we are using microarray analysis.

In *mex-1* mutant embryos, the maternal regulator SKN-1 (which activates MED-1,2 in EMS) becomes ectopically expressed in the AB lineage, leading to formation of extra MS-derived tissues. Conversely, in *skn-1* mutant embryos, MED-1,2 are not expressed, and no MS-derived tissue is made. We have used aRNA coupled with microarray analysis (performed by the Kim lab, Stanford) to identify transcripts upregulated in early *mex-1* embryos, and downregulated in *skn-1*. Our preliminary findings will be presented.

158. Genetic Analysis of Asymmetric LET-99 Localization

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Asymmetric cell division, which generates two daughter cells with different cell fates, is important for normal development. Two events are necessary for cells to divide asymmetrically. First, polarity has to be established to position cell fate determinants asymmetrically. Second, the mitotic spindle has to be properly oriented to correctly segregate cell fate determinants into different daughter cells.

In *C. elegans*, PAR proteins are required for generating polarity in the early embryo, and a subset of PAR proteins are necessary for establishing correct spindle orientation. For example, PAR-3 and PAR-2 are asymmetrically localized at the cell periphery in the P cells and are necessary for the asymmetric distribution of PAR-1 and downstream cell fate determinants. PAR-3 and PAR-2 are also required for the normal pattern of spindle orientation in the early embryo. Nevertheless, the precise relationship between these asymmetry cues and spindle orientation remains unclear.

The maternal lethal effect gene *let-99* plays an important role in spindle orientation. *let-99* mutant embryos have defects in nuclear rotation and anaphase spindle positioning. However, the asymmetric distribution of PAR proteins and P granules are unaltered in *let-99* mutants. We have found that the LET-99 protein is enriched as a band in cells undergoing asymmetric cell division. Furthermore, this localization pattern is PAR-3 and PAR-2 dependent. Therefore, LET-99 might act as a link between asymmetry cues and spindle orientation (see abstract by M-F. Tsou and L.Rose).

We are currently examining LET-99 localization in other mutant backgrounds to elucidate the pathway by which LET-99 is localized. Preliminary data suggest that LET-99 localization is normal in par-1 embryos. Taken together, these results suggest that LET-99 localization is in a PAR-1 independent pathway downstream of PAR-3 and PAR-2.

159. Mapping and cloning of generation vulvaless mutants 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*. In *P. pacificus*, seven ventral epidermal cells P(1-4,9-11).p undergo programmed cell death during late embryogenesis.P(5-7.p) form the vulva and P8.p remains epidermal but influences the fate of P(5-7).p. 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* . It has previously been shown that the *lin-39* homologue of *P. pacificus* prevents apoptosis of P(5-8).p and that mutations in *Ppa-lin-39* result in a generation vulvaless phenotype. Double mutants of *lin-39* with the cell death gene *ced-3* indicated that *Ppa lin-39* has an early role in vulva development in *P. pacificus*. It prevents cell death but is dispensable for vulva induction, which is in contrast to *Cel lin-39*.

Several genetic screens were carried out, to isolate new generation vulvaless mutants. We have identified seven novel mutations not allelic to *Ppa-lin-39*. These new mutations fall into 3 new complementation groups, *viz*. *ped-12*, *gev-1* and gev-2*. ped-12* animals are egg-laying defective and haplo-insufficient, i.e. heterozygous animals show a protruding vulva phenotype. Males show pleiotropic defects like absence of rays and spicules and a malformed rectum. Double mutants of *ped-12* with the cell death gene *ced-3* resulted in a vulvaless phenotype indicating that *Ppa ped-12* has both an early and a late role in vulva development in *P. pacificus.*

To facilitate the mapping and molecular characterization of these mutants, we constructed a genetic map of *Pristionchus pacificus*. (Srinivasan *et al*. 2002). *ped-12* mapped to Chromosome-I in *P.pacificus*. Fine mapping of *ped-12*placed it close to the BAC 21N20 containing the *P.pacificus* orthologue of the *lin-2*gene.To narrow down the region containing the gene , I looked for recombinants on both sides of the BAC 21N20. I obtained 3 recominants on 22D6, the neighbouring BAC on the left and 2 recombinants on 13C1, the BAC on the right. This indicates that *ped-12* lies in this region. Currently shotgun sequencing of the BAC 21N20 is in progress to find suitable candidate genes.

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160. Global synteny between *C. briggsae* and *C. elegans*

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Synteny is the evolutionary conservation of gene composition and collinearity between two genomes. We are conducting a global analysis of synteny between *C. elegans* and *C. briggsae* in an effort to understand the selective pressures that maintain or corrupt gene complement and genome structure. In particular, we are using synteny to define orthologs and paralogs, and are examining the extent and distribution of syntenic blocks and breaks in synteny within and between chromosomes.

We have utilized two complementary approaches to explore global synteny between *C. elegans* and *C. briggsae*. The first approach, at the gene level, uses sustained stretches of collinear ortholog pairs to delimit syntenic blocks. The second approach, at the nucleotide level, relies on sequence alignments generated with the WABA algorithm.

The ortholog-based approach to synteny begins with ab initio gene prediction, followed by assignment of ortholog pairs. Operationally, we conservatively assigned orthologs in two ways. First, we assigned genes to an ortholog pair if they were blastp best mutual matches and similar in length. A second approach (used by our collaborators Avril Coghlan and Marc Sohrmann) first "blessed" predicted *C. briggsae* exons that had a high degree of sequence identity, then assayed a combination of exon number, overall identity, and synteny between predicted genes and potential ortholog partners. The intersection of these two sets contains 7,378 primary ortholog pairs. Stretches of collinear orthologs were then used to define the limits of syntenic blocks and to score breaks in synteny. We are now expanding ortholog and paralog assignments using these syntenic blocks as a guide coupled with less-stringent metrics for identity than those used for the initial assignments. *C. briggsae* gene predictions and evidence for orthology will be available via WormBase (www.wormbase.org).

This approach to determining synteny rests heavily on both gene prediction and ortholog assignment algorithms, and will not reveal breaks in synteny contained entirely between two orthologs. To address these limitations, we also used a nucleotide-level similarity approach, exploiting sequence alignments generated using the WABA algorithm for cross species alignment of genomic sequence.

Both approaches reveal a similar degree and extent of synteny between the two genomes, with between 60-75% of the *C. elegans* genome contained within syntenic blocks. Breaks in synteny are far more likely to be due to local, intrachromosomal events than interchromosomal events. Intrachromosomal breaks occur more frequently on autosomes than on the X chromosome, and more frequently on chromosome arms than within the gene-rich clusters. Interchromosomal breaks are more likely to occur between autosomes than between autosomes and the X chromosome. In exploring the context of these breaks, preliminary observations suggest that regions rich in repeats are less likely to be covered by syntenic blocks.

In addition to exploring the global synteny between *C. elegans* and *C. briggsae*, we are characterizing the selective pressure across the genome both on ortholog pairs and paralogs, as well as exploring the expansion and contraction of gene families.

161. Analysis of Dauer Formation Mutations in *C. briggsae*

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During the normal development of the nematode *C. briggsae* and *C. elegans*, the eggs hatch into larvae, which go through a series of molts until they reach the adult stage. However, when faced with unfavorable conditions, young worms respond by following an alternate pathway to form dauers, a stage of developmental arrest capable of surviving relatively long periods of time until conditions become favorable again. Mutations affecting the dauer formation process cause worms to either form dauers even when conditions are permissible (Daf-c) or to lack the ability to form dauers in any condition (Daf-d).

We are interested in molecular mechanisms that underlie evolutionary changes in development, physiology, and behavior. Studying differences between *C. elegans* and *C. briggsae* can reveal the conservation and divergence of genes and pathways through evolution. Dauer formation is useful for this comparison, because it is a pathway shared by both *C. briggsae* and *C. elegans*, easy to isolate mutants, and well understood in *C. elegans*.

As one approach to studying dauer formation in *C. briggsae*, we have isolated new dauer mutants to gain insight into the genes involved in the pathway. A series of EMS mutagenesis screens were performed on wildtype *C. briggsae* to find Daf-c and Daf-d mutants. We have isolated 3 Daf-c mutants and 28 Daf-d mutants of which at least 7 are cilium structure mutants. Further studies will include characterization these mutants by assaying phenotypes under different conditions, mapping, complementation tests, and epistasis analysis.

162. Selection shapes patterns of neutral polymorphism in C. elegans' genome

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Natural selection can produce a correlation between local recombination rates and levels of neutral DNA polymorphism as a consequence of genetic hitchhiking and background selection. Theory suggests that selection at linked sites should affect patterns of neutral variation in partially selfing populations more dramatically than in outcrossing populations. However, empirical investigations of selection at linked sites have focused primarily on outcrossing species. To assess the potential role of selection as a determinant of neutral polymorphism in the context of partial self-fertilization, we conducted a multivariate analysis of single nucleotide polymorphism (SNP) density throughout the genome of the nematode Caenorhabditis elegans. We based the analysis on a published SNP data set (Wicks et al. 2001) and used a sliding-window approach to calculate SNP densities, recombination rates, and gene densities across all six chromosomes. Our analyses identify a strong, positive correlation between recombination rate and neutral polymorphism (as estimated by SNP density) across the genome of C. elegans. Furthermore, we find that levels of neutral polymorphism are lower in gene-dense regions than in gene-poor regions. Our results suggest that a neutral explanation alone cannot sufficiently explain the observed patterns. Consequently, we interpret these findings as evidence that natural selection shapes genome-wide patterns of neutral polymorphism in C. elegans. Our study provides the first demonstration of such an effect in a partially selfing animal. Explicit models of genetic hitchhiking and background selection can each adequately describe the relationship between recombination rate and SNP density, but only when they incorporate selfing rate. Non-linear regression fitting of genetic hitchhiking and background selection models to these data allow the estimation of parameters used in the models, such as outcrossing rate, genomic deleterious mutation rate, and average selection coefficient against deleterious mutations.

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163. Microarray analysis of ethanol effects in the nematode Caenorhabditis elegans identifies ethanol-sensitive genes

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In this report, we wanted to use a model organism and a genomic approach to study the gene network induced by ethanol stress. C. elegans shows responses to acute ethanol exposure that are similar to those observed in humans, mice, and Drosophila, namely hyperactivity followed by uncoordination and sedation. Therefore, we wanted to identify genes that show a difference in expression on ethanol exposure, and to functionally link those genes into an ethanol-induced gene network. After studying the changes in expression profile, further studies can be carried out more easily to elucidate the functions of individual genes in the response to ethanol. After analyszing the expression profiles of all C. elegans ORFs by 15 sets of microarray expeirments, we classified genes affected by ehtanol into five groups. Class I consists of genes whose trascription is induced early and maintained at high level. Class II consists of genes that show transcritional responses late. Class III include genes whose transcription is induced at early time points and then returns back to normal after longer exposure. Class IV genes are those whose transcription is repressed at early time points, and returns back to normal after longer exposure to ethanol. Class V consists of genes whose transcription is repressed at late time points. Genes in Class II and IV may represent those involved in adaptation to ethanol stress. The genes in class III and V may represent physiological consequences of ethanol exposure. We are most interested in genes in class I because these genes may represent those directly involved in ethanol response in C. elegans. These genes include heat shock protein genes, a glutamate receptor gene, a lipase homolog, and a few novel genes. Among ten probable glutamate receptor genes in C. elegans, glr-2 is the only gene to be induced by ethanol. We confirmed the microarray result by time-course Northern analysis. We propose that glr-2 is an ethanol-specific response gene, because we found no significant changes in the glr-2 transcription level in other microarray experiments when examined with 'History' option in the Stanford microarray database . The T28C12.4 gene encodes a novel protein with limited homology to human neuroligin. This gene was expressed in hypodermal cells and found to be specific to ethanol stress since it did not respond to heat shock, nor to high salt. We will present expression patterns of other genes and more updated results.

164. Analysis of Matrix Metalloproteinases in *C. elegans*

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Proteolytic remodeling of the ECM by matrix metalloproteinases (MMPs) plays important roles in the regulation of cellular behaviors including: shape change, migration and motility, growth, survival and differentiation. MMPs also cleave cell surface and pericellular proteins suggesting they can regulate cellular behavior through their control of intracellular signaling as well as their effects on the extracellular environment. Disruption or misregulation of MMP expression can result in developmental defects and phenotypes similar to those associated with various diseases. The MMPs and their endogenous inhibitors, the TIMPs, have been the focus of extensive analysis in the mouse model. However, the large number of MMP genes (>21 vertebrate MMPs) and the multiple layers of MMP regulation have hindered the identification of their exact in vivo function. *C. elegans* have at six potential MMPs as defined by their conserved zinc-binding motif (HEF/LGHS/ALGLXHS) and 'Met turn' (ALMYP). Three of the MMP genes have been demonstrated to produce protein products with protease activity (Wada, 1998). The relatively small number of MMP genes in *C. elegans* and the advantage of powerful genetic assays may allow a more precise elucidation of their function and regulation. I will characterize the expression and localization of all the *C. elegans* MMPs by both reporter assays and immunohistochemistry. Biochemical analysis will be performed using recombinant MMP expressed in *E. coli* to assess proteolytic activity and substrate specificity. RNAi experiments for many of the MMP genes have failed to produce an observable phenotype suggesting that these genes are resistant to targeting or perhaps share overlapping functions as seen in vertebrate models. I will report on attempts to target multiple MMP genes in combination using RNAi. Two genes in *C. elegans* possess similarity to the vertebrate TIMP genes and may encode modifiers of MMP activity. GFP reporter experiments suggest the TIMP genes are expressed in broad overlapping patterns. RNAi experiments have not yielded a phenotype when targeting either putative TIMP gene alone or in combination. The goal is to understand the role of the MMPs in *C. elegans* development including the identification of their substrates. I will report on directions we are taking to identify MMP targets and progress made in identifying deletion mutations in the MMP genes.

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165. Identifying male fertility genes by recombinant inbred mapping

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We have found that the Hawaiian race CB4856 exhibits higher male fertility than N2 Bristol. This difference is due (at least in part) to increased length of fertility in CB4856 compared to N2: Adult CB4856 males are typically fertile for six or more days, while adult N2 males are sterile after four. We are working to determine the physiological cause of this difference (sperm production, aging, etc.) as well as its genetic and molecular basis.

Increased mating efficiency is a property of CB4856 males rather than hermaphrodites. We tested the four possible matings between males and hermaphrodites of both races. Single L4 males were mated to three adult hermaphrodites and transferred daily to new hermaphrodites. Successful matings were scored by the presence of males. By the fourth day of mating N2 males were almost completely sterile (1/19 successful) while CB4856 males mated well (10/15 successful). The race of the hermaphrodite played no role in increased mating.

We tested heterozygous males and found that CB4856 male fertility was dominant to N2 infertility. Similarly, Hodgkin and Doniach (1) found that extended male fertility in the Stanford race CB4855 was dominant to the shorter fertility of N2. Thus, it is possible that short male fertility in N2 is due to mutations in N2 rather than a natural strain variation found in the wild. To test this idea we plan to determine the distribution of male fertility phenotypes in the complete set of *C. elegans* isolates.

To identify the genetic basis of extended male fertility, we first tested the candidate gene *npr-1.* The *npr-1* locus is a source of behavioral differences between N2 and CB4856 (2). Thus, we tested N2 males that carry the EMS-induced *npr-1(n1353)* allele. *npr-1(n1353)*animals, like CB4856, border and clump on bacterial lawns. However, *npr-1(n1353)* males did not have increased fertility compared to N2, suggesting that the *npr-1* pathway does not control male fertility.

We are now mapping the genes that control extended male fertility using recombinant inbred mapping. In recombinant inbred mapping a large number of self-progeny are cloned from a heterozygote between the two strains of interest. Single self-progeny are cloned from each animal, and this is repeated for 10 generations. Each line thus generated is homozygous for a random sample of parental alleles, approximately half of each. The phenotype and genotype of each line is then determined. Recombinant inbred mapping is ideal for potentially complex phenotypes because it can identify multiple genes simultaneously, including modifiers.

We have generated 225 inbred lines from N2/CB4856 heterozygotes and are currently characterizing them for male fertility and genotype. Our data thus far suggest that length of male fertility is a quantitative trait controlled by two or more genes, one of which appears to be a modifier.

Quantitative trait loci present a cloning challenge and few have been cloned from any species. However, due to the very large number of SNP markers between N2 and CB4856 (3), it should be feasible to clone quantitative loci for the male fertility difference. Further, the recombinant inbred lines and their associated genetic information will facilitate mapping any other phenotypic difference between the two strains. In the long run we hope to use this approach to understand the genetic and molecular nature of within-species differences.

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166. Sperm competition and reproductive interactions in *C. elegans*

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The success of sexual reproduction is influenced by interactions between a sperm cell and the female reproductive tract, the egg, and other sperm. We are using *C. elegans* to identify and characterize male factors that are involved in these interactions. *C. elegans* comes in two sexes, self-fertilizing hermaphrodites and males. Early in reproductive development, a hermaphrodite generates and stores a supply of sperm; she then switches permanently to making oocytes, which are fertilized by her "self" sperm. However, if a male mates with the hermaphrodite, his sperm must compete with her self sperm to fertilize eggs. In *C. elegans*, males always win: male sperm are used preferentially, and self sperm are not used until the supply of male sperm is exhausted. It is clearly advantageous for a male to ensure that his sperm is used whenever mating occurs, since in wild populations this opportunity is not always assured.

Male sperm cells apparently have an intrinsic selective advantage over hermaphrodite sperm. Successive mating experiments have shown that sperm from different males do not show any precedence relationship. A variety of experiments have suggested that large sperm may have a selective advantage, and male sperm tend to be larger than hermaphrodite sperm. However, the basis for this apparent size advantage is not clear. The reproducible precedence pattern of male *vs*. hermaphrodite sperm provides an opportunity for us to study how fertility can be modulated.

To identify factors that confer male sperm advantage, we are screening for mutant males that do not show the normal pattern of sperm precedence. We are assaying males from mutagenized *him-5* lines for lack of precedence in crosses to tester *spe-8; dpy-4* hermaphrodites. *spe-8* is important for sperm activation: mutant *spe-8* hermaphrodites make spermatids that are not activated prior to mating. *dpy-4* is used to distinguish self progeny from cross progeny. This screen can also identify mutants with a variety of other male-specific sperm defects. Crosses with males having sperm competition defects should generate a mixture of self and cross progeny, and crosses with males with defective sperm should generate only self progeny. So far we have completed a pilot screen and identified mutants that fall into a variety of phenotypic classes. One class has normal-looking sperm and may have defects in sperm precedence, based on results from mating assays. Another class shows very low or no male fertility (hermaphrodites are fertile); these mutants may represent male-specific spermatogenesis or spermiogenesis mutants. A third class has visible developmental abnormalities that may affect the ability of mutant males to mate efficiently. We are characterizing the mutants to determine which aspect of male fertility (*e.g.*, spermatogenesis, sperm motility, or sperm competition) is disrupted. Using SNP markers, we have identified rough map positions for several mutants.

167. Systematic analysis of post-embryonic roles of embryonic lethal genes by RNAi-by-L1-soaking

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In the development of multicellular organisms, some genes play multiple roles at different developmental stages. (For example, *glp-1* is required for specification of blastomere fates in the early embryo, as well as proliferation of germ cells in larval development.) Although mutant analysis is a powerful method to understand the function of a gene, in general, earlier mutant phenotypes prevent the analysis of later developmental events, which causes the difficulties in characterization of multiple gene functions. One way to circumvent this problem is the use of conditional alleles, but they are far from saturation.

In the conventional RNAi experiments that dsRNA is introduced into L4 larvae or adults, situations are similar in that embryonic RNAi phenotypes mask the later post-embryonic phenotypes. However, as reported by Kuroyanagi et al. (2000) and by some others, RNAi by soaking L1 larvae in dsRNA is an effective way to specifically inactivate post-embryonic gene functions. Therefore, we utilized this RNAi-by-L1-soaking method to systematically examine the potential additional functions of embryonic lethal genes. As an Emb gene set, we used a set of cDNAs that caused embryonic lethality in our previous systematic RNAi analysis with a non-redundant cDNA set. For each Emb cDNA, embryonic phenotypes (by L4-soaking) and post-embryonic phenotypes (by L1-soaking) are characterized with Nomarski microscopy. Approximately one third of embryonic lethal genes caused detectable defects in post-embryonic development, such as, gonadogenesis, gonad migration, germline proliferation, gametogenesis, and vulval development. These data will provide a new set of information to understand multiple developmental functions of each gene.

168. Identification of intestine-specific genes using mRNA-tagging

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The establishment and maintenance of polarity in epithelial cells is essential for their morphology, function and signaling. To generate a global overview of gene expression in the C. elegans intestine and provide a foundation for understanding how this tissue functions at the molecular level, we are profiling gene expression in the intestinal tissue of C. elegans using DNA microarrays. We are using mRNA tagging to identify intestine-expressed genes, and have generated transgenic worms expressing FLAG::PABP in the intestine using the ges-1 promoter. Immunohistochemical analyses has confirmed that FLAG::PABP is exclusively expressed in the intestinal cells from embryonic to adult stages. mRNAs bound to FLAG::PABP are then co-immunoprecipitated using anti-FLAG monoclonal antibody. The co-immunoprecipitated mRNA is used to prepare Cy-5-labeled cDNA, and RNA from the entire worm will be used to prepare Cy-3-labeled cDNA as reference. Both probes are simultaneously hybridized to DNA microarrays containing 17,871 genes (94% of the genome). The experiment will be repeated 6 times to measure experimental variability and statistically assess which genes are significantly enriched in the intestine.

We will then compare the list of intestine-expressed genes to muscle-expressed genes identified previously. Since muscle cells are not separated into apical and basolateral domains, genes involved in the specification or maintenance of cell polarity should be enriched in intestine versus muscle. Once we have a refined list of intestine-enriched genes, we will use RNAi to test the effects of reducing the activity of these genes by observing the general morphology of the intestinal cells and the integrity of the cell junction with the AJM-1::GFP marker. We anticipate identifying new genes that specifically affect intestinal cell polarity and hopefully general epithelial cell polarity.

169. Toward the identification of genes expressed in C. elegans GABAergic neurons

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Formation and maintenance of neuronal circuitry coordinating body movements in *C. elegans*require complex genetic interactions. Cellular components of this circuitry, such as GABAergic motor neurons, provide access to underlying genetic pathways. The functional states of component cells can be revealed by their pattern of gene expression including transcriptional programs executed primarily in them. In this study, we are using DNA microarrays to investigate genes and/or transcriptional programs (e.g. *unc-30*) differentially expressed in GABAergic neurons. A GABAergic neuronal reporter (unc-25::GFP) labels four RME and six DD neurons in the developing embryo. We isolated GFP-positive cells by flow activated cell sorting after acute dissociation of embryos. RNA was extracted from unsorted, sorted GFP-positive, or GFP-negative cell populations, and amplified to generate libraries representing cellular messages in each sample. Preliminary results indicated that higher representation of GABA-neuron specific markers was evident in the GFP-positive population, suggesting an enrichment of GABAergic neurons. In contrast, negative control markers for cholinergic neurons and muscle cells had lower representation in GFP-positive population as expected. Microarray hybridizations will be tested on GFP-positive enriched cells. A future goal is to identify downstream genes regulated by transcriptional factor UNC-30.

170. Identification of new endoderm regulators using an RNAi based genome wide screen

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We are conducting a genome-wide screen in Caenorhabditis elegans to identify genes involved in endoderm development. The endoderm of C. elegans, the only animal for which the entire cell lineage of is known, arises from a single cell, E, in the early embryo. After it is specified, the E cell undergoes up to 5 division rounds resulting in an intestine with 20 cells by the end of embryogenesis. Although several of the regulators of endoderm development have been identified, it is apparent that many steps in the pathway for constructing and maintaining the endoderm have yet to be elucidated. We have identified the primary endoderm-specifying genes, end-1and end-3, which encode GATA-type transcription factors. We found that, while elimination of these genes results in a complete failure to make endoderm, diminishing but not abolishing their activity results in an excess of gut cells; apparently the END-1 and -3 proteins not only specify the endoderm fate but also inhibit cell division in the E cell lineage. We have also found that a number of other mutations in regulators of end-1/3 gene expression similarly result in increased numbers of gut cells made from the E lineage. For example, while elimination of Wnt and MAP kinase signaling pathways, which activate endoderm development by intercellular signaling, results in the failure to specify endoderm, mutant worms with moderately diminished activity of these signaling pathways contain an excess of gut cells. These observations provide us with a sensitive method for identifying mutants that are defective in regulation of endoderm gene activity by looking for mutations that alter gut cell number.

We are performing the screen using RNAi (RNA interference), a procedure that abolishes the activity of a gene by introduction of double-stranded RNA (dsRNA) whose sequence is specific to the gene. We are using the Ahringer Lab's library of dsRNA-expressing bacteria which covers approximately 87% of the predicted C. elegans genome. The endoderm phenotype for each gene is assessed by simply feeding elt-7:GFP expressing worms each dsRNA-expressing bacterial strain and observing their progeny at the L1 stage with a dissecting fluorescence microscope. This approach allows us to quickly screen through the entire genome and immediately identify interesting genes. We have already screened through the entire first chromosome and have identified numerous candidate genes that affect gut cell number and elt-7 expression. We are continuing our screen with the other chromosomes. Using this method we will be able to identify new genes involved in specification of endoderm, gut development and patterning, and maintenance of gut cells.

171. Polyunsaturated Fatty Acids are Important for Normal *C. elegans* Cuticle Function

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The *C. elegans* cuticle plays a role in protection of the worm and determination of worm size and shape. We are studying two mutants with reduced levels of polyunsaturated fatty acids (PUFAs). The *fat-2* mutants lack Δ12 desaturase activity and as a result are devoid of 18- and 20-carbon PUFAs, while the *fat-3* mutants lack Δ6 desaturase activity and consequently lack 20-carbon PUFAs. The *fat-2* and *fat-3* mutants have 2.7 and 2.6 mol % of 20-carbon PUFAs respectively, whereas wild-type worms have 43.9%. Both *fat*-2 and *fat*-3 mutant worms disintegrate faster than wild-type worms when treated with an alkaline hypochlorite solution. In addition, both lines have a Dumpy (Dpy) phenotype. These observations indicate that the mutants are defective in cuticle function. In both mutant lines, phenotypic complementation of the mutant characteristics by addition of 20-carbon fatty acids to the growth medium establishes that the phenotypes are specifically a result of 20-carbon fatty acid deficiency. To investigate the consequences of fatty acid biosynthesis mutations on cuticle structure we used transmission electron microscopy (TEM) to examine *fat*-2 and *fat*-3 mutants. Cross-sections of *fat*-2 and *fat*-3 mutants, as well as wild-type worms revealed the presence of cortical, intermediate, fibrous, and basal layers. In addition to the total cuticle thickness, the cortical and fibrous layers were thinner in the *fat*-2 and *fat*-3 mutants than in wild-type worms.

172. Characterizing MEL-26, the post-meiotic inhibitor of MEI-1/MEI-2 microtubule severing.

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A number of genes have been identified that have an essential role in the oocyte meiotic and early mitotic spindle formation. We focus on *mei-1* and *mei-2*, which are essential for the meiotic spindle and *mel-26*, which is required for proper mitotic spindle formation. *mel-26* encodes a novel 44.5 kDa protein with the exception of two conserved domains. The N-terminal MATH (**m**eprin **a**nd **T**RAF **h**omology) domain is known to be required for proper folding of activable enzymes (1). The centrally located BTB (**B**ric a Brac, **T**ramtrack and **B**road Complex) domain, implicated in protein-protein interactions (2), has been shown to mediate both homomeric and heteromeric protein dimerisation (3).

Genetic evidence determined that *mel-26(+)* functions as a post-meiotic inhibitor of *mei-1* and *mei-2* (2). *mei-1* is similar to the catalytic p60 subunit of the sea urchin microtubule-severing protein, katanin, while *mei-2* encodes a protein similar to the p80 subunit of katanin (4,5). The sea urchin p80 subunit appears to function in targeting the heterodimeric katanin protein to the centrosome (4). In vitro microtubule severing assays indicate that MEI-1 and MEI-2 function as a complex to sever microtubules (5).

In wildtype embryos MEI-1 and MEI-2 are found in meiotic but not mitotic spindles. In *mel-26(lf)* and *mei-1(gf)* mutant embryos mitosis is abnormal due to ectopic MEI-1/MEI-2 localization (and presumably ectopic microtubule severing), resulting in small, misoriented mitotic spindles. Thus, MEL-26 functions to inhibit MEI-1/MEI-2 following meiosis, allowing the large microtubule asters of the mitotic spindle to assemble.

Kurz, T., *et al.*, (6), recently described an E1 activating enzyme, *rfl-1*, required for the *ned-8* ubiquitin-like (UBL) pathway in *C. elegans*. The MEI-1/MEI-2 katanin complex appears to be a target of this UBL pathway, suggesting that *mel-26* is a new component of this pathway as well.

A MEL-26 antibody is currently being characterized to further elucidate the function of this protein in the early embryo. Molecular characterization of MEL-26 by immunolocalization and western blotting will be described. Preliminary yeast-2-hybrid results indicate that MEI-1 interacts with itself and MEI-1*(gf)* in addition to both MEI-2 and MEL-26. However, the interaction between MEL-26 and MEI-1*(gf)* is abolished, likely preventing MEL-26 from acting as a post-meiotic inhibitor.

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173. Spindle Movements during Female Meiosis

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During female meiosis, a haploid chromosome number is achieved by sequentially segregating half of the homologous chromosomes, then half of the sister chromatids into polar bodies. Both meiotic segregation events require positioning a microtubule-based spindle at the cell cortex in an orientation perpendicular to the cortex. In many metazoans, this final spindle position is achieved after the meiotic spindle first translocates through the cytoplasm to the cortex and subsequently rotates to the perpendicular orientation. These spindle movements are critical for proper segregation of chromosomes into polar bodies, yet the molecular basis of these movements is largely unknown. We are using *C. elegans* as a model system to determine the molecules and mechanisms that drive these spindle movements. Meiotic spindle movements were observed by time-lapse imaging of intact, anesthetized worms expressing GFP-beta tubulin or GFP-histone H2B. Forty to fifty minutes elapsed between the start of meiosis at nuclear envelope breakdown and the end of meiosis marked by formation of the female pronucleus. After nuclear envelope breakdown, an 8 um long, anastral spindle assembled several um from the cell cortex. The spindle then translocated to the cortex with an average velocity of 1.5 um/min. The spindle initially adopted an orientation roughly parallel to the cell cortex and slid along the cortex for an average of 8.8 um before the metaphase-anaphase transition. At the metaphase-anaphase transition, the 8 um spindle first shortened to 6 um in length, then rotated perpendicular to the cortex. After rotation, separation of homologous chromosomes occurred as the spindle continued to shorten to a final length of 2.5 um. As the first polar body formed, the short post-anaphase spindle narrowed and lengthened into a midbody. Meiosis II proceeded in a similar fashion. To test whether microtubules are required for meiotic spindle movements, GFP-histone time lapse imaging was conducted on tubulin (RNAi) worms. Translocation of meiotic bivalents to the cortex was completely blocked in these worms indicating that microtubules are required for translocation of bivalent chromosomes to the cortex. The spindle checkpoint was apparently not activated by this treatment because homologs separated with normal timing. Diakinesis nuclei were also mispositioned relative to the oocyte cortex in tubulin (RNAi) worms, raising the possibility that the failure in bivalent translocation was an indirect effect of starting out too far from the cortex. *Zyg-9* null worms, however, also had mispositioned diakinesis nuclei but meiotic bivalents translocated to the cortex after maturation (9/9 worms). In addition, in worms null for *mei-1*, a microtubule regulator, bivalents failed to translocate to the cortex in 50% of matured oocytes (4/6 worms). These results indicate that microtubules are directly involved in translocation of bivalent chromosomes to the cell cortex. To test the role of F-actin in spindle movements, time-lapse imaging was conducted on worms treated with latrunculin A. All aspects of meiosis I spindle translocation and rotation were normal in latrunculin-treated worms but polar bodies did not form and the segregated chromosomes collapsed back together at the end of anaphase I. In addition, the narrowing of the post-anaphase spindle to a midbody was slow and incomplete. These results indicate that meiotic spindle translocation and rotation are less dependent on F-actin than is polar body formation. Further studies will concentrate on the motors and microtubule dynamics regulators involved in meiotic spindle translocation.

174. *hal-2* , a gene involved in pairing of homologous chromosomes during meiotic prophase

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To segregate away from each other at the meiosis I division, homologous chromosomes must first locate and recognize appropriate pairing partners within the nucleus. Few molecules involved in initial pairing and recognition of homologous chromosomes at the onset of meiotic prophase have been identified in any organism. To investigate the molecular requirements for the initial pairing of homologous chromosomes in *C. elegans*, we screened through a collection of meiotic segregation mutants to identify mutations that specifically disrupt this process. The spatio-temporal organization and distinguishable characteristics of distinct substages of meiotic prophase within germline nuclei of the *C. elegans* gonad facilitates analysis of meiotic events both because germline nuclei move proximally as they progress through prophase and chromosome organization is readily detectable within each nucleus. Therefore, the cytological appearance of the germline after DAPI staining can reveal alterations in specific stages of meiotic development.

This approach identified *hal-2* (homolog alignment) as essential for the establishment of pairing between homologs. In wild-type germlines, the entry into meiotic prophase is accompanied by a nuclear reorganization that is absent in *hal-2* mutant germlines. Additionally, chromosomes at the pachytene stage in *hal-2* mutant germlines are not properly aligned in parallel pairs. FISH experiments indicate this altered organization correlates with a defect in the initial establishment of paired associations in the *hal-2* mutant. Despite this early defect in meiotic prophase, the meiosis-specific axis component HIM-3 loads onto chromosomes in *hal-2* mutant germlines, indicating that nuclei have entered the meiotic program and that some aspects of chromosome axis morphogenesis are unaffected in *hal-2* mutants. Surprisingly, synaptonemal complex (SC) central region protein SYP1 loads extensively along the lengths of asynapsed chromosomes in *hal-2* mutant germlines, suggesting that HAL-2 activity may be important to prevent premature loading of SC components. Mapping of the *hal-2* mutation has localized it to a ~230 kb region on chromosome III between *rgs-1* and*div-1*. We will report on our efforts to identify the gene affected by the *hal-2* mutation.

175. Finding new components necessary for correct meiotic chromosome pairing
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Meiosis is an evolutionarily conserved mechanism responsible for the production of haploid gametes in organisms with sexual reproduction. During meiosis two rounds of chromosome segregation following a single round of DNA replication halve the diploid complement of chromosomes. For an orderly segregation of homologous chromosomes (homologues) during meiosis I, each chromosome must first recognize and then pair and recombine with its correct homologue. Genetic recombination results in the formation of chiasmata, the physical links that allow homologues to remain together (forming a structure known as bivalent) until they migrate to opposite poles during the first meiotic anaphase. Homologue pairing and alignment during meiotic prophase coincides in many organisms with a major reorganization of the nucleus involving chromosome movements and changes in chromatin condensation. The goal of our studies is to understand the mechanisms controlling the process of chromosome pairing during meiosis.

We have set up a genetic screen to isolate meiotic mutants displaying cytological defects at different stages of meiotic prophase. The screen uses a histone H2B-GFP fusion protein expressed in the germ line to allow visualization of chromosomes at all stages of meiotic prophase. We initially screened for mutants in two main phenotypic categories: 1) those with achiasmate chromosomes at diakinesis, the last stage of meiotic prophase (which could reflect a prior defect in homologue pairing, synapsis or recombination) or 2) those that fail to complete meiotic prophase. To detect defects in chromosome pairing we have analyzed the isolated mutants by fluorescence in-situ hybridization (FISH). Two mutants showing pairing defects have been isolated so far.

me84 shows a severe pairing defect at pachytene (the meiotic prophase stage at which homologues are fully aligned along their lengths) and produces 12 achiasmate chromosomes (univalents) at diakinesis (as opposed to 6 bivalents in N2). Surprisingly, DAPI staining reveals that chromosomes are synapsed, suggesting that synapsis is occurring between non-homologous chromosomes. Furthermore, by following individual chromosomes at pachytene it is possible to observe changes in pairing partner indicating that non-homologous chromosomes are synapsing. The components of the synaptonemal complex (SC) HIM-3 and SYP-2 are loaded onto chromosomes and they form linear structures similar in appearance to those seen in N2. However, SYP-2 (a central element component of the SC) is present at diakinesis between the sister chromatids of the univalents while in N2 SYP-2 localizes between homologues and not between sister chromatids. This suggests that the me84 mutant is defective in regulating chromosome association of SYP-2.

me85 shows a partial pairing defect at pachytene (not all the nuclei show lack of pairing), but the most striking phenotype is the presence throughout the gonad of endoreplicated nuclei. The size of these nuclei is many times that of normal diploid nuclei. These endoreplicated nuclei seem to be engaged in the meiotic program since they load SYP-2. Diakinesis nuclei contain a mixture of univalents and vibalents, showing that chiasma formation is not completely eliminated. Even though diakinesis chromosome morphology looks normal, nuclei in the embryos display irregular shapes and alterations in chromatin condensation resulting in embryo lethality.

Finally, we have developed a new method for cytological observation of meiotic nuclei. This method is based on the squashing of gonads, which produces a mono layer of flattened meiotic nuclei. This procedure facilitates analyses that involve tracing the entire length of a chromosome, or chromosome pair, which are difficult in intact gonads.

176. Direct screening for meiotic homologous pairing mutants in *C. elegans*

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In early meiotic prophase, chromosomes find their homologous partners and align along their entire lengths. Within this context, crossover recombination events take place between the DNA molecules of homologous chromosomes to produce chiasmata that ensure proper chromosome segregation at meiosis I. How homology is recognized and how homologues come together during the pairing process are not understood. The nematode *C. elegans* offers several advantages for investigating the molecular basis of meiotic homologous pairing: germline nuclei are visible in live animals, and they are arranged in spatial-temporal manner that permits examination of every stage in meiotic prophase. We are taking two distinct strategies to screen for pairing-defective mutants. One strategy uses a strain expressing GFP-tagged histone H2B to identify mutants with abnormal organization or morphology of chromosomes in early meiotic prophase and/or univalents in oocytes at diakinesis, the last stage of meiotic prophase. We have isolated several pairing-defective mutants in this screen. Among them,the *me80* mutant shows a strong pairing deficiency detected by FISH. The *me80* mutation is a new allele of *him-3*, which encodes a component of meiotic chromosome axes. Interestingly, the pairing defect in *him-3* (*me80*) hermaphrodite germ lines is much more severe for autosomes than for X chromosomes; the reduced amount of HIM-3 protein present in the mutant appears to load preferentially on the X chromosomes. Furthermore, in this mutant, one end of the X chromosome achieves stable pairing with normal kinetics while pairing at the other end is significantly delayed, suggesting that the requirement for HIM-3 for pairing may differ among chromosomal loci. Our second screening strategy uses LacI-GFP expressed in the germ line in conjunction with its binding target (multiple repeats of the lacO sequence) integrated into the genome. We have recently established this system and verified both normal meiotic pairing kinetics for the lacO loci in wild type worms and defective pairing in pachytene and diakinesis nuclei when synapsis is perturbed. We are now using this system to screen for pairing defects elicited either by RNAi or by EMS mutagenesis.

177. Using a Combination of Two Recombinases to Create Targeted Single-copy Genomic Insertion in *C. elegans*

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The most commonly used technique to create transgenic *C. elegans* animals has several disadvantages for a number of applications, such as promotor studies or studies on gene regulation. Extrachromosomal arrays created by microinjection contain, for instance, the transgene at a high copy number (1). This can cause silencing and/or misregulation of the transgene. The ideal technique for creating transgenic animals (at least for some applications) would be a technique that allows the insertion of a single copy of a transgene at a specific chromosomal location.

The target recombination site for the Cre recombinase is the *loxP* site and for the FLP recombinase the *FRT* site. We call a sequence flanked by a *loxP* site at the 5' end and by a *FRT* site at the 3' end a "Frox" element or a "froxed" sequence. It has been shown that the combination of Cre and FLP mediates the efficient exchange of a Frox element within the genome against a froxed DNA sequence located on a plasmid in ES-cells of mice (2). In order to use "Froxing" to create transgenic *C. elegans*, we tested whether Cre and FLP can mediate recombination in *C. elegans*. To that end, we created transgenic animals carrying an extrachromosomal array containing four different elements: a Frox element with the Neomycin resistance gene as a spacer between the two recombination sites, a froxed *gfp* coding sequence and the *cre* and *flp* coding sequences, each under the control of the *hsp16-2* promoter. Using a PCR-based assay, we confirmed that frox-mediated exchange took place in transgenic animals that had been heatshocked, however, not in animals that had not been heatshocked. From this we can conclude that froxing can be used to mediate exchange of DNA fragments in *C. elegans*.

To establish the froxing method for the creation of targeted, single-copy genomic insertions in *C. elegans*, we are in the process of constructing a strain with a single genomic Frox element. To create this single insertion of the Frox element, we want to use a modified MOS1 transposable element (3).

(1) Mello C et al. (1991) *EMBO J*, 10(12):3959-3970
(2) Lauth M and Meyer M, unpublished results
(3) Besseareau J-L, (2001) *Nature*, 413(6851):70-74

178. Developing new techniques

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We have been attempting to add a number of new technologies to the palette of worm genetic techniques. So far, all of these are under development. For the latest updates, or if you have any ideas, please come by my poster.

Homologous recombination: The ability to modify chromosomal sequences using homologous recombination would enable targeted knockouts as well as gene replacement. This technique requires the introduction of a linear fragment of DNA, containing free ends, into a germ cell nucleus. Recombination is induced by the free ends, so that the DNA is incorporated into the homologous position on the chromosome. Injection of linear DNA into the syncytial gonad arm never leads to homologous recombination, probably because the free ends are ligated before the DNA gets into a germ cell nucleus. We have tried using I-SceI to liberate a linear fragment from an array (a modification of the Rong and Golic (Science. 2000) approach from flies) with no success. We have also attempted to inject recombinogenic linear fragments directly into the nucleus. These fragments have been ends-in with large 3' overhangs. After a substantial number of injections, we have isolated a number of integrated transgenes, but no homologous events. We also hope to improve the efficiency of recombination by co-injecting the recombination enzyme RAD-51 with our linear DNA. Progress towards this and other approaches will be presented.

flp/FRT site specific recombination: The flp/FRT system has been used in many organisms to generate conditional expression of a gene, to generate chromosome rearrangements, and to insert single copies of a transgene into defined chromosomal positions. We are attempting to adapt the flp recombinase for these applications in worms. *Mos1* was used to introduce single copy FRT elements into the genome. We have not yet achieved a verified recombination between tandem FRT elements on a transgene, probably due to inefficient expression of the flp gene in the germline.

***Mos1* transposition:** Bessereau et al.(Nature. 2001) have shown that the mariner-like *Mos1* transposon can hop in worms. We are attempting to modify the *Mos1* element to expand its utility. Unfortunately, we have found that transposition of *Mos1* is very sensitive to the internal sequence of the element. Insertion of more than a few hundred base pairs completely eliminates the ability of the element to mobilize from an extrachromosomal array. This is consistent with observations of *Mos1* in *Drosophila*. This aspect of *Mos1* precludes its use for enhancer trap techniques. However, it would be useful to have a phenotypically visible marker in the *Mos1* element so that it could be followed during a mutagenesis. We are attempting to find a small gene that can be inserted into *Mos1* and retain its ability to mobilize. We have tried *lin-4*, *dpy-30*, *sup-7*, and *sup-21*. Each of these has unique problems that have stymied my efforts to date, but efforts to overcome these are ongoing. In addition, we are also attempting to use other mariner-like transposons that might be more amenable to internal modification.

179. A System for Measuring Worm Movement; Improvements and Applications.

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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, which reflects 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.

Our software analysis uses Matlab on a Windows platform. We have improved the software in the last year such that it is close to being exportable for worm biologists use. We have increased the number of metrics we can automatically calculate to include point velocities as well as centroid velocity. We have also added algorithms for data conditioning that allow us to interpolate across missing or erroneous data produced as a result of data collection. In addition, we have added some help functions for syntax, improved the graphical user interface, and have added algorithms to reduce data noise.

We are currently using our system to quantify changes in movement resulting from exposure of C. elegans to toxins. Nematodes respond to increasing doses of both aldicarb and arsenite by slowing their rate of movement as measured by a decrease in the instantaneous velocity of the centroid of the worm. There is a corresponding decrease in the frequency and amplitude metrics indicating reduced muscle activity in the presence of toxic agents. Our goal in quantifying nematode response to toxins is to obtain worms suitable for use in a remote biosensor capable of detecting toxins in the environment. A compact, integrated system has been developed at the Jet Propulsion Laboratory with funding from Dr. Alan Rudolph of the DARPA Activity Detection Technologies Project.

180. Worms on the International Space Station

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C. elegans was proposed as a model system for space biology studies in 1991 and has since flown on STS-42, STS-76, and STS-95. Data obtained from these flights have confirmed that *C. elegans* requires adequate in flight oxygenation and displays an increased rate of mutation, much like other organisms in space. Unlike vertebrates, *C. elegans* has been observed to successfully complete two continuous full life cycles in space without gross developmental abnormalities. These observations, coupled with the utility of *C. elegans* as a terrestrial, fully sequenced, model system make *C. elegans* a good candidate for long term research onboard the International Space Station (ISS).

We are currently working on technology to support biological studies aboard the ISS. A component of this effort is in the development of the Space Station Biological Research Program (SSBRP) Incubator which will be able to house organisms at constant temperature setpoints ranging from 4ºC to 45ºC. The SSBRP Incubator provides air exchange, power, data and video ports and, when placed in the collaborative NASA/NASDA 2.5M centrifuge rotor, will be capable of providing a 1*g* gravity control. Current plans for validation of the Incubator include video monitoring and periodic sampling of *C. elegans* in the Incubator onboard the ISS. Once returned to earth, samples will be distributed for analysis via a specimen sharing plan and analyzed for gene expression and other parameters of growth and development in space flight. These data should provide the *C. elegans* research community with a baseline from which to propose studies for future flights.

We have also been developing an appropriate method of culturing *C. elegans* in liquid media in order to remove the need for the crew to assure that strains are properly fed. Currently, we are growing strains in the chemically defined, axenic, media developed by Dr. Nancy Lu[1]. Wild-type animals complete multiple generations and appear generally healthy after being grown in unchanged media for a minimum of three to four weeks. To assure proper oxygenation we have chosen to grow animals in ten milliliter OptiCellsä. The choice of the OptiCelläalso allows for automation of culturing as demonstrated for cell cultures grown in the OptiCellä. Arrangements have been made for the liquid media to be commercially produced by Mediatech, Inc. and OptiCellsäare available from BioCrystal Ltd.

Reference:

[1]Lu, NC; Goetsch KM. Carbohydrate requirement of *Caenorhabditis elegans* and the final development of a chemically defined medium. *Nematologica* 39(3): 303-311, 1993

181. 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 AVD, 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. Previously, we showed that a GFP-tagged version of NAB-1, the *C. elegans* homolog of the vertebrate synaptic protein, neurabin, localizes to discrete puncta in axial nerve cords and on the inside surface of the nerve ring. Our evidence now suggests that these synapses are neuromuscular junctions and that NAB-1 is normally expressed in muscle cells and therefore postsynaptic to motor neuron inputs. Since we are seeking a synaptic marker expressed in motor neurons and therefore postsynaptic to interneuron inputs, NAB-1 is unlikely to be useful for this purpose. The non-NMDA glutamate receptor subunit, GLR-4, is expressed in B-class motor neurons[1] and is therefore an attractive candidate. The goal now is to determine if GLR-4 localizes to the motor neuron postsynaptic membrane and then to confirm that a GFP-tagged version of GLR-4 can be used to score specific synapses between command interneurons and motor neurons in the ventral nerve cord.

We have recently expanded our pallette of GFP markers to include dsRed2. We have optimized a configuration of laser lines and fluoresence filter sets in the confocal microscope to produce three-color images of CFP and YFP-labeled motor neurons and dsRed2 expressing body wall muscle cells. In the future, we will test dsRed2 expression in neurons and as a synaptic protein tag.

[1] see Von Stetina et al. (this meeting)

182. Alae Mutant Defects Assessed by SEM and TEM

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We previously reported movement defects in adult C. elegans after RNAi (dsRNA feeding) with the gene PKC4, which encodes a novel Ser/Thr protein kinase (Ren et al., 2001). The PKC4 protein is expressed highly in the seam cells in late-stage embryos and again in late L4 stage, at the time of morphogenesis for seam-cell derived cuticular structures, the alae. It seemed likely that the movement defects, a sloppy zig-zag body motion of variable amplitude when placed on standard agar plates, were the result of absent or poorly formed alae. We have fixed adult animals for examination by scanning electron microscopy (SEM) and transmission electron microscopy (TEM) and compared these PKC4 RNAi animals to adult wild type. We will show SEM evidence for the normal extent of adult alae and several other hallmark features of the body surface, and TEM data for the corresponding seam cell cytology which underlies the alae.

As predicted, the "knockout" animals often show alae defects or complete loss of alae locally, with a penetrance which agrees well with the prevalence of movement defects. The most common defect is a merger of the tripartite ala structure into a large smooth ridge of cuticle, or less often, a zone of smooth cuticle with no ridge at all. In severely affected PKC4 RNAi animals, some tissue defects can be seen in seam cells, which can wander away from the midline, or in the local hypodermis, which can show local swelling . Misassembled plaques of secreted material are sometimes seen in the cuticle in regions corresponding to the missing alae.

183. Modeling of human mitochondrial diseases in the nematode *Caenorhabditis elegans*

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The primary function of mitochondria is to provide energy for the cell via the process of oxidative phosphorylation. This process is achieved by the mitochondrial respiratory chain (MRC). The MRC is composed of four electron-transporting protein complexes (I through IV) that generate a proton gradient across the mitochondrial inner membrane, and the ATP synthase (complex V) that uses the proton gradient for ATP synthesis. Defects in one or more of these complexes can cause a variety of multisystemic diseases. Mitochondrial dysfunction occurs with an estimated incidence of 1 in 10,000 live births, and is often caused by a deficiency in complex I, the NADH:ubiquinone oxidoreductase. Complex I dysfunction is associated with a diverse group of disorders including myopathies, encephalomyopathies, and neurodegenerative disorders such as Parkinson's disease. The human *NDUFV1* gene encodes a 51-kDa subunit of complex I that forms the enzyme's active site. Two independent mutations (Ala341Val and Thr423Met) were found to cause myoclonic epilepsy and muscular hypotonia; the latter mutation resulted in infantile death.

To investigate the molecular mechanisms responsible for this complex I disorder, we introduced the *NDUFV1* mutations in the *C. elegans* homologue *nuo-1*, which encodes a protein with 75% amino acid identity to the human protein. The mutant *nuo-1* genes were transformed into a *nuo-1* knockout strain to create transgenic lines with extrachromosomal arrays. Mutant transgenes *nuo-1*^{A352V} and *nuo-1*^{T434M} both complemented the *nuo-1* knockout phenotype of third larval stage arrest, but these transgenic animals were clearly unhealthy with severe reproductive defects. Two arrays carrying the *nuo-1*^{A352V} transgene were integrated into the genome by UV-irradiation to stabilize transmission and expression.

The integrated *nuo-1*^{A352V} transgenic lines demonstrated a number of abnormal phenotypes: an extended larval developmental period, decreased life-span, abnormal gonad development and oocyte production, abnormal vulval development causing egg-laying defects, and premature tissue degeneration. To identify defects in metabolic pathways biochemical supplements were added to the growth medium. The nature of the supplements that produce beneficial effects could provide clues as to the molecular basis of pathogenesis caused by the mutation. Our results indicated that activators of the enzyme pyruvate dehydrogenase (PDH) have significant beneficial effects on mutant animals by dramatically increasing their reproductive capabilities and moderately extending their life-span. PDH activators have been used clinically to treat patients with lactic acidosis caused by mitochondrial dysfunction. Other compounds, such as the free-radical scavenger ascorbic acid, also have significant beneficial effects. These compounds may help to decrease the NADH/NAD^+ and/or lactate/pyruvate ratios, which are known to increase due to complex I dysfunction. We hope the results of our investigation will allow us to develop novel avenues of therapy for specific mutations of complex I.

184. The endophilin-related protein ERP-1 regulates mitochondrial division

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Mitochondria form a dynamic tubular network that undergoes both fission and fusion. Mitochondrial division is necessary in order to distribute mitochondria to progenitor cells and also plays a role in apoptosis. We think that there are two different mitochondrial division mechanisms, one for the inner membrane and one from the outer membrane. Our hypothesis is that the inner membrane division mechanism is more closely related to that of bacterial division while the outer membrane division mechanism is similar to that of endocytosis. The scope of this investigation is to explore the function of variants of endocytosis proteins in mitochondrial division.

The role of dynamin in endocytic vesicle formation is mirrored by the role of dynamin-related protein DRP-1 in mitochondrial division. We now provide evidence that endophilin, a protein that induces membrane curvature at the necks of budding endocytic vesicles, also has a mitochondrial counterpart, which we call ERP-1 in *C. elegans*. We find that loss of ERP-1 function in *C. elegans* muscle cells selectively inhibits mitochondrial division, causing the mitochondria to be nearly contiguously networked. In contrast, overexpression of ERP-1 causes mitochondria to fragment. Time lapse photography shows that an ERP-1::YFP fusion protein colocalizes with CFP::DRP-1 in spots on mitochondria. We conclude that ERP-1 cooperates with DRP-1 during mitochondrial division. The discovery that two very different endocytic proteins have relatives required for division of the mitochondrial outer membrane suggests that the machineries for these two processes share a common evolutionary origin.

It has recently also been shown that Drp1 is required for apoptosis and cytochrome C release in mammalian cells. We are currently investigating whether ERP-1 is also required for apoptosis and which, if any, of the programmed cell death genes in *C. elegans* affect mitochondrial division.

185. Search for Proteins Affecting Division of the Mitochondrial Inner Membrane

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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. Although we suspect that DRP-1 acts as a part of a larger protein complex, we know of no factors affecting the inner membrane. Here,we looked for novel factors that control inner membrane division, utilizing various bioinformatic techniques: presence of mitochondrial leader sequence; use of phylogenetic profiles and the gene expression map of C.elegans or homologues of candidate genes identified in other organisms, such as a recently published yeast deletion library screen. We came up with ten candidate proteins which were tested by injecting antisense constructs under control of the myo-3 promoter along with GFP targeted to mitochondria. Two of these proteins, E02H1.2 and F58G11.1a showed promising phenotypes and were further tested by overexpression and localization analysis. Our preliminary results suggest that these proteins affect division of the mitochondrial inner membrane.

186. Nematodes with Mitochondrial Diseases

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The mitochondrial respiratory chain (MRC) is composed of five protein complexes capable of generating ATP for most tissues. Its biogenesis requires the coordinate expression of genes from both the nuclear and the mitochondrial genomes. A defective MRC has been implicated in a wide variety of human diseases including diabetes, myopathies, neuromuscular and heart diseases.

We are developing the nematode, *Caenorhabditis elegans* as a model system for mitochondrial diseases and for understanding the relationship between genotype and phenotype. We have isolated 2 nuclear MRC mutations in the *nuo-1* and in the *atp-2* genes encoding the active site subunits of complexes I and V, respectively. Both mutations are homozygous lethal and result in developmental arrest at the third larval stage (L3). We have also isolated a mitochondrial DNA mutation affecting four MRC genes; however, this mutation is aphenotypic. Interestingly, when the replication or expression of the mitochondrial genome is impaired with ethidium bromide, chloramphenicol or doxycycline, nematodes arrest at L3 and are phenotypically similar to the nuclear mutants.

We conclude that MRC dysfunction in *C. elegans* is lethal and produces a distinctive L3 arrest phenotype. The phenotype can be elicited by interfering with nuclear or mitochondrial genes encoding subunits of the MRC. Since approximately 200 gene products are needed for MRC assembly and mitochondrial DNA replication, transcription and translation, we predict that L3 arrest will be characteristic of mutations in these genes.

187. Serotonin acts through G_o to modulate the timing of calcium transients in *C. elegans* egg-laying muscles

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Neuromodulation, the modification of an excitable cell's state through the activation of G-protein coupled receptors, is a topic that has been studied extensively in *C. elegans*. In particular, a number of research groups have focused on the neuronal signal transduction network involving two G-proteins, GOA-1 (most closely related to mammalian G_o) and EGL-30 (similar to mammalian G_q). The functional activity of this signaling network has typically been assessed using egg-laying and locomotion phenotypes: loss of GOA-1 function or gain of EGL-30 function leads to constitutive egg-laying and hyperactive locomotion, while loss of EGL-30 function or gain of GOA-1 function leads to defective egg-laying and sluggish locomotion (Segalat et al, 1995; Mendel et al, 1995; Brundage et al, 1996). These phenotypic criteria have led to the identification of a large number of putative regulators and effectors of G-protein signaling in neurons, including RGS molecules, a CaM kinase II homologue, and components of the phospholipase C signaling pathway (Koelle et al, 1996; Hajdu-Cronin et al, 1999; Robatzek et al, 2000; Lackner et al, 1999; Nurrish et al, 1999).

Despite the extensive genetic analyses that have been performed to date and the important insights they have yielded into the nature of G-protein signaling pathways, the specific effects of these pathways on the functional properties of individual excitable cells has been more difficult to examine.

In this study, we have investigated the effects of the neuromodulator, serotonin (5HT), and G_o signaling on an individual excitable cell-type, the vulval muscles, through *in vivo* calcium imaging. In this way, we have found that 5HT stimulates egg-laying at least in part by altering the calcium dynamics of the vulval muscles, switching them from a state in which transients are sporadic and clustered to a state in which there is a more sustained train of events. We have also found that this action of serotonin is independent of the neurons that innervate the vulval muscles, indicating a direct action of serotonin on muscle calcium dynamics. While the *goa-1* reduction-of-function allele, *n1134*, enhances vulval muscle calcium influx through the removal of G_o's inhibitory influence in neurons, a serotonin-resistance phenotype is simultaneously produced in the vulval muscles, suggesting that muscle G_o, in contrast to neuronal G_o, may be a direct effector of serotonin's stimulatory action on egg-laying behavior.

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Hajdu-Cronin YM et al. *Genes Dev* **13**:1780-1793.
Koelle MR et al. *Cell* **84**:115-125.
Lackner MR et al. *Neuron* **24**:335-346.
Mendel JE et al. *Science* **267**:1652-1655.
Nurrish S et al. *Neuron* **24**:231-242.
Robatzek M et al. *Genetics* **156**:1069-82.
Segalat L et al. *Science* **267**:1648-1651.

188. Voltage-gated calcium channel and Rac-like GTPase genetically interact with rpm-1 to coordinate synapse formation

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Presynaptic terminals are highly specialized subcellular structures to facilitate neurotransmitter release. In a presynaptic terminal, synaptic vesicles are docked at or localized in the vicinity of the active zone, an electron dense structure at the presynaptic plasma membrane and facilitating synaptic vesicle docking and fusion. The periactive zone surrounds the vesicle clustering and active zone region. In *rpm-1* mutants, the presynaptic terminals at the GABAergic neuromuscular junctions (NMJs) have multiple active zones within a single presynaptic terminal (Neuron. 26:331-43.). The RPM-1 protein has an RCC1-like guanine nucleotide exchange factor domain and RING-H2 finger. RPM-1 protein appears to be localized to periactive zone.

To identify genes that function in RPM-1 signaling or in other parallel pathways in synapse formation, we have performed genetic enhancer and suppressor screens of *rpm-1*. We have isolated alleles of *unc-2* and *mig-2* as enhancers of *rpm-1*. *unc-2* encodes the alpha1 subunit of voltage-gated calcium channel (VGCC) (non-L-type). VGCC is localized at the active zone of synapse and required for neurotransmitter release. *unc-36,* which encodes the alpha2 subunit of VGCC, also enhances *rpm-1*. However, *egl-19*, which encodes the alpha1 subunit of VGCC (L-type) and is expressed mainly in muscles, does not enhance *rpm-1*. Other mutations in active zone proteins including *C. elegans unc-10*/RIM and *unc-64*/syntaxin do not enhance *rpm-1*. These results suggest that the effects of VGCC on synapse formation are probably not a consequence of its effects on synaptic transmission rather may reveal that VGCC is required for maintaining the presynaptic architecture.

We found that null mutation of *mig-2*, which encodes Rac-like GTPase, enhances *rpm-1*. But mutations in *ced-10* and *rac-2*, the other two genes encoding *C. elegans*Rac-like GTPases, do not enhance *rpm-1*. Moreover, *rpm-1; mig-2* double mutants show abnormal branching of commissures of DD and VD neurons. These results suggest that *rpm-1* and *mig-2* may function in parallel pathways in synapse formation. We are also characterizing a dominant suppressor of *rpm-1*, *ju91*. We have mapped *ju91* between +1.21 and +3.21 on chromosome X. Further mapping and cloning of *ju91* will be presented.

189. Synapse morphology is disrupted by a gain-of-function mutation in the C. elegans alpha-tubulin gene tba-1

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We isolated a gain-of-function mutation, *ju89* (also named *syd-5),*that alters the size and morphology of GABAergic synapses in *C. elegans.* We mapped *ju89* to chromosome I between hp6 and Y67A6 SNPs and were able to rescue the synaptic defects of *ju89* with either cosmid F26E12 or the fosmid H01I7. We further defined the minimal rescuing region to a 4.5kb subclone that contains the *C.elegans* alpha-tubulin gene *tba-1* (F26E4.8). *ju89* is a missense mutation in the C-terminus of TBA-1, a region involved in binding of microtubules to MAPs and motor proteins. *tba-1* is one of two alpha-tubulin genes in the *C. elegans* genome and is expressed throughout the *C. elegans* nervous system during development (Fukushige et al., 1995). Null mutations in *tba-1* have not been identified, however, RNAi experiments (Fraser et al., 2000) suggest complete loss of function of *tba-1*may result in severe embryonic defects or lethality. By contrast, *ju89* mutant animals are viable and have no apparent defects in axon extension or pathfinding of the GABAergic neurons. Instead, *ju89* mutant worms exhibit defects in synapse shape and size. The synaptic vesicle marker SNB::GFP is expressed as abnormally small or large puncta in *ju89* worms and is also diffused weakly along neuron processes and commissures. A similar pattern of reduced and oversized synapses is observed in the mutants using antisera to the presynaptic active zone proteins SYD-2 and UNC-10. These defects suggest the *ju89* lesion does not disrupt microtubule assembly, but is likely to affect specific aspects of microtubule function or regulation that are essential for synapse growth or stabilization. EM analysis of synapse ultrastructure of *ju89* mutants and mapping and characterization of suppressors of *ju89* are in progress.

190. unc-35/talin is required for left/right commissural growth cone decisions

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Several classes of ventral cord motor neurons extend circumferential commissures to the dorsal cord with stereotypic trajectories that extend either on the left or on the right side of the animal (1). For example, most DD and VD neuron commissures exit the ventral cord on the right side, while most DA commissures extend to the left side. By laser ablation of the AVG interneuron precursor, R.Durbin observed that some commissures of DD and VD neurons exit the ventral cord on the inappropriate side, suggesting that AVG provides a guidance cue for the left/right commissural growth cone decision (2). Although several genes, including *unc-71*, *unc-73*, and *cle-1*, have been shown to affect this left/right asymmetry, the molecular mechanisms involved in this decision are not understood.

We carried out a screen to identify sterile mutants with axon guidance defects using a P*unc-25*::GFP reporter that labels the 19 DD and VD neurons. One mutant, *ju453,* shows striking defects in the left/right commissure growth cone guidance. In *ju453* mutants, 25% of the D axons exit the ventral cord on the incorrect side, of these, 70% do not reach the dorsal cord. By contrast, only 30% of the commissures that exit on the correct side fail to reach the dorsal cord. The commissures of DA and DB neurons stop 3/4 of the way to the dorsal cord and extend laterally. We mapped *ju453*mutation to the left arm of LGI and found that *ju453* is an allele of *unc-35*.

unc-35 encodes the *C. elegans* homologue of talin, which contains a FERM and an ILWEQ domain. Of the four known *unc-35* alleles (*e259, zd28, ky196, ju453*), only *ju453* causes complete sterility. RNAi experiments suggest that the null phenotype of *unc-35*is likely embryonic lethality (3, our unpublished results). Antibodies to talin (4) and P*unc-35*::GFP transcriptional reporters detect expression only in muscles. We are performing mosaic analysis to determine where *unc-35* function is required for the left/right growth cone decision.

Talin provides a link between integrin receptors and the actin cytoskeleton. *C. elegans* has two α-integrins and two β-integrins. *ina-1*mutations cause a weak left/right asymmetry defect (5). We are carrying out double mutant analyses to determine which integrins are required for *unc-35* function in the left/right decision.

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191. UNConventional molecules: saga of two axonal guidance genes of *Caenorhabditis elegans*

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Development of a functional nervous system requires that individual axons and dendrites be guided to their proper destinations. We have found a novel gene, *unc-69* which is critical for axonal outgrowth, targeting and fasciculation in *C. elegans.* Loss of *unc-69* function caused pleiotropic effects on axonal outgrowth and fasciculation in nearly all of the neurons. In a previous yeast-two-hybrid screen using UNC-69 as a bait, we found, among 24 interactors, that the previously characterized axonal guidance molecule UNC-76 associates physically with UNC-69. *In vitro* GST pull-down studies demonstrated that the interaction between UNC-69 and UNC-76 is specific. Epistatic data tentatively place *unc-69* in parallel to *unc-76*, and it is very likely that they form a protein complex*in vivo* that might mediate axonal guidance signals. We propose that UNC-69 acts by integrating and transducing guidance cues to downstream effectors via its association with UNC-76, possibly through their respective coiled-coil domains.

We are performing a forward genetics screen for suppressors of the Unc-69 locomotion defect. So far, we have screened ~210,000 haploid genomes and found five suppressors, which are currently being mapped. We are also using a TAP (tandem affinity purification)/mass spectrometry approach to identify proteins participating in the UNC-69/UNC-76 protein complex. In addition, the functional relevance of the other yeast-two-hybrid candidates will be addressed by a RNAi approach.

192. Investigating the role of *pdl-1*

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pdl-1 (phosphodiesterase 6 delta like) is the only known C. elegans homologue of *unc-119*, a gene involved in axon outgrowth and maintenance. Like *unc-119*, *pdl-1* appears to be expressed throughout the nervous system beginning early in embryogenesis and continuing through adulthood. Both *pdl-1* and *unc-119* have highly conserved mammalian homologues which show enriched expression in the retina. We are studying *pdl-1* in order to better understand the role(s) of this protein family.

The mammalian homologue of *pdl-1*, rod cGMP phosphodiesterase 6 delta (PDE6D) has been well characterized biochemically. Originally identified as the delta subunit of rod cGMP phosphodiesterase, this protein has since been shown to interact with a number of proteins including several members of the ras family. In general, PDE6D interacts with the prenylated ends of proteins, releasing them from the membrane. This solubilization may play regulatory or transport roles, although its biological significance is not yet understood.

We have been trying to determine a biological role for *pdl-1*. To this end we have tried to determine the *pdl-1* phenotype using PCR deletion screening, RNAi, and genetic screens. We have so far been unable to determine a phenotype, although RNAi experiments are ongoing in *rrf-3* worms which show enhanced sensitivity to RNAi. As well, we are looking for genetic interaction with *unc-119* using worms hemizygous for *pdl-1*.

In addition to our attempts at genetic characterization, we are studying the PDL-1 protein. We have performed a yeast 2-hybrid screen to identify potential interactors and have used domain analysis to determine which region(s) of PDL-1 are necessary for these interactions. Finally we have produced antibodies against PDL-1 and are trying to use these for immunostaining to determine the subcellular localization the protein.

193. zig genes and maintenance of the nervous system.

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A genome wide analysis of Ig domain containing proteins has revealed a family of eight genes called *zig* genes which are constituted simply of two Ig domains (O. Hobert). So far only *zig-4* has been partially characterized. Studies of a loss of function allele showed that *zig-4* is required to maintain the structural integrity of the ventral nerve cord (VNC) in post-embryonic stages through its expression in the pioneer neuron PVT (Aurelio, 2002). Our goal now is to further characterize neuronal defects of the *zig-4* loss of function, and investigate the functions of the other *zig* genes.

First, we would like to refine the cellular focus of action of *zig-4*. So far only the organization of VNC axons has been shown to be affected in *zig-4* null mutants. Interestingly, PVT projects its axon not only in the VNC but also into the nerve ring (WBG Oct., 1999). Therefore, we are going to investigate whether axons of the nerve ring are also affected by the loss of *zig-4* activity. To observe whether *zig-4* and PVT act to maintain the stereotyped relative positioning of nerve ring axons, we will use cell-specific gfp and rfp co-labeling and compare the organization of the nerve ring in wild type animals, *zig-4* mutants and PVT ablated animals.

Second, in order to continue the functional characterization of the *zig* genes, we are trying to generate a knock-out allele of *zig-1* by excision of a Tc1 insertion positioned 1.7kb upstream of *zig-1* (strain provided by L. Segalat). *zig-1* is an atypical member of the *zig* gene family. It is the only ZIG protein that contains a transmembrane domain whereas all other ZIGs seem to be secreted. In addition, whereas 5 *zig* genes are expressed exclusively in neurons and 2 exclusively in non neuronal tissue, *zig-1* is expressed both in a large number of neurons and in body wall muscles.

Third, in the absence of loss of function alleles for most *zig* genes, we have decided to undertake an extensive mis/overexpression study with the entire set of neuronaly expressed *zig* genes. For this study we have selected promoter fragments driving expression in the body wall muscles, hypodermal cells and different classes of neurons that project their axons along the VNC. We have shown already that expression of *zig-4* in both fascicles of the VNC using the *flp-1* promoter generates defects similar to the loss of *zig-4* function. In contrast, expression in the right fascicle using the *unc-4* promoter rescues *zig-4* defects and has no effect in a wild type background. We are currently investigating whether this apparent asymmetric requirement of *zig-4* reflects a physiological difference in the requirement of ZIG-4 in the right rather than left VNC fascicle.

194. *sax-1* and *sax-2* maintain neuron morphology in larval and adult stages in *C. elegans.*

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To generate a neural circuit, neurons send a characteristic number of axons and dendrites along stereotyped paths towards their final targets. Once these targets are reached, mature neurons increase in size with the animal, but stop sending out new processes and maintain a stable morphology. Evidence suggests that the maintenance of neuronal morphology is actively regulated throughout adulthood. For example, mature neurons from the peripheral nervous system (PNS) of vertebrates can switch from a state of maintenance to a state of re-growth following axotomy. What are the molecular mechanisms that initiate and maintain the transition from neurite growth to stability?

Clues on how neuron morphology can be stabilized have been provided by the analysis of two mutants in *C. elegans, sax-1 and sax-2.* Initial axon outgrowth and guidance are normal in *sax-1* and *sax-2* mutant animals, but at later larval stages and in the adult, additional neurites emerge from the cell body (defined as secondary neurites). Thus *sax-1* and *sax-2* are required to inhibit inappropriate neurite outgrowth at late developmental stages.

Recent analysis suggests that *sax-1* and *sax-2* also play a role in setting the boundaries of neurite outgrowth during the first stages of neuronal development. The PLM mechanosensory neurons extend neurites along the body wall to reach their targets soon after hatching. In *sax-1* and *sax-2* mutants, the neurite of the PLM neuron fails to terminate at its proper point along the anterior/posterior axis of the animal. Therefore, *sax-1* and *sax-2* mutations can lead to over-extension of a primary neurite, in addition to the outgrowth of secondary neurites. We are currently observing PLM development in real time to ask whether *sax-1* and *sax-2* act primarily at the step of neurite initiation, growth rate, termination, or pruning.

sax-1 encodes a S/T kinase most closely related to the NDR kinases, which include members from both unicellular and multicellular organisms. Interestingly, *tricornered*, the NDR kinase in flies, is required in epidermal cells for maintaining a single cellular extension (or outgrowth) (Geng et al., 2000). These cellular outgrowths are like neurites in the sense that they contain both actin and microtubules. To learn more about the *sax-1/sax-2* pathway, we cloned *sax-2. sax-2* encodes a novel, conserved protein of 2915 amino acids and lacks any recognizable motifs (by BLAST and SMART analysis). In parallel work, the Adler lab cloned the *sax-2* ortholog, *furry*, a *Drosophila* mutant with phenotypic similarity to *tricornered* (Cong et al., 2001). Since double mutant analysis places *sax-1/tricornered* in the same genetic pathway as *sax-2*/*furry* (respectively), the function of the *sax-1/sax-2* pathway itself may be conserved throughout evolution.

Progress in the molecular and phenotypic analysis of *sax-2* will be presented.

195. Suppressors of *unc-34* cell migration and axon outgrowth defects

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Neurons and their axons encounter a complex environment of signals telling them to go, turn, or stop, as they migrate towards their final destinations. These signals are received by guidance receptors whose output is rapidly transduced to the actin cytoskeleton. Within the last ten years, many guidance molecules and their receptors have been implicated in this process, such as UNC-6/UNC-5/UNC-40 and SLT-1/SAX-3. However, little is known about how this guidance information is transduced to the actin cytoskeleton. One molecule that may play a role in this process is UNC-34. Work done in both *C. elegans* and *Drosophila* implicates UNC-34/Ena as a mediator of the repulsive output of the SAX-3/Robo receptor, which it binds to *in vitro* (1,2).

Mutations in *unc-34*, a member of the Ena/VASP family of actin-binding proteins, display many defects in neuronal migrations and axon outgrowth. For example, both the CAN and the HSN, cells which migrate from the head and tail, respectively, to the midbody, undermigrate 40-60% of the time in *unc-34* mutants. In addition, the axons of the DD motorneurons fail to extend from the ventral nerve cord and branch properly in the dorsal nerve cord >70% of the time.

In order to isolate molecules that may function with UNC-34 to mediate guidance decisions, we have undertaken a genetic screen for suppressors of *unc-34*. We screened approximately 3000 haploid genomes, and isolated four strong and ten weak suppressors. We are currently mapping and characterizing the suppressor mutations.

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2. Bashaw et al. (2000) Cell 101: 703-715.

196. Functional analysis of UNC-33 involved in neural development

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Neurons have two types of highly polarized cell structures, axons and dendrites. We previously reported that human CRMP-2 (Collapsin response mediator protein-2) plays pivotal roles for the axon outgrowth and neural polarity. CRMP-2 is highly conserved from worms to mammals. However, the molecular mechanisms that CRMP-2 contributes to axon formation remain to be clarified. We have used the nematode *Caenorhabditis elegans* as a model organism to examine *in vivo* functions of CRMP-2.

In *C. elegans*, UNC-33 encodes a homologue of CRMP-2. *unc-33* mutants show the severe defects in axon outgrowth and guidance, suggesting the essential functions of UNC-33 in nervous system. To examine the molecular functions of UNC-33, we performed the screening of interacting molecules with UNC-33 using the yeast two-hybrid system. UNC-33 has three splicing forms (UNC-33L, UNC-33M, and UNC-33S) with extension at N-terminus. From the yeast two-hybrid screen with UNC-33S as bait, we identified two interacting proteins. One is UNC-33 itself, and the other is UNC-43, a *C. elegans* homologue of calcium-calmodulin dependent kinase II (CaMKII). The mutations in the *unc-43* gene are reported to cause the morphological defects in sensory neurons and reduction of the number of GLR-1-containing synapse. We confirmed that the C-terminal domain of UNC-43 interacts with full length of UNC-33 *in vitro*. Using the immunostaining with anti-UNC-33 antibody, we found that the localization of UNC-33 was unaffected by loss-of-function mutants of the *unc-43* gene. Possibly, UNC-33 may regulate the intracellular localization of UNC-43. By the yeast two-hybrid screen with UNC-33L as bait, we identified three interacting molecules, HSP-1/HSP70, UBQ-1/ubiquitin, and C01G8.5/ERM-1. Both, HSP-1 and UBQ-1 are involved in protein stability or degradation pathway, suggesting the presence of regulation of UNC-33 stability in neurons. From these results, we propose that UNC-33 regulates the neural morphologies by coupling with the UNC-33-interacting molecules.

197. Isolation and Characterization of Genes Involved in Ectopic Neurite Outgrowth Defects

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Blockade of communication between neurons or between neurons and muscle has been shown to cause ectopic axonal outgrowth in vertebrates and more recently in *C. elegans* (Holland and Brown 1980, Nonet et al. 2000). The ectopic axonal outgrowth or sprouting phenotype indicates that neurons are able to monitor and make compensatory attempts to maintain functional innervation of the target. Such activity-dependent remodeling may be caused by retrograde signaling from the target to the neuron leading to cytoskeletal rearrangements.

In order to understand the nature of neuromuscular communication, we have chosen the DVB neuron as our model system. DVB is a postembryonically born GABAergic motor neuron that mediates the expulsion step of the defecation motor program. Disruptions in neural differentiation (*lim-6*), activity (*unc-25, unc-104*), and axogenesis (*unc-44, unc-34*) lead to ectopic outgrowth defects in DVB. Furthermore, silencing of the enteric muscle by *egl-2(gf)*, a hyperactivated *eag*-like K^+ channel, or lack of enteric muscle formation by *hlh-8*, the Twist homolog, also causes DVB ectopic outgrowth defects. This suggests that neuronal innervation and activation of muscle may promote the feedback of a retrograde signal to prevent further axonal outgrowth.

To decipher the mechanisms behind this bidirectional neuromuscular communication, we have conducted a screen for mutants that display ectopic neurite outgrowth defects (the *eno* phenotype) in DVB. Isolated mutants can represent genes involved in DVB functional development, maturation, or maintenance. A transgenic strain expressing *unc47::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 the *eno* phenotype. 12 *eno* mutants have been retrieved and placed into 11 complementation groups.

We have started to clone and characterize *eno-1*. In addition to DVB sprouting, *eno-1* also shows ectopic outgrowth defects in the AVL motor neuron, which has redundant functions with DVB in mediating the expulsion step of defecation. *Eno-1* has a stong hypersensitive phenotype on aldicarb, suggesting that it may be involved in negative regulation of neurotransmission. *Eno-1* has been mapped between *lin-31* and *rol-6* on chromosome II. By snip-SNP mapping, *eno-1* has been narrowed down to 9 cosmids. Further mapping results will be presented.

198. Does UNC-119 mediate a 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.[1] Mutants have a variety of neurite defects. The VNC is defasciculated to varying degrees in different individuals. Both commissure and nerve ring axons end prematurely in aberrant terminal arbors which do not contact normal synaptic partners in 10% to 50% of examined neurites.

Examination of *unc-119* mutant L1 larvae suggests that fully-elongated axons are initially extended but are subsequently retracted.[2] Initial axon elongation may appear to be along the correct pathway (as is the case for DD and VD neurons) or misguided (as with ASI neurons). In either case supernumerary branching follows retraction of the original neurite. Terminal arbors occur largely at significant choice points such as on the ventral side of body wall muscle quadrants (for motor neurons) or at the ventral entry of the VNC into the nerve ring (for chemosensory neurons).

Localized expression of an UNC-119::GFP fusion protein in neural subsets rescues the neural defects only in the cells in which the protein is expressed. Ectopic expression in muscles does not rescue any structural or behavioral defects. Thus UNC-119 acts cell-autonomously.

Yeast two-hybrid experiments indicate that the carboxyl third of the UNC-119 protein interacts with the NC1 (non-collagenous) domain of the basement membrane collagen LET-2. This NC1 domain has 50% identity and 75% similarity to the human angiogenic inhibitors/tumor suppressors arresten, canstatin and tumstatin but no receptors are yet known. The NC1 domain of the unrelated basement-membrane collagen *cle-1* (which contains the worm homolog of endostatin) is required for proper axon guidance but the mechanism of action is not known.[3] Because all known *let-2* alleles with an observable phenotype are embryonic lethal (due to a failure to assemble a basement membrane) any later role in nervous system organization is masked. We reasoned that we should be able to rescue the lethal phenotype by expressing only the collagenous domain of the *let-2* gene in a *let-2* mutant background but that this should reveal any possible role of the NC1 domain in axon guidance. The results of this experiment will be discussed.

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199. Investigations into the mechanisms of SAX-3 mediated axon guidance

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The transmembrane receptor SAX-3 is involved in the guidance decisions of many cells and axons of *C. elegans*. Like its fly ortholog, Roundabout (Robo), SAX-3 is involved in guidance at the ventral midline, where it prevents aberrant midline crossing of ventral cord axons. In addition, SAX-3 is involved in long-range dorsal-ventral guidance of the axon of the AVM neuron, in anterior-posterior placement of axons in the nerve ring, and in the posterior migration of the CAN neurons. One ligand for SAX-3 is the secreted molecule SLT-1, which directs the ventral guidance of the AVM neuron. SLT-1 is expressed in dorsal muscles, where it acts as a repellent for axons expressing SAX-3 to direct them ventrally. SLT-1 is also expressed in the head of the animal, where it could potentially play a role in nerve ring guidance. Interestingly, however, SAX-3 is more than just a receptor for responding to SLT-1, because mutations in *sax-3* result in a number of phenotypes that are not present in animals with null mutations in *slt-1*. In *sax-3* mutants there are axon guidance defects in the nerve ring, lethality, and notched head phenotypes. These phenotypes are absent in *slt-1* mutants. These *slt-1*-independent phenotypes of *sax-3* could be explained by a second ligand for SAX-3 or by ligand-independent functions of SAX-3.

We're interested in understanding the *sax-3* phenotypes that are absent in the *slt-1* mutant. Previous genetic screens in have failed to identify mutants other than *sax-3* with the characteristic anterior nerve ring axon guidance defect, suggesting that there is not one discrete nerve ring ligand for SAX-3. Screens can often miss genes that function redundantly, and perhaps this is the case in nerve ring axon guidance. If a second ligand functions redundantly with SLT-1 it should be possible to reveal a nerve ring axon guidance phenotype by screening for *sax-3*-like phenotypes in a *slt-1* mutant background. We're conducting a screen for genes that have a nerve ring axon guidance phenotype in a *slt-1* background. We're also testing the hypothesis that *sax-3* functions as a homophilic receptor.

200. More axon guidance mutants

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Growth cones can migrate long distances along stereotypic pathways to find their targets. To identify genes involved in growth cone guidance, we undertook several screens for mutants with defects in axon outgrowth and pathfinding. Our focus has been on neurons that extend axons along the anteroposterior body axis, such as AVA, AVB, AVD, ALM, PLM, AVM, PVM and PVQ.

To identify mutants, we treated either *glr-1::gfp*, *mec-4::gfp* or *sra-6::gfp* animals with either EMS or ENU, isolated behavioral mutants in the F2 generation and then examined their progeny for axon defects. Although most mutants were identified using this enrichment strategy, some were found by direct visualization of F2 animals for axon defects. Mutants with a variety of axon outgrowth, branching and pathfinding defects as well as mutants with either axonal degeneration, cell fate, cell migration, cell position or programmed cell death defects were recovered. To date, over 160 mutants have been identified that define over 40 genes. We will present a phenotypic description of the different types of mutants recovered in our screens as well as our observations regarding the differences in mutational frequency using EMS and ENU.

The molecular characterization of several genes identified in our screens is described in abstracts by Chiu, Squires & Clark and Yam, Chiu & Clark. One such gene, *zag-1,* encodes a Zn finger and homeodomain-containing protein that is involved in neuronal differentiation and axonal development. We will present our analyses of the *zag-1* promoter and the promoters of other genes regulated by *zag-1*.

201. vab-8 and unc-51 interaction mediates posteriorly directed 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 the 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 both in yeast and in vitro. Several observations suggest that VAB-8 and UNC-51 also interact in C. elegans. First, vab-8 and unc-51 mutants have been shown to display axon outgrowth defects. We used a ceh-23::GFP transgene to assay the axons of CAN neurons, which extend growth cones both anteriorly and posteriorly. In vab-8 mutants, only posteriorly directed axon outgrowth was defective, whereas in unc-51 mutants, both anteriorly and posteriorly directed outgrowth were defective. Second, both genes have been shown to be expressed in neurons that require vab-8 function. Using the ceh-23 promoter to drive vab-8 or unc-51 expression, we have shown that both genes act cell autonomously for CAN axon migrations. Finally, misexpression of the UNC-51-binding domain of VAB-8 under control of the ceh-23 promoter resulted in a highly penetrant CAN posterior growth cone migration defect, a Vab-8 phenotype, presumably by interfering with UNC-51 binding to wild-type VAB-8L. Misexpression of the VAB-8-binding domain of UNC-51 also produced CAN axon outgrowth defects. The misexpression phenotypes are suppressed by simultaneous misexpression of both protein fragments. We propose that in this experiment the VAB-8 and UNC-51 fragments associate with each other, thereby allowing endogenous full-length VAB-8 and UNC-51 to interact and carry out their function in directing axon outgrowth. 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 unc-51 mutants, indicating a positive regulatory relationship between the two proteins. The same vab-8 overexpression array also enhanced the anterior axon outgrowth defects of unc-51. We are also taking biochemical approaches to determine the relevance of VAB-8 and UNC-51 interaction. We have demonstrated that VAB-8 is phosphorylated when co-expressed with UNC-51 in COS cells. We are testing whether VAB-8 is phosphorylated directly by UNC-51, and whether VAB-8 phosphorylation is important for its function in directing axon migration.

Ogura et al. (1994) Genes & Dev. 8: 2389-2400. Ogura et al. (1997) Genes & Dev. 11: 1801-1811.

202. The role of SNAREs in exocytosis

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Neurotransmission at a chemical synapse occurs via the controlled fusion of synaptic vesicles with the plasma membrane. Central players in this process are the SNARE proteins. In *C. elegans* the SNARE proteins are encoded by *unc-64, snb-1* , and *ric-4* which correspond to syntaxin, synaptobrevin, and SNAP-25 respectively. Due to their central and conserved role in membrane fusion, the SNARE proteins have been widely studied. Yet despite extensive study, heated debate still remains concerning their function.

In *C. elegans* both *unc-64* and *snb-1* null animals die as L1 larvae, making electrophysiological studies of these animals impossible. In order to conduct electrophysiological studies on the SNARE mutants we wish to rescue the animals to adulthood while maintaining a null phenotype at the neuromuscular junction, from which we routinely record. Toward this end we have taken two alternate approaches. First, we are reintroducing the SNAREs into those cells that we believe are the focus of L1 lethality. Second, we are working on a method of selectively knocking out the SNAREs in the motor neurons.

In the first approach, we are reintroducing UNC-64 and SNB-1 into the M4 neurons and the CAN neurons, which have been shown from laser ablation studies to be required for viability. We are using the *pha-4* promoter for selective expression in the M4 neurons of the pharynx and the *ceh-23* promoter for expression in the CAN neurons. By expressing the SNAREs in these two cell types we hope to rescue the L1 lethality while maintaining a null phenotype at the neuromuscular junction.

In the second approach, we are developing a site specific recombinase based system that should allow us to selectively delete the SNAREs from the motor neurons. Briefly, we will flank the SNARE coding regions with FLP recombinase target (FRT) sites. The FRT flanked SNAREs will be reintroduced into the corresponding null background. By expressing the FLP recombinase we should be able to induce recombination between the FRT sites, thus eliminating the ability to produce SNAREs. By putting the FLP recombinase under the control of the *acr-2* promoter we should be able to eliminate the SNAREs from the motor neurons.

Generation of these strains should allow us to determine possible roles of the SNAREs in docking, priming, and fusion.

203. MOLECULAR CLONING AND FUNCTIONAL CHARACTERIZATION OF A NOVEL CLASS OF VOLTAGE-GATED ION CHANNELS

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Utilizing the *Caenorhabditis elegans* genome database, we have characterized two novel four-domain voltage-gated ion channel (VGIC) genes. The *C. elegans* C11D2.6 and C27F2.3 gene products (called *nca-1* and *nca-2*, respectively, for "novel ion channel alpha-1 subunit") are similar to that of other four-domain VGICs such as sodium and calcium channels although their pore regions reveal a unique ion selectivity motif. Our efforts to date have focused on characterizing the properties of the NCA-1 and NCA-2 gene products. We have determined the cellular expression patterns of *nca-1* and *nca-2* by using GFP-promoter fusion constructs. The *nca-1* transgene was found to be primarily expressed in phayngeal neurons, neurons of the nerve ring, motorneurons of the ventral nerve chord, and neurons in the tail, whereas the *nca-2* transgene was found to be expressed primarily in the neurons of the nerve ring. Gene knock-outs of *nca-1* and *nca-2* indicate that neither mutant strain shows any overt morphological phenotype. Efforts are underway to further characterize the phenotypes of these mutant strains. In addition, full length cDNAs of *nca-1* and *nca-2* have been isolated in order to determine the electrophysiological and pharmacological properties of the NCA-1 and NCA-2 channels.

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204. *unc-74* encodes a protein disulfide isomerase required for levamisole receptor function.

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Acetylcholine gated ion channels are heteropentameric receptors that mediate fast chemical synaptic transmission. Formation of these receptors requires folding of individual subunits and assembly of folded subunits into a pentamer with a specific subunit order and stoichiometry. Folding and assembly occur in the endoplasmic reticulum prior to trafficking of the mature receptor to post-synaptic specializations.

The neuromuscular junctions of *C. elegans* possess at least two pharmacologically distinct acetylcholine gated ion channels, based on whether the receptor can be activated by levamisole (the levamisole receptor) or not (the nicotine-sensitive receptor). Mutants resistant to the paralytic effects of levamisole have been isolated and four loci *(unc-29, unc-38, unc-63* and *lev-1)*, encode subunits of the levamisole receptor.

unc-74 mutants are also resistant to levamisole and display an uncoordinated phenotype that is similar to receptor subunit mutants. The levamisole-induced current is abolished in *unc-74* animals when assayed electrophysiologically by whole cell voltage clamp. Interestingly, the nicotine-sensitive receptor response is normal in the absence of *unc-74*. This result suggests that *unc-74* is specifically required for levamisole receptor function.

Using *Mos1* mutagenesis, we obtained an allele of *unc-74*. The transcript encoded by *unc-74* was determined by sequencing the region flanking the *Mos1* insertion and rescuing the mutant by microinjection. In addition, other alleles of *unc-74* have been sequenced. UNC-74 is likely to function as a protein disulfide isomerase. The predicted protein sequence contains a thioredoxin domain, a transmembrane domain and a putative endoplasmic reticulum retention signal. The UNC-74 thioredoxin domain contains the conserved active site sequence C-X-Y-C that is required for catalyzing reduction and oxidation of disulfide bonds. Thus, UNC-74 is likely to exhibit thioredoxin activity.

What role might UNC-74, a putative protein disulfide isomerase, have in the function of levamisole receptors? All acetylcholine gated ion channels belong to the cysteine-loop family of ligand gated ion channels, which includes 5-HT_3, $GABA_a$, $GABA_c$ and glycine receptors. This family is defined by a conserved extacellular cysteine loop formed by a disulfide bond between two cysteine residues located 13 residues apart. Although the precise functional significance of this conserved structural feature is unknown, experiments in heterologous expression systems indicate the cysteine-loop has a role in receptor assembly. Consistent with this observation the crystal structure of acetylcholine binding protein suggests the cysteine-loop is important for conformational stability and may interact with the plasma membrane.

Our working model is that UNC-74 facilitates assembly of levamisole receptor by catalyzing cysteine-loop formation on individual levamisole receptor subunits. If UNC-74 is required to assemble levamisole receptors prior to receptor trafficking to the plasma membrane, then levamisole receptor subunits should be retained in the endoplasmic reticulum in *unc-74* mutants. To test this prediction, we compared the subcellular localization of UNC-38 in *unc-74* mutants to wild type. Confocal micrographs were collected of animals expressing UNC-38::GFP. In contrast to wild-type animals, UNC-38::GFP is not localized to the synapse in *unc-74* animals, but is concentrated in a corona around the nucleus, which may be the endoplasmic reticulum. These data are consistent with the hypothesis that UNC-74 function is required for levamisole receptor assembly.

In summary, *unc-74* encodes a putative protein disulfide isomerase required for levamisole receptor function. Our hypothesis is that UNC-74 is required for assembly of levamisole receptors by facilitating cysteine-loop formation on a levamisole receptor subunit.

205. Characterization of TRPC channels in *C. elegans*

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The *trp* genes encode a superfamily of cation channels conserved from worms to humans, comprising three major subfamilies, TRP-Canonical (TRPC), TRP-Vanilloid (TRPV) and TRP-Melastatin (TRPM). The canonical TRP (TRPC) channels possess both ankyrin repeats and a TRP domain, and are most closely related to the *Drosophila* TRP and TRPL, the founding members of the TRP superfamily. In contrast, the TRPV and TRPM channels lack either the TRP domain or the ankyrin repeats and are therefore more distantly related to the *Drosophila* TRP and TRPL. While the biological functions and gating mechanisms of the TRPV and TRPM channels are emerging, little is known about the TRPC channels. Although it has been well established that the *Drosophila* TRP and TRPL form the light-sensitive channels in photoreceptor cells, their TRPC homologues in other organisms do not seem to be required in visual transduction. This suggests that TRP and TRPL may have been functionally dedicated to fly vision during evolution and their TRPC counterparts in other organisms may play currently unknown functions. Furthermore, the signaling pathways leading to the activation of the TRPC channels also remain obscure. To address these questions, we have begun to use worms as a model system. The *C. elegans* genome encodes three TRPC genes, *trp-1*, *trp-2* and *trp-3*. We have generated deletion mutants of all the three TRPC genes, each with two independent alleles. Thus, *C. elegans* represents the first organism, in which a complete set of TRPC mutants have been isolated. *trp-1* and *trp-2* are co-expressed in several classes of excitable cells, whereas *trp-3* is enriched in the sperm cells. Phenotypic characterization will be presented.

206. *unc-58* ENCODES 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. Loss-of-function *unc-58* alleles have been isolated as revertants of the *unc-58(dm)*phenotype. These alleles have no strong phenotype on their own, suggesting that *unc-58(dm)* mutants result in an inappropriately activated gene product. *unc-58(dm)* animals also frequently flip around their longitudinal axis

We have cloned *unc-58* and found that it encodes a member of the TWIK potassium channel family. TWIKs are distinct from other potassium channels because they have four transmembrane domains (M1 to M4) and two pore domains. Potassium-selective currents have been recorded from TWIKs in both mammals and worms.

In contrast to *unc-58(dm)*, gain-of-function mutations in other *C.elegans* potassium channels cause flaccid paralysis and muscle relaxation. It is possible that the *unc-58(dm)* hypercontraction is caused by excessive potassium currents in the inhibitory motorneurons. However, this does not account for the hypercontraction of the egg-laying muscle, which has no known inhibitory motorneurons. Alternatively, UNC-58(dm) may act in excitatory neurons and/or muscle to conduct a current other than potassium. A precedent for that is the Ih channel in the human heart, which is permeable to both potassium and sodium [Ludwig et al, Nature 393, 587-591]. These two possibilities predict different sites of action and different ion selectivity profiles for the UNC-58 channel. We are currently expressing UNC-58(dm) in *Xenopus laevis* oocytes to determine its ion selectivity, and determining the UNC-58 site of action using GFP fusions and tissue-specific promoters. We will present data for both sets of experiments.

Both the flipping and the hypercontraction 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). The rescue is allele specific, and is not affected by mutants in GABA-ergic neurotransmission. We hope to use electrophysiology to determine whether this rescue is by direct channel block.

207. EXAMINING THE FUNCTIONAL PROPERTIES OF THE UNC-2 AND CCA-1 VOLTAGE-GATED CALCIUM CHANNELS

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With the ultimate goal of identifying modulators of voltage gated calcium channels (VGCCs), our research has focused on two genes in *C. elegans* namely, *unc-2* and *cca-1*. The *unc-2* gene encodes an alpha1-like subunit most similar to mammalian P/Q and N-type channels. Mutant animals show a number of different phenotypes including resistance to aldicarb suggesting a presynaptic function for the channel. Consistent with this hypothesis an *unc-2*::GFP transgene is expressed in a number of head and tail sensory neurons, the touch receptor cells and most motor neurons. Molecular lesions for 23 *unc-2* mutants have been identified and using a battery of behavioural assays we are attempting to determine UNC-2 functional activity for each mutation. Electropharyngeograms (EPGs) generated from the putative *unc-2* null mutant, *ra605*, show reduced activity of the M3 inhibitory motor neuron which normally contributes to repolarization and relaxation of the pharyngeal muscle. Advances in *in situ* patch clamping neurons and culturing of worm cells are being exploited to more fully characterize the electrophysiological properties of the individual UNC-2 mutant channels. Genetic analysis towards identifying genes that modulate the activity of UNC-2 are also being conducted, both with existing mutants in genes known to affect alpha1-like subunit function, as well as screens for novel modulators.

The *cca-1* gene (C54D2.5) encodes the only known *C. elegans* protein with strong homology to T-type VGCCs. *cca-1* mutants display no overt morphological defects, but mutants carrying either of two deletion alleles display abnormalities in feeding. Pharyngeal pumping is slow and EPGs show small, delayed excitation phase spikes following EPSPs from the MC pharyngeal motor neuron. CCA-1 appears to assist in converting an EPSP into a full membrane depolarization and a pharyngeal muscle contraction. Expression of a *cca-1*::GFP transgene in the MC motor neuron supports this model. Expression is also observed in a number of head neurons, the anterior-most body wall muscles, the ventral nerve cord and cells in the tail region. We are currently attempting to identify additional phenotypes so as to be able to screen for new mutations with which to probe the function of this VGCC.

208. Developing a screen for HERG blocking drugs using the *C. elegans* pharynx

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The HERG (*human ether-a-go-go related gene*) potassium channel is expressed in the human heart and is responsible for proper repolarization of cardiac muscle at the end of the action potential. Compromising HERG function gives rise to Long QT Syndrome, a disorder in which one is predisposed to ventricular fibrillation and sudden death. Many drugs, including Seldane (an anti-histamine) and Propulsid (an acid reflux inhibitor), block HERG as an unwanted side effect and consequently have been taken off of the market.

To develop a system for drug screening, our initial objective was to determine whether HERG could functionally replace EXP-2. EXP-2 is a voltage-gated potassium channel responsible for rapid repolarization of the pharynx, similar to the function HERG plays in the human heart. In addition, HERG and EXP-2 share functionally similar gating kinetics, although these two channels are structurally unrelated.

To determine whether HERG could functionally replace EXP-2, we expressed a HERG::GFP fusion protein 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* mutants lack both M3-mediated IPSPs and the repolarization spike. Unfortunately, HERG-expressing transgenic progeny show no rapid negative spike currents by EPG, which suggests that HERG cannot replace EXP-2 in *C. elegans*.

We have also expressed a non-selective and inactivation defective form of HERG (HERG (N629D)) in the pharynxes of wild-type worms and *eat-4*; *exp-2* mutants. Wild-type worms expressing HERG(N629D) in pharyngeal muscle are starved in appearance and exhibit a growth rate delay when grown on HB101 bacteria. When grown on DA837, this growth delay is enhanced and, in addition, ~50% of transgenic progeny arrest as L1 larvae. This L1 arrest is further enhanced when HERG(N629D) is expressed in *eat-4*; *exp-2*. In adult animals, contractions of the terminal bulb muscles appear to be uncoordinated and/or shallow, the grinder does not function properly and the gut becomes filled with unground bacteria. Surprisingly, we do not see any striking alterations in the EPG or intracellular recordings, given the severity of the feeding defect. In L1 larvae, the pumping rate of HERG(N629D) expressing worms is variably, but greatly reduced compared to wild-type. In addition, pharyngeal pumping appears to be shallow.

It is possible that the defects in adults are secondary- resulting from an inability of worms to clear bacteria before each molt. Therefore, we are currently focusing on the defects seen in L1s. Specifically, we are testing whether HERG blocking drugs can block the phenotypes we have observed.

209. Cameleon Imaging of Calcium Transients in Cultured Mechanosensory Neurons

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We have used primary *C.elegans* cell cultures and the genetically encoded calcium sensor cameleon to study the molecules involved in producing calcium transients in mechanosensory neurons in response to depolarization with a high K solution. The cultured neurons are amenable to genetic manipulations and to pharmacogical agents, which have been difficult to test in intact worms.

We have done experiments on an integrated strain expressing the yc2.12 cameleon under the *mec-4* promoter. After 48 hours of culturing, the cameleon expressing neurons were placed in extracellular saline in a flow chamber and the cameleon response to transient 8s high K^+ induced depolarizations were observed. In order to determine which ion channels and/ or intracellular stores are required for inducing calcium transients we varied the K^+ concentration and the extracellular calcium concentration. The amplitude of the cameleon response shows a graded response to depolarizations induced by K^+ concentrations ranging from 20 - 100 mM K^+ and to extracellular calcium concentrations from 0-5 mM. The rise in intracellular calcium can be reversibly blocked with the three different classes of L-type calcium blockers: Nifedipine (a dihydropyridine), verapamil (a phenylalkylamine) and diltiazem (a benzothiazepine). The only known L-type voltage gated calcium channel in the *C.elegans* genome is *egl-19* and homology search shows that the gene contains the residues thought to correspond to the three different drug binding sites. We are in the process of looking at egl-19 gain of function allele *egl-19 (n2368*) and loss of function allele *egl-19 (ad1006).*

We are also studying the role of the $\alpha_2\delta$ subunit UNC-36 and the possible interaction with EGL-19. An UNC-36 promotor GFP fusion shows broad expression in both muscle and neurons, and flourescence has been verified by Nomarski in AVM, ALM and PVM. A promotor GFP construct of T24F1.6, the other putative $\alpha_2\delta$ subunit in *C.elegans*, shows no expression in AVM, ALM or PVM.

We have also looked at the release of calcium from intracellular stores. 20 mM Caffeine induces a transient rise in intracellular calcium. We are currently trying block the IP_3 receptor and the ryanodine receptor separately with 2-APB and ryanodine, to determine their separate contributions to the K^+ evoked calcium transients. We are also in the process of doing experiments with the IP_3 receptor loss of function allele *itr-1(sa73)*and the rynanodine receptor loss of function allele *unc-68 (r1158).*

In order to check the integrety of the cameleon sensor and calibrate the dynamic range we have done an *in vivo* calcium calibration with free calcium in the range from 10 nM to 100 uM. We gained access to the cells with the non-flourescent ionophore Br-A23187. It was necessary to block the cells' energy production with rotenone and 2-deoxy-D-glucose to reach a stable baseline for the low calcium range. The *in vivo* results show a half maximal ratio change at 1uM free calcium, a biphasic calcium dependence and a maximal ratio change of approx. 80%.

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210. *nic-1* encodes a glycosyltransferase that affects nAChR activity

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Exposure to nicotinic agonists such as nicotine and levamisole leads to body muscle hypercontraction and paralysis, increased pharyngeal pumping, spicule protraction in males, and stimulation of egg laying under conditions inhibitory for egg laying. Long-term treatment with nicotine leads to 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. The molecular mechanisms underlying these processes involving the nicotinic acetylcholine receptor are not well understood in any organism. To address this question, we have been investigating the mechanisms of nicotinic receptor regulation in *C. elegans*; one way we have been doing this is by searching for genes that confer resistance or hypersensitivity to the effects of nicotine.

We have found that a mutation in *nic-1*, a gene we have isolated through a screen for potential negative regulators of the nAChR, causes hypersensitivity to nicotine and exhibits phenotypes that support *nic-1*'s possible role in the regulation of cholinergic neurotransmission. For example, *nic-1* mutants underwent paralysis and were stimulated to lay eggs at doses of nicotine three-fold lower than wild type, and were also 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. *nic-1* appears to effect the activity of nAChRs in both muscle and neurons: a mutation in *unc-38*, a *C. elegans* α-type nAChR subunit, causes resistance to nicotine; nic-*1* caused nicotine hypersensitivity in both muscle-specific and neuron-specific rescue of wild type *unc-38* in *unc-38* mutants. The effect of *nic-1* appears to be specific to the activity of ligand-gated ion channels such as the nAChR and the GABA receptor: while *nic-1* mutants are strongly hypersensitive to nicotine and to muscimol, a GABA receptor agonist, they displayed no apparent difference from wild type in their response to other drugs such as serotonin and dantrolene.

We have identified that *nic-1* encodes a homolog of the yeast glycosyltransferase gene *alg2*. *Alg2p* catalyzes the addition of a mannose in one of the initial steps in the building of a mannose-rich lipid linked oligosaccharide in the ER that is ultimately transferred onto an asparagine residue of a nascent protein. Analysis of the *nic-1* sequence showed conserved sites for glycosyltransferases, and RT-PCR revealed the presence of alternatively spliced variants. Mass spectroscopy of *nic-1* glycopeptide extracts revealed a drastic reduction of PNGase F-released high-mannose type glycans compared to wild type, while the levels of truncated glycans and PNGase A-released glycans remained comparable to wild type. These results support NIC-1's role as one similar to that of *Alg2p*, in the same pathway leading to N-linked glycosylation. This result raises the question of how nAChRs are affected by glycosylation. Are nAChRs, known to be glycoproteins, directly affected in its glycosylation, or are they affected indirectly, through some secondary glycoprotein? These are some questions we would like to explore in the coming months.

211. Mutations of a glutamate-gated chloride channel in *C. elegans* affect short-term memory in an ISI dependant manner, long-term memory, and foraging behaviour

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avr-14 encodes a alpha-type subunit of a glutamate-gated chloride channel (GluCls) in *C. elegans*, a receptor closely related to mammalian glycine-gated chloride channels. *avr-14* is expressed on the glutamatergic sensory neurons of the tap-withdrawal response (TWR) (Dent et al., 2000). *avr-14* receptors may act as an autoreceptors regulating sensory neuron activity. Worms with a functional null mutation of *avr-14* were tested for short-term habituation of the TWR at 10s, 30s, 45s, and 60 s ISI's. It was found that worms with this mutation showed more rapid habituation than wild-type at shorter ISIs (10s and 30s) while showing habituation similar to wild-type at long ISIs (45s and 60s). Worms with the same mutation appear to show enhanced long-term memory compared to wild-type controls. *avr-14* mutants show an increased frequency and a decreased magnitude of spontaneous reversals compared to wild-type. Spontaneous reversals are thought to be part of a foraging strategy in *C. elegans*. The hypothesis that the altered frequency and magnitude of spontaneous reversal would affect foraging strategy was supported by an experiment is which *avr-14* worms were unable to negotiate a simple copper sulfate maze to reach food while wild-type worms easily reached the food.

Reference: Dent, J.A., Smith, M.M., Vassilatis, D.K., & Avery, L. (2000). The genetics of Ivermectin resistance in *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. (USA), 97, 2674-2679.

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212. The Ras-MAPK pathway acts in interneurons for olfactory plasticity

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We have previously reported that the Ras-MAPK pathway is important for olfaction. The pathway is activated by odorant stimulus in mature olfactory neurons. Recently we found that the pathway might function in interneurons as well as in sensory neurons.

We observed that *C. elegans* shows behavioral plasticity in chemotaxis to volatile odorants. After pre-exposure to 10^{-4} dilution of odorants for 5 min, wild-type worms exhibit decreased chemotaxis to the same odorants at the normally attractive concentration. This plasticity, which we consider an early phase of adaptation, is observed for isoamylalcohol, benzaldehyde (both of which are sensed by AWC sensory neurons) and pyrazine (which is sensed by AWA). Interestingly, chemotaxis to AWC-sensed odorants is decreased after pre-exposure to AWA-sensed pyrazine. These results suggest that interneurons that have synaptic inputs from both AWA and AWC might be involved in this behavioral plasticity. The *ttx-3* mutants, which have defective AIY, exhibit severe deficits in adaptation in all combinations of odorants tested. Thus early phase of adaptation requires interneurons, and depends on neural network. The *let-60(n1046gf)*, *let-60(n2021lf)* and *mek-2(n2678lf)* mutants show defects in this behavioral plasticity. But they exhibit normal adaptation after longer exposure (30 min, 1 hr) to odorants. In immunofluorescence experiments, the activation of MAPK was detected in AIY as well as in AWC after application of 10^{-4} dilution of isoamylalcohol for 5 min. This result shows the possibility that the Ras-MAPK pathway functions also in AIY interneurons. To test this possibility, we induced AIY-specific expression of *let-60ras* using the *ttx-3* promoter and assayed for adaptation. AIY-specific expression of *let-60(gf)* in wild-type impaired adaptation. Similarly, *ttx-3::let-60(+)* rescued the defect of the *let-60(lf)* mutants. These results indicate that functions of *let-60* in AIY interneurons are important for adaptation.

213. Sensory Signal Integration in the Nervous System

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C. elegans has a dynamic ability to sense and respond to its environment, often integrating multiple sensory signals to generate one response. Previous studies have demonstrated the importance of sensory integration in modulating responses such as development, behavior, and gene expression. One of the best-characterized examples of signal integration is dauer formation. Dauer formation is induced by conditions of high dauer pheromone levels (crowding), starvation, and high temperature and is regulated by the activity of the chemosensory neurons ASI, ADF, and ASJ. We are interested in asking how distinct signals are sensed by these chemosensory neurons and how these signals are integrated to generate a response.

From laser ablation studies, both ASI and ADF have been implicated in suppressing dauer formation. ASI constitutively expresses DAF-7, a TGF-ß homolog, to maintain normal reproductive growth. High pheromone levels induce dauer formation and downregulate the expression of *daf-7* and several chemosensory receptors in ASI. ADF is one of two pairs of serotonergic cells in the head. They may act to transduce a food signal through release of serotonin, because exogenous serotonin is able to substitute for food in several behaviors. In addition to their role in dauer formation, ASI and ADF have a minor role in chemotaxis to water-soluble attractants, a behavior that is suppressed by crowding. These results suggest that ASI and ADF may sense pheromone and food to modulate dauer formation, behavior, and gene expression.

To refine our understanding of this process, we are generating transgenic animals that allow selective activation and inactivation of ASI and ADF. Cell-specific promoters for ASI and ADF are being used to drive expression of four proteins that affect neuronal activity: VR1, ODR-10, tetanus toxin light chain (TeTxLC), and mutant K^+ channels. VR1, the mammalian capsaicin receptor, and ODR-10, the *C. elegans* diacetyl receptor, can be activated by their respective ligands in a temporally controlled manner to regulate neuronal activity. TeTxLC may suppress evoked synaptic release, whereas gain of function K^+ channels such as *egl-2* (*A478V*) may block excitation by opening at inappropriately low voltages. These transgenic animals will be exposed to pheromone and food signals to see how ASI and ADF modulate responses to these stimuli.

214. Mutations that Cause Food-Deprived Behavior in Well-Fed Animals

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Ā̄cute food deprivation causes N2 animals to exhibit a serotonin-dependent slowing response when entering a bacterial lawn. This *enhanced slowing response* is absent in well-fed animals which exhibit a dopamine-dependent *basal slowing response* upon entering a bacterial lawn. Acute food deprivation has no significant effect on N2 locomotion in the absence of food. Thus, the condition of being acutely food deprived causes animals to react very differently to a food stimulus. Our laboratory is interested in determining what genes are involved in this food-dependent modulation of locomotion.

To find genes involved in establishing and responding to a food-deprived state in *C. elegans*, we have performed a screen, using EMS mutagenesis, for mutants that inappropriately exhibit a *constitutive enhanced slowing response* in the absence of acute food deprivation. Our starting strain includes a null mutation in the serotonin reuptake transporter *mod-5(n3314)*. This mutation causes a *hyper-enhanced slowing response*, so that *mod-5(n3314)* animals become temporarily paralyzed upon entering a bacterial lawn specifically after acute food-deprivation. We screened for animals that exhibit a food-dependent paralysis in the absence of acute food deprivation. We then tested for serotonin dependence of this paralysis. Six of the 33 mutants isolated in the screen regain mobility when pre-treated with the serotonin receptor antagonist methiothepin, which antagonizes the serotonin-gated chloride channel MOD-1, a key protein in the enhanced slowing response. These six mutants grow at a normal rate.

Currently we are screening for constitutive hyper-enhanced slowing mutants using Mos 1 transposon mutagenesis and have isolated several mutants. Through the identification of genes that underlie the enhanced slowing response, we hope to understand more about the generation, storage, and retrieval of potential hunger signals in *C. elegans*.

215. The analysis of anterior convulsions in *C. elegans*.

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Loss-of-function mutations in *unc-43*, which encodes the *C. elegans* CaMKII, cause full body convulsions when exposed to neurostimulants such as pentylenetetrazol (PTZ) and pilocarpine. A full body convulsion is the repeated contraction and relaxation of the dorsal and ventral body wall muscles along the entire length of the body. During screens for mutants with convulsions on PTZ, the anterior convulsion phenotype was discovered: it is similar to the full body convulsion phenotype, except the convulsion occurs only in the anterior region of *C. elegans*. Many of the anterior convulsion mutants found in the screen are GABA deficient mutants (*unc-25*, *unc-46*, etc.). We also identified *unc-10*, which is involved in general synaptic transmission. Based on finding *unc-10*, we tested other general synaptic transmission mutants (*unc-26*, *unc-64*, etc.) on neurostimulants and have found that some of these mutants also have anterior convulsions.

We are investigating the cause of this anterior convulsion phenotype. A simple hypothesis is that a deficiency in GABA neurotransmission is responsible. To test this hypothesis, we plan to drive the expression of a general synaptic transmission gene with a GABA-specific promoter to see whether the anterior convulsion phenotype is rescued. We are also completing the characterization of other anterior convulsion mutants found in our screens by genetic mapping and complementation testing.

216. EGL-46 regulates the specific characteristics of hook sensory neuron HOB for its function in *C. elegans* male mating

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The hook sensory neuron HOB mediates vulva location behavior during *Caenorhabditis elegans* male mating. We screened for mutations that affect HOB gene expression and isolated an allele of zinc finger transcription factor *egl-46*. Mutations in *egl-46* are defective in vulva location, indicating a disruption of HOB function. Establishment of primary neuronal fate of HOB, cell position and morphology, and dendritic process to the male hook are normal in the *egl-46(-)* males. EGL-46 regulates HOB-specific expression of polycystins *lov-1* and *pkd-2*, homeodomain gene *ceh-26*, and neuropeptide-like protein *nlp-8*, but is not required for expression of general cilium structure genes *osm-5* and *osm-6* in the HOB neuron. By contrast, RFX-like transcription factor *daf-19* is not only an upstream key regulator in general cilium formation pathway, but also affects the expression of *pkd-2*, *ceh-26*, and *nlp-8* in a non-HOB-specific manner. Therefore, *egl-46* regulates a cell-specific program to define sensory specificity for HOB neuronal function. This program might be dependant upon execution of the ciliogenic pathway.

217. Simulating neural networks for spatial orientation in C. elegans

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In C. elegans, spatial orientation behavior in a chemical gradient (chemotaxis) involves a series of turns (pirouettes) whose frequency is modulated by the change of attractant concentration. Ablation of identified neurons has delineated a candidate neural network for chemotaxis in C. elegans (C. Bargmann, UCSF, unpublished data). The aim of our research is to generate testable models of how this network computes behavioral state and consequently turning frequency in response to changes in concentration.

Neurons were modeled as passive, iso-potential electrical compartments with a sigmoidal input-output function. One neuron behaved as the chemosensory neuron, another as the output neuron which represented the behavioral state, with remaining neurons set as interneurons. Connection strengths in the model were iteratively trained via simulated annealing to reproduce idealized output from the biological network during a chemotaxis assay.

Networks with 4 to 18 interneurons were sufficient to generalize the input-output function. Trained networks also performed well when presented with novel input data (generalization). Larger networks could be reduced to networks with fewer dynamic interneurons without loss of generalization. This indicates that only small patterns of interneurons are necessary to simulate chemotaxis behavior.

We plan to use the model to predict and interpret the results of neuronal ablations in the biological network. Together, these results will provide insights into C. elegans' chemotaxis behavior and neural computational strategies.

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218. Simulation of food transport in the pharynx

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C elegans is a filter-feeder: it takes in liquid with suspended particles (bacteria), then spits out the liquid, while trapping the particles. When one analyzes videotaped motions of bacteria or latex beads in the pharyngeal lumen, they seem to move backward with the liquid during the pharyngeal contraction, as you would expect. However, when the muscle relaxes, the liquid rushes forward and out the mouth, while the bacteria seem not to move very far anterior. The net result is that bacteria are transported posteriorly, toward the intestine. Unfortunately, the relaxation is very fast, only a few milliseconds, so that the detailed motions can't be seen. I decided to test whether I could simulate the transport of bacteria by simple hydrodynamic mechanisms.

The simulation incorporates three assumptions. Two are not controversial:

1. When the diameter of the pharyngeal lumen is less than the diameter of the bacterium, the bacterium is held in place by the lumen walls.
2. When the diameter of the lumen is greater than the diameter of the bacterium, the bacterium moves with the fluid.

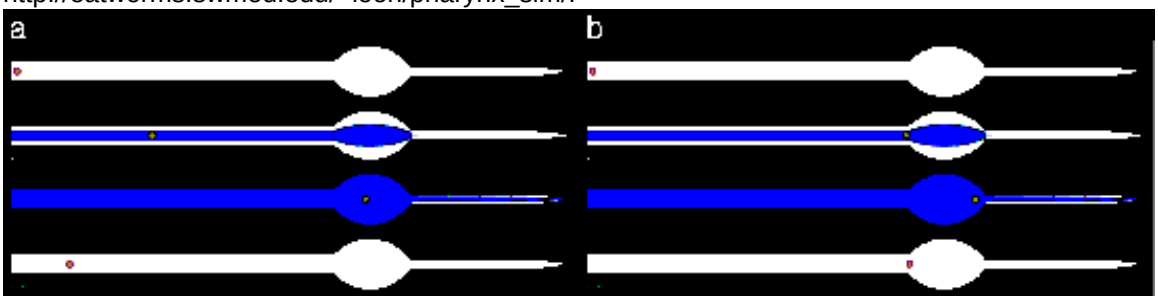
Under these assumptions, there would be no net transport of bacteria if the pharynx contracted simultaneously along its entire length. In this case the relaxation would be a simple reverse of the contraction, and during relaxation the bacteria would precisely reverse the motions they underwent during contraction, ending up where they started. In fact, analysis of videotapes shows that pharyngeal motions are not synchronized along the entire length of the pharynx: the anterior isthmus begins its contraction and relaxation slightly after the corresponding motions of the corpus. When this slight delay is included in the simulation, there is net posterior transport of bacteria but it is very inefficient (part a of figure below).

The last assumption that went into the simulation is less obvious than the first two, but is plausible given the triradiate shape of the pharyngeal lumen:

3. Bacteria are pushed to the center of the pharyngeal lumen when it closes. (Videotapes confirm this assumption.)

Fluid moves faster at the center of a tube than near the walls, so that under this assumption bacteria at the center move at 2.2 to 3.2 times the mean fluid velocity. With this assumption, the simulation not only transport particles, but does so with an efficiency similar to that of real worms (part b of figure below). These results suggest that simple hydrodynamics are indeed sufficient to explain the trapping and transport of bacteria within the pharynx.

The simulation, along with a more detailed explanation, is available as a Java applet at http://eatworms.swmed.edu/~leon/pharynx_sim/.



These pictures represent the motions of a bacterium in the lumen of the corpus and anterior half of the isthmus of the pharynx. In panel a the particle moves at the mean fluid velocity; in b, it moves at the center fluid velocity. The four snapshots on each side are taken at 0, 70, 167, and 305 ms after contraction begins. White represents the maximal possible opening of the pharyngeal lumen at all times, and blue the open of the lumen at the particular time represented by that picture. The bacterium is shown as a small brown circle.

219. Modulation of C. elegans egg-laying behavior by the environment and experience
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The egg-laying behavior of *C. elegans* provides a powerful system for the study of neural circuits at a cellular and molecular level. While the egg-laying neuromusculature is simple and well-characterized, little is known about how egg-laying behavior is regulated by the environment or experience. We are interested in characterizing two types of modulation of egg-laying behavior. First, when a well-fed hermaphrodite is removed from a food source, the animal stops laying eggs. More recently, we have also observed that upon return to food, the frequency of egg-laying events in food-deprived animals increases over that of animals left on food, and the magnitude of this increase is a function of the duration of time spent away from food.

To identify mutants defective in the modulation of egg-laying, we have begun to examine the existing set of Egl mutants, in particular class C, D, and E Egl mutants, which have HSNs with apparently normal morphology, have functional sex muscles, and have a normal vulva, yet lay fewer eggs than wild-type animals. These mutants, like wild-type animals, lay eggs in response to exogenous serotonin and in response to the serotonin reuptake inhibitor imipramine, which is thought to potentiate the signaling from the serotonergic HSNs to the sex muscles. The mutations underlying the egg-laying defects in serotinin- and imipramine-responsive Egl mutants may constitutively activate or alter the properties of pathways whose normal role is to modulate egg-laying.

Our preliminary survey of serotonin- and imipramine-responsive Egl mutants has identified *egl-6(n592)* and *unc-31(e928)* as mutants that fail to inhibit egg-laying in the absence of food. We also identified *egl-7(n595)* as a mutant that fails to up-regulate egg-laying after a period of food deprivation.*unc-31* has been cloned by others and encodes a CAPS-like protein that is implicated in the exocytosis of dense-core granules. *egl-6* and *egl-7* have not been molecularly characterized. We are pursuing the cloning and further characterization of these two genes.

220. Forward locomotion depends on expression of the Stomatin like protein, UNC-24, in *C. elegans* neurons.

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The membrane protein, stomatin, was first discovered in human erythrocytes and derives its name from the observation that stomatin protein levels are reduced in patients suffering from the hereditary cation transport disease, stomatocytosis. Stomatin Like Proteins (SLPs) in *C. elegans* include UNC-1 and MEC-2, both of which are expressed in neurons where they localize to the cytoplasmic membrane. Recent work from the Chalfie lab has shown that MEC-2 is required for DEG/ENaC cation transport function in touch cell mechanosensory receptors. In addition, genetic experiments have detected interactions between UNC-1 and UNC-8, a DEG/ENaC protein expressed in ventral cord motor neurons. *unc-24* encodes a novel SLP that includes a C-terminal domain resembling the non-specific Lipid Transfer Protein (nsLTP) in addition to an N-terminal stomatin-like sequence. Interestingly, hSLP-1, the human homologue of UNC-24 is expressed primarily in the brain including the basal ganglia which regulates vertebrate locomotion.

Although the function of UNC-24 is unknown, recent work has shown that UNC-24 is required for the cell surface localization and stability of UNC-1. In *unc-24* mutants, UNC-1 protein levels are substantially reduced and largely restricted to perinuclear dots in the vicinity of the Golgi apparatus (Sedensky et al., 2001). Our studies have now shown that a transgene encoding GFP-tagged UNC-24 protein under the control of the *unc-24* promoter is widely expressed in *C. elegans* neurons and is sufficient to rescue the Unc-24 forward movement defect. UNC-24::GFP protein is primarily localized to bright juxtanuclear puncta; however, weaker yet detectable localization to neuronal processes is evident in some cells (e.g. touch neurons). These results suggest that UNC-24 primarily resides in the Golgi compartment where it may be involved in the processing or sorting of UNC-1. We have prepared an affinity-purified UNC-24 polyclonal antibody to establish the subcellular localization of native UNC-24.

Strong extragenic suppressors of the Unc-24 movement defect were first isolated over twenty years ago (Riddle and Brenner, 1978), but these mutations have not been genetically mapped nor molecularly defined. We have isolated five new recessive extragenic *unc-24* suppressor alleles. Two of these alleles, *wd31* and *wd46*, map to chromosome X and I, respectively. A dominant *unc-24* suppressor, *m48* (Riddle and Brenner, 1978), maps to chromosome III. Thus, our preliminary data are indicative of a potentially large family of genes that can be mutated to confer Unc-24 suppression. Ongoing SNP mapping with the Hawaiian strain, CB4856, has positioned *wd31* to an interval ~ 3 m.u. to the right of *dpy-7*. We expect that molecular characterization of *unc-24* suppressor loci is likely to provide crucial clues to the mechanism of UNC-24 function in *C. elegans* neurons and that of its mammalian homolog, hSLP1, in the human brain.

221. Functional analysis of the *C.elegans* homologue of *Mblk-1*, a transcription factor expressed in the honeybee brain

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Sensory processing is mediated by interneurons in various animal species. In insect, interneurons of the mushroom bodies (MBs) of the brain are responsible for sensory integration, memory and learning. We have previously identified a honeybee gene, termed *Mblk-1*, encoding a transcription factor that is expressed preferentially in the MB-interneurons of the honeybee brain. Homology search revealed that the DNA binding motif of Mblk-1 has significant sequence homology with those encoded by genes from various animal species, including the nematoda, fruit fly, mouse and human, suggesting that the functions of these proteins in neural system are conserved among these animals.

To verify the role of *Mblk-1* in neural system, we performed reverse genetic analysis using nematoda *C.elegans*. We identified a single gene, termed *mbr-1* (MBlk-1 Related factor), two DNA-binding domains of which had significant sequence homologies with those of honeybee Mblk-1 (70% and 75%, respectively). The *mbr-1* expression was examined using animals bearing extrachromosomal array of *mbr-1 promoter::GFP*. GFP expression was observed in restricted pairs of head neurons, including AIZL/R, which is required for sensory processing in thermotaxis and also possibly in chemotaxis, raising the possibility that *mbr-1* plays a role in those behaviors.

To examine whether *mbr-1* is involved in chemotaxis, *mbr-1* deletion mutant was isolated by TMP/UV method and tested in several chemotaxis assays. *mbr-1* mutant animals showed a defect in chemotaxis toward 2-nonanone, a repellent sensed by AWB (presynaptic to AIZ). In contrast, they showed no significant defects in chemotaxis to all tested odorants sensed by AWA, AWC or ADL. *C.elegans* exhibits a behavioral plasticity, early phase adaptation, in which wild-type animals show declined chemotaxis toward odorants by exposed to the same odorants for 5 min in advance (Hirotsu et al. personal communication). *mbr-1* mutant animals were defective in this early phase adaptation to benzaldehyde, while they showed normal adaptation to isoamylalcohol and pyrazine. These results strongly suggested that *mbr-1* is involved in the chemotaxis and early phase adaptation. Considering that both *Mblk-1* and *mbr-1* are expressed selectively in interneurons, *Mblk-1* (and *mbr-1*) homologues may function in sensory processing beyond animal species.

222. AIY-Mediated Temperature Modulation of Behavior

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Thermotaxis has served as one of the key behavioral paradigms in *C. elegans* since its initial characterization in 1975 (Hedgecock and Russell, PNAS, 1975. 72(10):4061-5). With the neuronal pathway mediating thermosensation established, there has been comparatively little advance in understanding how temperature input might relate to other known *C. elegans* behaviors (Mori and Ohshima, Nature. 1995. 376:344-348).

We have begun a preliminary analysis of how various behaviors are modulated by temperature as well as what role the AIY interneuron class plays in this modulation. By testing various mutant backgrounds as well as laser ablation studies, we have looked at known behaviors such as defecation, chemotaxis and radial locomotion. We have also analyzed a new behavior, which we call thermokinesis and define as the temperature and experience modulated movement of an animal with no specified body-axis orientation. This behavior is based on the previously described thrashing of worms in liquid media (Miller et al., PNAS, 1996. 93(22):12593-8), (Tsalik and Hobert, 2001 International *C. elegans* Meeting).

223. The step response of spatial orientation behaviors in *C. elegans*.

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C. elegans exhibits two forms of spatial orientation behavior: migration to the peak of a chemical gradient (chemotaxis) and migration to cultivation temperature in a thermal gradient (thermotaxis). Both behaviors involve successive comparisons of stimulus strength through time, a process analogous to computing the time derivative of the stimulus. To investigate the neuronal basis of this computation, we devised a means of delivering rapid, step-like changes in chemical concentration or temperature to freely moving worms.

Individual worms were placed on a thin agarose film suspended over a chamber filled with a buffer solution containing 50 mM NaCl. After a baseline observation period, we quickly replaced the first chamber with a second one that contained 0 mM NaCl, to deliver a concentration step to the worm. We then observed the behavior of the worm for an additional few minutes.

In wild type worms, a decrease in NaCl concentration resulted in an increase in turning behavior. This increase was transient (~30s) and followed by a prolonged (several tens of seconds) suppression of turning. In order to understand the neuronal and genetic basis of this response, experiments will be conducted using chemotaxis-deficient mutants, and laser ablation of individual neurons.

In a second set of experiments, we tested the response of worms to step changes in temperature. The same apparatus and protocol was used, but the 2 chambers contained solutions at different temperatures (20 and 23^{o}C respectively), instead of different NaCl concentrations. When we shifted wild type worms to the higher temperature, we observed a transient increase in turning, followed by a prolonged suppression of turning. Thermotaxis-deficient mutants (ttx-1, ttx-3, unc-86) displayed very specific alterations in their response, alterations consistent with their phenotype.

Therefore, worms respond to a decrease in NaCl concentration and to an increase in temperature in a similar way: they initially increase turning, then suppress it. On a spatial gradient, this "turn-and-run" strategy would promote dispersal away from the aversive stimulus (low NaCl concentration or high temperature). Therefore these two different sensory modalities converge, at least partly, on a similar behavioral strategy. Notably, the time constant of the responses to concentration and temperature shifts was on the order of tens of seconds-a range that places significant constraints on the physiological basis of the derivative computation, to be addressed in future experiments.

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224. Role of the *egl-2* potassium channel in chemotaxis.

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egl-2, an eag-like potassium channel, is expressed in a subset of neurons and muscles. No phenotype has been reported for the loss-of-function (lof) mutation, and the deficits in egg-laying and chemotaxis observed in gain-of-function (gof) mutants have been attributed to hyperpolarization of the vulval muscle or chemosensory neurons respectively, due to inappropriate activation of the channel. Using behavioral assays and computer simulations, we sought to refine the functional role of *egl-2*. As *egl-2* is expressed in the ASE chemosensory neurons, responsible for salt sensation, we tested WT and *egl-2* mutants for NaCl chemotaxis.

We used a population assay, the quadrant assay, to identify possible chemotaxis defects in *egl-2* mutants. Both *egl-2* gof and lof mutants displayed a lower chemotaxis index than WT. In order to understand the origin of this deficit, we designed a new assay, allowing delivery of step changes in NaCl concentration to individual worms. Worms were restrained by glueing their head, leaving the tail free, and immersed in a chamber containing 50mM NaCl. Under such conditions, forward swimming, reversals and omega bends were clearly recognizable. The time of occurrence of these behaviors was recorded before and after replacement of the bath solution by 0mM NaCl. WT worms responded to this decrease in NaCl concentration by an initial increase in turning, followed by a prolonged (~2 min.) suppression of turning. *egl-2* lof and gof mutants displayed alterations of opposite sign in this response, the turning part being prolonged in gof mutants, whereas the suppression of turning was prolonged in the lof mutants. In order to understand if these alterations in the time course of the response were sufficient to account for the chemotaxis deficit, we build a kinetic model, in which transition rates between different behaviors were modulated by the chemosensory stimulus. When placed in a quadrant-like environment, model worms displayed a chemotaxis index similar to that of real worms. Moreover, changing the time course of WT transition rates reproduced the chemotaxis deficit observed in both lof and gof mutants.

Therefore *egl-2* appears to play a role in setting the time course of the response to chemosensory stimulation.

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225. Characterization of the sperm transfer step of male mating behavior of *Caenorhabditis elegans* .

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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). These sub-behaviors are: Response to hermaphrodite, backing, turning, vulval location, spicule insertion and sperm transfer. We are analyzing the genetic control of this stereotyped behavior as it may provide insight into sensory perception and nervous system function.

One of our foci has been on the sperm transfer sub-behavior. Preliminary observation of wild-type sperm transfer has defined four steps in this process: initiation, release, continuation and cessation of transfer. To begin to answer fundamental questions about how sperm transfer is regulated, we are currently doing a genetic screen designed to isolate males defective for this process.

Using an F_2 clonal screening approach, 14,000 EMS mutagenized *plg-1(2001d) him-5(e1490)* F_3 populations were scored for plugs. Plates that contain no hermaphrodites with mating plugs indicate a possible defect in one or more of the above mentioned sub-behaviors. These plug-less lines were further characterized and screened for males defective for sperm transfer. Thus far our screening has produced one mutant defective in the initiation step of sperm transfer *(sy671)* and one mutant defective for both initiation and sperm transfer continuation *(sy672)*. These isolated mutants appear morphologically normal and their phenotypic characterization will be discussed.

In order to characterize *sy671* at the molecular level, we are using positional mapping techniques to clone this gene. Molecular and phenotypic markers have been used to place *sy671* in a five cosmid interval on the X chromosome. The sperm transfer defect in *sy671* was rescued through injection of one of these cosmids and further delineation of the locus is in progress.

226. Characterization of the early steps of male mating behavior of *C. elegans*

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C. elegans male mating behavior can be organized into at least six steps: response to the hermaphrodite, backing, turning, vulval location, spicule insertion and sperm transfer. Although the signals that trigger these steps are unknown they are likely to include chemosensory, mechanosensory and proprioceptive cues. Male mating provides a basis for understanding how multiple sensory inputs can be processed to coordinate a series of behaviors. A complete understanding of this process will first require knowledge of the regulation of each individual step of mating. One of our foci is on the earliest steps of mating, response, backing and turning.

We have carried out an F2 clonal screen to isolate mutations that disrupt genes required for mating behavior. EMS mutagenized *plg-1*; *him-5* lines were first assayed for their ability to form a copulatory plug over the vulva of hermaphrodite siblings. The mating behavior of lines consistently failing to form a copulatory plug was then analyzed in depth to determine if any of the steps of mating were disrupted. Of 14,000 F2 lines screened approximately 5.9% failed to show copulatory plugs. Within this group were lines that disrupted subsets of each of the steps of mating. We chose a group of six defective lines based on the high penetrance and consistency of their phenotypes for further analysis. No mutations were isolated that only effected response, backing or turning individually. This could suggest redundancy of pathways controlling the steps of mating and/or that the screening procedure disfavored isolation of step specific mutants. Three mutant lines show defects in response and vulval location behavior. Two mutant lines show defects in response, turning and vulval location, and one shows defects in response and turning. The fact that we have isolated mutations disrupting different subsets of behaviors may provide us with insight into the similarities and differences in the regulation of the steps of mating behavior. Further characterization and mapping of these mutations is in progress.

The phenotypes in two of the lines with response and vulval location defects result from a mutation in a single gene and the mutations are recessive. Complementation tests and initial mapping experiments indicate that the two mutations are likely to be alleles of *pkd-2*, a gene found previously in the Sternberg lab to be necessary for response and vulval location. *pkd-2* encodes the *C. elegans* homolog to one of the two major genes disrupted in human autosomal dominant polycystic kidney disease. We are currently sequencing these alleles to determine the molecular nature of the mutations. These mutations may provide further insight into important functional regions of PKD2.

The mutation *cod-5* was previously isolated in our lab in a screen for lines with reduced mating efficiency. We found that in addition to having strong turning defects,*cod-5* mutants are also defective in response and vulval location. Cloning of *cod-5,* is in progress.

227. The role of chb-3 in sensory regulation of body size and locomotory state

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Mutants with defects in sensory cilium structure are unable to respond to sensory stimuli and also exhibit complex and unexpected changes in development and behavior. Lewis and Hodgkin initially reported that cilium-defective mutants such as che-2 and che-3 are smaller than wild-type animals (1977). We have previously demonstrated rescue of the che-2 body size defect by expressing the che-2 cDNA in amphid sensory neurons. These observations lead us to the hypothesis that an animal regulates its body size by sensing an environmental cue such as food. We have also demonstrated that cilium-defective mutants exhibit distinctive tracking patterns on food, although the mutants are not uncoordinated. Computerized analysis of locomotion revealed that a wild-type animal has two distinct locomotory states on food, dwelling and roaming, defined based on turning rate and speed. The roaming state is significantly decreased in cilium-structure mutants.

In order to analyze the putative neural regulation of body size, we isolated suppressor mutants of the che-2 small body size phenotype (chb). None of chb mutations we isolated suppress the dye-filling defect of che-2. Among them, chb-1 (4 alleles) proved to be allelic with egl-4 which was originally isolated as an egg-laying defective mutant. Interestingly, egl-4 also suppresses the che-2 locomotory change. We found that egl-4 encodes a cGMP-dependent protein kinase. We expressed the cDNA of this gene using a panneuronal promoter as well as an amphid promoter (tax-4), and found rescue of these egl-4 phenotypes (suppression of the che-2 small body size and locomotory changes). These results suggest that EGL-4 cGMP-dependent kinase acts in sensory neurons and regulates body size and locomotory states by processing of sensory information.

We are currently analyzing another chb mutant, chb-3(eg52). chb-3 also suppresses not only the che-2 small body size but also the che-2 locomotory change, suggesting a possible role in the egl-4 pathway. chb-3 exhibits pleiotropic phenotypes including cheomotaxis defects to both odorants (isoamyl alchol, benzaldehyde and diacetyl) and soluble attractants (Na+ and lysine), an avoidance defect to a repellent (nonanone), a relatively weak defect in osmotic avoidance, and inappropriate dauer formation (Daf-c; 8~% at 20C, 90~% at 27C). We could not find any gross anatomical defects in sensory neurons examined by DiI staining. We mapped this gene to a small region on LGI (left arm), by using the snip-SNP method based on the Daf-c phenotype. A YAC (Y2F4) covering this region rescues chb-3. A PCR fragment covering a gene (Y48G1A.3) on the YAC also rescues chb-3. Sequencing analysis of chb-3(eg52) showed a mutation in a splice donor site of the 1st intron of this gene. This gene encodes a novel protein with an ANK motif and a zf-MYND domain, which is often found in DNA binding proteins. A BLAST search revealed homologous proteins in human, mouse and Drosophila. A GFP promoter fusion gives expression in several amphid neurons, two other neurons (PQR, AQR) and weakly in intestine.

We are now further characterizing the functional role of chb-3. The Daf-c phenotype of chb-3 is suppressed by a cilium-defective mutant but not by daf-3, suggesting this mutant is a member of Group I Daf-c. We are also investigating the interaction between chb-3 and egl-4.

228. 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). An unknown signal or signals from the intestine initiates the process of defecation, but how that signal is transmitted to activate the aBoc and Expulsion is unknown. 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 have 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 and the enteric muscles that activates the aBoc and enteric muscle contractions.

We are taking two approaches to identify other proteins functioning in the AEX-2 pathway. First, we are looking for suppression of the Con phenotype in strains containing mutations in both *aex-2* and other G proteins in an attempt to identify the proteins functioning immediately downstream of *aex-2*. Second, we are screening for suppressors of the *aex-2* expulsion defect. We have identified 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 alleles of a novel class that occasionally execute more than one expulsion per cycle. None of these mutations suppresses the aBoc phenotype of *aex-2*, suggesting that while the aBoc and Exp phenotypes are caused by one mutation, they are functioning via separate downstream pathways.

1. Dal Santo P, et al., 1999. Cell 98: 757-67.
2. Thacker C and AM Rose, 2000. Bioessays 22: 1-9.
3. Carey, T. and J.M. Kaplan International Worm Meeting Abstract, 2001.

229. Analyses of mechanosensory transduction by *in vivo* calcium imaging

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In *C.elegans*, genetic analyses of gentle body touch sensation have lead to identification of the molecular components required for the mechanosensory transduction. However, characterizations of molecular events carried out by these molecules have been limited by the lack of techniques to detect such events *in vivo*. Direct electrophysiology is precluded by the tough cuticle and the unique environment surrounding the touch neurons, which also limit pharmacological characterization.

We have developed imaging methods to monitor in vivo calcium transients in neurons and muscle cells by using a gene-encodable calcium indicator, yellow cameleon (Miyawaki, et al., *PNAS* 96:2135-2140, Kerr et al., *Neuron* 26:583-594). We can monitor *in vivo* calcium transients by measuring the fluorescent emission of yellow cameleon through the transparent cuticle.

Several improvements to our method have been made since our preliminary study (IWM 2001, abstract 659). 1) reconstruction of *mec-4* promoter/cameleon reporter 2) integration of the transgene and repeated outcrossings 3) employment of Piezo electronic devices for our mechanical stimulator to precisely deliver gentle touch stimuli 4) programming the movement of the stimulator (speed, traveling-distance, interstimulus interval, etc)

Delivery of a stimulus consisting of a 10 micron deflection lasting 150msec(75msec forward travel, 75msec backward travel) on the sensory process of a touch neuron (ALM, AVM or PLM) evokes a rapid increase in intracellular calcium followed by a gradual decrease. A variety of other "gentle touch stimuli" with different movements were tried, and the size of the calcium transients corresponded to the duration of motion during stimulation. However, a motionless probe pressed against the worm failed to cause or maintain a calcium transient. These observations suggest that touch neurons primarily sense the motion of the applied mechanical stimulation.

Several types of "gentle touch stimuli" were applied to *mec* mutants (*mec*hanosensory abnormal) identified by the genetic analyses. We have tested *mec-4* (for a candidate mechanically-gated ion channel), *mec-2* (for a stomatin-like protein that has been shown to potentiate MEC-4 channel activity) and *mec-6* (neeed for stable MEC-4 channel expression) and none of the mutants have responded to the "gentle touch stimuli".

We have also employed a recently developed primary culture method of *C.elegans* embryonic cells (Christensen et al., *Neuron* 33: 503-514). The cells were depolarized with high potassium saline, and the calcium transients were imaged the same way as the intact worms. The cultured mechanosensory neurons from the wild-type and *mec-2* and *-4* mutants showed essentially indistinguishable calcium transients, confirming the intactness of the indicator and the calcium influx/ release mechanisms in the mutant worms. These observations also suggest that MEC-2 and MEC-4 are required to depolarize the touch neurons in response to mechanical stimulation, presumably by sodium influx via MEC-4 channel potentiated by MEC-2.

Voltage-gated calcium channels (VGCC) are good candidates for the calcium entry in response to mechanosensation. Apparently wild-type calcium transients have been observed in *unc-2* (non L-type VGCC) null alleles. Other sources of cytosolic calcium transients are under investigation, including egl-19 (L-type VGCC), *unc-68* (ryanodine receptor) and *itr-1* (inositol 3-phosphate receptor).

We are grateful to Dr. Atsushi Miyawaki for his kind supply of recent versions of cameleons.

230. A suppressor screen of flp-1 gene

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FMRFamide (Phe-Met-Arg-Phe-amide)-related neuropeptides are involved in feeding, muscular control, cardioregulation, and learning. In C.elegans, 22 flp (FMRFamide-like peptide) genes are expressed in distinct but overlapping sets of neurons. They encode 59 distinct predicted FMRFamide-related peptides. A deletion mutation caused by Tc1 insertion in flp-1 gene caused motor and behavior defects, indicating some distinct function of the gene.

Vital dye Nile red (9-diethylamino-5H-benzo[a]phenoxazine-5-one) is used to visualize fat droplets in living worms. flp-1(yn2) animals, when grown on plates with nile red has reduced nile red staining, compared with wild type animals. This indicates that the neuropeptides encoded by flp-1 gene might also be involved in fat metabolism in C.elegans.

We did a screen of 20,000 F1s for suppressors of flp-1. Six mutants from F1 and twenty from F2, brought the reduced nile red staining of flp-1(yn2) back to wild-type level. We are currently mapping the mutations for further characterization.

231. The *cat-4*/GTP cyclohydrolase I /F32G8.6 gene of *C. elegans*

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We are characterizing genes used by serotonergic neurons in *C. elegans* to learn how they are regulated; among these is the *cat-4* gene. Worms with mutations in the *cat-4* gene lack serotonin and dopamine, and are hypersensitive to a variety of agents. The *cat-4* gene appears to correspond to the predicted gene F32G8.6, which encodes GTP Cyclohydrolase I (GCH), an enzyme necessary for synthesis of biopterin. Biopterin is a cofactor required by (among others) all aromatic amino acid hydroxylases (AAAHs); these include the enzymes catalyzing synthesis of serotonin, dopamine and tyrosine. We have found that the original *cat-4* mutant allele *(e1141)* has a missense mutation in the GCH coding sequence altering a highly conserved amino acid. A new mutant (kindly provided by the C.e. Gene Knockout Consortium) that deletes the F32G8.6/GCH gene is also serotonin-deficient, hypersensitive, and fails to complement *cat-4(e1141)*. We hypothesize that hypersensitivity of *cat-4* mutants is caused by tyrosine deficiency during cuticle synthesis, leading to a reduction in tyrosine cross-linking in cuticle proteins, and a 'leaky' cuticle. We are continuing characterization of the new *cat-4* deletion allele, including comparing the presence of dityrosine cross-links in the cuticles of mutant vs. wild type worms.

232. Characterization of the aromatic amino acid decarboxylase *bas-1* in serotonergic neurons

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Several genes required for the production of the neurotransmitter serotonin have been identified in *C elegans*. In particular, *bas-1* (biogenic amine synthesis abnormal) is necessary for the synthesis of serotonin and dopamine as demonstrated by an absence of serotonin immunoreactivity (serotonin-IR) and formaldehyde induced fluorescence in *bas-1* mutants. *Bas-1* mutants exhibit a number of behavioral defects that reflect a deficit in signaling by serotonergic neurons, including abnormal male turning behavior and a subtle alteration in egg-laying behavior. We have shown previously that *bas-1* encodes a serotonin and dopamine synthetic amino acid decarboxylase (AAADC) corresponding to the predicted gene CO5D2.4. We are continuing to define the regulatory regions of *bas-1* by deletion constructs and are investigating *bas-1* expression. RT-PCR has revealed three different alternatively spliced forms of CO5D2.4. Northern blot analysis as well as an ongoing attempt to generate an antibody should further define the *bas-1* expression pattern through all stages of development.

233. Genetic Analysis of the Behavioral Effects of Amphetamine in *C. elegans*.
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Amphetamine as well as other psychostimulants such as cocaine and methylphenidate have a high potential for abuse. Their primary mechanism of action is the inhibition of the clearance of dopamine from the synapse via the dopamine plasma membrane transporter (DAT). The CeDAT has been cloned and shown *in vitro* to bind both amphetamine and cocaine (LD Jayanthi *et al.*, Mol Pharm 54:601-9, 1998). There is also evidence that even a single exposure to psychostimulants can produce long term potentiation on the mesolimbic dopamine system (Ungless *et al.*, Nature 411:583-587, 2001) which is part of the reward pathway activated by drugs of abuse. These synaptic changes may be important for the subsequent addiction to these types of drugs. We are interested in characterizing further the mechanisms that contribute to the effects of amphetamine modulation on dopaminergic pathways.

To examine the effects of amphetamine on synaptic transmission in *C. elegans* we pretreated worms with amphetamine in liquid culture prior to transferring animals to agar plates for behavioral analysis. We found that amphetamine pretreatment resulted in suppression of locomotion, egg laying, and pharyngeal pumping in a dose dependent manner. These behavioral effects are similar to those produced by exposing animals to exogenous dopamine, suggesting amphetamine is acting on dopaminergic pathways. We then initiated a genetic screen to identify mutants that showed reduced sensitivity to the effects of amphetamine on locomotion. We have identified eight candidate mutants and are in the process of mapping and cloning the genes responsible for their resistance. Most of these mutants are visibly wild type with the exception of two of the mutants which appeared hyperactive for locomotion. This hyperactivity was confirmed by assaying for aldicarb sensitivity. The other mutants were found to be either wild-type or resistant to the effect of aldicarb on movement. One of these mutants also displayed reduced sensitivity to the effects of amphetamine on pharyngeal pumping and two other mutants displayed reduced sensitivity to the effects of amphetamine on egg laying. We are characterizing the mutants from our screen for defects in dopamine mediated behaviors. Preliminary results indicate that two of these mutants are defective in the dopamine dependent basal slowing response on bacteria (Sawin *et al.*, Neuron 26:619-631, 2000).

234. Serotonin promotes Go-dependent neuronal migration in Caenorhabditis elegans

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The directed migration of neurons during development requires attractive and repulsive cues, which control the direction of migration, as well as permissive cues that potentiate cell motility and responsiveness to guidance molecules. We have found that the neurotransmitter serotonin functions as a permissive signal for embryonic and post-embryonic neuronal migration in the nematode C. elegans. In serotonin-deficient mutants (tph-1), the migrations of the ALM, BDU, SDQR and AVM neurons were often foreshortened or misdirected, indicating a serotonin requirement for normal migration. Moreover, exogenous serotonin could restore motility to AVM neurons in serotonin-deficient mutants as well as induce AVM-like migrations in the normally non-motile neuron PVM, indicating that serotonin was functioning as a permissive cure to enable neuronal motility. The migration defects of serotonin deficient mutants were mimicked by ablations of serotonergic neuroendocrine cells, implicating humoral release of serotonin in these processes. Mutants defective in Gq and Go signaling, or in N-type voltage-gated calcium channels, showed migration phenotypes similar to serotonin-deficient mutants, and these molecules appeared to genetically function downstream of serotonin in the control of neuronal migration. Thus, serotonin is important for promoting directed neuronal migration in the developing C. elegans nervous system. We hypothesize that serotonin may promote cell motility through G-protein-dependent modulation of voltage-gated calcium channels in the migrating cell.

235. A genetic dissection of OSM-9 and OCR-2 signal transduction pathways in *C. elegans*

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Members of the TRP ion channel family mediate sensory signal transduction in a wide variety of organisms. Prominent family members include the *Drosophila*phototransduction channel TRP, the mammalian capsaicin receptor TRPV1, and the *C. elegans* sensory channel *osm-9*. A precise understanding of the molecules that regulate TRPV function remains elusive. By using *C. elegans* genetics, we hope to identify genes and molecules essential for *osm-9*/TRPV channel function.

Together with the related channel OCR-2, OSM-9 facilitates multiple sensory behaviors, including chemotaxis to AWA-sensed attractive odorants and repulsion from ASH-sensed noxious stimuli. These behavioral assays provide a set of tools for understanding *osm-9/ocr-2* signaling pathways in discrete neurons.

Several recent papers suggest that polyunsaturated fatty acids and other lipids directly modulate TRP and TRPV channels. Mutations that alter the composition of fatty acids in *C. elegans* have been identified through biochemical screens[i]. Some of these *fat* mutants show AWA and ASH behavioral defects, suggesting that distinct 18- and 20-carbon polyunsaturated fatty acids may modulate OSM-9/OCR-2 signaling pathways.

Activity of the OSM-9 and OCR-2 channels modulates AWA expression of the diacetyl receptor ODR-10: In *osm-9* or *ocr-2* mutants, expression of an *odr-10*::GFP reporter transgene is reduced. *fat* mutants also affect *odr-10::*GFP expression, consistent with the idea that lipids regulate OSM-9 and OCR-2 activity. The Gα protein ODR-3 also promotes *odr-10::*GFP expression; ODR-3 is likely to couple signaling from G protein-coupled receptors in AWA and ASH to the OSM-9/OCR-2 channels. The receptor-ODR-3-OSM-9/OCR-2 pathway appears to be a sensory activity-dependent pathway for modulation of gene expression in AWA.

To further characterize regulatory pathways for AWA gene expression, we have identified suppressors of the *odr-10::*GFP expression defect in *osm-9* mutants. Either dauer formation or mutations in the insulin receptor *daf-2* suppress *osm-9* loss-of-function alleles, suggesting that components of dauer development/insulin signaling pathways impinge upon AWA signal transduction or gene expression pathways.

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[i] Watts, J.L., and Browse, J. PNAS 99:5854-5859.

236. Uncovering Sensory Roles for TRP Channels in Male Mating Behavior

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The mating behavior of male *C. elegans* is arguably the most complex behavior exhibited by the worm and as such represents an ideal model to study the genetic and neurophysiological bases of complex sensorimotor behaviors. Mating can be considered a sensorimotor program that proceeds through the following stereotypical, behavioral sub-steps: response to hermaphrodite contact; backing; turning; location of vulva (LOV) - stop; spicule insertion; and sperm transfer (Liu and Sternberg, 1995). The complexity of this behavioral program is reflected in the male nervous system, as males possess an additional 79 neurons compared to hermaphrodites. 75 of the male-specific neurons are associated with the copulatory structures in the specialized male tail and many send ciliated projections to the environment where they serve as sensory neurons for detecting hermaphrodite specific cues. We are interested in the signaling mechanisms employed by which male specific neurons function as sensory neurons.

TRP ion channels perform sensory functions in a diverse range of sensory systems and organisms, and we have set out to uncover potential sensory roles for *C.elegans* TRP genes in male tail sensory neurons and mating behavior. We have surveyed the expression of all 12 *C.elegans* TRP family members (Harteneck et al., 2000) using promoter::gfp translational fusion reporter transgenes. We have identified a subset of TRP channel genes, including *osm-9* and *ocr-2*, which are expressed in male specific sensory neurons, such as ray and spicule neurons. However, many TRP genes are expressed in various male specific cells, including interneurons and structural cells specific to male copulatory structures. We examined the male mating behavior of existing TRP mutants and found that only *osm-9* and *ocr-2* show sub-step specific mating defects. Combined, expression and behavioral data supports a role for these channels in the spicule neurons. We are further characterizing the function(s) of *osm-9* and *ocr-2* in the male nervous system and speculate that OSM-9 and OCR-2 may support a mechanosensory property of the spicules. Both *osm-9* and *ocr-2* have been shown to mediate sensory, including mechanosensory, functions of ASH and other head neurons (Tobin et al., in press). We are presently designing genetic strategies to dissect the functional circuitry of spicule sensory neurons to understand the nature of the signal that OSM-9 and OCR-2 receive or relay.

Harteneck, C., Plant TD, and Schultz, G. (2000) From worm to man: three subfamilies of TRP channels. TINS. 23 (4): 159-166.

Liu, KS and Sternberg PW. (1995). Sensory regulation of male mating behvior in Caenorhabditis elegans. Neuron, Jan 14 (1): 79-89.

Tobin et al., Neuron, in press.

237. Guanylate Cyclase Beta 2 Subunit: A Novel NO-insensitive, Heme-bound Cyclase?

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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 alpha 1 and beta 1 subunits. Nitric oxide (NO) binds to the heme, which is ligated to the beta 1 subunit, and stimulates the activity of the enzyme 400fold. A beta 1 homologue, beta 2, has been cloned from rat and human kidney cDNA libraries and appears to be expressed in the kidney collecting duct cells. However, the heme binding status, sensitivity to NO, and intracellular localization are not well-characterized. We would like to elucidate the structure and function of this protein.

Analysis of the *C. elegans* genome predicted seven putative sGC beta subunits, including five beta 2 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 beta 2 homologues in *C. elegans* are receptors in a cGMP-dependent signaling system.

Our data are consistent with this hypothesis. Characterization of the bacterially-expressed N-termini from *gcy-35* and from beta 2 demonstrate that they bind heme. Promoter::green fluorescent protein (GFP) fusion studies localize all of the beta 2 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 beta 2 is expressed in the kidney.

Does NO activate beta 2 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. Preliminary analyses of the heme domains of GCY-35 and beta 2 do not exclude the possibility of an oxidized heme. This would preclude NO binding and would be consistent with a novel class of NO-insensitive cyclases. Current biochemical studies are focused on the heme environment of these subunits and whether they bind NO.

238. Investigation of a cyclic-GMP dependent kinase's effects on chemosensory gene transcription and adaptation.

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Neurons of many different origins possess the ability to down-regulate their activity in the presence of an overabundant stimulus. This process, known as adaptation, is crucial to numerous organisms and requires further investigation. Fortunately, olfactory adaptation in C. elegans is a robust and genetically tractable process. Worms that are initially exposed to an attractive odor will, after prolonged exposure, fail to respond to this odor. This process is both odor-specific and endures for periods of time that correlate with duration of the exposure. For example, 30' exposures to butanone will render wild-type worms refractive to butanone chemotaxis for half and hour. They will, however, still chemotax to other odors such as benzaldehyde. This is particularly notable because one neuron, AWC, senses both odors.

One model explains chemosensation within the AWC olfactory neurons as a process that begins with activation of transmembrane G protein coupled receptors and culminates in the opening of cGMP-gated cation channels. Channel opening presumably depolarizes the neuron. Several genes have been identified as playing a role in adaptation of this process in AWC, these are: osm-9, adp-1, tax-6, and over-expressed odr-1. Thus far, there are few insights into to how their products affect adaptation of AWC.

We found that EGL-4, a cyclic-GMP dependant kinase, appears to make a dual contribution to this process. Phosphorylation of TAX-2, the beta subunit of the cGMP-gated channel at a consensus PKG/A phosphorylation site is required for adaptation to short (~30') exposures to odor but is not required for adaptation to prolonged exposures (> 60'). EGL-4 mutants are defective for adaptation to both short and long odor-exposures. Our hypothesis is that brief odor exposure down regulates neuronal activity by eliciting phosphorylation of the signal transduction machinery within the sensory cilia, but that adaptation to longer exposures requires additional changes within AWC. In this manner, cGMP both stimulates and represses the neuronal response to specific chemicals by activating two separate pathways, one that opens the cGMP-gated channel and the other that stems from activation of EGL-4 which may inhibit channel opening.

We theorize that prolonged (>60') odor-exposure increases cGMP levels and that cGMP binding exposes a nuclear localization signal (NLS) within EGL-4(Gudi et al., 1997). Once in the nucleus, EGL-4 may alter transcription of chemosensory genes. To test this, we have produced strains expressing EGL-4 with a mutated NLS sequence in a null background. If adaptation to prolonged odor exposure requires nuclear translocation, these strains should adapt to 30' exposures but be unable to adapt to >60' exposures. Additionally, we are producing GFP labeled constructs to assess localization changes of EGL-4 upon long-term exposure. We are currently designing experiments to determine how transcriptional changes might induce odor-specific adaptation.

Gudi, T., Lohmann, S., anfd Pilz, R.B. (1997) Molec. Cell Biol. 17:5244-5254.

239. Expression and Functional Analysis of RPM-1 in Synapse Formation

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The formation of a functional synapse requires a highly organized array of cellular machinery. RPM-1 (for regulator of presynaptic morphology) has been shown to be important for the gross organization of nerve terminals in *C. elegans*[1]. In wild type animals each presynaptic terminal of the GABAergic neuromuscular junction usually contains one active zone surrounded by a cluster of synaptic vesicles. In *rpm-1* mutant animals, a single presynaptic terminal often contains multiple active zones. The RPM-1 protein is homologous in structure to mammalian PAM and *Drosophila* Highwire. All three are large proteins with over 3,000 amino acids, and contain RCC1 domains, PHR domains, a B-box Zn finger domain and a RING-H2 E_3 ligase domain.

A functional RPM-1::GFP appears to localize to synapses at a specific sub-cellular domain called the periactive zone. In *Drosophila* NMJs, the periactive zones contain molecules that are important for synaptic growth [2]. To further examine the spatial organization of synapses, we have generated antibodies to RPM-1 and have developed a modified fixation method that improves the visualization of endogenous levels of RPM-1 in wild type animals. In addition, we are investigating the functional significance of different domains in RPM-1. To this end we are using a transgenic approach and expressing various functional domains of RPM-1 in wild type and *rpm-1* mutant animals.

1. Zhen M, et al. (2000) Neuron. 26:331-43. 2. Sone M, et. al. (2000) Development. 127:4157-68.

240. UNC-16, the C. elegans JIP3, plays kinesin-1-dependent and independent roles in directing vesicular traffic

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The proper localization of synaptic components is a regulated process that requires the pairing of multiple cargo vesicles with different motor proteins. Despite the identification of many motors, the specific interactions of cargoes and motors are poorly understood. We have previously shown that loss-of-function mutations in *unc-16*, the *C. elegans* homolog of JSAP1/JIP3/dSYD, cause mislocalization of synaptic vesicle and glutamate receptor markers (Neuron 32: 787-800). Moreover, mutations in *unc-16*partially suppress the synaptic vesicle retention defect in *unc-104* KIF1A mutants. UNC-16 physically interacts with the KLC-2/UNC-116 kinesin-1 complex via KLC-2. Mutations in *unc-116* KHC/KIF5 and *klc-2* share a similar synaptic vesicle mislocalization phenotype with *unc-16* mutants, and mislocalize a functional UNC-16::GFP. Conversely, UNC-116::GFP is mislocalized in *unc-16* and *klc-2* mutants. These results are consistent with a direct interaction of UNC-16 with the kinesin-1 motor complex and support an evolutionarily conserved function of UNC-16/JIP3 in mediating kinesin-1 transport.

However, the function of UNC-16 may be more complex than simply serving to regulate kinesin-1-cargo interactions because UNC-16 and kinesin-1 have separable functions. For example, mutations in *unc-116*do not suppress the *unc-104*synaptic vesicle retention defect; *unc-116* mutants, but not *unc-16*, have axon outgrowth defects; and GLR-1::GFP is mislocalized in *unc-16* mutants, but not in *unc-116*, *jnk-1*, or *unc-104* mutants. Therefore, we propose that UNC-16 likely has kinesin-1-independent functions.

241. Microarray experiments to identify *unc-4* target genes in FACS-isolated *unc-4*::GFP embryonic motor neurons.

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The UNC-4 homeoprotein is expressed in A-type motor neurons (DA, VA, SAB) 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, VAs show reduced numbers of synaptic vesicles and are miswired with inputs normally reserved for their lineal sister cells, the VB motor neurons. We have proposed that these defects arise from the failure of UNC-4 and its transcriptional cofactor, UNC-37/Groucho to repress B-motor neuron specific genes. Here we describe a new strategy that relies upon physical isolation of *unc-4*::GFP cells to identify these genes.

*unc-4::*GFP is initially detected midway through embryonic development (~400 min) and is expressed in 13 embryonically-derived motor neurons (9 DAs, 3 SABs, 1 I5). Although these neurons are not accessible in the intact animal they can be generated from dissociated preparations of embryonic precursor cells grown in culture. *unc-4*::GFP cells are not evident in freshly prepared blastomeres but appear to differentiate normally and approach the expected frequency (~3%) after one day.[1] Embryonic cells adhere loosely to poly-L-lysine coated plates and can be readily removed by gentle trituration after 18-24 hr in culture. Individual GFP^+ cells are collected by Fluorescence Activated Cell Sorting (FACS) after passage through a 5 micron filter to remove undissociated clumps of cells. Dead cells (~20%) are excluded by propidium iodide staining. *unc-4::*GFP neurons are enriched to ~90% of the FACS-isolated cell population. RNA is readily isolated from these cells with a yield of 1 pg total RNA/cell. Due to the limited amount of starting material (e.g., 100 ng total RNA from 10^5 *unc-4*::GFP cells), the polyA+ RNA fraction is subjected to two rounds of amplification before generating labeled cDNAs for microarray experiments. A *C. elegans* microarray will be queried with cDNA probes from the wildtype and from *unc-4 and unc-37* mutant backgrounds. Genes that are negatively regulated by *unc-4*, such as the known target gene, *acr-5*, are expected to show elevated levels of expression in *unc-4::GFP* cells isolated from *unc-4* and *unc-37* mutants.

[1] Christensen et al. (2002) Neuron 33:503-514.

242. GFP screens for regulators of motor neuron differentiation.

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A-type (DA,VA) and B-type (DB,VB) *C. elegans* motor neurons are morphologically distinct and receive inputs from separate sets of command interneurons. The UNC-4 homeodomain is expressed in A-type motor neurons where it functions with its Groucho-like cofactor protein, UNC-37, to block expression of B-type genes such as *acr-5* (nicotinic acetylcholine receptor). This UNC-4 repressor function is essential to the fidelity of the motor neuron network; mutations that disrupt *unc-4* or *unc-37* result in the miswiring of VA motor neurons with VB-type inputs. Conversely, ectopic expression of UNC-4 in VB motor neurons inhibits expression of *acr-5* and may miswire VB motor neurons with VA-type inputs (J. Ross & D. M.M. unpublished). Thus, proper regulation of *unc-4* expression is clearly important to the creation of a functional motor neuron circuit. We have utilized a reporter gene and *unc-4* target, *acr-5*::YFP, to conduct a visual screen for upstream regulators of *unc-4*. *acr-5*::YFP is normally expressed in B-type motor neurons but is also de-repressed in the A-type motor neurons in *unc-4* and *unc-37* mutants. The net two-fold increase in the number of YFP-expressing neurons in the ventral nerve cord (VNC) can be easily observed in a fluorescence stereodissecting microscope. (*acr-5*::YFP is also expressed in neurons in head and tail ganglia where it is not regulated by *unc-4*.) A clonal screen of 2200 F1 mutant worms revealed two new *unc-4* alleles, but did not detect mutations in other genes that also result in ectopic *acr-5*::YFP expression in the VBs. Our screen did uncover, however, seven mutants with decreased *acr-5*::YFP expression in the VNC. One particular isolate, *wd54*, lacks *acr-5*::YFP expression in the ventral cord but shows normal expression in head and tail neurons. *wd54* animals also display a forward movement defect. Although *wd54* does not affect *unc-4* expression, it appears to be required for proper differentiation of B-class motor neurons.

Recently, Union Biometrica presented a demo at Vanderbilt of the COPAS Biosort. This machine is capable of sorting worms by size (i.e, age) and fluorescence. Scatter plots of *acr-5*::YFP animals in wildtype and *unc-4* backgrounds were virtually indistinguishable, suggesting that this configuration of the Biosort would not detect mutations in activators of *unc-4*. However, wildtype and *wd54* populations, in which *acr-5*::YFP is absent from the VNC, were readily separable. We used the Biosort to screen F2 progeny of 6400 mutagenized F1 animals. Twenty-two new mutants showing altered or decreased *acr-5*::YFP expression were isolated. Two of these isolates mimic *wd54*. The speed and accuracy of the Biosort substantially enhanced our ability to isolate rare mutants affecting *C. elegans* motor neuron differentiation.

243. In search of UNC-4 response elements (U4REs)

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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. De-repression of these VB genes in *unc-4* mutant VA motor neurons presumptively imposes VB-type inputs. GFP reporters constructed from two genes, *del-1* (DEG/ENaC sodium channel subunit) and *acr-5* (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 in both VA and VB 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. Similar U4REs should also be located in other UNC-4 target genes and therefore could provide a basis for their identification. A comparison of the *C. elegans* (Ce) and *C. briggsae* (Cb) *del-1* upstream regions has revealed regions of sequence identity. A 900 bp upstream region spanning two of these conserved domains retains normal *del-1*::GFP expression. These motifs will be targeted for deletion and effects on *del-1* expression will be determined; removal of U4REs should result in *del-1*::GFP expression in VA motor neurons in wildtype animals. A comparison of the Ce and Cb *acr-5* upstream regions has not revealed obvious blocks of conserved sequence. However, deletion analysis of the Ce *acr-5* promoter has detected 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). 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 UNC-4 binding.

We recently determined that *glr-4*, a non-NMDA glutamate receptor subunit, is also regulated by *unc-4*. *glr-4*::GFP is expressed in several head/tail neurons and in the DB[1] and VB (this work) motor neurons. When placed in an *unc-4* background, *glr-4*::GFP is ectopically expressed in VA and DA motor neurons. We will compare the Ce and Cb *glr-4* promoters to detect conserved sequences and use deletion analysis to identify U4REs. A *glr-4* knockout mutant will be used to assess the potential role of *glr-4* in *unc-4* dependent synaptic specificity.

[1]Brockie, et al. (2001) *J Neurosci* . 27(5):1510-1522.

244. In search of UNC-4 targets.

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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 three genes, *del-1* (DEG/ENaC sodium channel subunit), *acr-5* (nicotinic acetylcholine receptor subunit), and *glr-4*[1] (non-NMDA glutamate receptor subunit) are ectopically expressed in the VA motor neurons in *unc-4* and *unc-37* mutants. 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 in both VA and VB neurons. The target genes that are responsible for synaptic choice have remained elusive, despite extensive genetic screens. We will now utilize a whole genome approach to identify the full complement of UNC-4 target genes and test those targets for a role in synaptogenesis.

We will compare the expression profile of wildtype worms vs. those of *unc-4* and *unc-37* animals to identify genes that are de-repressed in the mutants. Initially we obtained mRNA from synchronized wildtype, *unc-4*, and *unc-37* L2 larvae, the stage at which *unc-4* functions. The *C. elegans* microarray in the Kim lab was probed to detect differences between wildtype vs. *unc-4* (3 replicates) and wildtype vs. *unc-37* (3 replicates). Differences in expression of the known target genes (*acr-5, del-1, glr-4*) were not detected, presumably because these genes are also normally expressed in other cells where they are not regulated by *unc-4*. To obviate this problem, we have adopted a strategy to isolate VA-specific mRNAs in order to identify UNC-4 target genes. We have generated a transgenic line expressing FLAG-tagged poly-A binding protein (FLAG::PAB-1) under the control of the *unc-4* promoter. Wildtype, *unc-4,* and *unc-37* animals carrying the *unc-4*::FLAG::PAB-1 transgene will be homogenized, and the cell-specific mRNAs (bound to PAB-1) will be immunoprecipitated using an anti-FLAG antibody. Labeled cDNAs derived from amplified mRNAs will be used to probe the *C. elegans* microarray. UNC-4 target genes should be expressed at higher levels in *unc-4* and *unc-37* derived samples compared to wildtype. *In vivo* expression of these candidate genes and their regulation by *unc-4* and *unc-37* will be determined using promoter::GFP fusions. These authentic UNC-4 targets will then be genetically tested for a potential role in synaptic specificity.

[1]see Von Stetina et al., this meeting.

245. A screen to identify factors involved in active zone formation

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Neurons send out axons that form synapses with target tissues. Within these synapses, close apposition of the presynaptic and postsynaptic membranes is established. At the EM level, specialized presynaptic electron dense structures called active zones can be observed. The active zone represents the location where vesicles containing neurotransmitters fuse with the presynaptic membrane, releasing neurotransmitters to the post-synaptic membrane. While molecules have been identified that localize to the active zone, only a few have been implicated in active zone establishment, differentiation or maintenance. One example is SYD-2/liprin which has been demonstrated to be localized to active zones and is involved in regulating the size of active zones (Zhen and Jin, 1999; Kaufmann et al., 2002).

We have performed a GFP screen to identify additional factors involved in the formation of the active zone. To accomplish this, a SYD-2::GFP marker was generated and placed under the promoter of *unc-25* which expresses in GABAergic neurons. This GFP fusion protein allows for the visualization of active zones in vivo. An EMS mutagenesis screen was then performed looking for mutations affecting the formation of the active zone. From 2500 haploid genomes, 41 putative mutants have been identified. Initial characterization and phenotypic analysis will be presented on a subset of these mutants. It is our hope that the molecular characterization of these mutants will provide information on factors involved in the establishment, differentiation, and maintenance of active zones. Ultimately, this information will provide insight into the genetic pathways used to establish chemical synapses in general.

246. Mapping a new class of Ric mutants using an improved SNP mapping technique

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In an effort to identify additional molecules that function in synaptic transmission we have conducted several large screens for Aldicarb resistance. Aldicarb is an inhibitor of acetylcholinesterase and mutants that secrete less neurotransmitter are resistant to this drug. The mutants obtained from these screens fall into two classes: those that are uncoordinated, and those that are not uncoordinated.

We have mapped a large number of the uncoordinated mutations and have found that all or almost all are alleles of genes previously identified in aldicarb-resistance screens. This result suggests that all proteins involved in basal synaptic transmission in *C. elegans* that can be mutated to an uncoordinated phenotype have already been identified by mutation.

We are now investigating the second class of mutants. Since these animals are not uncoordinated, the mutated genes are probably not major components of the basal synaptic transmission machinery. One possibility is that they represent regulatory genes or minor components of the synapse, and that Aldicarb is a more sensitive assay than behavior. A second possibility is that they specifically counteract the effects of Aldicarb, rather than generally affecting synaptic transmission.

To distinguish between these possibilities and to learn more about this second class of genes, we plan to map and clone them. To facilitate this process we have improved the SNP mapping technique described by Wicks et al.(1). We have identified four SNPs per chromosome that alter a Dra I cleavage site. We have designed and tested a new set of primers that robustly amplify these regions. Finally, we have arrayed these primers in pairs into a microtiter plate, and pin-replicated them into a PCR master mix for bulk segregant analysis.

This combined technique reduces pipetting steps (and pipetting errors) many-fold, and enables the rapid simultaneous mapping of a very large number of mutants, with high resolution. We expect it to supersede other mapping techniques in our lab. At the meeting we will present the results of our mapping experiments on the Aldicarb resistant, non-Unc mutants. We will also make available the primer sequences and the protocol for improved SNP mapping.

1. Wicks, S *et al., Nature Genetics* 28, 2001.

247. The Ror Receptor Tyrosine Kinase CAM-1/KIN-8 functions at the *C. elegans* neuromuscular junction via a kinase-independent mechanism

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The receptor tyrosine kinase CAM-1/KIN-8 plays a critical role in a variety of cellular processes including cell polarity decisions and patterning of cell migration (Forrester et al., 1999, Nature 400(6747):881-5; Koga et al., 1999, Development(23):5387-98). Our laboratory has independently generated a *cam-1/kin-8* deletion mutant, *ak37*, and identified a characteristic set of locomotory defects associated with mutation of this gene. Using full-length GFP fusions, we have shown that CAM-1/KIN-8 is highly expressed at the neuromuscular junction. We hypothesize that CAM-1 may regulate the structure or function of neuromuscular synapses and are currently evaluating the effects of deletion of *cam-1* on synaptic transmission at the *C. elegans* neuromuscular junction using electrophysiology.

Using whole-cell patch clamp recording techniques, we have examined post-synaptic acetylcholine receptor (AChR) function in body wall muscles. Responses to application of cholinergic agonists are not significantly decreased in *cam-1(ak37)* mutants. However, evoked responses elicited by stimulation of the ventral nerve cord are significantly decreased in *cam-1(ak37)* animals. This result indicates a deficit in evoked neurotransmitter release. Consistent with this observation, the frequency of miniature excitatory post-synaptic currents (mEPSCs) appears reduced in *cam-1(ak37)* mutants. These deficits are rescued in transgenic worms expressing the full-length CAM-1/KIN-8 protein.

Interestingly, the behavioral and electrophysiological deficits in *cam-1(ak37)* mutants are also rescued in transgenic worms expressing truncated forms of CAM-1/KIN-8 lacking the kinase domain. This observation suggests that CAM-1/KIN-8 may provide a scaffold for organization of synaptic specializations. We are currently evaluating the expression requirements for rescue by tissue-specific expression of full-length and truncated forms of CAM-1 in either motor neurons or muscles.

Our results suggest that CAM-1/KIN-8 regulates function of the *C. elegans* neuromuscular junction by directing the organization of synaptic sites via a kinase-independent mechanism.

248. Identification of genes that contribute to the regulation of glutamate receptor function in *C. elegans*

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We have undertaken a genetic approach in *C. elegans* to find gene products that regulate glutamate receptor function at synapses.

We have previously shown that the pattern of movement is altered in transgenic worms that express a mutated constitutively open *C. elegans* glutamate receptor (GLR-1 (A/T)) in interneurons. These transgenic worms rapidly alternate between forward and backward movement, therefore not moving long distances over time. This lurching phenotype was used for a screen to search for suppressors with more normal movement. Mutated gene products causing the suppressor phenotype affect either membrane insertions of GLR-1(A/T) or decrease the stability of GLR-1(A/T) at synapses. We obtained several suppressor worms, two of which we are currently analysing. One suppressor expresses a mutated protein SOL-1, which we identified as a novel CUB-domain rich protein which is coexpressed with the glutamate receptor subunit GLR-1 and which is required for glutamate receptor function. The second suppressor is defined by *sol-2*. By characterizing the expression of a GLR-1:GFP fusion in *sol-2* mutants, we have shown that SOL-2 is not required for GLR-1 expression. Interestingly, like *glr-1* mutants, *sol-2* worms are nose touch defective. However, in contrast to *glr-1* mutants, we also noticed a defect in osmotic avoidance in the ring osm assay. Both of these observations suggest that *sol-2* is expressed in the polymodal ASH sensory circuit, which controls a backing avoidance response to both tactile inputs to the nose and osmotic signals. We are currently trying to map *sol-2* and are investigating further the behavioral phenotypes as well as the electrophysiological characteristics of *sol-2* worms.

249. C. elegans Nidogen, LAR and SYD-2/Liprin Function Coordinately During Neuromuscular Junction Formation

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Synaptic basal laminae are compositionally distinct from extrasynaptic basal laminae and are required for proper synapse formation. How the basal lamina is patterned with respect to the synapse and how it functions there remain unclear. Two *C. elegans* basement membrane proteins, NID-1 and CLE-1, homologues of nidogen and collagen XVIII respectively, are in the neural basal lamina and concentrated adjacent to presynaptic zones. Using a synaptobrevin (SNB)::GFP marker for the GABAergic type D motor neurons, we found that the presynaptic zones in *nid-1* null animals are smaller and more closely spaced than wild type, with an overall diffuse appearance along axons. These defects appear similar to, but slightly weaker than, those in *syd-2* mutants. Mutations in *syd-2*, which encodes the *C. elegans* α-Liprin, result in diffused and crowded synaptic GFP puncta[1].

The vertebrate Leukocyte-common Antigen Related Receptor Protein Tyrosine Phosphatase (LAR-RPTP or LAR) has been shown to bind the laminin-nidogen complex, via its extracellular domain[2], and α-liprin (LAR interacting protein) via its intracellular domain[3]. PTP-3, the *C. elegans* LAR homologue[4], has two isoforms that differ in the extracellular domain. Using an antibody common to both isoforms we found that endogenous PTP-3 is present along the nerve cord in a punctate pattern, indicating it is in a position to affect synaptogenesis. Thus we hypothesized PTP-3 could link *nid-1* and *syd-2* signaling and examined the role of *ptp-3* in synaptogenesis.

There are currently two alleles of *ptp-3*. One, *tm352*, is a deletion that specifically affects the long isoform, including the domain known to bind laminin-nidogen in vertebrates. The second, *op147*, is a Tc1 insertion in the first phosphatase domain. *tm352*animals have subtle changes in SNB::GFP similar to, but weaker than, those seen in *nid-1*animals. *op147*animals appear to have normal SNB::GFP patterns. *nid-1; tm352* double mutants appear more similar to *tm352*single mutants, suggesting that *tm352* is epistatic to *nid-1.* Interestingly, *nid-1; op147*double mutants appear more like *syd-2* animals than either single mutant. We also constructed *nid-1;syd-2* double mutants and observed a *syd-2* like phenotype, indicating that *syd-2*may be epistatic to *nid-1. nid-1;ptp-3(tm352* or *op147);syd-2* triple mutants are similar to *syd-2*single mutants, indicating that *syd-2* is epistatic to both *nid-1* and *ptp-3.* We can propose at least two possible models. First, SYD-2 may act through PTP-3 to pattern NID-1, which subsequently acts to somehow pattern the synapse. Alternatively, NID-1 may bind PTP-3 to define boundaries for SYD-2 within the synapse. We are investigating the localization of each of these molecules in the mutant backgrounds to differentiate these possibilities.

1. Zhen, M. & Jin, Y. *Nature* **401**, 371-5 (1999).
2. O'Grady, P., Thai, T. C. & Saito, H. *J Cell Biol* **141**, 1675-84 (1998).
3. Serra-Pages, C. et al. *Embo J* **14**, 2827-38. (1995).
4. Harrington, R. J., Gutch, M. J., Hengartner, M. O., Tonks, N. K. & Chisholm, A. D. *Development* **129**, 2141-53. (2002).

250. Synaptic remodeling of the DD motor neurons in C. elegans

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Neuronal dendritic-axonal asymmetry, once established, is normally stabilized in order to maintain information flow. However, changes in neuronal polarity can occur in response to developmental changes or experimental manipulations. The six *C. elegans* GABAergic DD motor neurons remodel their synaptic connectivity patterns as the worm matures from L1 to L2 stage (1). In L1 worms the DD motor neurons form neuromuscular junctions (NMJs) to ventral muscles. In L2 through adult stages the DD form NMJs along dorsal muscles. We use a synaptobrevin::GFP (SNB-1::GFP) marker to visualize this remodeling event (2, 3). Previous work has shown that DD remodeling does not involve any changes in neuronal morphology, and that *lin-14* controls the timing of DD synaptic remodeling (1, 2).

In order to identify additional genes involved in DD remodeling, we performed a genetic screen using a DD-specific SNB-1::GFP marker and isolated several mutants that retain synaptic-like puncta in the ventral processes of the adult DD neurons (also see Meir et al., this meeting). These mutants may be defective in completion of DD remodeling. We report the analysis of two mutations, *ju386* and *ju387*. Mutant animals homozygous for both mutations are healthy and move indistinguishably from wild-type animals. We mapped *ju386* to LGI, and found that it is an allele of *unc-11*, which encodes the *C. elegans* homolog of AP180 (4). The identification of this mutation suggests that the removal of old synapses from the ventral processes of juvenile DDs may require clathrin-mediated endocytosis. In the L2 through adult *ju387*mutant animals, DD neurons retain a significant number of synaptic-like puncta along the ventral cord, approaching the number of the puncta along the dorsal cord. The mutant phenotype of *ju387* appears to have a maternal contribution since the progeny of homozygous mutant animals show stronger DD remodeling defects than those of heterozygous animals. We have mapped *ju387* to LGV between *mec-9* and *egl-3*by deficiency mapping, and are attempting to determine the identity of this gene by germline transformation.

1. White JG, et al. *Nature* **271**, 764-766 (1978)
2. Hallam SJ and JinY *Nature* **395**, 78-82(1998)
3. Nonet ML *Journal of Neuroscience Methods* **89**, 33-40 (1999)
4. Nonet ML et al. *Molecular Biology of the Cell* **10**, 2343-2360 (1999)
5. Meir et al. poster, this meeting

251. Characterization of mutants regulating synaptic remodeling of DD motor neurons

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Information flow of the neural network depends on maintaining the polarity of individual neurons as well as on precise selection of synaptic partners. Certain neurons, however, re-specify their synaptic targets in response to developmental or environmental cues. The development of DD motor neuron circuitry in *C. elegans*accounts for a striking example of neural synaptic remodeling (1). DD neurons are born in embryo, and initially form synapses on ventral muscles in L1 stage. During the L1 to L2 transition, DD neurons remove their ventral synapses and re-establish synaptic connection to dorsal muscles without changing their cell morphology. Therefore, DD motor neurons re-specify their synaptic partners by way of reversing their neural polarity.

Using a synaptobrevin::gfp fusion marker driven by a DD specific promoter (a generous gift of Chris Li) (2), we are able to follow DD synaptic remodeling event and to search for mutants disrupting the remodeling process. We isolated two mutants, *ju298*and *ju334*, both of which have synapse-like puncta along both dorsal and ventral processes of DD neurons in older larvae and adults. Phenotypic analysis of these two mutants suggests that the establishment of DD neuron polarity may require two independent steps separated by the synaptic remodeling event. In L1 larvae of *ju298* homozygote, the DD neurons show partial defect in their synaptic pattern, suggesting that *ju298* may be required for the polarity establishment in both pre- and post-DD synaptic remodeling. *ju298* maps to the center of chromosome I. In *ju334* mutant, although the distribution of the synapse-like puncta in adult DD neurons is similar to that in *ju298*, the polarity of DD neurons in L1 larvae and the onset of DD remodeling appear to be normal. *ju334* may be specifically required for DD neuron polarity establishment in the post- but not pre-DD synaptic remodeling. We mapped *ju334*between -2.66 and -2.95 on chromosome X, and are currently performing transgenic rescuing experiments.

1.White, J. G. et al., (1978) Nature 271: 764-766.
2.Nonet, M. L., (1999) J. Neurosci. Methods, 89(1): 33-40.

252. *wly-1* mutations cause axonal degeneration

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Axons undergo a characteristic degeneration following axotomy (Wallerian degeneration) or in some neurodegenerative diseases (dying back degeneration). Raff, Whitmore and Finn have suggested that the elimination of axon branches during normal development might involve the same axonal degeneration mechanism, of which the molecular basis is poorly understood.

Mutations in *wly-1* (Wallerian-like decay) cause the distal half of the PVQ axons to degenerate. The PVQs are located in the tail and each extends a single axon that runs along the ventral nerve cord to the nerve ring. Using the *sra-6::gfp* reporter to visualize the PVQs, the PVQ axons extend to the nerve ring and appear normal in young *wly-1* larvae, whereas these axons are truncated at various positions between the vulva and posterior bulb of the pharynx in L4 and older animals. Several GFP-labeled blobs are typically observed in the ventral nerve cord anterior to the PVQ axon stump, suggesting a catastrophic degeneration of the distal half of the PVQ axon. *wly-1* double mutants with *ced-3* or *ced-4* mutations, which block programmed cell death, exhibit a similar PVQ axonal degeneration phenotype as *wly-1* single mutants. The sisters of PVQ die during wildtype development; in these *wly; ced* double mutants, the zombie PVQ sisters exhibit a similar axonal degeneration phenotype as PVQ. To examine the PVQ axonal degeneration in greater detail, we are generating reagents to visualize axonal cytoskeletal structures as well as analyzing the time course of the decay. We are also investigating the relationship of the *wly-1*-induced degeneration to various axonal degeneration phenomena observed in vertebrates.

We cloned *wly-1* and found that it encodes a protein with no recognizable homologues in other organisms, aside from nematodes. Both *wly-1* alleles are recessive, nonsense mutations, suggesting that they do not produce neurotoxic products *per se*. A *wly-1::gfp* transcriptional reporter is expressed broadly in muscle and neurons and a WLY-1-GFP translational fusion protein is detected in the cytoplasm of neurons when expressed under the *wly-1* promoter. The *wly-1* cDNA expressed under the *sra-6* promoter, which drives expression in PVQ, ASH and ASI, completely rescued the axonal degeneration phenotype, suggesting that *wly-1* functions cell autonomously. Although the sequence of *wly-1* is presently unenlightening, the *wly-1*mutant should be useful in the investigation of axonal degeneration in *C. elegans*.

253. Human beta amyloid peptide expressed in C. elegans muscles affects cellular trafficking possibly by altering chaperone-dependent ER-cytoplasm retrograde transport.

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Despite its great medical importance, the function of the beta amyloid peptide (Abeta) derived from the amyloid precursor protein (APP) remains a mystery. In humans, Abeta is generated within an ER-Golgi compartment by two proteolytic cleavages of APP. We have engineered a C. elegans model in which Abeta, expressed in muscles, is routed to the ER independently of the beta and gamma secretase cleavages normally required for APP processing in mammalian systems.

We have recently demonstrated by co-immunoprecipitation that Abeta interacts with both ER and cytoplasmic chaperones, probably as a consequence of retrograde transport. To determine if this intrinsic intra-cytoplasmic retention is a specific property of muscle cells loaded with Abeta aggregates we co-expressed ER-sorted reporter GFP constructs. We find that expression of human Abeta 1-42 dramatically alters the distribution of these reporters, indicating that this peptide generally affects protein and/or membrane sorting. To determine the role of Abeta-interacting chaperones on protein sorting in general, we examined the effect of RNAi against these chaperone proteins on the cellular distribution of the ER-sorted reporter GFP constructs. Our results suggest that Abeta is inducing the ER-cytoplasm retrograde transport by coordinated involvement of both ER and cytoplasmic chaperones controlled by two non-redundant genetic regulatory pathways. One interpretation of this result is that aggregating APP derivatives might naturally act as regulatory molecules involved in the modulation of vesicular transport of membrane and/or secretory protein trafficking in conditions favoring the formation of aberrant proteins within the ER-Golgi compartment. In this view, the pathological changes observed in human diseases associated with increased intracellular load of APP derived peptides result from induction of the non-adaptive ER-cytoplasm retrograde transport affecting cellular trafficking.

254. Infection of *Drosophila melanogaster* with *Heterorhabditis bacteriophora*

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Heterorhabditis bacteriophora is a parasitic nematode capable of infecting a wide variety of insects. The infectious juvenile (IJ, equivalent to the *C. elegans* dauer) harbors symbiotic bacterium *Photorhabdus luminescens,* which is used to kill the host. This is a potentially useful system for studying of bacteria/nematode symbiosis, bacteria/insect pathogenesis and nematode/insect parasitism.

We have found that that the insect *Drosophila melanogaster* can be infected with *Heterorhabditis bacteriophora*. This allows the extensive tools of *Drosophila* genetics and molecular biology to bear on these interactions. We have investigated the induction of insect immune system using a diptericin-lacZ reporter. Upon infection by *Photorhabdus luminescens*, there is a significant induction of lacZ expression, suggesting that the immune response is active during infection.

We are currently attempting to isolate morphological mutations in *Heterorhabditis*. Once the genetics of this organism is established, we will screen for mutations that affect its ability to infect the insect.

255. 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 eukaryotic organisms. 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 roughly 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 ÒarmsÓ) and there is a low frequency of exchanges in the central 50% (the Òcentral gene clusterÓ). The reduced rate of exchange in the central cluster may be intrinsic Ð due to the presence or absence of specific sequence elements in the region Ð or may result from chromosome-wide mechanisms that favor Òoff-centerÓ exchanges Ð *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. Evidence for chromosome-wide regulation of crossing over comes from determination of the total genetic map lengths of end-to-end chromosome fusions. mnT12 and eT6 are homozygous-viable end-to-end fusions of X and IV that differ in the orientation of IV. 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. We have also characterized the meiotic behavior of a ÒmegasomeÓ, a fusion of three complete chromosomes (III;X;IV); in strains homozygous for this fusion, almost half of the genome is contained within a single chromosome. Preliminary results reveal that the three-chromosome fusion also has a genetic length of roughly 50 cM, indicating that the forces that exert chromosome-wide crossover regulation are capable of acting over half the genome. Evidence for sequence-intrinsic effects on crossover distribution is provided by a comparison of the genetic maps of two-chromosome fusions and the cognate unfused chromosomes. The relative distribution of exchanges along the fused and unfused chromosomes is similar, indicating that the observed crossover distribution is largely due to sequence-intrinsic effects.

Further experiments have investigated the nature of the functional unit upon which chromosome-wide crossover control mechanisms operate. Our results suggest that chromosomal regions that fail to assemble contiguous axes and/or synaptonemal complex do not propagate interference. Taken together, these results suggest a model wherein the unit over which chromosome-wide crossover control mechanisms act may correspond to chromosome regions with contiguous chromosome axes and/or contiguous homologous synapsis. We are testing this hypothesis by examining the effect upon crossover control of non-null mutations affecting components of meiotic chromosome structures (*e.g. him-3 (me80)*, see abstract by K. Nabeshima).

256. Systemic RNAi: Form and Function

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Three complementation groups, *sid-1*, *sid-2*, and *sid-3*, were identified in a screen for mutants defective in systemic RNAi (Winston *et al*., 2002). The resultant mutants all retain *rde-1*-dependent cell autonomous RNAi function, suggesting that these loci contribute to the systemic nature of RNAi but do not interfere with RNAi processivity. We will discuss recent progress in mapping and/or characterization of the Sid loci.

1. SID-1 does not appear to be expressed in neurons, and neurons are frequently resistant to RNAi. Therefore, we expressed SID-1 in neurons to determine if RNAi of neuronal target genes could be enhanced. However, expression of a translational fusion between SID-1 and GFP did not provide sensitivity to RNAi of neuronal targets when dsRNA was introduced by injection or bacterial expression. We are developing a cell culture system in which to address the function of SID-1, a putative multipass transmembrane protein. Preliminary results indicate that *sid-1* mutant cells in culture are resistant to dsRNA in the medium. Additional current results will be presented.

2. *sid-2* RNAi resistance has been characterized, and *sid-2* worms appear to be resistant to bacterially-mediated RNAi, but not injected dsRNA. Current progress in mapping and cloning will be presented.

3. *sid-3* maps to the far "plus" end of LGX. We are in the process of cloning *sid-3* and hope to reveal its molecular identity at the meeting. *sid-3*, like *sid-1*, appears to be resistant to RNAi induced by both bacterially-mediated and injected dsRNA. Current phenotypic characterization results will be presented.

References

Winston W.M., Molodowitch C., Hunter C.P. (2002) Systemic RNAi in *C. elegans* Requires the Putative Transmembrane Protein SID-1. Science **295**(5564): 2456-9.

257. RNAi and cosuppresion in the *C elegans* germ line.

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RNA interference and cosuppression are both functional gene silencing phenomena; however, they exhibit some fundamental differences: 1) RNAi is the specific degradation of a target mRNA triggered by the introduction of homologous dsRNA. Cosuppression in contrast, is triggered by the introduction of transgenic DNA, which results in the silencing of the transgene as well as the endogenous copy. 2) RNAi can occur in the germ line as readily as in the soma, while cosuppression is almost exclusively seen in the germ line. 3) The mutator genes: *mut-2, mut-7, mut-8, and mut-9* are required for both silencing processes (1). However, *rde-1*, an essential RNAi gene, is not required for cosuppression (1,2). These differences indicate that although the mechanisms of RNAi and cosuppression share some genetic components and have similar functional silencing effects, they are distinct processes.

To understand the mechanisms that establish and maintain RNAi and cosuppression, we have developed an assay using a gain of function allele of *oma-1* (oocyte maturation defective*)*. *oma-1* is a non-essential germ line gene initially characterized and kindly provided to us by Rueyling Lin (3). Using the *oma-1* assay, we were able to perpetuate RNAi with no loss of efficacy for 15 generations. In our cosuppression studies, we first established cosuppression and found that the continued presence of the transgene is generally required to maintain the cosuppressed state. These results indicate that the germ line is capable of establishing and maintaining a variety of silenced states.

We are currently investigating the molecular character of the trigger for each process. We are also testing whether the silenced states reflect amplification of dsRNA or changes in chromatin structure.

1) Ketting and Plasterk Nature (2000) 404, 296-298.

2) Dernburg et al. Genes & Dev (200) 14,1578-1583

3) Detwiler MR et al. Dev Cell. 2001 Aug;1(2):187-99.

258. Searching for the Autosomal Sex Signal

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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. The X portion of the sex signal is comprised of several X-signal elements (XSE) that repress the activity of *xol-1* in a dose-dependent manner. 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 so, 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. One allele, *y356*, is a recessive suppressor of *fox-1 sex-1* and maps to a 1 cM interval on LGII. This mutation can suppress the XX Dpy phenotype and lethality caused by mutations in all other known XSEs, but cannot suppress the XX lethality caused by a null mutation in *sdc-2*, a gene downstream of *xol-1*. Fulfilling another important criterion of ASEs, *y356* acts as a dose-dependent enhancer of the male lethality caused by duplicating XSEs. We are currently characterizing this and other suppressors to determine whether any are ASEs.

259. The ONECUT homologue *ceh-39* is involved in sex determination in *C elegans*

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In *C. elegans*, the primary signal that determines sexual fate is the ratio of X chromosomes to sets of autosomes, the X:A ratio. The X component of the ratio consists of X-signal elements (XSEs) that act in a synergistic and dose-dependent manner to repress the master sex switch gene *xol-1*. In XX animals, the double dose of XSEs efficiently represses *xol-1*, promoting the hermaphrodite fate and initiating dosage compensation. In XO animals, with just a single dose of XSEs, *xol-1* is robustly expressed, promoting male fate. Duplications bearing extra copies of XSEs cause repression of *xol-1* in XO animals, resulting in lethality. These same duplications have no effect on XX animals. By contrast, decreasing the dose of XSEs has the reciprocal effect: XO animals have no phenotype, but XX animals are Dpy, Tra, or dead.

Thus far, two XSEs, *sex-1* and *fox-1,* have been characterized; *sex-1* acts transcriptionally, and *fox-1* acts posttranscriptionally. Another less well characterized mutation, *y323,* may also define an XSE. In addition, other X-signal elements must exist because duplications of X regions outside *sex-1* and *fox-1*, such as *yDp14*, cause XO-specific lethality. An RNAi based screen of genes in one such region was implemented to identify additional XSEs. RNAi of one gene, *ceh-39,* almost completely rescued the XO lethality of *yDp14* animals. This rescue is comparable to that seen with RNAi of *sex-1* or *fox-1*. *ceh-39* encodes a protein similar to the ONECUT class of homeodomain proteins. This class of proteins is involved in transcriptional regulation in vertebrates and invertebrates.

Synergy is a defining characteristic of XSEs. For example, while *fox-1* XX animals have no phenotype and *sex-1* XX animals are Dpy, Tra and 65% viable, virtually all *fox-1 sex-1* XX animals are dead. *fox-1* also synergies with *y323* in XX animals resulting in mildly Dpy but fully viable animals. *ceh-39* RNAi causes synergistic XX lethality with *sex-1* but not *fox-1*. *ceh-39* RNAi also enhances the severity of the Dpy phenotype in *fox-1 y323* animals but does not cause lethality. The fact that no synergy was seen in combination with *fox-1* alone indicates that *ceh-39* may function at a similar point in the sex determination pathway. One possibility is that *ceh-39* affects *fox-1* expression. However, RNAi of *ceh-39* did not affect *fox-1* protein levels as measured by western analysis. Other possibilities are being addressed. Epistasis analysis with *xol-1* will further pin down where *ceh-39* functions.

Another consequence of reducing XSE dose in XX animals is sexual transformation. A male-specific *pkd-2::gfp* marker was used to determine whether any transformation was present in XSE mutant XX animals. *pkd-2* is normally expressed only in male-specific neurons in the head and tail. Jennifer Powell observed that *sex-1* but not *fox-1, y323*, or *fox-1 y323* XX animals have a low penetrance of *pkd-2::gfp* expression in several neurons in either the head or tail. *ceh-39* RNAi causes *fox-1 y323* XX animals to express *pkd-2::gfp* in several neurons in the tail, demonstrating that *ceh-39* affects sex determination, as expected for an XSE.

GFP tagged CEH-39 is expressed from the 20 to 500 cell stage of embryogenesis, consistent with the temporal expression of other XSEs. It is expressed throughout the cell cycle and appears to be associated with DNA during mitosis. Though *ceh-39* shares many of the characteristic of an X-signal element the mechanism of its role in sex determination has yet to be elucidated.

260. Assaying C. elegans sex ratio in natural populations

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C. elegans natural populations are comprised of self-fertilizing hermaphrodites and an unknown, but presumably small, percentage of males. Males arise as a consequence of rare X-chromosome non-disjunction events during meiosis in hermaphrodites or via outcrossing with males (Hodgkin, Horvitz, and Brenner 1979). Laboratory studies have identified variation among wild-caught strains in X-chromosome non-disjunction rates and male reproductive success (Hodgkin and Doniach 1997). However, like much of C. elegans ecology and natural history, the incidence of males and of outcrossing in natural populations remains unknown. We are working to address the question of sex ratio in natural populations by developing a molecular approach to assay male frequency. Our method utilizes semi-quantitative rtPCR to describe the abundance of a male-specific mRNA transcript relative to a control transcript that is expressed in both sexes. We are currently (1) constructing a standard curve of relative expression levels from lab samples of known sex ratio, from which we will be able to infer the frequency of males in unknown laboratory or natural samples, and (2) ascertaining the species specificity of the primers that we are employing in this method.

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261. Characterization of tyramine receptors from *Caenorhabditis elegans*

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Octopamine (OA) plays an important role in the regulation of a number of key processes in nematodes, including pharyngeal pumping, locomotion and egg-laying. However, while putative OA receptors have been tentatively identified in the *C. elegans* database, no OA receptors have been functionally characterized from any nematode. We have isolated two cDNAs, ser-2 and ser-2a, previously identified by others as putative *C. elegans* serotonin/OA receptors (C02D4.2, ser-2). The sequences of these cDNAs differ from that predicted by GeneFinder and lack 42 bp of exon 2. In addition, ser-2a appears to be alternatively-spliced and lacks a predicted 23 amino acids in the third intracellular loop. COS-7 cells expressing SER-2 or SER-2a both bind [^{3}H]-LSD in the low nM range and SER-2 exhibits Kis for tyramine, octopamine and serotonin of 0.07, 2 and 13.7 micromolar, respectively. Significantly, tyramine reduces forskolin-stimulated cAMP levels in HEK293 cells stably expressing SER-2 or SER-2a with an IC_{50} of about 360 nM for SER-2. Taken together, these results suggest that both SER-2 and SER-2a encode functional tyramine receptors that couple to $Galpha_{i/o}$ and reduce cAMP levels. These studies are continuing to more fully characterize the pharmacology and coupling of SER-2a to determine if they differ from SER-2. This work was supported by NIH Grant AI145147 to RWK.

262. Alternatively-spliced nematode 5-HT$_2$-like receptor isoforms: interaction of the C-terminus with a multi-PDZ domain organizer protein (C52A11.4).

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5-HT receptors play key neuromodulatory roles in the regulation of both pharyngeal pumping and muscle contraction in nematodes. Previously we identified multiple isoforms of a 5-HT$_2$-like receptor in the parasitic nematode, *Ascaris suum* that appeared to be differentially expressed. AS1 and AS2 exhibited nearly identical pharmacological profiles and coupling, but AS1 lacked a consensus PDZ binding motif at its C-terminus, suggesting that the differential splicing might play a role in the localization of the individual isoforms to specific subcellular locations. Both AS2 and its closely related *C. elegans* homologue, 5-HT$_{2CE}$, contain the identical PDZ binding motif (TFL) at their C-termini. Therefore, using the C-terminus of 5-HT$_{2CE}$ as a bait for yeast-two hybrid screening, we identified a *C. elegans* protein (C52A11.4) which contains 9 predicted PDZ domains and exhibits significant identity to a multiple PDZ domain binding partner of the human 5-HT$_2$ receptor (FEBS Letters, 1998, 424: 63-68). Further analysis of the individual PDZ domains by additional yeast-two screening and direct protein/protein interaction studies suggests that only PDZ domain 7 binds to the TFL motif. Preliminary localization studies using a GFP/NLS fusion construct with the putative C52A11.4 promoter suggests that the expression of C52A11.4 is confined exclusively to the nervous system of *C. elegans*, i.e., several neurons in the nerve ring, 4 neurons in the body (AVM, SDQR, PVM, SDQL) and three neurons in the tail (PVCL, PVNR, PVQL). More importantly, the expression of predicted PDZ domains 7/8 fused to GFP under the control of the C52A.11.4 promoter yields a punctate staining along processes of the individual neurons, suggesting that C52A11.4 is localized to synapses. As predicted, all of the neurons expressing 5-HT$_{2CE}$ also express C52A11.4. These studies are continuing with the intention of identifying additional binding partners in the putative signaling complexes assembled by C52A11.4. This work was supported by NIH Grant AI145147 to RWK.

263. MAPK activation by acetylcholine in C. elegans neurons

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In mammalian neurons the importance of acetylcholine neurotransmission in memory is implied in Alzheimer's disease, where massive loss of cholinergic neurons causes retrograde memory loss and eventually death. One of the suggested mechanisms of acetylcholine transmission in mammalian neurons is activating the MAP kinases ERK-1/2, whose activation to modify the synaptic plasticity is essential in fundamental neuronal functions such as learning and memory. We are studying the role of MAPK in acetylcholine signaling in C. elegans. The C. elegans ortholog of mammalian ERK1/2, MPK-1, has been shown to be part of the conserved ras-raf-mek-mapk signaling pathway in cell fate determination during vulval development. MPK-1::GFP translational fusion expression shows that MPK-1 is expressed predominantly in neurons, including head neurons in the nerve ring, ventral cord neurons and tail neurons. Furthermore, 30min treatment of worms with 1mM arecoline, a muscarinic agonist, activates MPK-1 by increasing its phosphorylation. This activation can be blocked by treatment with atropine, a muscarinic receptor antagonist. The significant reduction of MPK-1 activation by arecoline in ras partial loss of function mutants and the complete blockage of MPK-1 activation by treatment with the MEK-1 inhibitor U0126 suggest that acetylcholine signaling in MPK-1 activation is through the conventional ras-raf-mek-mapk pathway. We think MPK-1 activation by arecoline probably happens in the neurons because 1) MPK-1::GFP fusion protein is expressed in neurons, 2) preliminary data from whole worm antibody staining shows bright staining in the head neurons by phospho-ERK antibody and 3) the three muscarinic receptors in C.elegans (GAR-1, GAR-2 and GAR-3) are expressed mostly in neurons.

So far our results suggest that 1) MPK-1 can be activated by acetylcholine through muscarinic receptors and 2) This activation is through the conventional ras-MAPK pathway. Currently, we are trying to further dissect the signaling pathway between the muscarinic receptor and MPK-1 and figure out the physiological significance of this MPK-1 activation by acetylcholine.

264. Identification of proteins involved in *C. elegans* sperm chromosome condensation.

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The transfer of genetic material to subsequent generations requires the efficient packaging of DNA into chromatin during gamete formation. Spermatogenesis in *C. elegans* provides an ideal system to study the mechanisms of chromatin condensation during the processes of mitosis, meiosis, and spermiogenesis. Sperm chromatin is tightly compacted after meiosis and remains transcriptionally inactive in the spermatid until after fertilization, when it is decondensed. The proteins and mechanisms involved in this efficient packaging and subsequent disassembly of specialized chromatin are unknown. We are interested in characterizing proteins that are required specifically for sperm chromatin condensation. A proteomic approach is being utilized in which gamete chromatin is purified and associated proteins identified using a multidimensional protein identification technique (MudPIT). MudPIT utilizes two tandem liquid chromatography steps to provide resolution of complicated peptide mixtures before peptide identification through tandem mass spectrometry. Computer comparison against a predicted peptide database from the *C. elegans* genome is used to identify corresponding proteins. Progress in identification of candidate proteins and assessment of function in sperm formation will be presented.

265. Characterizing the function of FER-1, a muscular dystrophy-related gene involved in membrane fusion during *C. elegans* spermiogenesis

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Fer-1 was identified because mutations cause defective sperm in which the membranous organelles (MOs) fail to fuse with the plasma membrane and a motile pseudopod fails to form. The FER-1 protein is similar to dysferlin, a human gene that when mutated causes limb girdle muscular dystrophy type 2B. Fer-1 is part of the "ferlin" family of genes, highly conserved over most of their length, containing at least three C2 calcium-binding domains, a transmembrane domain at the C-terminus, and weighing approximately 240kD. The similarity between fer-1 and dysferlin suggests that the worm and human proteins have similar functions. We hypothesize that the mechanism of fusion of the MOs and the membrane recycling mechanism used during sperm crawling at the leading edge resembles the vesicle-associated repair mechanisms used by injured cells, which have been suggested to be defective in the muscle cells of patients with dysferlin-related muscular dystrophies.

A polyclonal antibody to FER-1 has been used to determine the cellular localization of FER-1. Preliminary results show that FER-1 is localized to both the cell body and the motile pseudopod. This supports a role for FER-1 in both MO fusion and pseudopod extension. Currently, coimmunoprecipitations and a yeast two-hybrid screen are being performed to determine the proteins interact with FER-1.

266. 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 *egl-5* as well as 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 results in partially penetrant P12 to P11 or P11 to P12 transformations. Animals with mutations in both EGF and Wnt pathway components exhibit significantly enhanced transformation frequencies. 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 an approximately 13 kb. promoter construct provided by Ferreira and Emmons, we have identified an approximately 1.3 kb. sub-fragment of the *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 that may participate in signal integration

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267. Visualizing PHA-4 binding to target DNA during pharynx organogenesis

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The FoxA transcription factor PHA-4 specifies pharyngeal identity during embryogenesis. It does so by directly binding and activating hundreds of target genes via an AT rich consensus sequence TRTTKRY (R=A/G, K=T/G, Y=T/C) (Gaudet *et al.*, 2002). This sequence exists tens of thousands of times in the genome. In some cases, the sequence is bound by other Fox family members (Nash, *et al.*, 2000), of which there are 15 in *C. elegans*. In other cases, the sequence resides in DNA that is not regulatory and presumably is not recognized by any Fox protein. The identification of hundreds of PHA-4 binding sites against a backdrop of tens of thousands of potential sites raises the question of what constitutes a PHA-4 binding site in vivo.

We would like to understand the relationship between PHA-4 binding and target gene expression. Does PHA-4 bind its site at all times and in all cells, even for genes whose expression is temporally or spatially restricted? Or is binding a regulated event, similar to what has been observed for other transcription factors such as MyoD during muscle development (Bergstrom et al., 2002) or RAP1 in yeast (Lieb et al., 2002)?

To answer these questions, we are examining PHA-4 binding to its recognition sequence *in vivo*, and correlating binding with transcription of one of its downstream targets. The nuclear dot assay was devised by the Sternberg and Meyer labs (Gonzales-Serrichio *et al*., 1997, Carmi, *et al.*, 1998) to visualize a protein bound to an extrachromosomal array *in vivo*. We are adapting this technique to observe PHA-4 binding to target promoters. Extrachromosomal arrays bearing lac operator (*lacO*) and *pha-4* target promoter sequence are introduced into *LacI::CFP; pha-4::YFP* transgenic worms. LacI::CFP binds *lacO*, thereby allowing for detection of arrays. When PHA::YFP binds directly to target gene promoter sequence on arrays, then co-localization of LacI::CFP and PHA-4::YFP results in merged green (cyan + yellow) "dots" in cell nuclei. This assay enables visualization of PHA-4 binding to its target in the developing pharynx, in real-time, and allows us to investigate and understand how binding site occupancy is utilized as a mechanism for regulation of gene expression.

268. cis-Regulatory Control of Vulval Cell-Fate Markers in *Caenorhabditis elegans*

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Caenorhabditis elegans vulval precursor cells (VPCs) undergo up to three rounds of cell division before adopting one of the final vulA, B1, B2, C, D, E and F fates. *egl-17*, *zmp-1*, *cdh-3* are expressed differentially in the developing vulva cells, providing a potential readout for different signaling pathways. To understand how different signaling pathways interact to specify vulval cell types in a precise pattern, we have identified upstream cis-regulatory regions that are sufficient for their ability to confer vulval cell type specific regulation when fused in cis to the basal *pes-10* promoter. In the *egl-17* promoter, we have identified a 143 bp region that drives vulC and vulD expression, and a 102 bp region that is sufficient to drive the early expression in presumptive vulE and vulF cells. In the *zmp-1* promoter, we have identified a 300 base pair region that is sufficient to drive expression in vulE, vulA and the anchor cell. In the *cdh-3* promoter, we have identified a 689 bp region sufficient to drive expression in the anchor cell and vulE, vulF, vulD and vulC, a 155 bp region sufficient to drive only anchor cell expression, and a separate 563 bp region that was also sufficient to drive expression in these vulval cells. We have identified the *C. briggsae* homologs of these three genes, and the corresponding control regions, and tested these regions in both *C. elegans* and *C. briggsae*. We conclude that the regions conserved in *C. elegans* and *C. briggsae* upstream of *egl-17*, *zmp-1*, *cdh-3* promote expression in vulval cells. We also conclude that although these cis-regulatory elements promote cell-specificity in gene regulation, they probably do so by using distinct transcription factors.

269. The *cis*-regulatory logic of transcriptional control during neuronal subtype specification in *C. elegans*

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The control of gene expression in multicellular organisms is determined by a complex network of *cis*- and *trans*-acting transcriptional regulators. The *cis*-regulatory regions, which are comprised of the DNA sequences of the promoters and enhancers of individual genes, serve as a sort of "hardwiring" of the network, delimiting all possible patterns of gene expression for a given organism. The ultimate pattern of gene expression is then determined by the interaction of transcription factors, or the "software" of the network, with this "hardwiring" of *cis*-regulatory sequences. This programming, which is based on the regulation of *trans*-acting factors by signaling and lineage cues acting on *cis*-regulatory sequences, is thus able to create the complex patterns of gene expression seen in multicellular organisms.

To understand this logic of the control of gene expression in the nervous system of *C. elegans*, we are studying the transcriptional control of several genes with differential but overlapping gene expression in the nervous system. Each of these genes; *ttx-3, ceh-23, kal-1, ser-2, unc-17, sra-11*, and *hen-1*; display expression patterns that overlap in the AIY class of interneurons, where their expression has been shown to be genetically downstream of the LIM homeobox transcription factor gene, *ttx-3*. Through promoter deletion analysis we have found the promoters of these genes to be highly modular in nature, with distinct enhancer regions often driving the expression of *gfp* in single cell types.

In addition, we have identified AIY specific enhancers that contain putative TTX-3 binding sites in the enhancer regions of the *ttx-3, ceh-23, kal-1, ser-2, and sra-11* genes. Comparison of these sequences with homologous *C. briggsae* sequences has revealed the conservation of these sites across species. Further, through transcription factor misexpression studies, we are beginning to determine which transcription factors are necessary and sufficient for the transcriptional control of expression driven by each of these enhancer elements.

270. The Role of TLF in C. elegans 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 individual promoters should depend on either TBP or TLF, but not both. Alternatively, TLF could have co-opted the TBP DNA binding domain for a new purpose, such as functioning as an enhancer-binding activator that recruits co-activators or basal machinery. 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 determine the role of TLF binding in vivo. RNAi against TLF or a TBP-specific TAF (TAF250) both abolish or severely reduce *pes-10*::GFP expression at the earliest detectable stage, suggesting both proteins are required for *pes-10* transcription. These data support the second model, suggesting TLF has co-opted the TBP-like DNA binding domain for some other purpose. We made site-specific mutations in the *pes-10* promoter from 300bp upstream of the ATG through the trans-splice site and analyzed the effects on GFP expression at the earliest detectable stage, the 28-cell stage. We have identified specific sequences within the *pes-10* promoter that affect *pes-10*::GFP expression at the 28-cell stage. We hypothesize that one of these sequences is a TLF binding site. We have previously shown TLF associates with the *pes-10* promoter in vivo using a nuclear spot assay (1). We are using wild-type and mutated versions of these sequences in the nuclear spot assay to determine if TLF or TBP bind these sites in vivo.

1. Kaltenbach et al., Molecular Cell, 6, 705-713 (2000).

271. Functional Evolution of Diverged Nuclear Hormone Receptors in C. elegans

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A major focus of our work is to understand the evolution of the NHR expansion and diversification in nematodes. Our goals are to retrace the ancestral origin of these diverged NHRs, to pinpoint the genomic events that led to the expansion, and to understand the evolution of their biological function and structural mechanisms. In order to carry out these studies, we have developed a classification scheme defined by the degree of divergence of the P-box element from the "conserved" worm receptors. The P-box is the fundamental structural unit of NHR DNA binding, and is the most conserved structural element across the NHR family. We are addressing NHR evolution both physiologically and structurally. Here I will present our physiological and functional investigations of the most homologous set of the divergent NHRs.

RT PCR of a mixed-stage library indicates that all of the NHRs we have investigated in this work are expressed at comparable levels. Simple RNAi studies reveal no distinct physiological role for the majority of these NHRs, however some receptors, including NHR-31 and NHR-49 do have interesting biological functions. We are currently characterizing NHR-31 and NHR-49 activity and working to reveal the physiological roles of the other NHRs that have no apparent RNAi phenotype. In order to characterize worm NHR functional evolution, we have focused on the DNA binding mechanism, as the NHR-DNA binding interaction is the most well conserved of C. elegans NHR properties. Our data reveal that some of the "divergent" NHRs employ similar mechanisms to that of their "conserved" NHR relatives, while others do not, suggesting that they either have evolved new DNA reponse element specificity, employ a different type of DNA binding mechanism, or do not bind DNA at all. Interestingly, the level of functional conservation does not necessarily correlate with the level of sequence identity. Further investigation of these NHRs requires the identification of the novel DNA binding sites for these functionally "diverged" worm NHRs.

272. Biochemical characterization of PLP-1, a regulator of mesendoderm development

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The zygotic genes end-1 and end-3 together are required to specify the endoderm progenitor, the E cell. Their expression is restricted to the E blastomere and ectopic expression of either gene is sufficient to convert non-E lineages to an E-like fate. Genetic screens have identified maternal factors, including SKN-1 and POP-1, that are involved in mesendoderm specification and correct end-1,3 expression. To isolate regulators of end-1 that were missed in genetic screens, we used mass spectrometry to identify a transcription factor, PLP-1 (pur alpha like protein) that directly binds to the end-1 promoter and that is similar to the mammalian transcription factor pur alpha. RNAi of plp-1 results in embryos that fail to form a pharynx. Some of the arrested embryos produce extra intestine, suggesting a transformation of non-E lineages to an E-like fate. A very impenetrant gutless phenotype is also observed, which is greatly enhanced in mutants defective in the endoderm-inducing Wnt pathway. However, plp-1 RNAi does not enhance the gutless phenotype observed in mutants in the MAP kinase pathway, which converges with the Wnt pathway to regulate endoderm development, suggesting that plp-1 may function in the latter pathway. To understand how plp-1 might act both positively and negatively on end-1 expression, we have examined the in vitro binding characterisics of PLP-1 and its possible post-translational modifications. PLP-1 binds to a putative Lef-1 binding site, which is required for activation of a minimal end-1 promoter. Surrounding this region, the end-1 DNA apparently adopts a secondary structure in vitro that can be cleaved with an endonuclease specific to cruciform structures. Deletion of the Lef-1 site results in loss of this secondary structure and PLP-1 binding activity. The affinity of PLP-1 for this site appears to be regulated by phosphorylation, as demonstrated by the loss of PLP-1 binding activity when early embryonic nuclear extract is treated with a non-specific phosphatase and by the ability of the extract to phosphorylate recombinant PLP-1 protein. A candidate kinase that might phosphorylate PLP-1 is the nemo-like kinase LIT-1, since it is a component of the MAPK pathway. Consistent with this possibility, we find that in lit-1 mutants, PLP-1 only weakly localizes to the nucleus with a more diffuse staining pattern throughout the cell, unlike in normal embryos in which it is strongly nuclear-localized.

273. Characterization of *Mos1* transposition.

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We have mobilized the *Mos1* transposable element in the germline of *C. elegans*. *Mos1* insertions constitute unique sequence tags that can be used to rapidly determine the site of gene disruption. Thus, *Mos1* mutagenesis allows genes to be rapidly cloned.

Our system involves two different extrachromosomal arrays. The enzyme array contains the *Mos1* transposase under control of a heat shock promoter. The substrate array has multiple copies of the *Mos1* transposable element. These two arrays are crossed together to generate double transgenic animals, which are heat shocked as young adults to induce expression of the transposase. The transposase enzyme then catalyzes the transposition of a *Mos1* element from the substrate array into the worm genome.

In an effort to characterize when transposition occurs, the time course of transposition was assayed. Transposition events were first detected in F1 animals laid 12-18 hours after heat shock. Peak transposition rates were observed around 24-30 hours after heat shock before dropping rapidly. Insertions detected in animals that were laid 12-18 hours after heat shock may have occurred either in mature sperm, or proximal oocytes that have entered meiosis. However, the majority of events probably occur prior to entry into meiosis since the transposition frequency peaks 24 to 30 hours after heat shock.

Thus, transposition of *Mos1* in the germ line largely occurs in nuclei of the gonad syncytium during mitotic proliferation. During these stages, the germ cell nuclei may be mosaic for the either the transposase array or the substrate array. Are both arrays required in an individual germ cell nucleus for transposition to occur? By assaying the presence or absence of the arrays in progeny that have an insertion we can determine whether the substrate or enzyme arrays were likely to be present in the germ cell during transposition. The transposition frequency was identical regardless of whether or not F1 animals had the enzyme array. Specifically 31% (114/366) of the F1s with the enzyme array had an insertion compared to 29% (85/293) of the F1s without the enzyme array. This result suggests that the transposase protein can diffuse throughout the syncytial gonad and catalyze transposition in germ cell nuclei that do not contain the enzyme array.

In contrast to the transposase enzyme, the presence of the substrate array element is probably required in germ cells in which transposition takes place. F1s with the substrate array exhibit a higher transposition frequency than F1s without the substrate array (45% (129/286) vs. 19% (70/370)). This result supports the hypothesis that the substrate array is required in germ cell nuclei that undergo transposition. Interestingly, insertions have been detected in F1s that do not have the substrate array. This suggests that transposition happens prior to loss of the array, which could occur during nuclear divisions or as a result of transposase-generated cleavage of the substrate array.

The substrate array has been observed to undergo "silencing", based on a decreased transposition frequency over time (Bessereau and Boulin, 2002 European Worm Meeting). In an effort to "desilence" the substrate array, we tested whether maintaining substrate array animals at different temperatures has any effect on transposition frequency. A "silenced" substrate array was propagated at either 20°C or 25°C for at least 10 generations and then the transposition frequency was determined. The transposition frequency was not significantly different regardless of whether the substrate array was maintained at 20°C or 25°C (35% ±7, n = 4 P0 at 20°C vs. 45% ±7, n = 8 P0 at 25°C).

In summary, the highest frequency of insertions are detected in F1 animals that are laid 20 to 30 hours after heat shock, the transposase can diffuse throughout the gonad, transposition probably occurs during mitotic proliferation, and maintaining the substrate at an elevated temperature did not cause a dramatic increase in hop rate.

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