1. Regulation of Cul-3 Neddylation is essential for SCFCul-3 activity during
2. sas-1, a gene required for centrosome duplication in one-cell stage C.elegans
3. The Ran GTPase cycle is essential for spindle formation and nuclear
4. The role of lin-5 in chromosome segregation and cleavage plane
5. apo-5, a paternal gene involved in mitotic spindle orientation and
6. Orientation and polarization of the mitotic spindle in the embryonic cell P1
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11. The putative actin binding protein ERM-1 is required for correct
12. Multiple regulators of actin dynamics modulate cadherin function during
13. plexin-1 is required for attraction and repulsion of cells in the epidermis of C. elegans
14. One gene for two distinct plakins that provide outer and inner resistance
15. Evidence of a role for the C. elegans COPII complex in cuticle secretion
16. Role of C. elegans pdi-3 in extracellular matrix assembly.
17. Specific aspartyl and calpain proteases are required for neurodegeneration
18. The Snail-like zinc finger DNA-binding protein CES-1 is a direct regulator
19. Regulation of programmed cell death by the two C. elegans BH3-only
20. Caenorhabditis elegans HUS-1 is a DNA damage checkpoint protein
21. Does the DNA damage checkpoint shorten lifespan in C. elegans?
22. Characterisation of MUS-81, an XPF-like endonuclease required for
23. Recent changes and improvements to the WormBase database
24. Access to C. elegans resources at the EBI.
25. Sequence analysis supports the interpretation, based on reporter gene
26. The C. elegans orfeome cloning project : version 1.0
27. A genome wide RNAi screen
28. Characterization of Mos1-mediated mutagenesis.
29. Improvement of the biolistic transformation of C. elegans
30. Homologous gene targeting in C. elegans.
31. rrf-3, a C. elegans strain with increased sensitivity to RNAi
32. Enhanced Analytical Performance of the C. elegans Flow Sorter COPAS
33. The rhomboid homolog rom-1 is involved in the anchor cell-independent
34. The Pumilio protein puf-8 negatively regulates vulval induction
35. LIN-39 represses EFF-1-dependent cell membrane fusion in C. elegans
36. spr-1, a suppressor of presenilin, encodes a conserved transcriptional
37. Loss of spr-5 bypasses sel-12 defects by stage-specific derepression of hop-1.
38. The Axin-like protein PRY-1 is a negative regulator of a canonical Wnt
39. Maintenance of embryonic stem cell identity in the germline blastomeres
40. t1530 affects the development of pharynx and body wall muscle and the
41. Genetic analysis of mab-9 regulation during embryonic development
42. Evolution of Hox gene function in C. elegans
43. Ray formation and the evolution of nematode Hox proteins.
44. Evolution of vulvalpatterning mechanisms: studies in Oscheius sp. 1
45. Phylogeny and ontogeny of free-living and parasitic nematodes
46. Zag-1, a zinc finger homeobox transcription factor acting late in neuronal

48. Classical cadherin-catenin complexes and the developing C. elegans
49. A motoneuron-derived signal is required for differentiation of post-
50. Long chain polyunsaturated fatty acids are required for efficient
51. Cellular and genetic analysis of salt adaptation in C. elegans
52. tpa-1, a gene encoding a protein kinase C subunit, is a downstream target
53. C. elegans CREB mutants show defects in dauer formation and in behaviors
54. Antagonistic signalling pathways in neurons exposed to the body fluid
55. In vivo imaging of mechanoreceptor function.
56. Phenotypic plasticity of dauer larva formation in Caenorhabditis elegans.
57. Sex differences in the neuroendocrine regulation of development and
58. DIN-1, a putative DAF-12 coregulator important for the C.elegans dauer
59. Molecular Identification of Transcriptional Targets of the DAF-16
60. The circadian clock of C. elegans:
61. Analysis of Caenorhabditis elegans mutants resistant to infection by
62. Mutations in a cyclic nucleotide gated channel alter the response to
63. Inducible innate immune defences in C. elegans; a TGF connection.
64. Genetic analysis of systemic RNAi in C. elegans - An RDE-1/Argonaute
65. The roles of developmental arrest, phosphoinositides, Type II PIP kinase
66. Xenobiotically induced cytochrome P450 gene expression in
67. The C. elegans MAP kinase phosphatase LIP-1 is required for the G2/M
68. A putative LET-418 interacting protein plays a role during germ line
69. The role of cdc-25.2 during embryogenesis
70. The role of chk-1 during early development
71. Identification of genes involved in dauer larva morphogenesis by
72. Getting into the function of the nematode homologue of Eps8
73. Formal Modeling, Simulation and Analysis of C. elegans Development
74. Studying telomere replication in C. elegans
75. Searching for mechanisms of neuronal synchrony in the convulsing worm
76. Genetic and biochemical approaches to study modulation of
77. Gonadal and hormonal signals that regulate life span in C. elegans
78. DNA damage transcriptional responses in C.elegans
79. MEI-3, a novel kinesin-like protein is essential for female meiotic spindle
80. Development of a bio-monitoring screening system based on the
81. Mutational analysis of tbb-2, a beta-tubulin required for proper P0 spindle
82. rad-51 gene in meiosis and in soma
83. A glr-2 channel pore domain mutant with phenotypic effects only in the
84. Heat Shock Factor in Caenorhabditis elegans and in parasitic nematodes
85. A transcription factor and a hsp70 homologue required for cuticle
86. The unique forms of the prolyl 4-hydroxylase complex found in C. elegans
87. Functional conservation and specificity of GATA transcription factors.
88. FEM-3 is a cytoplasmic protein that is expressed in sperm and
89. The C. elegans Heterochromatin Protein 1 homologue HPL-2 acts in
90. A C. elegans model of neuronal dysfunction in Huntington's Disease:
91. The Ubiquitin-like Nedd8 protein modification pathway regulates
92. bHLH transcription factors are involved in specifying the cell-death fate
93. Differing susceptibilities to systemic RNAi within the Caenorhabditis clade

94. Exploring the role of sterol-sensing domain proteins in C. elegans
95. The Leucine-Rich Repeats of LAP proteins mediate basolateral
96. Identification and characterization of the pvl-1 gene, a member of the
97. Early cell migrations in the Caenorhabditis elegans embryo
98. The HOX gene mab-5 has a conserved function in vulva formation in C.
100. Studies on the anthelmintic effects of inhibitors of the glycosphingolipid
101. Genetic analysis of the Rap1-pathway in C. elegans
102. Positive selection of mutants with increased resistance and longevity.
103. Functional characterisation of mammalian syndecan homologue in the
104. Sensory neuron Ca++ imaging in response to water-soluble chemical
105. Investigating the functional role of candidate neuropeptide activated
106. Maternal EMB-29 is required during early development
107. Investigating the COP9 signalosome using C. elegans and S. pombe
108. Characterization of Neuropeptide Receptors in Caenorhabditis elegans.
109. Identification in Caenorhabditis elegans of cross-reacting carbohydrate epitopes
110. The human and C.elegans p66 NuRD chromatin remodelling
111. A role for embryonic polarity components in the development of the
112. Identification of genes implicated in ubiquinone biosynthesis in C.
113. Dissecting the complexities of C. elegans DAF-2 insulin/IGF like receptor
114. The EH network in C.elegans
115. A multifaceted approach to elucidate the regulation of ciliogenesis in C.
116. Ten-1 is involved in sperm development in C. elegans.
117. The Mi-2 chromatin-remodelling proteins LET-418 and CHD-3 act
118. Analysis of early ceh-13 expression and anterior embryonic patterning
119. Screens to identify positive and negative regulators of neurotransmission
120. Identifying regulators of DGK-1
121. An investigation into the mechanism of action of the novel anthelmintic
122. dyf-8 encodes a type I trans-membrane receptor with a zona-pellucida
126. Mutations in glutamate-gated chloride channel genes affect C. elegans locomotion
127. Xenobiotically induced cytochrome P450 gene expression in
128. Dna polymorphism in Pristionchus pacificus
129. Four Classes of Vulvaless Mutants in Pristionchus pacificus
130. Genetic and molecular analysis of the vulval patterning mutants ped-7
133. Identification of a new mutant defective for DNA damage responses in
134. Genome-wide RNAi screen for genes that control germ cell apoptosis in
135. A direct interaction between C. elegans IP3 receptors and a LIN-15B
136. Characterisation of a C. elegans calexcitin.
137. Transient disruption of IP3 receptor function in C. elegans.
139. TRPm channel function in the defecation rhythm of Caenorhabditis
140. Variation in parasite resistance between natural isolates of
141. C. elegans whole genome microarray: An update
142. Novel drug targets: the identification and characterisation of candidate
143. Specificity of G-protein mediated signaling in odorant detection by C.
144. Using a Combination of Two Recombinases to Create Targeted Single-
145. Annotation of C.elegans protein sequences in SWISS-PROT.
146. Identification of factors specifically involved in the regulation of

147. Identification of an AAA ATPase as an interacting partner of C.elegans
148. Multi-parametric & Dual Color Fluorescent Analysis and Flow Sorting of C. elegans
149. Enhanced One-step Nematode Recognition on micrographs of Living C. elegans
150. Identifying transcriptional targets of the DAF-12 nuclear hormone
151. Proteome analysis reveals distinct molecular differences in
152. A new screen to identify negative regulators of Egfr signalling in C.
153. Functional analysis of a collection of maternal effect mutants of C.elegans
154. Characterization and identification of genes involved in transposon
155. Homologous gene targeting in C. elegans.
156. Genes involved in genome stability in C. elegans
157. DYSTROPHIN AND ASSOCIATED PROTEINS IN C. elegans
158. DYC-1 a dystrophin related protein of C. elegans
160. Systematic analysis of cell specific enhancers in C. elegans
161. Novel downstream targets of G-protein -subunits in C. elegans
162. Genome analysis of the effect of the host immune response on
163. The role of protein turnover in caloric restriction and aging
164. Functional analysis of the ACR-13 nicotinic acetylcholine receptor
165. The function and interactions of the C. elegans orthologue of the SMN
166. The _-catulin gene ctn-1 is alternatively spliced and encodes a component
167. A comparison of the action of ivermectin and emodepside on C.elegans
168. The nicotinic acetylcholine receptor family of Caenorhabditis elegans
169. Stage-specific effects of the cholinergic anthelmintic drugs, levamisole,
170. Towards a two-component based enhancer trap system in C. elegans: a
171. Y74C10AM.1 and W09D6.6 are the Caenorhabditis. elegans orthologues
172. Characterisation of the Caenorhabditis elegans orthologue of the
173. Homology modelling of nicotinic acetylcholine receptors in
174. Cloning of a novel regulator of the LET-23 EGFR/ LET-60 RAS/ MPK-1
175. The mammalian FGFs belonging to the 9, 16 and 20 subfamily but
176. Somatic cell fusion and fertility in C. elegans:
177. New players in RNA interference: Implication of rde-1 homologues.
178. C. elegans as a model organism for lysosomal storage disorders
179. The lin-26 gene is regulated by tissue-specific cis-elements
180. Olfactive learning and memory in nematodes
181. feh-1 and apl-1, the orthologues of Fe65 and Alzheimer's -amyloid
182. Screening for paternal-effect mutations involved in the establishment of
183. Double strand break repair induced by transposon excision
184. Patch-clamp study of the cationic currents in the pharyngeal muscle cells
185. Resistance to the nicotinic agonist DMPP defines a new class of genes.
186. Characterisation of two EF-1a homologues: translational elongation
187. A novel conserved rna-binding domain protein, Rbd-1, is essential and
188. Microtubule-dependent processes in the one-cell embryo of C. elegans.
189. Identification of novel downstream effectors of Rap1 and Rap2.
190. Update on warthog genes
191. Genetic analysis of Ras-like GTPases in C. elegans
192. Genetic interactions between lin-25 and C. elegans Mediator components
193. The C. elegans Mi-2 orthologs LET-418 and CHD-3 are together

194. Cln-3.3, one of the C. elegans homologues of the human CLN3 gene
195. zyg-11 and cul-2 are required for meiotic cell cycle progression and AP
196. C. elegans cut superclass genes, ceh-44, ceh-32 and ceh-37
197. A functional analysis of the extradenticle ortholog ceh-40
198. Phenotypic Plasticity In Nematodes.
199. Functional Analysis of Acyl-Binding Proteins in C. elegans
200. Functional analysis of the micro RNA genes of C. elegans
201. CUT-1-like proteins involved in the formation of the alae of L1 and
202. The C. elegans F47F2.1 gene encodes a cyclic AMP-dependent protein
203. C.elegans genes coding for proteins involved in the biosynthetic pathway
204. Analysis of the G protein coupled receptors family to identify candidates
205. Functional conservation between the human and nematode KAL proteins
206. cow-1 is required to spatially restrict contraction of the actin cytoskeleton
207. The human PTEN tumor suppressor regulates diapause and longevity in
208. Cytological characterization of DNA damage response pathways in C.
209. ccf-1, a putative deadenylase encoding gene, is necessary for meiotic
210. Functional characterisation of the Caenorhabditis elegans homologue of
211. An RNAi screen for axon guidance genes
212. Axon guidance in the ventral cord of C. elegans
213. Searching for target genes of the nuclear receptor NHR-25 in C. elegans
214. Functional analysis of IgCAMs expressed in the nervous system
215. Ast-1 , a novel gene important for fasciculation of ventral cord axons
216. Expression and function of ftz-f1/nhr-25 in Caenorhabditis elegans
217. Expression and characterisation of the C. elegans NEP-like gene
218. The RAD27/FEN1 homolog is essential during early development in
219. The C.elegans Forkhead gene F26B1.7.
220. Identifying ligands for NPR-1
221. Embryonic development of the free-living, marine nematode Pellioditis
222. Suppressors of social feeding behaviour
223. Essential roles for four cytoplasmic intermediate filament proteins in
224. Characterization of M. nematophilum resistant mutants bus-3 and bus-5
225. Molecular cloning of C. elegans bus-6 using snip/SNP mapping
226. C. elegans ≠ M. nematophilum interaction: Genetic and molecular
227. Cloning and characterization of srf-3
229. Characterization of mau-8 mutants
230. mab-9/Tbx20 orthologues and the generation of morphological diversity.
231. WormBase: a web-accessible database for C. elegans biology
232. Caenorhabditis elegans as a model for infantile neuronal ceroid
233. Genetic and molecular characterisation of two new dumpy genes: dpy-31
235. Functional analysis of the C. elegans T-box gene family
236. Taste perception by the nematode Caenorhabditis elegans
237. Cloning of the mab-2 gene of C.elegans using RNAi
238. Caenorhabditis elegans as a model organism in the study of nucleoside
239. Genomic RNAi screens for genes influencing C. elegans diapause and
240. Environmental and genetic inputs regulate DAF-9 cytochrome P450
241. A positive role for lin-1 in C. elegans vulval development?

242. The Caenorhabditis elegans homologue of human angiotensin converting
244. Computer prediction of cis-acting elements from co-regulated genes.
245. Cathepsin L cysteine protease is essential for embryogenesis in C. elegans
246. The role of the DPY-7 cuticular collagen in the exoskeleton of
247. Genome organization of Microbacterium nematophilum and possible
248. A GFP-fusion to the cuticular collagen, COL-19, its expression pattern,
249. Characterisation of mutants with an altered E Lineage in Caenorhabditis
250. Genes regulating social feeding behaviour in C. elegans.
251. The mechanism of action of the neuropeptide AF1 in C.elegans
252. Functional role for glutamate-gated chloride channel subunits in the

European C. elegans Meeting 2002 - Program

Paestum, Hotel Ariston, May 18-21 2002

SATURDAY MAY 18

14.30 - 22.00 Registration and check-in

18.00 Welcome reception

18.30 Dinner

20.00 -22.30 **Session 1** **Early embryo**

Chair: Pierre Gonczy

Abstract 1 **Regulation of Cul-3 Neddylation is essential for SCF^{Cul-3} activity during mitosis in C. elegans** *(Lionel Pintard)*

Abstract 2 ***SAS-1*, a gene required for centrosome duplication in one-cell stage *C. elegans* embryos** *(Marie Delattre)*

Abstract 3 **The Ran GTPase cycle is essential for spindle formation and nuclear envelope assembly in living *Caenorhabditis elegans* embryos** *(Peter Askjaer)*

Abstract 4 **The role of *lin-5* in chromosome segregation and cleavage plane specification** *(Ridgely Fisk)*

21.00 - 21.15 Coffee break

Abstract 5 ***apo-5*, a paternal gene involved in mitotic spindle orientation and positioning in the one-cell *Caenorhabditis elegans* embryo** *(Sandra Encalada)*

Abstract 6 **Orientation and polarization of the mitotic spindle in the embryonic cell P1** *(José-Eduardo Gomes)*

Abstract 7 **ZYG-8 and TAC-1 are required for proper microtubule behaviour in one-cell stage *C. elegans* embryos.** *(Jean-Michel Bellanger)*

Abstract 8 **Positioning the cleavage furrow in *C. elegans* embryos** *(Reinhard Dechant)*

Abstract 9 ***chp-1*, a CHORD domain containing protein required for early embryonic polarity** *(Peder Zipperlen)*

SUNDAY MAY 19

7.30 - 8.30 breakfast

8.30 - 10.15 **Session 2 A** **Epithelia and cuticle**

Chair: Renaud Legouis

Abstract 10 **Identification of new genes required for apical sorting in epithelial cells** *(S. Liégeois)*

Abstract 11 **The putative actin binding protein ERM-1 is required for correct localization of the apical junction in the *C.elegans* intestine** *(Daniela van Fürden)*

Abstract 12 **Multiple regulators of actin dynamics modulate cadherin function during hypodermal morphogenesis** *(Jonathan Pettitt)*

Abstract 13 ***plexin-1* is required for attraction and repulsion of cells in the epidermis of *C.elegans*** *(Gratien Dalpé)*

Abstract 14 **One gene for two distinct plakins that provide outer and inner resistance to mechanical stress** *(Michel Labouesse)*

Abstract 15 **Evidence of a role for the *C. elegans* COPII complex in cuticle secretion** *(Brett Roberts)*

Abstract 16 **Role of *C. elegans pdi-3* in extracellular matrix assembly** *(Sylvain Eschenlauer)*

10.15 - 10.45 Coffee break

10.45 - 12.30 **Session 2B Cell death and DNA repair**

Chair: Anton Gartner

Abstract 17 **Specific aspartyl and calpain proteases are required for neurodegeneration in *C. elegans*** *(Popi Syntichaki)*

Abstract 18 **The Snail-like Zinc Finger DNA-binding Protein CES-1 is a Direct Regulator of *egl-1* Transcription** *(Marion Thellmann)*

Abstract 19 **Regulation of Programmed Cell Death by the Two *C. elegans* BH3-only Proteins EGL-1 and CED-13** *(Nicole Wittenburg)*

Abstract 20 ***C. elegans* HUS-1 is a DNA damage checkpoint protein required for genome stability and induction of EGL-1.** *(E. Randal Hofmann)*

Abstract 21 **Does the DNA damage checkpoint shorten lifespan in *C. elegans*?** *(Shawn Ahmed)*

Abstract 22 **Characterisation of MUS-81, an XPF-like endonuclease required for genome stability** *(Nigel J. O'Neil)*

12.45 Lunch

14.30 - 17.00 **Posters** Posters will be up for the whole length of the meeting

17.00 - 20.15 **Session 3 Part I** **Workshop on genome and resources**

Chair: Jonathan Hodgkin

Abstract 23 **Recent changes and improvements to the WormBase database** *(Keith Bradnam)*

Abstract 24 **Access to *C. elegans* resources at the EBI** *(Eleanor Whitfield)*

Abstract 25 **Sequence analysis supports the interpretation, based on reporter gene data, that there are many pseudogenes amongst the annotated *C.elegans* genes** *(Ian A. Hope)*

Abstract 26 **The *C. elegans* Orfeome Cloning Project : Version 1.0** *(Jérôme Reboul)*

Abstract 27 **A genome wide RNAi screen** *(Julie Ahringer)*

Coffee Break

Session 3 Part II **Workshop on techniques and technology**

Chair Ronald Plasterk

Abstract 28 **Characterization of *Mos1*-mediated mutagenesis** *(Jean-Louis Bessereau)*

Abstract 29 **Improvement of the biolistic transformation of *C. elegans*** *(Ralf Schnabel)*

Abstract 30 **Homologous gene targeting in *C. elegans*** *(Eugene Berezikov)*

Abstract 31 ***rrf-3*, a *C. elegans* strain with increased sensitivity to RNAi** *(Femke Simmer)*

Abstract 32 **Enhanced Analytical Performance of the *C. elegans* Flow Sorter COPAS *BIOSORT*: Automated Re-analysis of Populations in Multi-well Plates and Reading of Axially Distributed Positional Fluorescent Signals** *(Johan Geysen)*

20.30 **Dinner + free time**

MONDAY MAY 20

7.30 - 8.30 breakfast

8.30 - 10.15 **Session 4, Part** I **Cell specification**

Chair Alison Woollard

Abstract 33 **The rhomboid homolog *rom-1* is involved in the anchor cell-independent induction of vulval cell fates** *(Amit Dutt)*

Abstract 34 **The pumilio protein *puf-8* negatively regulates vulval induction** *(Gopal Battu)*

Abstract 35 **LIN-39 represses EFF-1-dependent cell membrane fusion in *C. elegans*** *(Benjamin Podbilewicz)*

Abstract 36 ***spr-1*, a suppressor of presenilin, encodes a conserved transcriptional repressor that may play a general role in LIN-12/Notch signalling** *(Sophie Jarriault)*

Abstract 37 **Loss of *spr-5* bypasses *sel-12* defects by stage-specific derepression of *hop-1*** *(S Eimer)*

Abstract 38 **The Axin-like protein PRY-1 is a negative regulator of a canonical Wnt pathway in *C. elegans*** *(Damien Y.M. Coudreuse)*

10.15 - 10.45 Coffe break

10.45 -12.30 **Session 4 Part II** **Cell specification**

Chair Gert Jansen

Abstract 39 **Maintenance of embryonic stem cell identity in the germline blastomeres of *C. elegans*** *(Ciosk R)*

Abstract 40 ***t1530* affects the development of pharynx and body wall muscle and the phagocytosis of cell death** *(Ingo Büssing)*

Abstract 41 **Genetic analysis of *mab-9* regulation during embryonic development** *(Roger Pocock)*

	Abstract 42	**Evolution of Hox gene function in *C. elegans*** *(Tibor Vellai)*
	Abstract 43	**Ray formation and the evolution of nematode hox proteins** *(Arturo Gutierrez)*
	Abstract 44	**Evolution of vulval patterning mechanisms: studies in *Oscheius* sp. 1 CEW1** *(M.-A. Félix)*
	Abstract 45	**Phylogeny and ontogeny of free-living and parasitic nematodes** *(Einhard Schierenberg)*
12.30		**lunch bag next to swimming pool** **13.00 → bus for Visit to the Paestum Temples**
16.15		
13,00	lunch	
17.00- 20.15	**Session 5**	**Neurobiology**
	Chair	Stephen Nurrish
	Abstract 46	**Zag-1, a zinc finger homeobox transcription factor acting late in neuronal differentiation** *(Irene Wacker)*
	Abstract 47	**The axon trajectory of the M2 pharyngeal neurons may be established in part via a growth cone-independent mechanism** *(Marc Pilon)*
	Abstract 48	**Classical cadherin-catenin complexes and the developing *C. elegans* nervous system** *(Ian D. Broadbent)*
	Abstract 49	**GABAergic neuromuscular junctions in *C. elegans*** *(Christelle Gally)*
		Molecular interactions at the synapse *(Erik Jorgensen)*
18.30 - 19.00	Coffe break	
	Abstract 50	**Long chain polyunsaturated fatty acids are required for efficient neurotransmission in *C. elegans*** *(Giovanni M. Lesa)*
	Abstract 51	**Cellular and genetic analysis of salt adaptation in *C. elegans*** *(Gert Jansen)*
	Abstract 52	***tpa-1*, a gene encoding a protein kinase C subunit, is a downstream target of G12-mediated signaling in *C. elegans*** *(Celine Moorman)*
	Abstract 53	***C. elegans* CREB mutants show defects in dauer formation and in behaviors that are coupled to food sensation** *(Mark Alkema)*
	Abstract 54	**Antagonistic signalling pathways in neurons exposed to the body fluid regulate social feeding in *C. elegans*** *(Mario de Bono)*
	Abstract 55	**In vivo imaging of mechanoreceptor function** *(William Schafer)*
20.30	**Gala Dinner and Music**	

TUESDAY MAY 21

7.30- 8-30	Breakfast	
8.30 - 10.15	**Session 6**	**Physiology**
	Chair	Bernard Lakowski

Abstract 56 **Phenotypic plasticity of dauer larva formation in *Caenorhabditis elegans*** *(Mark E. Viney)*

Abstract 57 **Sex differences in the neuroendocrine regulation of development and lifespan** *(Diana McCulloch)*

Abstract 58 **DIN-1, a putative DAF-12 coregulator important for the *C.elegans* dauer diapause** *(Andreas H. Ludewig)*

Abstract 59 **Molecular Identification of transcriptional targets of the DAF-16 winged helix transcription factor** *(Joshua J. McElwee)*

10.00-10.30 Coffee break

Abstract 61 **Analysis of *Caenorhabditis elegans* mutants resistant to infection by *Microbacterium nematophilum*** *(Hannah Nicholas)*

Abstract 62 **Mutations in a cyclic nucleotide gated channel alter the response to pathogenic *M. nematophilum* bacteria** *(Karen J. Yook)*

Abstract 63 **Inducible innate immune defences in *C. elegans*; a TGF connection** *(C. Léopold Kurz)*

Abstract 64 **Genetic analysis of systemic RNAi in *C. elegans* - An RDE-1/Argonaute protein defective in a natural isolate of *C. elegans*** *(Marcel Tijsterman)*

Abstract 65 **The roles of developmental arrest, phosphoinositides, Type II PIP kinase and DAF-16 in the larval response to oxidative stress** *(David Weinkove)*

Abstract 66 **Xenobiotically induced cytochrome P450 gene expression in *Caenorhabditis elegans*** *(Ralph Menzel)*

12.00 lunch

Departure

ORAL PRESENTATIONS

1-66

1. Regulation of Cul-3 Neddylation is essential for SCF^{Cul-3} activity during mitosis in C. elegans

Lionel Pintard [1], Thimo Kurz [2], Sarah Glaser [1], John Willis [2], Bruce Bowerman [2] and Matthias Peter [1]

[1]Swiss Institute of Experimental Cancer Research (ISREC), CH-1066 Lausanne, Switzerland
[2]Institute of Molecular Biology, University of Oregon, Eugene, OR 97403, USA

Several cell cycle transitions are regulated by ubiquitin-dependent degradation of specific proteins. This is achieved by a multi-step process whereby ubiquitin is first activated by an ubiquitin-activating enzyme (E1), then passed to an ubiquitin-conjugating enzyme (E2) before being transferred to the target protein by the ubiquitin-ligase (E3). Two types of E3 control cell cycle progression: the APC (Anaphase Promoting Complex) and the SCF (Skp1-Cullin-F-box). The E3 displays substrate specificity and recruits the E2 allowing poly-ubiquitinylation of the substrate and its subsequent degradation by the 26S proteasome. However, little is known about the regulation of E3 ligases. Within the SCF complex, the Cullin subunit was found to be neddylated. In a cascade of reactions similar to ubiquitination, the ubiquitin-like protein Nedd8 is transferred to the Cullin and it is thought that neddylation positively regulates SCF activity by triggering the recruitment of the E2 within the SCF complex.
We recently discovered the Nedd8 pathway in *C. elegans* which is required for activation of the SCF^{Cul-3} complex and subsequent degradation of the Katanin-like protein Mei-1 at the meiosis to mitosis transition. Our results showed that neddylation of Cul-3 is essential for SCF^{Cul-3} activity in vivo. Indeed, Western blot experiments allowed us to confirm that Cul-3 is neddylated in vivo in wild–type but not in a mutant of the neddylation pathway (*rfl-1*). To better understand the role of neddylation in the regulation of SCF activity, we searched for mutants that were defective in deneddylation. The multiprotein complex COP9/signalosome was found to contain such a deneddylase activity. The COP9/signalosome, originally identified in *A. thaliana* as a repressor of photomorphogenesis, is widely conserved in metazoans. Searches in the databases allowed us to identify six subunits with significant homology in *C. elegans*. Interestingly inactivation of these subunits by RNAi leads to an accumulation of the neddylated form of Cul-3 and inhibition of the SCF^{Cul-3} activity. As a result, the SCF^{Cul-3} substrate Mei-1 is not degraded and severs the microtubules during mitosis. Most importantly we found that the lethality observed in neddylation deficient mutant (*rf1-1*) grown at semi-permissive temperature can be suppressed by the inactivation of deneddylation through RNAi of the signalosome subunits. Our results are in favour of a model where cycles of neddylation-deneddylation are essential for activity of SCF complexes.

2. *sas-1*, a gene required for centrosome duplication in one-cell stage *C.elegans* embryos

Marie Delattre and Pierre Gönczy

ISREC. Chemin des Boveresses, 155. EPALINGES/LAUSANNE 1066. SWITZERLAND.

Centrosome duplication is essential for the proper division of animal cells, yet remains a poorly understood process. We have started a cellular and molecular dissection of centrosome duplication in *C. elegans*. In wild-type one-cell stage *C. elegans* embryo, the sperm contributes the only pair of centrioles; the single centrosome then duplicates shortly after fertilization and forms a bipolar spindle. Among a large set of parental-effect embryonic lethal mutations on chromosome III (Gönczy & *al*. JCB 1999), we identified mutations in two loci, *sas-1* and *sas-2*, that result in defective bipolar spindle assembly in early embryos. Here, we focus on our analysis of two alleles of *sas-1*.

We performed immunofluorescence on fixed specimens using antibodies against *a*-tubulin, the pericentriolar material component ZYG-9 and the presumptive centriolar component PLK-1, to further characterize the *sas-1* mutant phenotype. Strinkingly, this analysis revealed that one-cell stage embryos possess a single centrosome, which nucleates microtubules correctly but does not duplicate. As a result, a bipolar spindle does not assemble, leading to failure in chromosome segregation and cytokinesis. These observations were confirmed using time-lapse fluorescence microscopy with transgenic animals carrying β-tubulin::GFP and γ-tubulin::GFP fusion proteins. Moreover, we showed that progression through S phase is not affected in *sas-1* mutants, suggesting that the failure of centrosome duplication is not a consequence of a more general cell cycle defect. Ultrastructural analysis is under way to understand which precise step of the centrosome duplication cycle is affected in *sas-1* mutant embryos,

Using a combination of time-lapse DIC and fluorescence microscopy, we found that the centrosome never duplicates in 20% of *sas-1* mutant embryos. In contrast, while the centrosome fails to duplicate during the first cell cycle, it does duplicate at the onset of the second or the third cell cycle in the remaining 80% mutant embryos. These observations suggest that *sas-1* function may be required strictly early during development. These two phenotypes are reminiscent of those that can be observed in *zyg-1* mutant embryos (O'Connell & *al.* Cell 2001). Interestingly, the authors showed that the first duplication of centrosomes in the *C. elegans* embryo is under the control of paternally contributed *zyg-1*, whereas subsequent rounds of duplication are under the control of maternally contributed *zyg-1*. Taken together, these findings raised the possibility that *sas-1* is a paternal gene. To test this hypothesis we crossed wild-type males with homozygous mutant *sas-1* hermaphrodites and showed that the cross-progeny is 100% viable for both alleles of *sas-1*. These results establish that the two alleles studied strictly affect a paternal contribution of *sas-1*.

We have initiated the cloning of *sas-1* using SNP mapping and have narrowed the search down to 36 candidate genes. Because RNAi does not appear to work in sperm, and because *sas-1* is most likely a paternal gene, we have initiated rescue experiments using cosmids and YACs covering the region of interest to identify the molecular nature of *sas-1*.

3. The Ran GTPase cycle is essential for spindle formation and nuclear envelope assembly in living *Caenorhabditis elegans* embryos

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The small GTPase Ran has been found to play pivotal roles in several aspects of the cell cycle. We have investigated in real time the role of the Ran GTPase cycle in spindle formation and nuclear envelope regeneration in dividing *Caenorhabditis elegans* embryos. We found that Ran and its co-factors RanBP2, RanGAP and RCC1 are all essential for reformation of the nuclear envelope after division. Knocking down the expression of any of these component of the Ran GTPase cycle by RNAi leads to strong extranuclear clustering of integral nuclear envelope proteins and nucleoporins. Ran, RanBP2, and RanGAP are also required for building a mitotic spindle, whereas astral microtubules are normal in the absence of these proteins. *RCC1(RNAi)* embryos have similar abnormalities in the initial phase of spindle formation, however, a bipolar spindle eventually forms. Irregular chromatin structures and chromatin bridges due to spindle failure and DNA condensation defects were frequently observed in embryos where the Ran cycle is perturbed. Finally, we have demonstrated that IMA-2, which is a homologue of vertebrate importin α, is essential for spindle formation in early embryos, presumably acting downstream of Ran.

4. The role of *lin-5* in chromosome segregation and cleavage plane specification

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The mitotic spindle provides force and directionality during chromosome segregation. In addition, the position of the mitotic spindle determines the plane and axis of cell division, as the cleavage furrow bisects the mitotic spindle perpendicularly. Thus, correct spindle function and position are critical for both genetic stability and the generation of cells with different developmental fates. To better understand these processes, we study the *lin-5* gene in *C. elegans*.

Previous work in our lab established a role for *lin-5* in both chromosome segregation and spindle positioning, as well as in coupling the S and M phases of the cell cycle[1]. We hypothesize that *lin-5*, which encodes a novel coiled-coil component of the spindle apparatus, affects spindle dynamics or force generation. However, the mechanisms of *lin-5* function remain unknown.

To gain further insight into the molecular function of *lin-5*, we took a biochemical approach to identify proteins that interact with LIN-5. Gel filtration chromatography of embryo lysates indicated that LIN-5 is part of a large protein complex over 600 kDa in size. Coiled-coil predictions and two-hybrid assays in yeast suggested that LIN-5 homodimerizes, accounting for part of the complex. Affinity purification of LIN-5 complexes with monoclonal antibodies revealed at least eight co-purified proteins. Tandem mass spectrometry identified LIN-5-associated proteins (LAP) of approximately 60 kDa in size as the product(s) of two paralogous genes, *lap-1/ags-3.3* and *lap-2/ags-3.2*.
The LAP-1 and LAP-2 proteins contain a potential Gα protein interacting motif, the GoLoco/GPR domain. Interestingly, the RNAi loss-of-function phenotypes for *lin-5*, *lap-1 and lap-2*, and the combination of two *C. elegans* Gα homologs, *goa-1 and gpa-16*[2], all show a high degree of similarity. In each case, RNAi does not affect spindle formation, but inhibits proper migration, rotation, and anaphase B movements of the spindle. Furthermore, the localizations of LIN-5, LAP-1/2, and GOA-1 all overlap at the cell cortex. We are further characterizing the interactions between LIN-5, LAP-1, LAP-2, GOA-1, and GPA-16, as well as their roles in chromosome segregation, spindle orientation, and cell polarity. Based on our results thus far, we propose that LIN-5 acts in a signal transduction pathway that involves heterotrimeric G-proteins and guides the migration of the spindle in response to polarity cues at the cell cortex.

[1] Lorson, M. A., Horvitz H. R., van den Heuvel S. 2000. LIN-5 is a novel component of the spindle apparatus required for chromosome segregation and cleavage plane specification in Caenorhabditis elegans. *J Cell Biol.* 148:73-86.
[2] Gotta, M. and Ahringer, J. 2001. Distinct roles for Gα and Gβγ in regulating spindle position and orientation in *Caenorhabditis elegans* embryos. *Nature Cell Biology*. 3:297-300, and Miller, K. G. and Rand, J. B. 2000. A role for RIC-8 (Synembryn) and GOA-1 (G(o)alpha) in regulating a subset of centrosome movements during early embryogenesis in Caenorhabditis elegans. *Genetics.* 156:1649-60.

5. *apo-5*, a paternal gene involved in mitotic spindle orientation and positioning in the one-cell *Caenorhabditis elegans* embryo

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During the first mitotic division of the one-cell *C. elegans* embryo, called P_0, the mitotic spindle aligns along the longer anterior-posterior (a-p) axis and moves slightly posteriorly during anaphase, resulting in an asymmetric division that produces a smaller posterior daughter (P_1), and a larger anterior daughter (AB). To understand how spindle positioning and orientation are regulated in the early worm embryo, we are characterizing *apo-5*, a temperature sensitive maternal- and paternal-effect embryonic lethal mutant with defects in the positioning and orientation of the first mitotic spindle along the a-p axis.

In *apo-5(or358*ts*)* mutant embryos, the nucleo-centrosomal complex often fails to rotate fully during the first division. As a result, the P_0 spindle does not always align with the a-p axis (range of rotation is between 0-86˚ relative to the a-p axis). Prior to spindle elongation, the nucleo-centrosomal complex hyper-displaces posteriorly, and the spindle then elongates either transversely or eventually flips to orient with the a-p axis. Furthermore, elongation of the P_0 spindle is delayed in *apo-5* mutant embryos, and the poles do not move as far apart as in wild-type. Overall antero-posterior polarity appears unaffected in *apo-5* mutant embryos, based on the correct distribution of P-granules in the one-cell stage embryo. Moreover, *apo-5; par-3* double mutant embryos resemble *par-3* embryos, indicating that the exaggerated posterior displacement of the nucleo-centrosomal complex in *apo-5* mutant embryos requires *par*-mediated polarity cues. Staining of fixed embryos with an antibody against α-tubulin shows that some one-, two-, and four-cell stage *apo-5* mutant embryos have microtubule nucleation defects. Furthermore, a β-tubulin::GFP line crossed into *apo-5* also revealed that in some embryonic cells centrosomes are dissociated from the nucleus. These results suggest that *apo-5* mutant embryos may have a centrosomal defect that influences spindle function and orientation. Mapping of *apo-5* positions it on linkage group (LG) I, at approximately –8.7 map units. Progress on the positional cloning of *apo-5* will be reported.

Intriguingly, genetic analyses indicate that mutations in *apo-5* result in a paternal-effect embryonic lethal phenotype: fertilization of wild-type hermaphrodites and *fem* females with sperm from homozygous mutant males results in partial embryonic lethality (~36% embryos are dead). These data, together with the microtubule nucleation defects observed in *apo-5* mutant embryos suggest that a possible role for *apo-5* is in centrosomal function. Thus we believe that *apo-5* will provide insights into the role of the sperm-donated centrosome during early embryogenesis in *C. elegans*.

6. Orientation and polarization of the mitotic spindle in the embryonic cell P_1

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The orientation of the mitotic spindle can be important for proper segregation of cytoplasmic determinants during asymmetric cell divisions. Nonetheless, the mechanisms controlling spindle orientation remain poorly understood. The posterior blastomere of the 2-cell stage *C. elegans* embryo, called P_1, undergoes an asymmetric cell division that involves rotation of the mitotic spindle. After duplication and migration, the centrosomes in P_1 are positioned transversely to the anterior-posterior (a-p) axis of the embryo. Subsequently the forming spindle rotates about 90 degrees, orienting longitudinally. It has been shown that this rotation involves interactions between astral microtubules and a remnant site from Cytokinesis, although the underlying mechanism remains unknown. We have identified maternal effect embryonic lethal mutations in a gene we call *spn-4*, which is required for orientation of the P_1 mitotic spindle. In *spn-4* mutant embryos the P_1 spindle stays transverse to the a-p axis. Moreover the ectopic a-p orientation of the spindles observed in *par-3* mutants at the 2-cell stage requires the function of the *spn-4* gene. *spn-4* encodes a putative RNA binding protein containing a single RNA Recognition Motif (RRM), and mediates a subset of asymmetries, likely downstream of the polarity established by the PARs. Although spindle orientation is abnormal in P_1, this cell division remains remarkably asymmetric: the daughter blastomeres are unequal in size, the larger daughter divides before the smaller one, and P granules and PIE-1 protein are segregated to the smaller daughter, as in wild type. Furthermore P granules and PAR-2 are re-localized during mitosis, accumulating laterally near one pole of the transversely oriented mitotic spindle. We suggest that the P_1 mitotic spindle itself becomes polarized in and reorients the axis of polarity in *spn-4* mutants. Furthermore, we propose that the P_1 spindle maintains the asymmetry during the division of P_1 in the wild type. Preliminary results indicate that DEP domain protein LET-99 is required for polarization of P_1 mitotic spindle in *spn-4* embryos. We are currently investigating the role of microtubules and of the heterotrimeric G proteins in polarization of the P_1 mitotic spindle in *spn-4* mutant embryos. We propose that the mitotic spindle of the P_1 cell is polarized, and this polarization requires the *let-99* gene, and may require the heterotrimeric G proteins.

7. ZYG-8 and TAC-1 are required for proper microtubule behaviour in one-cell stage *C.elegans* embryos.

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Proper spindle positioning is essential for spatial control of cell division. We have previously reported that *zyg-8* is a key component directing anaphase spindle positioning in one-cell stage *C. elegans* embryos (Dev Cell. 2001 Sep;1(3):363-75). ZYG-8 harbors a kinase domain and a domain related to the microtubule-associated protein (MAP) Doublecortin. We have shown that ZYG-8 is a MAP that protects microtubules against depolymerizing agents, suggesting that ZYG-8 directs anaphase spindle positioning in one-cell stage embryos by promoting microtubule stability.

Anaphase spindle positioning is mainly driven by pulling forces exerted on the spindle poles along astral microtubules. We probed the extent of these forces in *zyg-8* mutant embryos by severing spindle microtubules with a laser beam. Surprisingly, we found the extent of pulling forces to be like in wild-type. Therefore, *zyg-8* function is not required for the generation of pulling forces. Instead, these results indicate that *zyg-8* is necessary for spindle elongation in response to pulling forces exerted on spindle poles during anaphase.

In order to better understand how *zyg-8* promotes microtubule stability, we sought to identify molecular partners of ZYG-8 using the yeast two-hybrid system. Using the N-terminal 464 aa of ZYG-8 that encompass the Doublecortin domain as bait, we screened 0.75×10^6 clones and identified 76 interactors corresponding to 24 different open reading frames. We have begun to assess the biological significance of these interactions by inactivating the corresponding genes using RNAi in wild-type and *zyg-8* mutant animals. This approach allowed us to identify the only *C. elegans* counterpart of the mammalian Transforming and Acidic Coiled Coil family of proteins as a particularly interesting candidate. We name this gene *tac-1*.

TACC genes have been initially identified in human cancer cell lines where they are either overexpressed (Hs-TACC1 and Hs-TACC3) or underexpressed (Hs-TACC2). Subsequent work in *Drosophila* revealed that D-TACC is required for proper spindle and astral microtubule behaviour. D-TACC is localized to spindle poles and interacts with MAPs of the Msps/XMAP215/ZYG-9 family. Interestingly, we found that *tac-1* (RNAi) embryos in *C. elegans* have a pronuclear migration phenotype indistinguishable from that of *zyg-9* mutant embryos, characterized by compromised microtubules throughout the cell cycle. Taken together, these observations suggest that TAC-1 may interact with ZYG-9, in addition to ZYG-8, in one-cell stage *C. elegans* embryos. We are currently testing tenets of a working model in which TAC-1 is an essential centrosomal anchor for microtubule stabilizers like ZYG-9 and ZYG-8.

8. Positioning the cleavage furrow in *C. elegans* embryos

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To generate two cells with identical genetic material during mitosis, cells regulate cytokinesis with respect to mitotic events in space and time. One particularly interesting question concerns the mechanism of cleavage furrow positioning by the mitotic spindle. In order to understand this regulation at a molecular level, we performed a genetic screen for maternal effect embryonic lethal mutations with multinucleate blastomeres indicative of a failure in cytokinesis. In this screen we isolated one allele, *xs59*, that is defective in spatial regulation of cytokinesis.
In *xs59* mutant embryos, cleavage furrows ingress at the correct time and place, but as anaphase proceeds, ectopic furrows form and appear to cause the initial cleavage furrows to regress leading to the formation of multinucleate cells. Ectopic furrows are dependent on the same components as normal cleavage furrows, as their formation is dependent on both RhoA and non-muscle myosin. Interestingly, ectopic furrows are restricted to the anterior part of the embryo and can be modulated by interfering with general polarity of the early embryo. In *xs59; par-2(RNAi)* embryos ectopic furrows can be observed in the anterior and the posterior part of the embryo, whereas *xs59; par-3(RNAi)* embryos do not display ectopic furrows and the cytokinesis defect is partially rescued. PAR-2 and PAR-3 could regulate ectopic furrowing either by regulating cortical properties or they may indirectly affect microtubule (MT) density at the cell cortex. Immunofluorescence studies reveal that spindle morphology is altered, asters appear smaller and the central spindle appears weak, although the central spindle marker ZEN-4 could be detected in all embryos. Taken together, these data suggest that high MT density may prevent furrow formation at the poles of the cell.
More recently, we have investigated the role of the central spindle in furrow ingression. Embryos lacking a central spindle due to a mutation in the kinesin-like protein ZEN-4 form deeply ingressing cleavage furrows, but these furrows regress, indicating that the central spindle has a critical role in completion of cytokinesis. However, we have discovered that under certain conditions, the central spindle is essential for furrow initiation. This observation is reminiscent of the fact that the Drosophila ZEN-4 ortholog, Pavarotti, is required for furrow formation. These data indicate a surprising degree of plasticity in the spindle structures that lead to furrow initiation. Moreover, they indicate that the long standing controversy over the source of the signal for furrow initiation may depend greatly on the relative geometry of the spindle and the cell cortex.

9. *chp-1*, a CHORD domain containing protein required for early embryonic polarity

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Numerous genetic screens for maternal effect embryonic lethal mutations resulted in the identification of many genes required for early embryonic polarity. Amongst them, the most important ones are the PAR genes that result in a symmetric first cleavage when knocked out. However, it is still unclear how, for example, the mutual exclusive cortical localisation of the PAR-1/PAR-2 and PAR-3/PAR-6/PKC-3 complexes is initially established at opposite poles of the 1-cell embryo and how the PAR proteins signal to downstream effectors. To identify new genes required for these processes, the phenotypes of 147 chromosome I embryonic lethal loci have been analysed using time-lapse video-microscopy (1). We identified *chp-1* as a gene resulting in a *par*-like mutant phenotype. It has been shown in plants (barley) that the homologous gene RAR-1 is required for disease resistance (2). CHP-1 contains two zinc-binding domains called CHORD I and CHORD II (*c*ysteine and *h*istidine *r*ich *d*omain). The metazoan versions have a C-terminal extension that shows weak similarity to yeast SGT-1, a supressor of a G2 allele of yeast *skp-1*. *chp-1(RNAi)* embryos show a symmetric first cleavage, complete or partial rotation of the AB spindle in 90% of the cases, and near-synchronous division of AB and P1. Like in *par-3* or *par-6* mutants, PAR-2 is found all around the cortex of 1 and 2 cell *chp-1(RNAi)* embryos. As a consequence, PAR-3 and PAR-6 cortical localisation is reduced. Consistent with CHP-1 being required for embryonic polarity, P-granules are mislocalised at the 4 cell stage. Alongside its role in early embryonic polarity, CHP-1 also plays a role in germline development as *chp-1(RNAi)* animals develop a strong sterility phenotype. At the meeting we will present progress on the phenotypic characterisation of *chp-1(RNAi)* embryos and discuss possible modes of action.

1. P. Zipperlen, A. G. Fraser, R. S. Kamath, M. Martinez-Campos, J. Ahringer, *Embo J* ***20****, 3984-92. (2001).*
2. K. Shirasu *et al.*, *Cell* **99**, 355-66. (1999).

10. Identification of new genes required for apical sorting in epithelial cells

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We are interested in membrane trafficking in *C. elegans* epithelial cells. We previously showed that the sterol-sensing domain protein CHE-14, which is related to Patched and Dispatched (two proteins required for Hedgehog signalling), is involved in apical sorting in ectodermal epithelial cells (*Curr Biol*, 10 : 1098). *che-14* mutants display what we called the Rdy phenotype, which associates osmotic failure leading to **r**od-like larval lethality and **dy**e-filling defect (due to abnormalities in support cells associated with chemosensory organs).

In order to identify potential CHE-14 partners and more generally other genes involved in membrane trafficking, we searched for new mutations with a Rdy phenotype and identified eight such mutations. One of them proved to be a new *che-14* allele, which validates our screening strategy. So far, we cloned two of the genes defined by these mutations. Two allelic mutations affect a gene named *rdy-1*, that encodes an *a* subunit of the Vo sector of (H+)V-ATPase. Two other allelic mutations affect a second gene named *rdy-2*, which encodes a new protein with four putative transmembrane domains. Using rescuing GFP-fusions, we found that RDY-1 and RDY-2 proteins are localised at the apical membrane of several ectodermal epithelial cells, like the CHE-14 protein. The Vo sector of (H+)V-ATPase has been recently shown to act in membrane fusion by biochemical experiments (*Nature*, 409 : 581). Indeed, electron microscopy (EM) analysis indicates that *rdy-1* mutants have secretory defects in the hypodermis, in part similar to those observed in *che-14* mutants. These results thus provide genetic support in favour of an implication of the Vo sector in exocytosis, and raise the possibility that *rdy-1* and *che-14* act in the same pathway.

In order to explore the possibility that RDY-1, RDY-2 and CHE-14 could be part of a protein complex, we performed fluorescence resonance energy transfer (FRET) experiments, using CFP and YFP fusion proteins. Preliminary results show that FRET occurs between RDY-1 and RDY-2, at least in the hypodermis and in the excretory canal, leading to the possibility that RDY-1 and RDY-2 interact in these epithelial cells.

In conclusion, we have identified two new genes that are potentially involved in membrane trafficking in epithelial cells. Ongoing FRET, EM and genetic experiments should better define at which step RDY-1, RDY-2 and CHE-14 act in apical sorting, and whether they are part of one or more complex.

11. The putative actin binding protein ERM-1 is required for correct localization of the apical junction in the *C.elegans* intestine

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At present, several proteins are known to affect epithelial cell polarity during development. However, their signalling pathways and mechanisms are still elusive. This is in part due to the abundance of adaptor molecules which mediate the interaction of polarity cues with the executive cytoskeleton. We have started in-depth analysis of *erm-1* (ezrin-radixin-moesin), a band-4.1/ERM protein that is defined as membrane-cytoskeleton linker. *erm-1* has an essential function during embryonic development of the apical junction in the gut epithelium of *C.elegans*. ERM-1 is predominantly expressed on the apical membrane domain and depletion of the protein causes larval lethality. In *erm-1(RNAi)* embryos subapical and adherens junction components (e.g. CRB-1/HMP-1 and DLG-1/AJM-1, respectively) remain clustered apically instead of forming a continuous belt around the apex of the intestinal cells. Additional FITC phalloidin staining reveals a disorganized actin cytoskeleton in the cortex of gut epithelial cells.
Interactions of cell-cell complexes, e.g. catenin/cadherin with actin filaments either directly[1] or through actin-associated proteins, such as a-actinin [2] has been studied biochemically and a relationship between the actin cytoskeleton and the development of cadherin-mediated cellcell adhesion has been proposed [3]. However, a correlation between band-4.1/ERM proteins and final position of a junction was completely unexpected. At the moment, we are considering the possiblity that ERM-1 is needed structurally to anchor adherens junction components directly or indirectly to the barbed ends of actin filaments, thereby triggering movement to the correct position along the apico-basal axis.

1. Rimm DL, Koslov ER, Kebriaei P, Cianci CD, Morrow JS: Alpha 1(E)-catenin is an actin-binding and -bundling protein mediating the attachment of F-actin to the membrane adhesion complex. *Proc Natl Acad Sci U S A* 1995, 92:8813-8817.
2. Knudsen KA, Soler AP, Johnson KR, Wheelock MJ: Interaction of alpha-actinin with the cadherin/catenin cell-cell adhesion complex via alpha-catenin. *J Cell Biol* 1995, 130:67-77.
3. Adams CL, Chen YT, Smith SJ, Nelson WJ: Mechanisms of epithelial cell-cell adhesion and cell compaction revealed by high-resolution tracking of E-cadheringreen fluorescent protein. *J Cell Biol* 1998, 142:1105-1119.

12. Multiple regulators of actin dynamics modulate cadherin function during hypodermal morphogenesis

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Recent work in a variety of model systems has revealed that changes in actin cytoskeleton organisation are pivotal to the stabilisation and maturation of cadherin-based adherens junctions. However the mechanisms that co-ordinate changes in cell adhesion with cytoskeletal organisation are only just beginning to emerge, and little information has been obtained in the context of a developing animal. We have recently identified a novel maternal-effect Hmp mutation, *fe4*, which is a weak hypomorphic allele of *hmp-1* (the *C. elegans* alpha-catenin gene). Homozygous mutant progeny of *fe4* heterozygotes display only minor defects in the shape of the tail, whereas the progeny of these homozygotes display more severe morphological phenotypes. The molecular nature of the *fe4* mutation, coupled with its relatively weak phenotype, suggests that HMP-1 function is compromised, but not abolished in *fe4* homozygotes, and is supported by the fact HMP-1 expression and localisation is not obviously affected in these animals. The variable nature of the phenotype suggests that the hypodermal cells of *fe4* mutants are sensitive to stochastic fluctuations in cadherin-catenin function. We have used RNAi, and previously identified mutations to identify genes whose loss of function enhances the *fe4* mutant phenotype, assuming that such genes will encode molecules that have roles in the formation and function of cadherin adhesion junctions.

In cultured keratinocytes members of the Ena/VASP family, which regulate actin remodelling, localise to nascent cadherin junctions, where they regulate changes in the actin cytoskeleton that occur during adherens junction formation. The *unc-34* gene encodes the sole *C. elegans* member of the Ena/VASP family, and we therefore sought to examine its role in cadherin function. None of the available alleles of *unc-34* display any obvious defects in epithelial morphogenesis. However *unc-34 fe4* double mutant combinations result in 100% maternal-effect embryonic lethality, with all embryos displaying either ventral enclosure, or elongation defects. This indicates that despite not being essential for hypodermal morphogenesis in animals with a wild-type cadherin-catenin complex, UNC-34 nevertheless contributes to the modulation of cadherin adhesion.

The other group of candidate genes that we have examined are the Rho family of small GTPases, which have previously been shown to regulate cadherin adhesion in cell culture, as well as a variety of morphogenetic events *in vivo*. Loss-of-function mutations in the Rac genes *mig-2* and *ced-10*, as well their putative guanine-nucleotide exchange factor, *unc-73*, enhance the penetrance of ventral enclosure defects in combination with *fe4*, indicating that Rac signalling is involved in the regulation of the formation and/or function of cadherin-catenin complexes in *C. elegans*.

Taken together these results suggest that UNC-34 and MIG-2/CED-10 act in parallel to regulate the cadherin-catenin complex activity required for ventral enclosure. In support of this we find that *ced-10*/*mig-2(RNAi)* in *unc-34* mutants results in ventral enclosure defects. We are currently exploring the molecular basis of these observations, to attempt to identify the targets of MIG-2/CED-10.

13. *plexin-1* is required for attraction and repulsion of cells in the epidermis of *C.elegans*

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Plexins are semaphorin receptors conserved in vertebrates and invertebrates. However, their *in vivo* role in axon guidance, cell migration and embryonic development is not yet understood. Here we report the isolation of a mutation in the *C.elegans plexin-1* (*CEL-plx- 1(ev724)*). Mutant animals have ventral enclosure defects and the first male ray is anteriorly positioned. Mutant ray 1 precursor cells are misplaced anteriorly during male tail development. *semaphorin-1* mutants *(smp-1/2(ev715, ev709))* have an identical male phenotype and we show that they function in the same genetic pathway as *plx-1*. *CEL-plx- 1* shares high similarity with the C-terminal domain of human and *D.melanogaster* plexins, in which the presence of a RAC^{GTP} binding domain has been demonstrated (Hu (2001)). We find that known mutants for *C.elegans rac* genes (*ced-10, mig-2*) and a small GTPase activator *(unc-73)* have a ray 1 positioning defect similar to *plx-1(ev724)*. *mig-2* and *ced-10* genetically act in parallel for this phenotype and these genes are likely activated by *unc-73*. Our genetic data suggest that *plexin-1* and *sema-1* are part of a pathway modulating the *unc-73/mig-2/ced-10* pathway for ray 1 positioning. This model is consistent with known biochemical interactions between PLX-1 and RAC^{GTP} activated form (Vikis (2000); Driessens (2001); Hu (2001)), suggesting that upon SMP-1/2 binding the function of the PLEXIN-1/RAC complex is modulated.

•Vikis et al. (2000), PNAS 97(23), p12457
•Driessens et al.(2001), Curr Biol 11(5), p339
•Hu et al.(2001), Neuron 32, p39

14. One gene for two distinct plakins that provide outer and inner resistance to mechanical stress

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To identify genes required for epidermal integrity and embryonic morphogenesis, we previously performed a chromosomal deficiency screen looking for embryos that failed to elongate (*Dev Dyn* **210**:19-32). We next focused on a promising deficiency, *hDf17*, and identified an embryonic lethal mutation, *h1356*, mapping under *hDf17* that recapitulates part of its phenotype. We showed that *h1356* corresponds to a putative null allele of the gene *vab-10*, previously defined by the viable allele *e698*, and identified another strong allele, *ju281* which failed to complement *h1356* and *e698*. We showed that *vab-10* encodes two distinct plakins via a complex pattern of alternative splicing. Plakins are large proteins thought to bridge different cytoskeletal elements. Specifically, all VAB-10 isoforms share a common N-terminal domain with a predicted actin-binding domain. Their C-terminal domains are either similar to plectin, a protein known to bind actin and intermediate filaments, or MACF, a protein known to bind actin and microtubules (we refer to these isoforms as CePlectin and CeMACF, respectively). Sequencing of the three *vab-10* alleles shows that *h1356* affects the common N-terminal region, while *ju281* and *e698* are missense mutations affecting the unique CePlectin region. Using polyclonal antibodies against CePlectin, we found that it is a likely component of fibrous organelles, a structure found in the epidermis that provides a mechanical link between muscles and the cuticle across the epidermis. Similarly using two antisera against CeMACF we found that CeMACF forms a pattern of parallel bands in the epidermis that is interspersed with fibrous organelles.

Genetic and RNAi experiments, coupled with electron and confocal analysis, showed that CePlectin is essential for muscle anchoring and fibrous organelle integrity. Indeed, when CePlectin activity is affected, embryonic elongation does not progress beyond the 1.5-fold stage (after RNAi) or the 2-fold stage (most *ju281* mutants raised at 25°C) and muscle cells fail to attach to the cuticle. This detachment depends on muscle activity. EM studies revealed a strong reduction of the number of fibrous organelles, and in parallel a detachment of the epidermis from the cuticle and from muscle cells. The phenotype of *h1356* is very similar to that of embryos lacking CePlectin. Similar studies suggest that CeMACF plays a partially functionally redundant role, and that it is critical to maintain a regular thickness of the epidermal layer. Indeed, RNAi against CeMACF results in partially elongated embryos that hatch to produce lumpy larvae that generally do not reach adulthood. EM analysis showed that fibrous organelles are initially present in CeMACF-deficient embryos, but that the thickness of epidermal cells in areas of muscle-epidermis contact can reach 40 times the normal thickness (i.e. ≈100 nm). In addition, we found that actin filaments are misoriented in CePlectin-deficient or shorter in CeMACF-deficient embryos, while intermediate filaments (recognised by the mAb MH4) are strongly disorganised in CePlectin-deficient embryos. We conclude that *vab-10* provides resistance to mechanical stress in two different ways, organises the cytoskeleton, and that coupling between muscles and the epidermis is essential for embryonic elongation.

15. Evidence of a role for the *C. elegans* COPII complex in cuticle secretion

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The *C. elegans* cuticle is a complex extracellular structure that is synthesised and secreted by the underlying hypodermis. The cuticle is composed primarily of a group of small collagen-like proteins which are under strict temporal and spatial control. Genetic screens were performed on *C. elegans* strains carrying collagen gene/GFP transgenes to isolate embryonic lethal mutants defective in cuticle synthesis and secretion. *ij13*, an embryonic lethal allele isolated from this screen encodes the *C. elegans* homologue of the yeast *Sec23* gene.

Sec-23 encodes a protein involved in transport of secretory proteins, in COPII-coated vesicles, from the endoplasmic reticulum to the Golgi. The SEC-23 protein binds with SEC-24 and several other proteins to form these COPII complexes which are required for vesicle formation and packaging of cargo in the ER and subsequent transportion to the Golgi. It has been hypothesized that, in vertebrates, the large size of the procollagen molecules prohibits their packaging into small COPII vesicles. However, we have evidence that the SEC-23/SEC-24 complex is critical for cuticle collagen secretion. We demonstrate that loss of *sec-23* results in intracellular perinuclear accumulation of cuticular collagen and failure to secrete a cuticle. Restoration of *sec-23* function specifically within the hypodermis by its expression from a hypodermal-specific promoter, restored the ability of mutant embryos to secrete a cuticle. We have also demonstrated, by RNAi and immunofluorescence, that the SEC-23 protein plays a role in other developmental pathways and is essential for oogenesis.

There are two *C. elegans* homologues of the yeast *Sec24* gene which we have termed *sec-24a* and *sec-24b*. We have demonstrated that they have distinct biological functions. Reduction of *C. elegans sec-24a* by RNAi results in cuticle secretion defects and defects in oogenesis, similar to that seen for *sec-23*. RNAi of *C. elegans sec-24b* results primarily in early embryonic defects.

Although we demonstrated that the *C. elegans* COPII complex is essential for cuticle collagen secretion, our data suggest it may not be required for secretion of basement membrane.

16. Role of *C. elegans pdi-3* in extracellular matrix assembly.

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Nematodes extracellular matrix (ECM) is essential for the maintenance of the post-embryonic body shape, locomotion and protection from the environment. It is mainly composed of collagens and its assembly requires extensive post-translational modifications. Such modifications are carried out by many enzymes such as protein disulfide isomerase (PDI). To date two PDI from *C. elegans* have been studied: PDI-1 and PDI-2. Both enzymes are involved in ECM assembly and modification. *pdi-1* is expressed in an operon with *cyp-9* (Page (1997) DNA and Cell Biology, 16, 1335-1343). Both PDI-1 and CYP-9 may contribute to ECM assembly through the chaperone and disulfide bond formation activity of PDI-1 and the peptidyl-prolyl-cic/trans isomerase activity of CYP-9. PDI-2 is the β-subunit of the prolyl 4-hydroxylase complex in *C. elegans* (Winter and Page (2000) Molecular and Cellular Biology, 20, 4084-4093). PDI-2 plays an essential role in ECM assembly as part of the prolyl 4-hydroxylase complex, which is responsible for hydroxylation of proline in collagen molecules.

An additional predicted *pdi* with homology to *pdi-1* and *pdi-2* was identified from the *C. elegans* genome (H06o01.1). We cloned and expressed this predicted *pdi*. *In vitro*, the enzyme was able to refold denatured RNAase A, which confirmed it was PDI and we named the enzyme PDI-3.

We studied the effects of *pdi-3* RNA mediated interference on nematode morphology and the localisation pattern of two collagen proteins (DPY-7 and COL-19). The cuticle surface was also observed by scanning electron microscopy following *pdi-3* RNAi. Worm morphology and localisation of the two studied collagen markers DPY-7 and COL-19 were affected by *pdi-3* RNAi.

The relative abundance of *pdi-3* transcript during the *C. elegans* life cycle was determined by semi-quantitative RT-PCR. The expression of *pdi-3* cycled with the nematode moulting cycle, and displayed highest expression levels between moults. This expression pattern was similar to that of many collagen genes, including *dpy-7*.

The spatial expression pattern of *pdi-3* was examined via *lacZ/gfp* promoterless reporter transgene analysis and further localisation studies were carried out using a PDI-3 specific polyclonal antibody. Both techniques showed that *pdi-3* was expressed in many tissues including the hypodermis.

The RNAi results, and the temporal and spatial expression patterns suggested a potential interaction between collagen expression and localisation and *pdi-3*, indicating that *pdi-3* may be involved in ECM assembly.

A homolog of *pdi-3* has been identified in the parasitic nematode *D. immitis* (Chandrashekar *et al.* (1998) PNAS, 95, 531-536). Unusually this enzyme has tranglutaminase activity. In human, tissue transglutaminase has been shown to be involved ECM cross-linking (Raghunath *et al.* (1996) J. Clin. Invest., 98, 1174-1184), via the formation of a covalent isopeptide bond between glutamine and lysine residues of two distinct proteins. *pdi-3* also has transglutaminase activity and may be involved in collagen cross-linking in the ECM.

17. Specific aspartyl and calpain proteases are required for neurodegeneration in *C. elegans*.

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Necrotic cell death underlies the pathology of numerous human neurodegenerative conditions. In the nematode *Caenorhabditis elegans*, gain-of-function mutations in specific ion channel genes such as the degenerins *deg-1* and *mec-4*, the acetylcholine receptor channel *deg-3*, and in the G-protein gene *gsa-1* evoke an analogous pattern of cell death (necrotic-like) in neurons expressing the mutant proteins. Increase of cytoplasmic calcium levels in dying cells, elicited either by extracellular calcium influx or by release of endoplasmic reticulum stores is thought to comprise a major death signaling event. However, the mechanisms by which calcium triggers cellular demise remain largely unknown. We report that neuronal degeneration inflicted by various genetic lesions in *C. elegans*, requires the activity of the calcium-regulated CLP-1 and TRA-3 calpain proteases and aspartyl proteases ASP-3 and ASP-4. Our findings demonstrate that two distinct classes of proteases are involved in necrotic cell death and suggest that perturbation of intracellular calcium levels may initiate neuronal degeneration by deregulating proteolysis.

The identification of two specific classes of proteases required for neurodegeneration in *C. elegans*, may provide insight into similar pathologies in mammals. Similar proteases may mediate necrotic cell death in humans: The lysosomal degradation system was found to be up-regulated in neurons of Alzheimer's disease patients and cathepsin D expression is induced under conditions of excitotoxic cell death. Furthermore, calpain inhibitors can be protective in certain cases of nerve or muscle degeneration. These findings suggest that similarly to apoptosis, necrotic cell death mechanisms are conserved from nematodes to humans, and highlight specific targets for therapeutic intervention in an effort to battle neurodegenerative disorders.

18. The Snail-like zinc finger DNA-binding protein CES-1 is a direct regulator of *egl-1* transcription

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Genetic and molecular analyses performed in *C. elegans* have defined a genetic pathway for programmed cell death (PCD), an important physiological process of animal development, which has been conserved through evolution (1).
Four genes, *egl-1* (*egl*, egg laying defective), *ced-9* (*ced*, cell death abnormal), *ced-4*, and *ced-3*, form the central pathway required for cell-death execution in *C. elegans*. Mutations in these genes can block most if not all somatic PCD events that occur during the development of *C. elegans*. A group of genes, the *ces* (*ces*, cell death specification) genes, appears to regulate the central pathway in a cell-type specific manner. Gain-of-function mutations in the gene *ces-1*, for example, specifically block the death of the NSM sister cells, the sisters of the NSMs, two serotonergic, neurosecretory motoneurons (2). *ces-1* encodes a Snail-like zinc finger DNA-binding protein (3), and negatively regulates the cell-death activator gene *egl-1*. *egl-1* represents the most upstream gene of the central cell-death pathway and is regulated at the transcriptional level at least in some cells, including the NSM sister cells (4, Hatzold, J. *et al.*; European *C. elegans* Meeting 2002, abstract). Moreover, the *egl-1* promoter contains four Snail family consensus sites, which are conserved in the *egl-1* locus of *C. briggsae*. Therefore, we propose that the CES-1 protein specifies the cell-death fate of the NSM sister cells by directly regulating *egl-1* expression.
To determine which part of the *egl-1* locus is essential for the death of the NSM sister cells, we analysed subclones of the *egl-1* locus for their potential to rescue this particular cell-death event in *egl-1*(lf) mutant animals. We could show that the region that spans the four Snail family consensus sites (Region B) is necessary and sufficient for the specification of the NSM sister cell death *in vivo*. Moreover, using gel mobility shift assays with bacterially expressed GST-CES-1 fusion protein, we could also show that GST-CES-1 binds to the four Snail family consensus sites in Region B in a specific and cooperative manner. These data suggest that in the surviving NSMs, CES-1 acts as a repressor of *egl-1* transcription. In addition, we have *in vivo* evidence indicating that CES-1 and a potential NSM-specific transcriptional activator may specify the NSM sister cell fate by competing for binding to the Snail family consensus sites in Region B. Genetic and molecular data suggest that this activator is a heterodimer of *C. elegans* Daughterless and Achaete-Scute like proteins (Hatzold, J. *et al.*; European *C. elegans* Meeting 2002, abstract), which belong to the family of basic helix-loop-helix (bHLH) transcription factors (5, 6).

1. M. M. Metzstein et al. (1998). TIG 14, 410416
2. R. E. Ellis and H. R. Horvitz. (1991). Development 112, 591-603
3. M. M. Metzstein and H. R. Horvitz. (1999). Molecular Cell 4, 309-319
4. B. Conradt and H. R. Horvitz. (1999). Cell 98, 317-327
5. J. E. Lee (1997). Curr Opin Neurobiol 7,13-20
6. W. R. Atchley (1997). Proc. Natl. Acad. Sci. 94, 5172-5176

19. Regulation of programmed cell death by the two *C. elegans* BH3-only proteins EGL-1 and CED-13

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During hermaphrodite development, programmed cell death is the fate of 131 of the 1090 somatic cells originally generated. Genetic studies of this process revealed a conserved central cell death machinery consisting of EGL-1 (BH3-only), CED-9 (Bcl-2), CED -4 (Apaf-1), and CED-3 (caspase). In addition to the lineage dependent cell deaths during development, cell death also occurs in the hermaphrodite germ line, where 50% of all germ cells die (physiological germ cell death). This process was found to be *egl-1* independent. In contrast, non-physiological germ cell death induced by DNA damage or infection with *Salmonella typhimyrium* have been shown to be at least partially dependent on *egl-1*. These findings suggested that there might be alternative or additional factors regulating germ cell death in the hermaphrodite germ line.

Besides EGL-1, the *C. elegans* genome encodes at least one more BH-3 only protein, CED-13 (formerly called CIP-1). Both proteins interact with CED-9 in a yeast two-hybrid assay and *in vitro*. When we expressed the *ced-13* cDNA under the control of a heat shock promoter, ectopic cell death was observed, indicating that CED-13 can act as a killer protein, similar to EGL-1. This effect was rescued by *ced-3*(lf), *ced-4*(lf) or *ced-9*(gf) mutations, suggesting that CED-13 encodes a cell death activator, which acts upstream of the central cell death pathway.

A *ced-13* deletion mutant, *ced-13(sv32)*, was isolated that deletes the entire coding region plus some upstream and downstream sequence.

Both *C. elegans* BH3-only proteins seem to have a function in the regulation of programmed cell death in the soma during development as well as in the regulation of non-physiological germ cell death induced by DNA damage. While the *egl-1*(*n1084 n3082*lf) mutation blocks most somatic cell death events and results in the survival of 11.1 extra cells in the anterior pharynx, *ced-13(sv32)* mutant animals only have 0.6 extra cells. However, *ced-13(sv32)* strongly enhances the Ced phenotype caused by a weak *ced-3(n2438*lf*)* mutation (1.7 versus 6.0 extra cells), indicating a minor role of *ced-13* in somatic tissue. In contrast, *ced-13(sv32)* completely blocks non-physiological germ cell death induced by DNA damage, while the process is only partially dependent on *egl-1*.

Interestingly, like *egl-1*, *ced-13* is not necessary for physiological germ cell death, which indicates that the regulation of germ cell death might be independent of BH3-only proteins. It is therefore likely that with respect to physiological germ cell death, CED-9 function is regulated by a different mechanism (see also P. Opitz *et al.* EWM2002).

20. *Caenorhabditis elegans* HUS-1 is a DNA damage checkpoint protein required for genome stability and induction of EGL-1.

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Tumorigenesis is characterized by the accumulation of genetic mutations, rearrangements, amplifications, and deletions, all of which drive the progressive transformation of normal cells into highly malignant derivatives. Therefore it is essential for cells to have precise and efficient means to maintain the integrity of the genome. Damage to DNA triggers checkpoint controls that result in cell cycle arrest and repair of the lesion. In metazoans, a second option is the programmed demise of the cell, possibly due to extensive damage that is not rectifiable. Loss of communication between the DNA lesion and the apoptotic program, which allows the persistence of cells with damaged and/or unstable genomes, can lead to tumorigenesis.
In *C. elegans*, gamma irradiation induces apoptotic cell death of meiotic germ cells as well as proliferation arrest of mitotic germ cells. DNA damage-mediated apoptosis is dependent on *ced-3*, *ced-4* and is negatively regulated by *ced-9*. The positive death regulator, *egl-1*, participates in, but is not essential for radiation-induced apoptosis.
Recently, three mutants - *op241, rad-5(mn159), and mrt-2(e2663)* - have been identified in *C. elegans* that block DNA damage induced apoptosis and cell cycle arrest[1-3].
We have recently mapped *op241* to the left arm of *LGI* between *unc-11* and *stu-4*. Sequence analysis revealed a mutation in the homologue for the *S. pombe hus1*$^{+}$ checkpoint gene. In addition, we have isolated a deletion mutant of *hus-1(op244)* from a deletion library. Characterization of *op244* reveals other phenotypes suggesting a role for *hus-1* in maintaining genome stability and telomere maintenance. HUS-1 is a nuclear protein that is expressed in the germline. Nuclear localization of HUS-1 is dependent on the checkpoint genes *mrt-2* and *hpr-9*, but not *rad-5*. Following DNA damage, HUS-1 re-localizes to putative sites of DNA damage. Re-localization of HUS-1 is enhanced in *rad-5 (nm159)* and *rad-51*(RNAi) backgrounds, suggesting a role for these proteins in DNA repair. Finally, *egl-1* is upregulated following DNA damage in a HUS-1 dependent manner.

1. Gartner, A., Milstein, S., Ahmed, S., Hodgkin, J. & Hengartner, M. O. *Mol Cell* 5, 435-43. (2000).
2. Ahmed, S. & Hodgkin, J. *Nature* 403, 159-64. (2000).
3. Ahmed, S., Alpi, A., Hengartner, M. O. & Gartner, A. *Curr Biol* 11, 1934-44. (2001).

21. Does the DNA damage checkpoint shorten lifespan in *C. elegans*?

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The DNA damage checkpoint is composed of proteins that sense DNA damage and then send a signal that DNA damage has occurred, which results in either cell cycle arrest and DNA repair (which eliminates the damage) or apoptosis (which eliminates the damaged cell). Recently, we cloned a novel DNA damage checkpoint gene, *rad-5/clk-2*, which is defined by two alleles, *rad-5(mn159)* and *clk-2(qm37)*, which are defective for both the DNA damage checkpoint and the S-phase replication checkpoint (1). One mutant allele of this gene, *clk-2(qm37)*, was previously characterized as one of four *clock* genes, which, when mutated, display maternal-effect slow growth (Gro), slowed biological rhythms, and an extended lifespan (2). It was suggested that these *clock* mutants might experience an extended lifespan as a result of slowed metabolic rates (2). Although *clk-2(qm37)* has a strong maternal-effect slow growth (Gro) phenotype, the *rad-5(mn159)* allele is only weakly Gro. However, both *rad-5(mn159)* and *clk-2(qm37)* are equally long-lived, suggesting that checkpoint inactivation and not metabolic rate may be the cause of the longevity experienced by these two mutants.

1. Ahmed, S., Alpi, A., Hengartner, M. O., and Gartner, A. (2001). *C. elegans* RAD-5/CLK-2 defines a new DNA damage checkpoint protein. Curr Biol *11*, 1934-1944.

2. Lakowski, B., and Hekimi, S. (1996). Determination of life-span in *Caenorhabditis elegans* by four clock genes. Science *272*, 1010-1013.

22. Characterisation of MUS-81, an XPF-like endonuclease required for genome stability

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Cell cycle checkpoints play essential roles in ensuring the faithful transmission of genetic information during cell division; disruption of these checkpoints can lead to genetic mutation or cell death. To further our understanding of cell cycle checkpoints in multicellular organisms, we are investigating the role of cell cycle checkpoint genes in *C. elegans*.

We have identified a *C. elegans* orthologue of the yeast protein Mus81 (Methyl methane sulphonate and UV Sensitive) and isolated a *C. elegans* cDNA. Mus81 was first identified because of its ability to interact with the both the cell cycle checkpoint kinase CDS1 and the DNA repair protein RAD54 in yeast 2-hybrid screens (1,2). Mus81 contains two helix-hairpin-helix domains and an XPF-endonuclease domain. The presence of an XPF-like endonuclease suggests that Mus81 is likely to play a role in DNA damage repair. Mutations in the XPF nucleotide excision repair endonuclease are responsible for causing xeroderma pigmentosum, a disease characterised by sensitivity to UV radiation and a propensity to develop certain cancers. Yeast that lack Mus81p are hypersensitive to UV irradiation and to chronic MMS exposure, but are not abnormally sensitive to X- irradiation. In addition, Mus81 mutants are sporulation defective and show synthetic lethality when they also carry a mutation in Sgs1, a member of the RecQ helicase family. Recently, Mus81 has been shown to act as a Holliday Junction resolvase in vitro (3,4) supporting the hypothesis that Mus81 functions to allow replication to proceed through collapsed replication forks.

Our RNAi studies in *C. elegans* indicate that MUS-81 is essential for conferring resistance to UV radiation and for promoting genome stability. When *mus-81(RNAi)* is performed by feeding for multiple generations, animals develop a late onset sterility; most lines become completely sterile between the 15th and 20th generation. This decrease in fertility is accompanied by evidence of chromosome breakage and fusion. Because the absence of both Mus81p and Sgs1p leads to synthetic lethality in yeast, we examined *him-6; mus-81(RNAi)* animals. *him-6* is one of 4 RecQ helicases in *C. elegans.* These mutants exhibited early onset genomic instability; most lines became sterile within 6 generations. This genomic instability is more severe than that observed for either *mus-81(RNAi)* or *him-6* alone. We interpret these findings to mean that MUS-81 and HIM-6 are each involved in maintaining genome integrity. We are presently investigating the expression of the *C. elegans* MUS-81 protein by immunocytochemistry. The implications of these results on genome maintenance will be discussed.

1. Boddy, MN et al. (2000) Mol Cell Biol 20: 8758-8766
2. Interthal, H and Heyer, WD (2000) Mol Gen Genet 263: 812-827
3. Chen XB et al. (2001) Mol Cell 8(5): 1117-27
4. Boddy MN et al. (2001) Cell 107(4): 537-48

23. Recent changes and improvements to the WormBase database

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WormBase is a repository of mapping, sequencing and phenotypic information for C. elegans and some closely related nematodes. New releases of the database are made freely available to the community every two weeks. The principal way in which researchers can access this data is through our website (www.wormbase.org).

The database is constantly evolving through the refinement of existing data (e.g. curation of gene models) and the inclusion of new datasets (large or small). Also, because it is a goal to make WormBase easier to use, we have made many improvements to the website, including the addition of new tools for data extraction and many advances to existing pages (particularly the genome browser). These and other recent developments will be discussed alongside an overview of the data that WormBase contains.

WormBase exists to serve the C. elegans and broader biomedical commuinity, and the WormBase Consortium thanks our many data contributors and collaborators, especially those providing their large-scale datasets, and those providing feedback. Comments, questions and suggestions are welcome and can be made by emailing wormbase-help@wormbase.org

24. Access to *C. elegans* resources at the EBI.

Eleanor Whitfield, and Rolf Apweiler.

The EMBL outstation – European Bioinformatics Institute (EBI) is a centre for research and services in bioinformatics. The EBI maintains a publicly accessible SRS6 server (http://srs6.ebi.ac.uk/) that holds in excess of 140 databases. These include databases concerned with DNA sequence (EMBL), protein sequence (SWISS-PROT, TrEMBL), protein domains (InterPro), literature (MEDLINE) and many more. SWISS-PROT and TrEMBL together contain all translations known in *C. elegans* either predicted or experimentally determined, and those in SWISS-PROT are fully annotated from literature for protein function, expression patterns and functional/non-functional sites on the protein. InterPro (http://www.ebi.ac.uk/interpro/) is a combined view of Pfam, PRINTS, PROSITE, ProDom, SMART and TIGRFAM representing 1068 domains, 3532 families, 74 repeats and 15 post-translational modification site. InterPro has been used to analyse the complete proteome of *C. elegans*. This complete non-redundant proteome (as defined byWormPep) is available at http://www.ebi.ac.uk/proteome/.
I would like to walk you through an SRS query on the SWISS-PROT and TrEMBL databases to discover the *C. elegans* sequences available, discuss the information in these databases and go on to demonstrate how InterPro can be used to help navigate and retrieve from these databases.

25. Sequence analysis supports the interpretation, based on reporter gene data, that there are many pseudogenes amongst the annotated *C.elegans* genes.

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Results obtained using reporter genes had suggested that one fifth of the annotated *C.elegans* genes may be pseudogenes (Mounsey *et al*. 2001 International Worm Meeting and Genome Research (in press)). Reporter gene fusions had been generated for a random sample of the annotated genes using a shotgun cloning procedure. Amongst the 364 genes assayed, reporter gene expression was much less frequently observed for those that had been duplicated recently in *C.elegans* evolution. Further consideration of the data suggested that this was due to an unexpectedly large number of pseudogenes in the *C.elegans genome.*

Additional evidence for this interpretation was sought in the genomic sequence. Recently duplicated genes for which no EST had been identified had the highest failure rate in the reporter gene studies and the annotation of these genes as functional units is based solely on computer analysis. The closest homologues of the 74 assayed genes, that were in this category and failed to give reporter gene expression, were identified and families of encoded protein predictions were aligned. Only those with high levels of sequence identity were examined, to be confident of the alignments. Alignments of encoded protein families readily revealed interruptions in the otherwise very close homologies. An explanation of the interruptions was sought in the genomic DNA sequence primarily using the Blixem presentations in ACeDB. Some of these interruptions appeared to result simply from errors in the intron/exon structure prediction. For others the only explanation appears to be that the gene has undergone a small deletion, a disruption of the translational reading frame or an introduction of a nonsense mutation, all in conserved regions, and all of which would be expected to have rendered the gene non-functional and therefore a pseudogene. In annotating these genes as real genes, the problem in the protein coding regions had been missed in the intron/exon structure prediction by incorporation of non-coding intron into an exon and/or by placement of coding region into an intron or intergenic region. Out of the 364 annotated genes that had been assayed with reporter gene fusions, sequence analysis suggests 13 are pseudogenes. While this extrapolates to only 1/30th of the genome, and not the 1/5th proposed from the reporter data, restricting the sequence analysis to those genes with high levels of identity means this must be an underestimate. Furthermore this sequence analysis does suggest that there are a large number of relative young pseudogenes in the *C.elegans* genome and genes rendered inactive by alterations that disrupt expression, rather than the protein-coding region, will not have been determined as pseudogenes in this analysis. The interpretation presented here raises many questions about how selection can have operated through evolution to generate such a high level of pseudogenes. Questions might also be raised about potential redundancy of the recently duplicated genes that as yet remain functional but might also be destined to become pseudogenes.

26. The *C. elegans* orfeome cloning project : version 1.0

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[2] Invitrogen Rockville, MD and Huntsville AL USA.

[3] Genome Therapeutics Corp. , GenomeVision™ Services, Waltham, MA USA

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.

27. A genome wide RNAi screen

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We have constructed a reusable feeding library of 16,757 bacterial strains capable of targeting 86% of the 19,427 predicted genes in *C. elegans* by RNAi; each chromosome has a similar coverage. We have screened this library in a wild-type N2 background, scoring for grossly observable phenotypes such as sterility, embryonic or larval lethality, slowed growth rate, and abnormal morphology or behaviour of surviving progeny. From this screen, we identified 1722 genes with a detectable RNAi phenotype. We will present a summary of these data along with global analyses that provide evidence for phenotypic clustering, the correlation of phenotype with domain type, and different evolutionary conservation of genes with different functions in the worm.

28. Characterization of *Mos1*-mediated mutagenesis.

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Mos1-mediated mutagenesis relies on the mobilization of the *Drosophila* transposon *Mos1* in the *C. elegans* germline (*Nature*, 2001, 413, 70-74). Since *Mos1* insertions are easily localized in the genome using inverse PCR, the transposon can be used as a molecular tag to rapidly identify a gene that has been mutated by a *Mos1* insertion.
The mobilization of *Mos1* requires two extrachromosomal arrays. *oxEx166[hsp::MosTransposase; unc-122::GFP]* is the "enzyme array" which drives the expression of the transposase under the control of a heat shock promoter. *oxEx229[Mos1; myo-2::GFP]* is the "substrate array" which contains multiple copies of the transposon. The two transgenes are maintained in independent strains because transposition efficiency decreases when double transgenic animals are propagated for many generations. Moreover, we observed that the substrate array can be "silenced" over time: a substrate array which had been propagated in the laboratory for about a year had a low transposition rate (10 ± 6 % of F1s with at least one insert, n = 3 experiments). When the original version of *oxEx229* was thawed and retested, we recovered high transposition rates (49 ± 15 %, n=13 experiments).
To measure the mutagenicity of *Mos1*, we performed three screens for mutants resistant to levamisole using the *Mos1* transposon. Of 13,940 *Mos1*-mutagenised F1s, we identified 6 levamisole resistant mutants. Under similar screening conditions, we recovered 8 mutants from 1,710 EMS-mutagenised F1s. There are 6 loci which can be mutated to confer strong levamisole resistance, and the observed rate of EMS-induced mutations is consistent with the known mutagenicity of EMS and the target size for levamisole resistance. Therefore, *Mos1* mediated mutagenesis is about 10 times less efficient than chemical mutagenesis.
Mobilization of transposons can generate mutants that do not contain a copy of a transposon ('hit and run' events). However, these events are rare with *Mos1*: among the mutants that were identified in different *Mos1*screens conducted in our laboratory, a *Mos1* insertion was present in 14 of 15 mutated genes.
In conclusion, *Mos1* is a less efficient mutagen than EMS but the identification of a mutated gene is extremely fast. Before using *Mos1* for mutagenesis, one must evaluate the trade-off between time spent screening for mutants versus the time spent mapping and rescuing a mutation.

29. Improvement of the biolistic transformation of *C. elegans*

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Transformation of the worm by microinjection is now a rate-limiting step in many labs during the analysis of genes. We find microinjection still quite unreliable and cumbersome. At least in our hands the transformation rate is in average quite low, we get only one stable array per 30 injected hermaphrodites. In 1999 we demonstrated that the worm can be reliably transformed using ballistic bombardment with gold particles using a helium gun. However, the efficiency by which stable arrays were formed was still rather low, approximately one stable line per ten shots was isolated. Meanwhile we improved the method by a factor of 100; typically we now isolate 10 stable arrays per shot. One or two of these lines show a stable transmission of the transformed DNA, indicating that integration into the genome occurred. The in and outs of biolistic transformation will be discussed.

30. Homologous gene targeting in *C. elegans*.

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In several model organisms including mouse, yeast and *E. coli*, homologous recombination is a method of choice for gene targeting and alteration. A few anecdotal cases of integration via homologous recombination after microinjection has been reported for *C. elegans*, however a low frequency of homologous recombination (~3% of integration events, 1) makes use of the method impractical in the worm. The limiting factor is production of hundreds of integrated lines, only a few of which could be a result of homologous recombination. Recently, a robust method to produce single and low-copy integrated lines by microparticle bombardment has been developed (2), and its application for homologous disruption gave one successful event (Shai Shaham, personal communication at 13th International *C. elegans* Meeting). We have explored the potential of this method for scaling up to produce amounts of integrated lines required to overcome the current numbers problem in homologous recombination technique.

The PDS-1000/He particle delivery system (Bio-Rad) with hepta adapter has been used to bombard 10-cm plates full of worms, and unc-119 gene has been used as a selection marker for transformation (2). This setup allowed us to routinely produce 10-18 independent transformants per bombardment, approximately half of which proved to be integrated lines. Next we made an "ends-in" construct to target the unc-22 gene. The construct contains 6-kb region of homology to unc-22 gene and a unique restriction site approximately in the middle of the region, allowing linearisation of the plasmid prior to bombardment. Using this construct, 400 independent transformants have been obtained in 28 bombardments. Progeny of the transformed worms has been analyzed, and 3 independent lines appeared to have unc-22 phenotype. PCR analysis (using construct-specific and unc-22 flank-specific primers) confirmed insertion of our construct in the homologous region, rather than an accidental non-homologous disruption of the unc-22 gene. We are currently also disrupting other genes, and preliminary results will be presented. Thus we provide evidence that ballistic transformation of worms could be a good approach to put homologous recombination into a powerful yet incomplete "*C. elegans* toolkit".

1. Broverman et al. 1993. Proc. Natl. Acad. Sci. USA, **90**, 4359-4363.
2. Praitis et al. 2001. Genetics, **157**, 1217-1226.

31. *rrf-3*, a *C. elegans* strain with increased sensitivity to RNAi

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Recent analysis on the RNA dependent RNA polymerase (RdRP) like genes of *C. elegans* hinted that mutations in the *rrf-3* gene cause increased sensitivity to RNAi (Sijen et al., 2001). We now studied the *rrf-3* RNAi phenotype more extensively. We selected 80 clones from the RNAi feeding library and compared *rrf-3* to wild type animals upon feeding with these clones. We looked at embryonic and postembryonic phenotypes. Of the 80 genes tested we found for *rrf-3* 48 genes that gave phenotypes whereas for wild type animals we found phenotypes for 25 genes. Several genes have more then one associated phenotype. In total we detect 74 phenotypes for *rrf-3* animals and 44 phenotypes for wild type animals. For example we could detect very clear postembryonic phenotypes for *dpy-18*, *lin-1* and *unc-73*. In wild type animals neuronally expressed genes appear to be more difficult to interfere with. We found for several of these genes, such as *unc-30* and *unc-33*, a phenotype upon feeding of the corresponding dsRNA to *rrf-3*, whereas we did not find a phenotype in wild type. This shows that the *rrf-3* mutant strain has a general enhancement in sensitivity to RNAi for genes in all categories.

Sijen, T., Fleenor, J., Simmer, F., Thijssen, K.L., Parrish, S., Timmons, L., Plasterk, R.H.A., and Fire, A. (2001). On the role of RNA amplification in dsRNA-triggered gene silencing. Cell, Vol. 107, 465-476.

32. Enhanced Analytical Performance of the *C. elegans* Flow Sorter COPAS *BIOSORT*: Automated Re-analysis of Populations in Multi-well Plates and Reading of Axially Distributed Positional Fluorescent Signals

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The COPAS *BIOSORT* system, used to analyze and sort *C. Elegans*, can replace visual inspection and manual picking of nematode populations by accurate, high speed, multi-parametric analysis followed by automated sorting and dispensing in multi-well plates or single recipients. Current COPAS applications include separating egg-to-adult life stages, fluorescent from non-fluorescent animals such as live from dead animals, males from hermaphrodites or those with (altered) expression levels of fluorescent proteins. COPAS also facilitates fast bulk isolation for biochemical or micro-array experiment and automation of genetic/enhancer/suppressor screens. Union Biometrica has developed add-on devices that expand the capacities of COPAS *BIOSORT*.

Automated re-analysis of populations in multi-well plates. The higher throughput that comes with COPAS in the compound screening lab and the larger numbers of mutants that can be isolated from bigger populations create a new bottleneck at the level of downstream analysis of multi-well plates. For this purpose, the COPAS *ReFLx* has been designed to facilitate re-analysis of populations incubated in 96-well plates. The ReFLx probe will enter the well and will gently mix and aspirates the population from the well. While being aspirated, the sample is washed and filtered in a 'bubble trap' to remove debris of the culture medium and air bubbles that would interfere with the analysis. The population of animals is then rerouted through the flow cell for analysis or for sorting of a desired sub-population. Finally, those animals that are not re-sorted are sent to waste and the selected animals are deposited in the corresponding well of a fresh plate. The ReFLx unit can re-analyze and sort a 96-well plate in less than 45 minutes with a yield of > 90%.

Reading axially distributed positional information in the C. elegans body. Inside the COPAS flow cell, co-axial 670 nm and 488 nm laser beams are optically focused to a vertical 10 micron stripe, giving rise to fluorescent emission profiles in which details the size of a single cell that can be resolved axially. To take advantage of this information in the analysis of animal populations and to use it to drive the sorting process, the COPAS *Profiler* has been developed. It records and stores fluorescent profiles for each animal for analysis purposes. Features in profiles, such as a *predefined* number of 'peaks' with *predefined* amplitude and width can be used as sort criteria.

The profiler thus allows the investigator to zoom in from total fluorescence at the organism level, to fluorescent features of organs, tissues, groups of cells and perhaps individual cells in the animal body. A salient feature of axial profiling is that weak fluorescent signals coming from a few cells will be accurately measured when these few cells are axially separated from 'bulk fluorescence' coming from major organs. In this way, much more sophisticated forms of analysis and sorting become possible with COPAS technology: as organs, tissues and cell groups are born, dividing, migrating, differentiating or dying in the nematode body.

It is a particularly challenging idea to determine whether with today's novel fluorescent proteins, the Profiler will be able to accurately discriminate individual cells or perhaps cellular processes that cross multiple optical sections along the A/P axis such as the elongating processes of the excretory canal cell or even axons.

33. The rhomboid homolog *rom-1* is involved in the anchor cell-independent induction of vulval cell fates

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During hermaphrodite vulval development, the gonadal anchor cell (AC) signal LIN-3 EGF activates the LET-23 EGFR/ LET-60 RAS/ MPK-1 MAPK signaling pathway in the neighboring vulval precursor cells (VPCs) to specify the vulval cell fates. In *Drosophila*, the Rhomboid transmembrane protease cleaves the membrane-bound SPITZ EGF growth factor cell-autonomously to activate the RTK/RAS/MAPK signaling pathway in the adjacent cells (Urban et al., 2001; Lee et al., 2001; Bang et al., 2000; Sturtevant et al., 1995). The *C. elegans* genome encodes four putative homologs of *Drosophila* Rhomboid, termed *rom-1* (F26F4.3), *rom-2* (C48B4.2), *rom-3* (Y116A8C.14) and *rom-4* (Y116A8C.16) (Wasserman, 2000). To investigate the role of Rhomboids during vulval development we have isolated a deletion mutant that is predicted to inactivate the *rom-1* gene. *rom-1(lf)* animals exhibit no phenotype as a single mutants. However, *rom-1(lf)* or *rom-1* RNAi partially suppresses the multivulva (Muv) phenotypes caused by over expression of LIN-3, by the *let-60(n1046*^{gf}) mutation or by expression of MPK-1 under control of the heat-shock promoter (HS-MPK-1). Around the time of vulval induction (in late L2 or early L3 larvae), a *rom-1::gfp* transcriptional fusion is expressed in the VPCs, while no *rom-1::gfp* expression can be detected in the AC. Likewise, the *rom-1(lf)* phenotype is rescued by expression of *rom-1(+)* under control of the Pn.p cell-specific *lin-31* promoter, suggesting that ROM-1 acts in the signal receiving VPCs rather than in the signal sending AC. Moreover, *rom-1(lf)* strongly suppresses the AC-independent induction of vulval cell fates in the distal VPCs (P3.p, P4.p & P8.p) that occurs in gonad-ablated *let-60(n1046*^{gf}) or HS-MPK-1 animals. Taken together, these results indicate that ROM-1 function is required in the VPCs for the production or transduction of an AC-independent signal that induces the vulval cell fates in the VPCs that are further away from the AC.

Bang et al., 2000; *Genes Dev*. 14: 177-186
Lee et al., 2001; *Cell* 107: 161–171
Sturtevant et al., 1995; *Development* 121: 785-801
Urban et al., 2001; *Cell* 107: 173–182
Wasserman, 2000; *Genes Dev*. 14: 1651-1663

34. The Pumilio protein *puf-8* negatively regulates vulval induction

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During the development of the hermaphrodite vulva, three out of six equivalent vulval precursor cells (VPCs) adopt vulval cell fates. The gonadal anchor cell (AC) induces the 1° vulval fate in the nearest VPC (P6.p). The AC produces a ligand (LIN-3) similar to the vertebrate epidermal growth factor that activates in P6.p the conserved LET-23 EGFR/LET-60 RAS/MPK-1 MAPK signaling cascade. P6.p then generates a lateral signal that activates the LIN-12 Notch pathway in the neighboring VPCs (P5.p and P7.p) to specify the 2° cell fate (1).
We are interested in the mechanisms that negatively regulate EGFR/RAS/MAPK signaling during vulval cell fate specification. Since single mutations in inhibitory genes often cause no obvious vulval phenotype, we performed a genetic screen for mutations that cause excess vulval induction in a sensitized genetic background. For this purpose, we mutagenized animals lacking the GTPase activating protein GAP-1 that inhibits LET-60 RAS and screened the F2 progeny for mutants displaying a Multivulva (Muv) phenotype. Using this approach, we isolated two mutations (*ga145* and *zh17*) that cause a penetrant (>80%) Muv phenotype in a *gap-1 (0)* background. The two mutations were mapped to a genomic region on LGII encoding the *puf-8* gene. PUF-8 belongs to a family of RNA-binding proteins characterized by the presence of eight FBF/Pumilio or PUF repeats. *zh17* animals contain a stop mutation truncating PUF-8 after residue 117 and the *ga145* mutation changes a conserved Gly in the fourth PUF repeat to Arg.
Moreover, a transgene carrying the *puf-8* ORF rescued the Muv phenotype of *puf-8(ga145); gap-1(0)* animals.
In *Drosophila*, a Pumilio forms a complex with nanos and Brain Tumor that binds to the NRE (Nanos-response-element) in the 3' UTR of the *hunchback* and *cyclinB* mRNAs to repress their translation (2). To identify possible downstream targets of PUF-8, we searched the *C. elegans* genome for genes that contain NRE-like elements in their 3' UTR. A *cyclinB* homolog that contains a conserved NRE in its 3'UTR may be a target of PUF-8 since RNAi against this *cyclinB* gene -but not against another *cyclinB* or the *hbl-1* hunchback gene- efficiently suppressed *the puf-8(ga145); gap-1(0)* Muv phenotype.
Thus, PUF-8 may control the cell-cycle progression and vulval fate specification in the VPCs by negatively regulation the translation of a *cyclinB* mRNA.

1. Wang M, Sternberg PW. (2001) Pattern formation during *C. elegans* vulval induction. Curr Top Dev Biol. 51:189-220.
2. Junichiro Sonoda and Robin P. Wharton (1999) Recruitment of Nanos to *hunchback* mRNA by Pumilio Genes & Dev. 13: 2704-2712.

35. LIN-39 represses EFF-1-dependent cell membrane fusion in *C. elegans*

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How Hox genes establish the regional identity in the anterior-posterior axis in metazoans is a central question in developmental biology. Hox genes must have targets that participate in cellular behaviors but very few of these effectors have been found. In *C. elegans* the Hox gene *lin-39* is essential for the formation of the vulva. *lin-39* is active in the six vulval precursor cells (VPCs) inhibiting them from fusing to the surrounding epidermis in the first larval stage (L1). Later in development, *lin-39* inhibits cell fusion by preventing three of the VPCs from fusing to the epidermis in the L3. We have recently identified and characterized *eff-1*, a "fusogene" that is essential for all vulval and epidermal cell fusion events in *C. elegans**. Here we asked whether *eff-1* is the "cell fusion effector" controlled by *lin-39* in the VPCs. In *lin-39(-)* null and temperature-sensitive (ts) mutants the VPCs fused in the L1. However, we found that these cells failed to fuse in *eff-1;lin-39(-)* and *eff-1;lin-39(ts)* double mutants showing that *eff-1* is epistatic to *lin-39*. The Vulvaless (Vul) phenotype of *lin-39(-)* was also suppressed in the L3 as not three but all of the VPCs escaped fusion in *eff-1;lin-39* double mutants. After escaping cell fusion the vulval cells did not divide but still formed ring-shaped cells that stacked resulting in a tubular structure that was abnormal and resulted in non-functional vulvae revealing that *lin-39* is needed not only to inhibit cell fusion but also for other function(s) during vulva formation. To exclude the possibility that *lin-39* may work in parallel to *eff-1* we investigated the pattern of expression of *eff-1* gene during vulva formation. We found that eff-1p::GFP was expressed only in the three VPCs committed to fusion in the wild-type. However, in *lin-39* null and ts mutants, all the VPCs expressed eff-1p::GFP showing that *lin-39* abolishes VPCs fusion to hyp7 by repressing *eff-1* expression.

Thus, LIN-39 transcription factor negatively regulates cell fusion in cells committed to form the vulva by repressing the expression of *eff-1* RNA in wild-type L1 and L3 hermaphrodites. During the later L3 we have identified and characterized a new defect in *lin-39* mutants that does not appear to be a result of homeotic transformation of the vulval to cell fusion fate. The *eff-1;lin-39(-)* and *eff-1;lin-39(ts)* double mutants show either (1) a block in VPCs proliferation or (2) a precocious appearance of vulval fates in P(3-8).p similar to the differentiated states of their great-granddaughters. The precocious production of P(5-7).pxxx-like vulval fates characteristic of ring-shaped or toroidal cells (vulA-vulF) suggests that in the absence of *eff-1* function the default state of the VPCs may be determined by the vulval program. However, in the *eff-1(hy21)* single mutant we only get 3% multivulva phenotype, suggesting that escaping the cell fusion fate is not sufficient for a transformation to vulval fate. We will also discuss our current model for the direct repression of *eff-1* activity by LIN-39 and co-repressors.

*Mohler, W. A., Shemer, G., et al. (2002). Dev. Cell 2, 355-362

36. *spr-1*, a suppressor of presenilin, encodes a conserved transcriptional repressor that may play a general role in LIN-12/Notch signalling

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The *C. elegans sel-12* gene was identified as a suppressor of a *lin-12* gain-of-function allele and found to encode a functional homolog of human presenilin (1, 2). Presenilin was also identified as a gene implicated in familial early-onset Alzheimer's disease. It is now believed that presenilin mediates transmembrane cleavage of LIN-12/Notch proteins, a critical step in signal transduction by these receptors, as well as transmembrane cleavage of beta-amyloid precursor protein, a critical step in the generation of a peptide that can cause Alzheimer's disease. Hermaphrodites carrying a *sel-12* loss-of-function mutation are unable to lay eggs. The Egl phenotype of *sel-12* null mutants resembles that of *lin-12* partial loss-of-function mutants, and it has been proposed that reduction in *lin-12* activity during Pi cells determination is the basis for the *sel-12(-)* Egl phenotype (3). A screen for suppressors of the Egl phenotype of *sel-12(-)* mutants was conducted (4) and defined at least four *spr* genes (for suppressor of presenilin). We have undertaken the genetic and molecular characterisation of *spr-1*.

Our genetic evidence suggests that *spr-1* does not bypass the need for presenilin activity, as *spr-1* is unable to suppress the *sel-12* mutant phenotype when the activity of the other C. elegans presenilin, *hop-1*, is removed. Furthermore, genetic interactions with various loss- or gain-of-function alleles of *lin-12*, as well as with allele of *glp-1* (the other C. elegans LIN-12/Notch gene), suggest that *spr-1* may play a general role in LIN-12/Notch activity.

We mapped *spr-1* between single nucleotide polymorphisms using the Hawaiian strain CB4856 (5) and identified the ORF corresponding to *spr-1* by sequencing the predicted ORFs in the region (5). Our analysis of cDNAs and RACE products revealed a more extensive ORF than originally predicted by Genefinder. The SPR-1 protein based on the corrected ORF appears to have orthologs in humans and Drosophila. The human ortholog of SPR-1 has been shown to be involved in transcriptional repression. Our finding that SPR-1 is a nuclear protein is consistent with a role for SPR-1 in transcriptional repression as well.

We have evidence that *spr-1* appears to function in Pi cells to negatively regulate the LIN-12/Notch pathway: expression of SPR-1 under the control of *cog-2* regulatory sequences restores the Egl defect of *spr-1;sel-12* hermaphrodites. Furthermore, the human homolog has some ability to replace SPR-1 in this assay.

At the meeting, we will discuss how a protein involved in transcriptional repression may function in LIN-12/Notch signalling, and why a gene encoding such a protein might be identified in genetic screens for suppression of the Egl phenotype of *sel-12(-)*.

1- Levitan, D. and Greenwald, I. (1995). Nature 377:351-354.
2- Levitan, D. et al. (1996). PNAS 93:14940-14944.
3- Cinar et al. (2001). Dev. Biol. 237:173-182.
4- Wen et al. (2000). PNAS 97:14524-14529.
5- Genome Sequencing Consortium (http://genome.wustl.edu/gsc/CEpolymorph/snp.shtml) and additional identified SNPs.

37. Loss of *spr-5* bypasses *sel-12* defects by stage-specific derepression of *hop-1*.

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sel-12 and *hop-1* are two *C. elegans* genes which are structurally and functionally homologous to the human presenilins, PS1 and PS2. Mutations in the human presenilins contribute to the majority of familial Alzheimer's disease cases. Work in *C. elegans* revealed that presenilins are also involved in Notch signaling, as *sel-12* was initially isolated as a suppressor of a *lin-12* gain-of-function allele (Levitan and Greenwald, 1995). Several lines of evidence suggest that presenilins mediate the transmembrane cleavage event that releases the LIN-12/Notch intracellular domain which is crucial for nuclear Notch signaling. As a consequence, a *sel-12*; *hop-1* double mutant causes phenotypes that resemble that of Notch (*lin-12* and *glp-1*) null mutants (Westlund et al., 1999).

However *sel-12* mutants display a highly penetrant egg-laying defect (Egl) which resembles the Egl defect of *lin-12* reduction-of-function mutants. This Egl phenotype is caused by two developmental defects: (i) a pi cell induction defect which results in blockage of the vulva-uterine connection (Cinar et al., 2001) and (ii) a sex-muscle patterning defect leading to morphologically abnormal and misattached vulva-muscles (Eimer et al., submitted).

In order to find new molecules that are able to influence presenilin mediated signaling we screened for extragenic suppressors of the *sel-12* Egl defect. In several screens we identified six alleles of *spr-5* (**s**uppressor of **pr**esenilin) which are able to suppress both *sel-12* defects in the egg-laying system. *spr-5* requires the activity of the second presenilin *hop-1* for *sel-12* suppression. A Tc3 induced allele of *spr-5* facilitated cloning of *spr-5*. Molecular analysis revealed that SPR-5 belongs to a conserved family of proteins found in co-repressor complexes that negatively regulate transcription. In *C. elegans* mutations in components of this co-repressor complex lead to a stage specific upregulation of *hop-1*. We will present a further analysis of *spr-5* function and its potential role in *sel-12* mediated LIN-12 signaling.

38. The Axin-like protein PRY-1 is a negative regulator of a canonical Wnt pathway in *C. elegans*.

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Correct temporal and spatial expression of Wnt target genes is mediated via tight control of the effector β-catenin. In the default state, β-catenin is targeted for degradation by a complex consisting of Axin, the kinase GSK3β, and APC. Wnt signaling inhibits this complex via Dishevelled (Dsh), allowing the release of β-catenin, which in turn interacts with Tcf transcription factors to activate Wnt target gene expression. In *C.elegans* a similar pathway relayed by EGL-20/Wnt, LIN-17/Frizzled, MIG-5/Dsh, BAR-1/β-catenin and POP-1/Tcf controls the expression of target genes such as *mab-5*. The mechanisms that control BAR-1 intracellular levels are unknown. Surprisingly, SGG-1/GSK3β and the APC-related protein APR-1 have been shown to have a positive, rather than negative, input in an alternative signaling cascade mediated by MOM-2/Wnt and WRM-1/β-catenin. Furthermore, an Axin-related protein has not been found by sequence based homology searches.
In the Q neuroblast lineage, a difference in sensitivity to EGL-20 restricts the activation of *mab-5* to QL and its daughter cells (QL.d - left side). This left/right asymmetric expression of *mab-5* is responsible for the difference in migration of the Q cells: the QL.d migrate towards the posterior, whereas on the right side, the QR.d migrate in the default anterior direction. *pry-1* has been described by Maloof et al. to negatively regulate the EGL-20/BAR-1 mediated control of *mab-5* in the Q lineage[1]. *pry-1* loss-of function mutants show Wnt activation phenotypes, such as ectopic expression of *mab-5* in the QR lineage and subsequent posterior migration of the QR daughter cells. To better understand the mechanism of BAR-1 regulation by the EGL-20 signal, we have characterized *pry-1*. We find that *pry-1* encodes a protein that contains a DIX and RGS domain and is distantly related to Axin. Despite this sequence divergence, we show that PRY-1 is a functional Axin homologue. As predicted for a potential Axin, PRY-1 physically interacts with BAR-1/β-catenin, SSG-1/GSK3β, APR-1 and MIG-5/Dsh. Furthermore, *pry-1* functions downstream of *egl-20* and *mig-5* but upstream of *bar-1*, *pop-1* and *mab-5*. Overexpression of *pry-1* inhibits *mab-5* expression in QL and results in anterior migration of its daughter cells, a phenotype similar to the *egl-20*/Wnt loss-of function phenotype. Finally, we show that overexpression of *pry-1* rescues the zebrafish *axin1* mutation *masterblind*, demonstrating the functional similarity of PRY-1 and Axin. In addition, we find that overexpression of *sgg-1* also results in a Wnt loss-of function phenotype in the Q lineage and inhibits the Wnt-1 induced activation of a Tcf-reporter gene in vertebrate cells. Recently, *apr-1*(RNAi) has been shown to strongly enhance the *pry-1* vulval phenotype[2] (a similar pathway controls the expression of *lin-39* in the vulva precursor cells). These data suggest an additional negative role for SGG-1 and APR-1 in the BAR-1 mediated Wnt pathway. We therefore propose that PRY-1 is a functional Axin homologue and that a highly divergent destruction complex consisting of PRY-1, SGG-1 and APR-1 regulates BAR-1 levels in *C. elegans.*

1. Maloof et al. *Development* 126: 37-49
2. Gleason et al. *Genes Dev* in press

39. Maintenance of embryonic stem cell identity in the germline blastomeres of *C. elegans*

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In the early *C. elegans* embryo, both the somatic blastomere EMS and the germline precursor P2 contain the maternally supplied transcription factor SKN-1 (Bowerman et al., 1993). SKN-1 is required for pharynx and gut development in the EMS descendants (Bowerman et al., 1992). In P2, SKN-1 activity appears to be inhibited by another maternal protein PIE-1. In *pie-1* mutants, P2 responds to SKN-1 and the P2 descendants produce pharynx and gut (Mello et al., 1992). Several lines of evidence suggest that PIE-1 maintains germline identity by inhibiting accumulation of RNA polymerase II-dependent mRNAs. This phenomenon is referred to as transcriptional inhibition, though the underlying mechanism has not been determined (Seydoux et al., 1996).

To identify potential targets of PIE-1 in maintaining germline identity, we purified PIE-1 from *C.elegans* extracts. We found that PIE-1 associated with three **P**IE-1 **a**ssociated **p**roteins (PAP-1-3). *pap-2* (RNAi) produced a gradient of phenotypes ranging from viable embryos, through dead embryos with well-differentiated tissues, to embryos that failed to undergo cell division. Among the embryos that die with well-differentiated tissues are many with pharyngeal defects. By looking at the expression of the pharyngeal marker *ceh-22::GFP*, we found that majority of embryos had either no or only a few CEH-22::GFP-positive cells. Although we detected gut cells in almost all *pap-2* (RNAi) embryos, intensity of the gut-specific birefringence was often reduced compared to the wild-type embryos. Despite the pharyngeal and intestinal defects, SKN-1 was present in *pap-2* (RNA-i) embryos. In wild-type embryogenesis, SKN-1 specifies pharynx and gut development by triggering zygotic expression of the *med-1* and *med-2* transcription factors (Maduro et al, 2001). We tested whether MED-1::GFP was present in *pap-2* (RNAi) embryos and found that MED-1::GFP was not expressed. Thus, SKN-1-dependent expression of *med-1/2* appears to require PAP-2. This finding raises a possibility that in the P2 blastomere, PIE-1 may directly inhibit PAP-2 activity and by doing so, prevent SKN-1 from triggering expression of *med-1/2*. However, PAP-2 is probably not the only target of PIE-1 in maintaining germline identity, as another early zygotic gene, *pes-10*, appears to be expressed in *pap-2* (RNAi) embryos.

Bowerman, B., Draper, B. W., Mello, C. C., and Priess, J. (1993). Cell *74*, 443-452.
Bowerman, B., Eaton, B. A., and Priess, J. (1992). Cell *68*, 1061-1075.
Maduro, M. F., Meneghini, M. D., Bowerman, B., Broitman-Maduro, G., and Rothman J. H. (2001). Molecular Cell *7*, 475-485.
Mello, C. C., Draper, B. W., Krause, M., Weintraub, H., and Priess, J. (1992). *Cell* 70, 163-176.
Seydoux, G., Mello, C. C., Pettitt, J., Wood, W. B., Priess, J., and Fire, A. (1996). Nature *382*, 713-716.

40. *t1530* affects the development of pharynx and body wall muscle and the phagocytosis of cell death

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We found in our collection of maternal effect mutants on LG III a mutant *t1530* with defects in pharynx and body wall muscle specification. The normal number of 21 pharyngeal cells stained with mAb 3NB12 are reduced to 6±2 cells. Instead of the 81 body wall muscle cells only 37±10 cells were found. In wild-type embryos the descendants of the ABa and MS blastomeres form the pharynx, where the ABa derived part of the pharynx is induced by the MS blastomere. The body wall muscle is derived from the MS, C and D lineage, which contribute 28, 32 and 29 body wall muscles respectively. A single cell is derived from the AB lineage. The observed pattern of differentiation in the mutant cannot be attributed to a loss of differentiation in a single founder cell. Using laser ablations we found that in this mutant still both ABa and MS contribute to the small pharynx cluster. This can be due to either a failure in specific sublineages derived from both founder cells or to only a partial induction of pharynx in the ABa lineage by a defective MS blastomere which can still produce some pharyngeal cells itself. Our lab observed such partial inductions earlier in studying pharyngeal induction. The lacking number of body wall muscles cannot be explained only by an absence of the muscles derived from the MS lineage. However, as shown earlier body wall muscle cells in the D lineage also depends on an induction from the MS blastomere. The average number of body wall muscles found in the mutant is also consistent with a partial induction of body wall muscles in D.
The mutant also shows a defect in the engulfment of cell corpse. The cell corpse are present for a longer time or not engulfed at all. This defect is stronger if the mutation is over the deficiency sDf121. Our current hypothesis is that *t1530* is a hypomorphic mutation in a gene primarily required for the specification of the MS founder cell, which has also a role in engulfment of the cell corpse. Since we isolated only one allele in a huge mutagenesis experiment we do not expect to get more alleles in a simple way.
Until now we got a rescue with the cosmid T10F2. This cosmid contains only 10 predicted ORFs. Using RNAi we were not able to phenocopy the mutation until now. We are trying to rescue the mutation by fragments of the cosmid to clone the gene by this way. No previously described gene involved either in the specification of the MS founder cell or the engulfment of cell corpse is found on the cosmid T10F2 indicating that we may define a new function in the specification of MS and in the engulfment of cell death.

41. Genetic analysis of *mab-9* regulation during embryonic development

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The *C. elegans* transcriptional activator *mab-9* is a member of the T-box gene family. T-box genes have been identified in a wide range of genomes from sea urchin to human with mutations resulting in severe developmental defects. *mab-9* mutants have severely abnormal male tails due to cell fate transformations of the B and F male-specific blast cells, which take on the fates of their anterior neighbours Y and U. *mab-9* function is also required in the hermaphrodite to maintain the structural integrity of the rectum, with *mab-9* mutants having weak constipation phenotype. *mab-9* mutants of both sexes are also weakly uncoordinated for backwards movement, suggesting that *mab-9* functions in the nervous system. Overproduction of *mab-9* also has a deleterious affect therefore precise control of *mab-9* expression is required for proper development of the worm to occur.

mab-9 expression analysis using GFP reporter constructs shows that MAB-9 is localized to the nuclei of a small subset of cells throughout development. *mab-9* expression first occurs at the 1.5-fold embryo stage in three nuclei of the presumptive rectum (B, F and hyp7). In the L1 larva, expression continues in B, F and hyp7 nuclei, along the ventral nerve cord and in a single nucleus of the head ganglion. Identification of genes that control such cell-specific expression of *mab-9* will help to elucidate this genes role during development and give novel insights into the regulation of T-box genes, about which surprisingly little is known in other organisms.

We have shown that the *C. elegans even-skipped* homologue *vab-7* acts as a repressor of *mab-9* expression. In a *vab-7* (*e1562*) mutant background *mab-9* is ectopically expressed in hyp7 nuclei at the 1.5-fold embryo stage. When two functionally redundant T-box genes T0C74.2 and T07C4.6 are simultaneously silenced by RNAi, *vab-7* expression is reduced in muscle cells. Thus, it was pertinent to investigate the effect of T07C4.2 and T07C4.6 RNAi on *mab-9* expression. When single RNAi is carried out with T07C4.2 there is no change in *mab-9* expression and there is no observable phenotypic change. Similarly, when single RNAi is carried out on T07C4.6 there is no change in *mab-9* expression, however there is a bobbed-tail phenotype similar to a weak *vab-7* phenotype. When T07C4.2 and T07C4.6 are silenced simultaneously embryos have a grossly deformed mid-region and die soon after hatching. A similar pattern of ectopic *mab-9* expression to that seen in a *vab-7* mutant background is observed in these embryos at the 1.5-fold embryo stage. In addition, when single T07C4.6 RNAi is performed on the *vab-7* (*e1562*) mutant an enhancement of the mutant phenotype is observed where the central region of the worm is deformed in addition to the characteristic bobbed tail phenotype. This suggests a genetic interaction between T07C4.6 and *vab-7*.

These results suggest that T07C4.2 and T07C4.6 may act as activators of *vab-7* which in turn represses the expression of *mab-9* in certain cells during embryonic development. Further studies on the interactions between these genes will lead to a better understanding of how T-box genes mediate developmental patterning in the worm.

42. Evolution of Hox gene function in *C. elegans*

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In many organisms, the Hox genes are organized along the chromosome in a genomic sequence that reflects their spatiotemporal activity during development, a phenomenon called colinearity. The *C. elegans* genome contains a total of six Hox genes. Three of them, the middle paralogs *lin-39* and *mab-5*, and a posterior paralog *egl-5*, have been shown to control cell identity in adjacent spatial domains. Generating functional GFP fusion proteins for each of the *C. elegans* Hox genes, we found this to be true also for the other Hox genes except for the anterior paralog *ceh-13*. The expression domain of *ceh-13* overlaps with that of each of the other Hox genes. Consistently with the expression data, our analysis shows that *ceh-13* genetically interacts with the other Hox genes (*php-3* has not yet been tested). This suggests that *ceh-13* shares overlapping functions with the other Hox paralogs during development. To test for functional redundancy, we performed overexpression experiments. Under these conditions, LIN-39 and MAB-5 can rescue parts of the Ceh-13 phenotype. Moreover, chimeric constructs containing the *ceh-13* promoter attached to the *lin-39* or *mab-5* coding region are functionally equivalent in their ability to partially replace *ceh-13*. In summary, our data reveal that *ceh-13* is a unique member among the *C. elegans* Hox genes in that it is expressed and acts all along the anteroposterior body axis rather than a distinct domain. Currently it is not clear whether this reflects a primitive or derived mechanism operating in *C. elegans*.

43. Ray formation and the evolution of nematode Hox proteins.

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Hox genes code for transcriptional regulators that play an important role in the evolution of animal morphology. In the phylogenetic lineage of nematodes, Hox gene number has been reduced, resulting in only four core members in *Caenorhabditis elegans*. Previous studies in *C. elegans* and *Pristionchus pacificus* indicated that Hox genes acquired new functions and that their sequences diverged substantially within the nematodes. For example, when MAB-5, LIN-39 and EGL-5 are compared between *C. elegans* and *P. pacificus*, sequence similarities are basically restricted to the homeodomain and the hexapeptide, whereas the rest of the protein differs tremendously. Are these sequence differences crucial for the acquisition of specific Hox functions?

To address this question, we used the male rays as an in vivo test system and compare ortologous, paralogous and chimeric Hox genes. Using *Cel-mab-5* mutants, we found that, under the endogenous *Cel-mab-5* regulatory sequences, cDNA cassettes containing *Cel-mab-5* and *Ppa-mab-5*, but not *Cel-lin-39 and Cel-egl- 5* can rescue *Cel-mab-5* function. By studying hybrid and deletion constructs, we show that the functional specificity resides within the homeodomain and the hexapeptide. Constructs, containing chimeric Cel-LIN-39 and Cel-MAB-5 proteins, support this observation. We are currently using in vitro mutagenesis experiments to fine map the functional specificity to single amino acids in helix I and II of the homeodomain. Together, our data indicate that the protein function and sequence of Hox transcription factors evolved differentially within nematodes. Furthermore, this and previous data on the evolution of the function of *lin-39* indicates different patterns of Hox protein evolution in nematodes and insects.

44. Evolution of vulvalpatterning mechanisms: studies in *Oscheius* sp. 1 CEW1

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We compare vulval patterning mechanismsbetween *Caenorhabditis elegans* and *Oscheius* sp. 1 CEW1. In *Caenorhabditis elegans*, the pattern of Pn.p vulval precursor cell fates is set by several cellinteractions: an inductive signal from the anchor cell and a lateral signalbetween Pn.p cells. In *Oscheius* sp. 1 CEW1, the same fate pattern is specified by two successive anchorcell inductions, first on P(5-7).p, and then on P6.p daughters.

In order to study vulval development in *Oscheius*, weperformed genetic screens for egg-laying mutants and found the threefollowing vulva development mutant categories. 1) We obtained numerous*dov* mutants, which show an abnormal number of division of vulval precursor cells (Dichtel etal., Genetics 2001). Similar mutants are scarce (and sterile) in *C. elegans*. 2) We also obtained many *cov* mutants (competence and/or centering of the vulva). 3) Unlike in *C. elegans*, we foundfew *iov* mutants (abnormal induction of vulval precursor cell fates), and they show different phenotypescompared to the *C. elegans* induction mutants. Thevulval-defective screens in *C. elegans* and *Oscheius* thus show strikingdifferences in the mutant phenotypes that were reached, which points todifferent evolutionary potentials. A corresponding greater variability inPn.p division number of the 3° cells was observed in natural populations of *Oscheius*species (Delattre and Félix, Curr. Biol. 2001). Thus, these celldivisions are less robust against genetic variations in *Oscheius*.

In *C. elegans*, vulval induction occurs through the RAS/MAPKinase pathway. In order to study the role of this pathway in *Oscheius*, we used U0126, an inhibitor of MEK (a downstream effector of RAS). U0126blocks the induction in *C. elegans*. In *Oscheius*sp. 1, both inductions are affected, suggesting that MEK could play a rolein both steps of vulval induction.

Using anchor cell ablation experiments in *dov* and *cov* mutants that show a partial penetrance, we observed that the anchor cellinfluences the competence and the divisions of the Pn.p cells, in additionto inducing the vulval fates. We will present the epistatic relationshipsof the *iov* mutations with the *cov/dov* mutations, and the possiblerole of MEK in these different anchor cell roles in *Oscheius*.

45. Phylogeny and ontogeny of free-living and parasitic nematodes

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Phylogenetic relationships between nematodes have always been discussed controversially. In recent years analysis of molecular data, particularly sequence data from genes coding for ribosomal RNA, have challenged some of the traditional taxonomic proposals based on morphological criteria. As a third means to identify characters of potential phylogenetic value, we are studying developmental processes during early embryogenesis. The study of free-living and parasitic represenatives of diverse taxa revealed differences in the establishement of axial polarity, separation of soma and germline, assignment of cell fates and the pattern of gastrulation. Some of the existing differences can be readily identified by comparative, light microscopical analysis while others require experimental interference. Our results support the notion that the classical separation into Secernentia and Adenophorea is artificial, and that at least three supertaxa should be defined. In some cases we identified major differences between species considered to be rather closely related, questioning their present position in the phylogenetic tree. To assess the phylogenetic value of specific developmental characters, cases are of particular interest where conclusions conflict with those from other approaches.

46. Zag-1, a zinc finger homeobox transcription factor acting late in neuronal differentiation

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Correct wiring of the nervous system requires that in the developing embryo each axon finds its proper position and interaction partners.
To identify new genes involved in axonal guidance and fasciculation a genetic screen was performed. Mutagenized worms expressing GFP in interneurons under the control of the glr-1 promoter were scored for fasciculation defects in the ventral cord. One of the mutations recovered was rh315.
It mapped to chromosome IV between unc-17 and an SNP-marker on cosmid W03D2. Rescue of the mutant phenotype was obtained with both of the overlapping cosmids T08C8 and F28F9 and with a 10.4 kB PCR fragment containing the predicted open reading frame F28F9.1. This gene encodes a transcription factor with a central homeodomain and two clusters of C2H2-type zincfingers (ZF) at either end (zag = **z**inc finger involved in **a**xon **g**uidance). Sequencing of the mutant revealed a stop codon in exon five which deletes the C-terminal ZFs. Sequencing of 6 cDNA clones from Y. Kohara´s lab revealed no alternative splice forms. Zag-1 has homologs in *D. melanogaster* (zfh-1), and vertebrates (eg. δEF1 in mouse). Common to all are N- and C-terminal ZF clusters, a homeobox and a corepressor binding site, while the number of ZFs within the clusters is not fully conserved. In *Drosophila* zfh-1 is expressed in muscle precursors and neurons during embryogenesis, mutants have subtle muscle defects. The mouse homolog has a similar expression pattern, with additional expression in tissues not present in invertebrates. Knock-out mutants have defects in thymus development and a variety of skeletal defects.
Zag-1 expression in *C. elegans* was analyzed using two different GFP reporter constucts: a putative zag-1 promoter (4.5kb upstream sequence) fusion and a translational fusion containing the same promoter plus the entire coding region. Transgenic lines carrying the integrated promoter construct express GFP in a number of head neurons, and in intestinal and anal depressor muscles from late embryo to adult stages. Expression of the ZAG-1::GFP fusion protein however, is very dynamic, starting at the comma stage in many neurons of head and tail. It is shut down in most neurons at hatching, but in late L1 it appears in the differentiating motorneurons of the ventral cord. Worms carrying the mutation zag-1(rh315) are phenotypically Unc. They are fairly active but tend to curl and change direction of movement quite often. Axon guidance defects can be detected with a variety of neuronal GFP markers. Defasciculation, i.e. crossing of ventral cord axons from right to left side is observed in some mutant worms. Motorneuron commissures sometimes fail to reach the dorsal cord and run laterally. In addition, one to several interneuron axons can be seen running dorsally and/or laterally. Another aspect of the zag-1(rh315) mutant phenotype is misexpression of neuronal markers. Some motorneuron markers are not expressed in all the cells they should be. Others are expressed in more cells than in the wildtype, eg. glr-1::GFP expression in PDB or in some cell(s) of the PDE cluster. Using various muscle GFP markers muscle defects in mutant animals could not be detected.
The dynamic expression of zag-1 at times when neurons differentiate and its effects on the expression of other neuronal markers indicate that it regulates late steps in neuronal differentiation.

47. The axon trajectory of the M2 pharyngeal neurons may be established in part via a growth cone-independent mechanism

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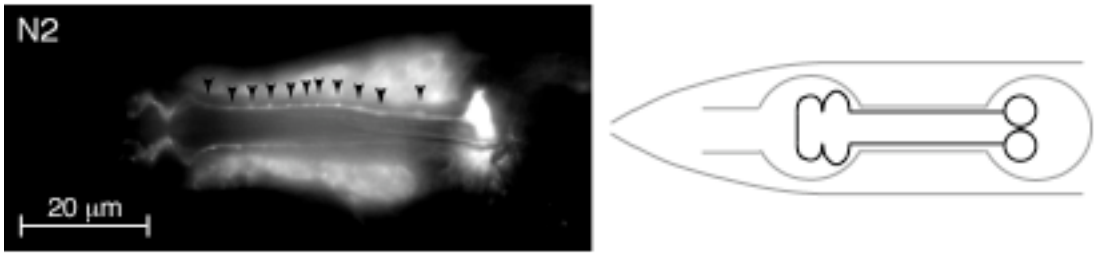

We have investigated the effects of various axon-guidance mutations on the trajectories of the M2 pharyngeal motor neurons which, in wild-type animals, have their cell bodies located in the posterior pharyngeal bulb and send axonal projections through the isthmus and into the metacorpus. To visualize the M2 neurons, we made use of a pRIC-19::GFP construct which we have chromosomally integrated to establish stable transgenic lines. The figure shows the M2 trajectories as seen under UV microscopy, and in cartoon form on the right. The arrowheads in the figure indicate the location of synapses that are revealed as varicosities in the M2 axons where it innervates the m5 muscle cells through which it travels. This study served three purposes: 1) To determine which part of the trajectories of these neurons involves growth cones; 2) To identify genetic backgrounds where abnormal connectivities of the M2 neurons may be correlated with pharyngeal functional defects; and 3) To determine the feasibility of a screen for axon guidance defects in the M2 neurons. We used our GFP reporter to visualize the M2 neurons in various cell-autonomous and cell-non-autonomous axon guidance mutant backgrounds, as well as in pharyngeal abnormal mutant backgrounds. No M2 projection abnormalities were ever observed in the proximal part of the neuronal trajectories, i.e. between the cell body and the metacorpus. Defects were however observed at the distal ends of the M2 axons, within the metacorpus, where abnormal "bead-like" terminations and inappropriate trajectories were observed in both cell autonomous and cell non-autonomous axon guidance mutant backgrounds, including *unc-5*, *unc-6*, *unc-51*, *unc-73* and *sax-3*. Our results show that the trajectories of the M2 axons are very robust in their proximal sections, that is between the cell body and the metacorpus, but quite sensitive to the effects of known axon guidance mutations in their more distal regions. We propose a model whereby the M2 neurons of the pharyngeal primordium form physical connections with neighboring cells, such as the sister cells M3, that are ultimately located within the metacorpus, and that these connections elongate to form the proximal M2 axon trajectories as the priomordium undergoes morphogenesis. In a screen of approximately 2500 mutagenized haploid genomes, we have found 5 mutants with defects in the distal M2 trajectories, and none affecting the proximal trajectory.

48. Classical cadherin-catenin complexes and the developing *C. elegans* nervous system.

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The cadherin superfamily of molecules has been implicated in many cellular developmental processes including adhesion, proliferation and morphogenesis. However, the mechanisms by which cadherins affect neuronal development remain enigmatic, particularly with respect to axon guidance and fasciculation, and the formation and maintenance of synapses.
The *C. elegans* cadherin superfamily is comprised of 12 loci, yet only the *hmr-1* locus is capable of encoding a "classical" cadherin, as defined by conserved regions in the cytoplasmic C-terminus responsible for binding to a set of cytoplasmic proteins, the catenins (1,2). In contrast, *Drosophila* possesses two distinct classical cadherin loci, encoding *D*E- and *D*N-cadherin. *D*E-cadherin is expressed in epithelial tissues, and is required for the maintenance of adherens junctions, whereas *D*N-cadherin is expressed in neuronal tissues, and is involved in fasciculation of axon bundles and pathfinding (3,4,5).
The *hmr-1* locus has been shown previously to encode an epithelial cadherin, HMR-1A, which is essential for epidermal morphogenesis during embryonic development (2). However, we have demonstrated recently that the *hmr-1* locus can also encode a second isoform, HMR-1B, via an unusual mechanism involving alternative splicing and an alternative promoter (6). Recent information from the *C. briggsae* genome project suggests that this mechanism is also conserved in the *C. briggsae hmr-1* locus.
In contrast to the mainly epithelial HMR-1A isoform, HMR-1B is expressed exclusively in the nervous system in a particular subset of neurons, and shows a striking degree of sequence similarity to *D*N-cadherin. We have selectively disrupted the functions of HMR-1B using a *hmr-1* null allele in conjunction with a HMR-1A rescuing construct, and a "snapback" RNAi construct designed to specifically target HMR-1B. Animals lacking HMR-1B function assayed by either of these approaches display defects in motorneuron pathfinding and ventral nerve cord fasciculation. We have also observed similar defects in animals carrying a null mutation in the *hmp-1* _-catenin gene that have been rescued for epidermal (but not neuronal) HMP-1 function. We are currently using this epidermally-rescued *hmp-1* background to identify molecules that act in concert with the cadherin-catenin complex to regulate nervous system development in *C. elegans*.

1) Hill *et al*., (2001) *J Mol Biol* **305**:1011-24
2) Costa *et al.*, (1998) *J Cell Biol* **141**:297-308
3) Uemura *et al.*, (1996) *Genes Dev*. **10**:659--671
4) Iwai *et al.*, (1997) *Neuron* **19**: 77-89
5) Lee *et al.*, (2001) *Neuron* **30**:437--450
6) Broadbent ID, Pettitt J (2002) *Curr Biol* **12**:59-63

49. A motoneuron-derived signal is required for differentiation of post-synaptic domains at GABAergic neuromuscular junctions in *C. elegans.*

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In *C. elegans*, most GABAergic synapses are formed between inhibitory motoneurons and body-wall muscle cells. Behavioral screens have identified 5 genes that are required for GABAergic neurotransmission. However, the mechanisms that are involved in the differentiation of post-synaptic GABA receptor clusters are not known. We developed new tools to analyze the formation of GABA receptor fields and we are undertaking a genetic approach to identify the genes that participate in the formation of GABAergic neuromuscular junctions.

First, we characterized the distribution of the muscle GABA receptor UNC-49 during development using an antibody that we raised against the N-terminal part of UNC-49. In L1 larvae, dorsal muscles do not receive any GABAergic innervation. At this stage, UNC-49 forms discrete clusters that are only present along the ventral cord of the worm. In L2 larvae, after the arrival of GABAergic inputs, we observed UNC-49 clusters along ventral and dorsal cords. In order to analyze in more details the relationship between motoneurons and muscle, we expressed in GABA neurons a synaptobrevin-CFP fusion and in muscle an UNC-49-YFP fusion. This enabled simultaneous visualization of pre- and post-synaptic compartments in the same animal. In wild-type adults, there was a strict correlation between the accumulation of pre-synaptic vesicles and the clusters of GABA receptor. During development, GABA receptors were detected dorsally soon after the differentiation of presynaptic varicosities.

Together, these results suggested that a signal provided by motoneurons was triggering postsynaptic
differentiation.

To test this hypothesis, we expressed *[unc-47::snb-1-CFP; unc-49-YFP]* in *unc-104(e1265).*

In this kinesin mutant, synaptic vesicles are no longer transported in neurites but remain concentrated in cell bodies (Hall and Hedgecock, 1991). No SNB-1-CFP nor UNC-49-YFP could be detected dorsally. GABA receptor clusters were observed ventrally directly in contact with motoneuron somas. Therefore, at least one neural factor seems to be transported by UNC-104 up to pre-synaptic sites to cause formation of GABA receptor post-synaptic clusters. We asked whether the neurotransmitter itself was this factor by examining UNC-49 distribution in *unc-25* mutants that do not synthesize any GABA. In *unc-25* background, UNC-49 distribution was wild-type. Together with the results of Jin *et al.* (J. Neurosc., 1999) who demonstrated that pre-synaptic differentiation is normal in *unc-25*, we concluded that GABA transmission *per se* is not required for GABAergic synaptogenesis.

To identify the proteins required for the differentiation of postsynaptic domains at GABA synapses, we have undertaken a visual screen for mutants with altered UNC-49-GFP distribution.

50. Long chain polyunsaturated fatty acids are required for efficient neurotransmission in *C. elegans.*

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Long-chain polyunsaturated fatty acids (LC-PUFA) are enriched in many neurones and are thought to be required for neuronal development and function. Indeed, alteration of LC-PUFA metabolism causes specific forms of at least two human diseases: mental retardation and retinal macular dystrophy. Furthermore, correlative evidence has linked LC-PUFA dietary intake with normal neonatal brain development. However, the precise role of LC-PUFA in the nervous system remains unclear since neither animals nor cultured neurones devoid of LC-PUFA have been analysed. To understand the role of LC-PUFA we generated a *C. elegans* strain lacking these fatty acids.
We isolated two deletions of the gene *fat-3*, which encodes a D6-desaturase, an enzyme essential for LC-PUFA biosynthesis. *fat-3* mutant animals display specific behavioural defects associated with neuronal impairment. In particular, they are defective in reversing direction and show an egglaying
phenotype reminiscent of that caused by mutations disrupting the Hermaphrodite Specific Neurones. Using a highly sensitive and quantitative method, we demonstrated that *fat-3* mutant animals are defective in LC-PUFA production. In addition, we showed that the behavioural defects of *fat-3(-)* animals are caused by reduced LC-PUFA levels. Expression of *fat-3(+)* under the control of the unc-119 promoter, which drives expression specifically in the entire nervous system, was sufficient to rescue the motility defects caused by loss of *fat-3* activity. Conversely, expression of the *fat-3* coding sequence under the control of the muscle-specific myo-3 promoter, did not rescue the behavioural defects associated with the *fat-3* mutation. Therefore, LC-PUFA are likely to be required in neurones for normal nervous system function. Deficiency of LC-PUFA caused functional rather than morphological defects in neurones since exogenous application of LC-PUFA rescued the behavioural deficits associated with adult *fat-3(-)* animals. In addition, we could not detect any morphological defect in neurones of *fat-3* mutant animals. This suggested that the behavioural defects seen in *fat-3(-)* animals derive from reduced neuronal functionality. To test this possibility, we measured the transmission efficiency of serotonergic neuromuscular junctions (NMJs), involved in egg-laying, as well as cholinergic NMJs, involved in body wall muscle contraction and movement. We found that both NMJs release neurotransmitter inefficiently in *fat-3* mutant animals. Since unsaturated fatty acids stimulate membrane fusion *in vitro* and a specific LC-PUFA induces endocytosis in an *in vitro* assay, it is likely that LC-PUFA affect neurotransmitter availability at the NMJ by interfering with synaptic vesicle fusion and/or endocytosis.

51. Cellular and genetic analysis of salt adaptation in *C. elegans*

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C. elegans shows strong chemotaxis to salts. However, prolonged exposure to salts abolishes chemoattraction to these same salts. This decrease in chemotaxis is time and concentration dependent, partially salt specific and reversible. Thus far we identified 3 loci that affect adaptation to salts: *gpc-1*, *adp-1* and *osm-9*. *gpc-1* encodes a G protein g subunit, specifically expressed in the amphid sensory neurons. Both gain- and loss-of-function mutations in this gene affect salt adaptation. We have not been able to find other defects in the *gpc-1* mutant animals. It is striking that G proteins are involved in taste perception, since this process is presumed to be mediated by channels. The gene affected in *adp-1* mutants has not been identified. This mutation also affects adaptation to odorants. The *osm-9* gene encodes a putative channel protein with limited similarity to the Drosophila TRP phototransduction channel and is expressed in a subset of sensory neurons. Mutations in this gene also affect odorant adaptation. It is unclear which cells mediate salt adaptation. The ASE neurons have been found to be the most important cells for salt detection. However, *gpc-1* is not expressed in these neurons. We are currently performing cell specific rescue and genetic cell ablation experiments to determine which cells are involved in salt detection and adaptation in our assay.
To identify more molecules involved in salt adaptation we have performed a genetic screen for salt adaptation mutants. Thus far, we have identified 10 different mutants. Genetic mapping using SNP analysis is ongoing.

52. *tpa-1*, a gene encoding a protein kinase C subunit, is a downstream target of G_{12}-mediated signaling in *C. elegans*.

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Heterotrimeric G proteins are signal transducing molecules that link extracellular signals received via transmembrane receptors to intracellular second messengers. In mammals, heterotrimeric G protein α-subunits are classified into four families: G_i/G_o; G_q; G_s and G_{12}. In this study, we characterize a *C. elegans* Gα-subunit gene, *gpa-12*, that is most similar to mammalian $G_{12}α$/ $G_{13}α$.

gpa-12 is expressed in pharyngeal muscle and hypodermal cells. Animals defective in *gpa-12* do not show any obvious phenotype. However, overexpression of a constitutively active form of *gpa-12* results in developmentally arrested larvae that eventually die. This developmental growth arrest is likely caused by a feeding defect, because the pharyngeal pumping rate of the arrested animals is almost reduced to zero.

To elucidate the molecular nature of the signaling pathways in which G_{12} participate, we screened for suppressors of the activated GPA-12 phenotype. Therefore**,** we expressed the constitutively active form of *gpa-12* from a heat-shock promoter. Heat-shock treatment of L1 larvae results in a developmental growth arrest from which the animals eventually recover. A 5 min heat-shock is sufficient to induce a growth arrest, but the recovery time is less than for longer heat-shock treatments (up to 2 hr). Using EMS mutagenesis, we identified 44 extragenic suppressors that suppress the developmental growth arrest induced by 2 hr heat-shock. All 44 suppressors have a mutation in *tpa-1*, a gene encoding a protein kinase C subunit. This strongly suggests that *tpa-1* is a mediator of G_{12} signaling.

tpa-1 encodes two isoforms, TPA-1A and TPA-1B. TPA-1A has four additional exons upstream compared to TPA-1B. Mutations in *tpa-1* were firstly identified to suppress the developmental growth arrest induced by the tumor-promoting phorbol ester PMA (Tabuse *et al.*, 1995). All mutations found in our screen disrupt both isoforms, whereas none is located in one of the four additional exons of TPA-1A. This suggests that wildtype TPA-1B is required for the G_{12}-induced growth arrest. This is consistent with the result that TPA-1B and GPA-12 are expressed in the same tissues.

We also isolated several mutants that suppress the developmental growth arrest induced by 5 min heat-shock, but that do not or only moderately suppress the growth arrest induced by 2 hr heat-shock. Surprisingly, one of these suppressors has a mutation in the part of TPA-1 that is only present in TPA-1A, thereby disrupting TPA-1A but not TPA-1B. Altogether, these results indicate that both isoforms of TPA-1 are involved in G_{12}-mediated signaling.

53. *C. elegans* CREB mutants show defects in dauer formation and in behaviors that are coupled to food sensation

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The cyclic AMP-response element binding protein CREB plays a central role in long-term memory in *Aplysia*, *Drosophila* and mice. *C. elegans* has a single *CREB*-homologous gene, *crh-1*. We characterized the *C. elegans* and *C. briggsae crh-1* genes and found that the *crh-1* gene contains four alternative promoters that give rise to four different CRH-1 isoforms. All CRH-1 isoforms contain a C-terminal DNA-binding bZIP domain; two isoforms contain a N-terminal cAMP-dependent kinase site. The bZIP domain and cAMP-dependent kinase site share high similarity with the respective domains in the *Aplysia*, *Drosophila* and mammalian CREB family members. CRH-1 can bind to cyclic AMP-response element (CRE) sites and can be phosophorylated by cAMP-dependent protein kinase (PKA) and Calmodulin-dependent protein kinase II (CaMKII) *in vitro*. Immunohistochemistry with an antibody that recognizes all four CRH-1 isoforms shows that CRH-1 is localized to nuclei and is ubiquitously expressed throughout development.

To determine *crh-1* function, we isolated three *crh-1* deletion. Western blot analysis shows that two deletion alleles, *crh-1(n3450)* and *crh-1(n3451)*, eliminate the expression of the two CRH-1 isoforms that contain the cAMP-dependent kinase site. A third deletion allele, *crh-1(n3315)*, eliminates the expression of all CRH-1 isoforms indicating that *crh-1(n3315)* is a null allele.

All *crh-1* mutants are viable and show no obvious abnormalities in brood size, locomotion, mechanosensation, chemotaxis or thermotaxis. However, *crh-1* mutants tend to form clumps of animals and burrow into the agar, reminiscent of wild-type worms after food has been exhausted. In addition, we found that *crh-1* mutants are dauer-constitutive (Daf-c) at 27˚C but not at 25˚C. Many mutants that have a similar Daf-c phenotype at 27˚C, like *unc-3*, *unc-31*, *unc-64*, have a synthetic Daf-c phenotype at 25˚C in double mutant combinations[1]. Double mutants between *crh-1* and either *unc-31* or *unc-64*, but not *unc-3*, show a strongly enhanced Daf-c phenotype at 25˚C. This observation suggests that *crh-1* and *unc-3* affect similar aspects of dauer formation. The decision to undergo dauer development is made in part by regulating the expression of the transforming growth factor (TGF)-beta homolog DAF-7 in the ASI chemosensory neurons in response to dauer pheromone, food availability, and temperature. *unc-3* encodes a transcription factor that is expressed in the ASI neurons and has been suggested to regulate the expression of *daf-7*[2]. The expression of a *daf-7::gfp* reporter is strongly reduced in *crh-1* mutants, indicating that *crh-1* is part of a sensory signal transduction cascade that regulates *daf-7* expression. Interestingly, *daf-7::gfp* expression is also strongly reduced in *tph-1* mutants which are defective in serotonin biosynthesis[3]. Serotonin modulates many behaviors of the worm in response to food. Our data suggest that *crh-1* mutants have defects in food sensation, and we hypothesize that *crh-1* functions downstream of serotonin in the long-term assessment of food availability.

1. Ailion and Thomas (2000). Genetics **156**, 1047-1067.
2. Ren, Qian, McCron, and Riddle (1998). Midwest Worm Meeting abstract, p. 30.
3. Sze, Victor, Loer, Shi, and Ruvkun (2000). Nature **403**, 560-564.

54. Antagonistic signalling pathways in neurons exposed to the body fluid regulate social feeding in *C. elegans*

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Wild isolates of *C. elegans* can feed either alone or in groups. This natural variation in behaviour is associated with a single residue difference in NPR-1, a predicted neuropeptide receptor related to NPY receptors. NPR-1 suppresses social feeding, since null mutations in *npr-1* make wild solitary feeders social feeders. A functional NPR-1::GFP fusion protein under the control of the *npr-1* promoter is expressed in about 20 neuron types. To identify the neurons where NPR-1 acts to suppress social feeding we expressed NPR-1::GFP in subsets of these neurons using heterologous promoters. Expression of the NPR-1 isoform associated with solitary feeding in the neurons AQR, PQR and URX is sufficient to suppress social feeding. Targetted expression of an activated K^+ channel in the same neurons also suppresses social feeding. AQR, PQR and URX are unusual neurons in *C. elegans*, being in direct contact with the body fluid of the animal. NPR-1 activity in these neurons may suppress social feeding by antagonising signalling through a cGMP-gated ion channel encoded by *tax-2* and *tax-4*. Mutations in *tax-2* or *tax-4* disrupt social feeding, and *tax-4* is required in several neurons for social feeding, including one or more of AQR, PQR and URX. One possibility is that NPR-1 antagonises cGMP ion channel activity in AQR, PQR, and URX by hyperpolarizing these neurons. Such an inhibitory role of *npr-1* would be analogous to the activity of mammalian NPY receptors, that hyperpolarise hippocampal neurons, and proopiomelanocortin-containing anorexigenic neurons in the hypothalamus. Our data suggest a model in which the AQR, PQR and URX neurons integrate antagonistic signals in the body fluid to regulate social versus solitary feeding.

55. *In vivo* imaging of mechanoreceptor function.

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To fully understand behavior at the molecular and cellular level, it is necessary to determine how specific gene products affect the activity of identified neurons, and to correlate the activity of these neurons with behavior. Genetically-encoded optical sensors, such as the FRET-based, ratiometric calcium-sensitive protein cameleon, have many potential advantages for cell-specific non-invasive neural imaging. The use of optical indicators is particularly attractive in *C. elegans* due to the animal's transparency, the ease with which transgenic animals can be generated, and the difficulty of electrophysiological methods. However, because of their relatively slow kinetics and small signal size, it has been difficult to use genetically-encoded sensors like cameleon in excitable cells. We have recently overcome these hurdles and developed imaging methods that have allowed us to detect and measure in vivo calcium transients in mechanosensory touch receptor neurons in response to sensory stimulation. Using this technique, we have begun to address the molecular basis for these neurons' response to touch stimuli. We have found that calcium influx occurs in both the cell body and sensory dendrite of the touch neuron, suggesting that calcium mediated action potentials may play a role in mechanotransduction. Recordings of calcium transients from mutants defective in the putative mechanotransduction channel MEC-4 showed occcasional calcium transients in response to touch, suggesting that the touch neurons may contain a mec-4-independent sensory modality, perhaps involved in harsh touch detection. Finally, we have observed that repeated high-frequency stimulation of the touch cells results in an accumulation of baseline calcium and a subsequent reduction in the calcium response to successive stimuli. Experiments to determine the mechanistic basis for these experience dependent changes in touch neuron function are in progress..

56. Phenotypic plasticity of dauer larva formation in *Caenorhabditis elegans*.

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Dauer larvae are formed when developing L1s and L2s experience conditions of low food availability and high con-specific population density; non-dauer L3s are formed under conditions of plenty. This developmental response to environmental conditions is an example of phenotypic plasticity, *i.e.* an environmentally induced change in phenotype. The form of plasticity of a phenotype (more formally considered as a reaction norm) can vary between different genotypes. Such different plasticities have evolved and are maintained by selection and are therefore, presumably, adaptations to the likely environment of that genotype. Thus, for example, plasticity of dauer, non-dauer development of *C. elegans* may vary between worms from different geographical locations, because different plasticities of dauer formation are most fit in different environments.

We have found extensive variation in phenotypic plasticity of dauer formation among wild isolates of *C. elegans.* These differences are, presumably, adaptations to enhance fitness under their (different), natural prevailing conditions. We extended this survey by looking in greater detail at the plasticity of dauer formation of N2 and another wild isolate (DR1350) over an extensive range of conditions. With a view to mapping the plasticity trait we have constructed N2 x DR1350 recombinant inbred lines (RIL) and assayed their phenotypic plasticity. These lines have a wide range of phenotypic plasticities, of a range greater than that set by the parental strains. We also consider the difficult issue of inter-assay variation in dauer formation assays.

57. Sex differences in the neuroendocrine regulation of development and lifespan

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Wild-type males, when maintained in isolation to prevent harmful interactions with other worms, are longer-lived than hermaphrodites. Solitary male lifespan is further enhanced by uncoordinated (*unc*) mutations, while hermaphrodite lifespan is largely unaffected (1). We have found that male lifespan is only increased by neuronal, and not by muscle *unc* mutations, indicating that diverse general defects affecting the nervous system retard ageing in males but not in hermaphrodites.

Males also form dauers more readily than hermaphrodites (2,3). Together, insulin/IGF signalling (IIS), TGF-β and cGMP signalling regulate entry into dauer. IIS (but not TGF-β signalling) also regulates longevity, with some IIS mutants showing increased lifespan and stress resistance. Using several approaches, we are investigating whether increased lifespan and dauer formation in males is due to reduced IIS.

Firstly, we have measured dauer formation in both sexes in IIS, TGF-β and cGMP mutants. Increased dauer formation occurs in TGF- β and cGMP mutant males, but not in IIS mutant males.

Secondly, survival analyses show that increased male longevity is no longer seen in *daf-16(0)* and class 1 *daf-2* mutants (4). Thirdly, wild-type males are more resistant than hermaphrodites to the free-radical generator paraquat. These results suggest constitutive down-regulation of IIS in males. Since IIS inactivates the transcription factor DAF-16 by localising it to the cytoplasm (5,6), we are currently examining the effect of gender on cellular localisation of *daf-16*::GFP.

Ageing in *C. elegans* is also regulated by gonadal signalling. Laser ablation studies have shown that a signal from the germline reduces hermaphrodite lifespan, while a counteracting signal from the somatic gonad increases it (7). This germline signal is dependent on activity of the gene encoding the nuclear hormone receptor *daf-12*, which is a convergence point downstream of IIS and TGF- β signalling during the dauer decision. We have investigated whether reproductive signalling regulates male lifespan in a similar way. Interestingly, we found no or very little effect on male longevity upon germline ablation. Examination of a *daf-12*::GFP strain showed that unlike in hermaphrodites, *daf-12* is not expressed in the male somatic gonad. Also, previous work has shown that *daf-9*, which encodes a cytochrome P450 thought to be responsible for synthesis of the DAF-12 ligand, is expressed strongly in the hermaphrodite somatic gonad but is not expressed at all in male somatic gonad (8).

These results demonstrate that there are major sex differences in the determination of longevity.

Why might this be so? To test the possibility that this is the result of the hermaphroditic mode of life, we are performing survival analyses for both sexes of a range of other nematode species, both hermaphroditic and dioecious.

(1) Gems & Riddle, *Genetics* 154: 1597; (2) Ailion & Thomas *Genetics* 156: 1047; (3) Vowels & Thomas *Genetics* 130: 105; (4) Gems *et al* . *Genetics* 150: 129; (5) Henderson & Johnson *Curr. Biol* 11: 1975; (6) Lin *et al* . *Nat. Genet*. 28: 139; (7) Hsin & Kenyon *Nature* 399: 362; (8) Gerisch *et al* . *Dev. Cell* 1: 840

58. DIN-1, a putative DAF-12 coregulator important for the *C.elegans* dauer diapause

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The *C. elegans* orphan nuclear receptor DAF-12 acts as key control gene at the intersection of heterochronic, dauer and aging pathways. A putative steroid hormone, metabolized by DAF-9/ cytochrome P450 likely regulates DAF-12. In addition, distinct transcriptional ensembles could mediate DAF-12 functional complexity. Some vertebrate nuclear receptors activate gene expression in the presence of cognate hormone, and repress in its absence, by assembling coactivator and corepressor complexes, respectively. This mechanism allows nuclear receptors to act as ligand-inducible logic switches.

To define such transcriptional complexes, we screened for DAF-12 interacting proteins by the Yeast-Two Hybrid method. Eight candidate interactors were identified. One of these, DIN-1 (DAF-12 Interacting Protein 1) is predicted to encode two large nuclear proteins of 2713 and 2784 amino acids based on cDNAs isolated by RT-PCR. DIN-1 contains several nuclear localization sites and three RNA recognition motifs. It also has several motifs typical of nuclear receptor corepressors, and most of these are located within the receptor interaction domain (RID). Recently, the human homolog, SHARP, was shown to act as a corepressor with a number of nuclear receptors.

In *C. elegans*, DIN-1(+) is important for the dauer diapause. A knockdown of DIN-1(+) activity by RNAi or conventional mutations produces no evident phenotype in an otherwise wild type background. However, *din-1* partly or completelv suppresses the dauer constitutive phenotypes (Daf-c) of various dauer pathway mutants, e.g. insulin like receptor *daf-2* (class I) alleles. It also suppresses the delayed heterochronic and Daf-c phenotypes of certain *daf-12* alleles. Interestingly, all *din-1* alleles identified to date map within the RID. *din-1* does not affect the expression or localization of *daf-12::GFP*. Taken together, these structural and functional data strongly suggest that DIN-1 is a DAF-12 coregulator. We propose that in the absence of reproductive hormone, DAF-12 assembles a corepressor complex containing DIN-1 and other components to mediate developmental arrest and diapause. Moreover, these complexes must be functionally redundant because DIN-1(+)is not essential for diapause under dauer inducing conditions.

59. Molecular Identification of Transcriptional Targets of the DAF-16 Winged HelixTranscription Factor

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Dauer arrest and longevity in *C. elegans* are controlled by an insulin-like signaling pathway transduced by the winged helix transcription factor DAF-16. Mutations in several genes within this pathway (*daf-2*, *age-1*, and *pdk-1*) result in constitutive dauer formation (Daf-c) and increased lifespan (Age) phenotypes. Both the Daf-c and Age phenotypes of these mutants are suppressed by loss of function mutations in *daf-16*. This suggests DAF-16 acts as the principal transcriptional output controlling both diapause and lifespan governed by this pathway. We are attempting to identify both direct and indirect transcriptional targets of DAF-16 utilizing two complementary molecular approaches. Recently, the DAF-16 consensus binding element (DBE) was identified by Furuyama et al (2000). Using the DBE sequence, we have searched the *C. elegans* genomic sequence and identified many genes that contain the DBE within regulatory regions. To refine this list of possible DAF-16 target genes, we have collaborated with the Kim lab at Stanford to examine *in vivo* differences in gene expression between *daf-2* and *daf-2;daf-16* strains using cDNA microarrays. These two approaches have yielded a small subset of genes that contain at least one DBE and show a reproducible expression difference, suggesting that they may be direct transcriptional targets of DAF-16. We have examined the *in vivo* role of a subset of putative DAF-16 target genes using RNAmediated interference. This analysis has identified one gene, encoding a putative protease, that is necessary for the lifespan extension observed in long-lived *daf-2* animals.

60. The circadian clock of *C. elegans:* towards a molecular genetic and pharmacological analysis

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We have recently described our discovery of a circadian clock in *C. elegans* [Current Biology 2002: 12, R47-R49]. Starved L1 larvae, which maintain viability over extended time spans, show a circadian rhythm in their resistance to hyperosmotic stress. This rhythm displays the canonical characteristics of a circadian clock: i), it persists under constant conditions with a period of about 24 hours, ii) it is phase shifted by light and temperature pulses and entrained by light/dark and temperature cycles, iii) the period is temperature compensated. The starved L1 larvae also show rhythms in the resistance to heat stress and in the expression of stress-related genes. The latter includes cytosolic catalase, a gene showing circadian rhythms in expression in many organisms.

In insects and vertebrates, the *period* clock genes are central components of the circadian oscillator. *lin-42* shows significant similarity to them and is considered a potential orthologue. Both a null mutation and RNAi of *lin-42* lead to the loss of circadian rhythmicity. Another *lin-42* allele, n1089, is a ts mutant: at 20ºC the larvae show close to normal circadian rhythms whereas at 25ºC rhythmicity is lost. Our results indicate that *lin-42* may indeed be the *period* orthologue and suggests that all metazoans may have related oscillator mechanisms. However, significant differences are expected since *C. elegans* does not have any cryptochromes which play important roles in insect and vertebrate circadian systems.

Since the starved L1 larvae are in a semi-dormant state, they can be targeted by RNAi of genes crucial for reproduction and development, and by pharmacological treatments that interfere with these processes. For example lithium, at concentrations that inhibit development, has little effect on the viability of the L1 but considerably lengthens the circadian period. Since lithium has a universal period-lengthening effect on circadian clocks, the observation that it does so also in *C. elegans* further supports the notion of the existence of components of the circadian system that are conserved throughout the eukaryotic world.

Remarkably, *lin-42* mutations and lithium exposure have parallel effects on the ultradian defecation oscillator. It thus appears that related mechanisms may be central to a very different pair of oscillators: a temperature-compensated circadian clock and an oscillator in the minute range whose period displays profound dependence on environmental conditions.

61. Analysis of *Caenorhabditis elegans* mutants resistant to infection by *Microbacterium nematophilum*

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Microbacterium nematophilum is a recently-identified bacterial pathogen of *C. elegans*. On adherence to the rectal and post-anal cuticle of the worm, these bacteria induce swelling of the underlying hypodermal tissue (deformed anal region, Dar). Screens for animals resistant to infection (bacterially unswollen, Bus) identified strains carrying mutations at 20 susceptibility loci.

It has been demonstrated that the resistance-causing mutation in one of these strains is the deletion of the posterior-patterning Hox gene, *egl-5*. Other existing *egl-5* mutant strains were tested and shown to be resistant to the pathogen, with the exception of a strain carrying a weak *egl-5* allele, *n1439*. The *egl-5* coding region in this strain was found to be wild type, an insertion of a repeat element approximately 13kb upstream of the start site of translation being the only identified mutation near the *egl-5* gene. The 37 bases preceding this insertion site are conserved between *C. elegans* and *C. briggsae* suggesting that there are important regulatory elements even at such a distance from the open reading frame. The insertion may therefore alter some tissue-specific expression of the *egl-5* gene.

In accordance with the posterior-patterning function of the *egl-5* gene, the EGL-5 protein is expressed in numerous cells arising in the posterior of the worm including the cells of the rectal epithelium, B, Y, U, F and K. Infection involves adhesion of *M. nematophilum* to the rectum, suggesting these cells as likely candidates for those in which EGL-5 function might be necessary for infection to proceed. A study of genetic mosaics has been undertaken to test this notion and investigate whether all 5 of these cells need *egl-5*, or whether the Dar phenotype has a single-cell focus. Results of this analysis will be reported.

In addition to studying the susceptibility loci identified in the screens for resistant mutants, we have been investigating the possible role of the *C. elegans* mitogen-activated protein (MAP) kinase, MPK-1, in response to infection. MAP kinases function in receptor tyrosine kinase/Ras signalling pathways to mediate cellular differentiation and proliferation. In *C. elegans*, MPK-1 plays a role, among others, in vulval development and olfaction, and the effect of both loss and gain of function of this protein and each of the upstream components of the pathway has been observed. One observation that remains unexplained is a hermaphrodite swollen tail abnormality present with gain of function of three elements of the pathway, *lin-45raf*, *mek-2* and *mpk-1*. The similarity of this abnormality to the Dar phenotype raises the possibility that the MAP kinase pathway is involved in response to *M. nematophilum*. Indeed an *mpk-1* loss-of-function mutant is resistant to infection. Loss-of-function mutants of the remaining pathway-components are currently being tested. Recently a MAP kinase signalling cascade was demonstrated to be involved in the resistance of *Arabidopsis* to bacterial and fungal pathogens, suggesting this pathway as an ancient element of the innate immune response.

62. Mutations in a cyclic nucleotide gated channel alter the response to pathogenic *M. nematophilum* bacteria

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The Gram-positive bacterium *Microbacterium nematophilum* adheres to the post-anal region of *C. elegans* causing constipation, swelling of the underlying hypodermis, and growth retardation (*1*). To identify worm factors that are involved in this host/pathogen interaction, we carried out a Mos1 mutagenesis screen looking for mutations that altered the response to *M. nematophilum* exposure. Mos1 is a Tc1/mariner family transposon isolated from *D. mauritiana* that has recently been developed as a tool for marking insertions in the *C. elegans* genome (*2*).

From 1061 F1's, we identified 1 mutation, *e2861*, that allow worms to grow on *M. nematophilum* better than wildtype worms. These mutants also exhibit less swelling of the post-anal region. On OP50 bacteria alone, *e2861* mutants tend to accumulate rings of cuticle in the head region. In addition, on starved plates, non-dauer worms develop cuticle bumps. Finally, *e2861* is Daf-c at 25°C.

We identified a Mos1 insertion in the *tax-4* locus through inverse PCR of outcrossed *e2861* mutants. *tax-4* encodes the α-like subunit of a cyclic nucleotide gated channel (*3*). *tax-4* along with *tax-2*, which encodes the β-like subunit of the channel, is required for olfaction, and plays a role in dauer formation (*4*, *5*). To see if the resistance of homozygote *e2861* mutants can be attributed to the altered function of the cyclic nucleotide gated channel, we tested known *tax-4* and *tax-2* mutant worms for resistance to *M. nematophilum*. Similar to *e2861*, *tax-4(pr678)* and *tax-2(pr671)* homozygote mutants exhibit a weaker response to *M. nematophilum* than wildtype worms.

Surprisingly, *tax-4(pr678)*, *tax-2(pr671)* and *e2861* homozygous mutants do not avoid *M. nematophilum*, which is the normal response displayed by wildtype worms. The avoidance of some pathogenic bacteria is also disrupted in worms with a deletion of *tol-1*, a homologue of Toll receptor (*6*). Like TOL-1, TAX-4 and TAX-2 could be involved in deterring the worm from harmful situations. However, mutations in *tax-4* and *tax-2* cause the worms to increase their exposure to *M. nematophilum* without becoming sicker than wildtype worms. This observation suggests that TAX-4 and TAX-2 may function to regulate more than just an avoidance response to a potential pathogen. We are currently exploring the importance of the chemotactic, dauer and cuticle defects displayed by *tax-4(e2861)* mutants in relation to *M. nematophilum* pathogenesis.

1. J. Hodgkin, P. E. Kuwabara, B. Corneliussen, *Curr. Biol.* **10**, 1615-1618 (2000).
2. J. L. Bessereau *et al.*, *Nature* **413**, 70-74 (Sep 6, 2001).
3. H. Komatsu, *et al.*, *Neuron* **17**, 707-718 (Oct, 1996).
4. C. M. Coburn, C. I. Bargmann, *Neuron* **17**, 695-706 (Oct, 1996).
5. M. Ailion, J. H. Thomas, *Genetics* **156**, 1047-1067 (Nov, 2000).
6. N. Pujol *et al.*, *Curr Biol* **11**, 809-821 (Jun 5, 2001).

63. Inducible innate immune defences in *C. elegans*; a TGF connection.

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C. elegans is emerging as a very promising model for the study of host-pathogen interactions (Aballay and Ausubel 2002; Ewbank 2002). In addition to its utilisation for the identification of bacterial virulence factors, it can be used to investigate conserved aspects of innate immunity. Both plants and vertebrates respond to infection by the production of antimicrobial proteins and peptides. To determine whether *C. elegans* possesses an analogous inducible system of defence, we used high-density cDNA arrays to look for genes that are induced upon infection with the Gram-negative bacterium *Serratia marcescens*.
We found that several hundred genes show significant alterations in their level of expression following infection. Among the most robustly induced are genes encoding lectins and lysozymes, known to be involved in immune responses in other organisms. Inactivation of the lysozyme gene *lys-1* by RNAi renders worms more susceptible to infection. Conversely, its over-expression augments the resistance of worms to *S. marcescens*.
Certain of the infection-inducible genes are under the control of the *dbl-1*/TGFb pathway, and it had previously been shown that their expression was abolished in *dbl-1* mutants (Mochii, Yoshida et al. 1999). Consistent with this, *dbl-1* mutants exhibit increased susceptibility to infection.
We therefore conclude that *C. elegans* does possess an inducible system of antibacterial defence, that in part rests on the TGFb signalling pathway. In our future work, we aim to determine the specificity of the response and the degree to which the underlying mechanisms have been conserved in other species.

Aballay, A. and F. M. Ausubel (2002). "*Caenorhabditis elegans* as a host for the study of host-pathogen interactions." Curr Opin Microbiol 5(1): 97-101.
Ewbank, J. J. (2002). "Tackling both sides of the host-pathogen equation with *Caenorhabditis elegans*." Microbes and Infection 4: 247-56.
Mochii, M., S. Yoshida, et al. (1999). "Identification of transforming growth factor-beta-regulated genes in *Caenorhabditis elegans* by differential hybridization of arrayed cDNAs." Proc Natl Acad Sci U S A 96(26): 15020-5.

64. Genetic analysis of systemic RNAi in *C. elegans* - An RDE-1/Argonaute protein defective in a natural isolate of *C. elegans*.

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One of the most striking aspects of RNAi in *C. elegans* is that it works not only when the dsRNA is directed to the target tissue, but also when the RNA is added to the food of the animal or when the animals are soaked in a solution of dsRNA. Apparently, dsRNA or RNAi can spread through the entire organism and can even exert its effect via the gonads on the next generation of animals. We tested if this is a universal property of RNAi in *C. elegans*, and found that most natural isolates are sensitive to feeding on dsRNA, while one strain from Hawaii is not (it is, however, sensitive to injected or transgene encoded dsRNA). We SNP-mapped an allele in the Hawaiian strain to a single locus that carries multiple mutations, suggestive of a null allele. Indeed, deleting this gene in the canonical *C. elegans* strain Bristol N2 makes it resistant to feeding dsRNA. Interestingly, this locus encodes a PAZ/PIWI domain containing protein that belongs to the RDE-1/Argonaute family, thus suggesting a broader role for these family members in RNAi.
In addition to this natural variant, we mutagenized an uptake proficient strain, and screened for mutants specifically defective for RNAi by feeding, or deficient in the spreading of RNAi to the target tissue. Several genes were cloned and the molecular functions of these are being analyzed.

65. The roles of developmental arrest, phosphoinositides, Type II PIP kinase and DAF-16 in the larval response to oxidative stress.

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Since the appearance of terrestrial oxygen, it has been essential to deal with oxidative stress. For a nematode, the important issue is to lay eggs without germline damage. Rather than study the response in adults, for which it is probably too late to do anything useful, we have investigated how L1 larvae respond (1) developmentally, (2) genetically and (3) biochemically to a burst of hydrogen peroxide.

(1) In our assay, starved L1s are exposed various concentrations of H_20_2 for 45 minutes, washed and allowed to recover on food. At and above a concentration of 2 mM, larvae permanently arrest at the L2 stage, show uncoordinated movement, and eat slowly. However, they stay alive for at least two weeks. After a 0.5 mM treatment around 20% N2 arrest permanently, 20% are unaffected, and 60% show a temporary developmental delay, taking one day longer at 20°C to reach adulthood. This delay may represent a strategy to avoid oxidative stress in the environment.

(2) We have isolated a deletion mutant in *ppk-2*, which encodes the only Type II PIP kinase *in C. elegans*. We find that *ppk-2(pk1343)* worms are more sensitive to H_20_2 than N2. We find that members of the *age-1/akt-1/daf-16* gene pathway are also important for H_20_2 sensitivity. Our double mutant analysis suggests that *ppk-2* acts upstream of *daf-16* parallel to *akt-1*.

(3) Because *ppk-2* encodes an enzyme which converts phosphatidylinositol (PI) 5-phosphate to $PI(4,5)P_2$, and *daf-18* encodes a homologue of PTEN, a PIP_3 3-phosphatase, we examined changes in phosphoinositides after H_20_2 treatment. Orthophosphate labelling of L1s and subsequent lipid analysis revealed that H_20_2 causes a increase in the production of PIP_3, PI(4)P, and PI(5)P but no change in $PI(4,5)P_2$ levels. The PIP_3 increase was abolished in *age-1* (Class I PI 3-kinase) mutants. The model that AGE-1 produces PIP_3 to activate AKT-1, which in turn inhibits DAF-16, fits the genetic data. *ppk-2* mutants show no difference from the wild type in the PIP3 response.
Together, the genetic and biochemical data suggest that *ppk*-2 lies in a parallel pathway to *age-1/daf-18/akt-1* upstream of *daf-16.*

66. Xenobiotically induced cytochrome P450 gene expression in *Caenorhabditis elegans*.

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Cytochromes P450 (CYP) are a super family of heme containing NADPH dependent monooxygenases. These enzymes play important roles in the biotransformation of many drugs, carcinogens, steroid hormones and environmental chemicals. CYP gene expression can be induced by the presence of these compounds in the environment. At high concentrations many of the inducers produce a wide range of biological effects on animals and other organisms, e.g. mortality or reproductive failure.

The nematode *Caenorhabditis elegans* possesses the remarkable number of 80 different cytochrome P450 genes. The functions of the encoding proteins are almost unknown. In order to study xenobiotically induced gene expression in *C. elegans* liquid cultures were exposed to different well-known xenobiotic inducers. The mRNA expression was detected by two different DNA arrays and semi-quantitative RT-PCR. In addition, using concentration-effect experiments, a classical reproduction test was compared with CYP gene expression screens.

β-naphthoflavone, PCB52, Lansoprazol and Fluoranthene were the most active CYP inducers. They mainly induced almost all CYP35 isoforms. The reproduction test with nine different xenobiotics revealed Tributylzinn, Endosulfan and Fluoranthene as most toxic substances. The threshold of the semi-quantitative RT-PCR experiments showed a significant higher sensitivity than the reproduction tests. Obviously, the xenobiotic inducible gene expression of *C. elegans* is a very sensitive tool to reveal defense mechanisms against potential toxic substances and can be used to develop a biomonitoring system.

Interestingly, the CYP35 isoforms promoter regions contain xenobiotic response elements (XRE) similar to mammalian CYP1 forms. A transgenic *C. elegans* line expressing GFP under control of the CYP35A2 promoter was constructed. With this tool it was possible to follow the GFP production *in vivo* depending on the added inducer. In the control worms a slight but distinct label was visible at the anterior and posterior part of the intestine. In β-naphthoflavone treated worms the total gut emitted a very strong GFP fluorescence.

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67. The *C. elegans* MAP kinase phosphatase LIP-1 is required for the G2/M cell cycle arrest in developing oocytes

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In the *C. elegans* hermaphrodite germ line, spatially restricted MAP kinase signaling controls the meiotic cell cycle. First, the MAP kinase signal is necessary for the germ cells to progress through pachytene of meiotic prophase I (Church et al.1995). As the germ cells exit pachytene and enter diplotene/diakinesis, the MAP kinase is inactivated and the developing oocytes arrest in diakinesis (G2/M arrest). During oocyte maturation, a signal from the sperm reactivates the MAP kinase to promote M-phase entry (Miller et al. 2000). We have previously reported that the dual-specificity phosphatase LIP-1 negatively regulates MAP kinase signaling during vulval induction (Berset et al. 2000). Here, we show that LIP-1 dephosphorylates the MAP kinase in germ cells that exit pachytene in order to maintain the MAP kinase in an inactive state in the developing oocytes that arrest in diakinesis. Germ cells lacking LIP-1 fail to arrest the cell cycle at the G2/M boundary and enter a mitotic cell cycle without fertilization. LIP-1 thus coordinates meiotic cell cycle progression and maturation with ovulation and fertilization.

68. A putative LET-418 interacting protein plays a role during germ line development

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The vertebrate NuRD (nucleosome remodeling and histone deacetylase) family of chromatin-remodeling complexes has been shown to play a crucial regulatory role in the control of gene expression. The Mi-2 protein is the central component of this NuRD complex. *C. elegan*s genome contains two Mi-2 orthologs, LET-418 and CHD-3. Mutations in the *let-418* gene display pleiotropic phenotypes, among them sterility, gonad migration defects, protruding vulvae, ectopically induced P8.p cells and in the absence of the maternal contribution, an L1 larval arrest (von Zelewsky *et al*., 2000). In contrast, *chd-3* seems not to be essential on its own. However, a putative null mutation of *chd*-3(*eh4*) enhances the Let-418 phenotype, suggesting that the two proteins have partially redundant functions during development (von Zelewsky *et al*., 2000). Yeast two-hybrid screens using the carboxyl termini of LET-418 and CHD-3 as baits have been performed. So far, several candidates were found, and interestingly, proteins interacting with LET-418 differ from the ones interacting with CHD-3, suggesting that, at least, two different Mi-2 containing complexes exist in *C. elegans*. Among the candidates interacting with LET-418, a putative transcriptional co-repressor and tumor suppressor protein was identified. RNAi interference experiments revealed a significant reduction of the brood size as well as of the number of viables. In addition, we also observed a high incidence of males among the viables. Preliminary cytological observations suggest that germ line proliferation is disrupted. Interestingly, these phenotypes are significantly enhanced in *chd-3*(*eh4*) worms. This synergistic interaction reinforces the hypothesis of the presence of distinct Mi-2 containing complexes. Moreover, a *gfp* fusion construct was generated and its expression pattern is currently analyzed. Finally, genetic and biochemical interactions will give us further insights into the understanding of the epigenetic code of *C. elegans*.

von Zelewsky *et al*., Development 127: 5277-5284 (2000).

69. The role of *cdc-25.2* during embryogenesis

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Cell divisions in *C. elegans* are tightly controlled both temporally and spatially. The controls of these divisions appear to use the same conserved regulators found in other organisms. For example, the cyclin-dependent kinase CDC-2 controls the entry into M-phase. Investigations into how CDC-2 is regulated may show how the different cell cycles are established and maintained. In other organisms CDC2 is activated by a dual specificity phosphatase called CDC25. Whereas other organisms are known to have between one and three cdc25 genes, four homologues have been identified in the *C. elegans* genome. We are currently focusing our attention on one of these genes called *cdc-25.2.*

Northern blot analysis shows that *cdc-25.2* has two transcripts. Both transcripts have the phosphatase domain while only the larger transcript has the putative regulation domain. Only the longer transcript is expressed in embryos, whereas the shorter transcript is present in adults. Perturbation of *cdc-25.2* by RNAi causes L1 larval lethality. This phenotype is only prominent if the animals are incubated at 15^{o}C. If both the expression of *cdc-25.2* and *cdc-25.3* is disrupted at the same time embryos fail to hatch and arrest at approximately the 150 cell stage. A potential *cdc-25.2* mutant, *emb-29*, maps extremely close to the *cdc-25.2* locus on the left arm of chromosome V. Indeed, *emb-29* alleles produce cell cycle phenotypes, and one allele has a point mutation substituting a serine to phenylalanine just outside the predicted CDC-25.2 catalytic domain. However, we will present evidence that suggests *emb-29* is not *cdc-25.2*, but is a novel cell cycle regulator. We are currently cloning *emb-29* and have narrowed the locus to two overlapping cosmids.

70. The role of *chk-1* during early development

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Much interest has been devoted in the last few years on how cells respond to DNA damage. Surveillance mechanisms have been found that detect DNA damage and activate checkpoint responses. These checkpoints can either temporally stop the cell cycle presumably to enable the damage to be repaired or cause the cell to undergo active cell death. This checkpoint related repair decreases the risk of defective DNA being inherited in subsequent cell generations. Defects in genes involved in these checkpoint pathways can lead to an increase in the susceptibility of cancer.

Most of the studies on these checkpoint proteins have been based on single cell experimental systems. However, little is known about the role of checkpoint genes in a complex multicellular organism. We have examined the role of a chk1 homologue in the model organism, *C. elegans*. Real-time RT-PCR analysis suggested that *chk-1* is predominantly expressed in embryos and the germline. Although we found no genetic mutant for *chk-1*, Chk-1 phenotypes were characterised by RNAi. First, germ cells treated with *chk-1* RNAi were unable to undergo DNA damage induced cycle arrest or apoptosis. The first few embryos to be laid after the dsRNA injections hatched, but developed into sterile adults suggesting a role for *chk-1* in germline proliferation and/or maintenance. The majority of embryos treated with double-stranded RNA failed to hatch. Upon further examination, defects in embryonic nuclei were observed very early on during development. Similar phenotypes were observed if embryos were exposed to DNA damaging radiation. The frequency of these defective cell cycles increased in embryos exposed to both RNAi and DNA damage. This is the strong evidence that *chk-1* is an essential gene and has a role during early development and the DNA damage checkpoint response.

71. Identification of genes involved in dauer larva morphogenesis by systematic RNA interference

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Under conditions of starvation and extreme crowding *C. elegans* enters the dauer larva, a duration stage alternative to the third larval stage. Dauer larva exhibit a unique morphology, an extended life span and are resistant to many environmental stresses. The decision to form dauer larva is controlled by TGF-beta and insulin signaling pathways, which are defined by a large number of *daf* mutants and well characterized. However, the subsequent genetic control and the molecular nature of the dramatic morphological changes characterizing dauer larva formation is largely unknown.
We used systematic RNA interference (1) (Julie Ahringer's Chromosome 1 RNAi library and further additional clones) in two different *daf-c* background strains (*daf-2(m41)* and *daf-1(m40)*) to identify genes specifically required for the dauer molt, dauer morphogenesis, or dauer recovery. Two genes were identified, which are essential for the survival of the dauer molt, a phenotype observed here for the first time. These are the patched related ligand *ptr-23* and the G-protein coupled chemorezeptor *srh-49*, which both are potentially involved in signaling events. Both depletion experiments do not cause phenotypes in *daf-c* backgrounds at the permissive temperature or in the wild type at 25°C.
Morphogenetically incomplete dauer larva developed when the p66 subunit of the Mi-2 histone deacetylase complex was depleted. The Mi-2 complex has been implicated in chromatin remodeling and transcriptional repression. This suggests that global transcriptional regulation caused by histone deacetylation and chromatin remodeling is important for dauer larva formation.
Depletion of the ras related protein *rab-11.2* interfered with the dauer specific shrinkage of the pharynx, whereas depletion of the developmentally regulated actin gene Y71F9AL.16 resulted in dauer larva with a twisted buccal cavity. These and further observations indicate that systematic RNA interference is an efficient and promising tool to identify genes involved in dauer morphogenesis.
Interestingly, we also identified genes, which were essential for hermaphrodite fertility when the animal was passed through the dauer stage, but which did not influence hermaphrodite fertility when the animal had developed as a normal L3 larva.

1. Fraser, A.G., Kamath, R.S., Zipperlen, P., Martinez-Campos, M., Sohrmann, M., Ahringer, J.: Functional genomic analysis of *C. elegans* chromosome I by systematic RNA interference. Nature 408:325-330 (2000)

72. Getting into the function of the nematode homologue of Eps8

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The small GTPases of the Rho subfamily (Rho, Rac and Cdc42) are key signalling molecules in the regulation of actin cytoskeletal rearrangement, which in turn plays a crucial role in many biological processes such as cell motility and attachment as well as cellular growth and development. Eps8, a protein originally identified as a substrate for the kinase activity of the epidermal growth factor receptor (EGFR), contains a putative PTB domain at its N-terminus, a SH3 domain and a C-terminal region responsible for the binding to actin and proper subcellular localisation. Eps8 is involved in the transduction of signals from Ras to Rac, leading to actin remodeling (Scita et *al.*, 1999), and in the regulation of endocytosis of EGFR (Lanzetti *et al*. 2000). Since actin cytoskeletal modifications are fundamental during development, we investigated the role of Eps8 in a complex organism. To overcome the problem of redundancy (in mammals there is a family of genes related to eps8), we turned to the nematode *C. elegans*.
In *C. elegans* genome there is only one homologue of eps8, the Y57G11C.24 gene (*ceeps8*), which has the same modular organisation as mammalian eps8. Northern blot and RT-PCR experiments showed that *ceeps8* gives rise to two alternative spliced products, a long and a short isoform, which differ for the presence of a C-terminal region in the long isoform.
The expression pattern of *ceeps8* gene was investigated both by immunoistochemistry and by studying transgenic worms carrying promoter/reporter gene fusions. The *ceeps8* gene was mainly expressed in mioepithelial cells of the pharynx and in the gut, where it seems to be enriched in the apical portion of intestinal cells.
An initial approach to define the functions of the *ceeps8* gene in *C. elegans* was based on reducing its expression by RNA interference (RNA*i*) (Fire *et al*., 1998). In RNA*i* experiments aimed at knocking out both the isoforms, we observed mainly larval lethality: worms died as necrotic larvae and showed defects in intestine (some embryonic lethality was also observed). We isolated a deletion mutant allele of *ceeps8* gene from an EMS mutagenised worm library.
The *ceeps8* mutation deletes 1500 bp of the genomic sequence, removing exons 8 to 12. Most likely *ceeps8* deletion allele is a null allele, since both the isoforms should be produced in a truncated version that retains only the N-terminal portion.
A detailed analysis of the phenotypes of *ceeps8* mutant is in progress and should provide information about its physiological role.

1. Scita G. *et al*., Eps8 and E3b1 transduce signals from Ras to Rac (1999). *Nature* **401**, 290-293.
2. Lanzetti L. *et al*., The Eps8 protein coordinates EGF receptor signaling through Rac and trafficking through Rab5 (2000). *Nature* **408**, 374-377.
3. Fire A. *et al.*, Potent and specific genetic interference by double-stranded RNA in *Caenorabditis elegans* (1998). *Nature* **391**, 706-811.

73. Formal Modeling, Simulation and Analysis of *C. elegans* Development

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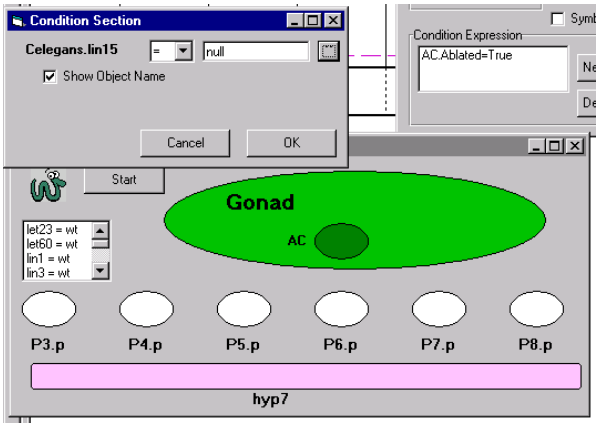
The field of developmental genetics is entering a new phase, in which the synthesis of information from many sources will be necessary to gain a deeper understanding of how various tissues, cells, biochemical interactions and genetic networks collaborate to form a functional organism. The purpose of this project is to model and rigorously simulate and analyze a particular biological system – the *C. elegans* egg-laying system – using languages, methodologies and tools developed by computer scientists for the reliable development of highly reactive computerized systems. The model will incorporate existing anatomical, genetic and biochemical data pertaining to the development and function of (i) the gonad, (ii) the vulva, (iii) the uterine and vulval musculature, and (iv) the hermaphrodite specific neurons (HSNs). We concentrate on an object-oriented approach using the visual language of *statecharts* for specifying behavior, and tools such as Rhapsody for model execution and analysis. In previous work, we have successfully applied this language and tool to the biological phenomenon of T cell activation. The T cell activation model served as a feasibility test and integrated phenomena associated with cell-cycle control, cell fate, cell behavior and location. We are now in the midst of a far more ambitious effort, involving more complex biological phenomena that will incorporate additional aspects of development, including cell fate acquisition, cell migration, axon guidance, and apoptosis. In principle, our model will eventually handle virtually all aspects of development, ultimately allowing our results to be extended to and used by the entire *C. elegans* community, and will apply to other systems too.

As a first stage, we will formalize the existing genetic, biochemical and anatomical data from the biological literature into a set of *live sequence charts* (LSCs). These LSCs capture the behavior of the system in terms of *inter-object behavior*, describing the interaction between objects as scenarios. LSCs enable the user to distinguish between scenarios that *can* occur in the system, scenarios that *must* occur in the system, and ones that are forbidden ("anti-scenarios"). Within this aim is the development of a graphical user interface (GUI) for the *C. elegans*. This part of the project will use a recently developed system called the Play-Engine, which enables the user to input the behavioral information in a user-friendly way, and to execute it too. Thus, non-computer scientists can enter biological data in ways in which they are accustomed to representing their system. This will become a critical point regarding one of the future plans of this project: enabling the entire *C. elegans* community to 'play-in' experimental data into a behavioral database of LSCs.



A GUI depicting VPC specification

For additional information, demonstration and references, see:
http://www.wisdom.weizmann.ac.il/~kam/

74. Studying telomere replication in *C. elegans*

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The stable length of telomeres, the native ends of linear chromosomes, is maintained by a specialized reverse transcriptase, telomerase. Whereas the telomerase catalytic subunit and its RNA component have been shown to be sufficient to confer the addition of repetitive sequences to chromosome ends *in vitro*, studies in yeast suggest that a larger protein complex is required to ensure access of telomerase *in vivo*. *mrt-2* was the first *C. elegans* mutant that exhibits progressive telomere shortening and eventually sterile mutant worms (Ahmed and Hodgkin, 2000), phenotypes that are similar to yeast and mouse telomerase mutants. Strikingly, mutating the *S. pombe* homologue of the *mrt-2* checkpoint gene does not abolish telomerase action and instead results in short but stable telomeres (Dahlen *et al*., 1998). Thus, we are interested in studying telomere replication in higher organisms like the nematode *C. elegans.*
We are currently characterizing two novel *mortal germline* mutants that are defective for telomere replication, *trt-1* and *mrt-1*. One mutant, *trt-1*, carries a mutation in DY3.4, a *C. elegans* reverse transcriptase that is similar to other telomerase catalytic subunits, although DY3.4 appears to lack the T-motif that is found in most other telomerases. A second mutant, *mrt-1*, maps closely to DY3.4, and we are trying to determine which gene is mutated in *mrt-1*. Furthermore, we have initiated a largescale screen to identify most genes that are involved in telomerase function in *C. elegans.*

Ahmed, S. and Hodgkin, J. (2000) MRT-2 checkpoint protein is required for germline immortality and telomere replication in C. elegans. *Nature*, **403**, 159-64. Dahlen, M., Olsson, T.,

Kanter-Smoler, G., Ramne, A. and Sunnerhagen, P. (1998) Regulation of telomere length by checkpoint genes in Schizosaccharomyces pombe. *Mol Biol Cell*, **9**, 611-21.

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

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Mutations in a few *C. elegans* genes can result in full body convulsions caused by the simultaneous contraction of dorsal and ventral body-wall muscles. *unc-43* encodes the only CaM Kinase II homologue in *C. elegans*. *unc-43(lf)* worms have rare spontaneous convulsions that are intensified in frequency and severity by exposure to neurostimulants (e.g. pentylenetetrazole [PTZ] and pilocarpine). Mutants affecting GABA function (*unc-25, 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. We will identify the specific neurons involved by using promoters that drive expression in all motor neurons, inhibitory motor neurons, or locomotory interneurons. We are presently injecting these constructs and will be assaying the convulsion profiles of transgenic lines of *unc-43(lf)*.

In addition, we have conducted mutagenesis screens to discover other genes able to confer convulsion susceptibility in worms. We have screened through 35,000 haploid genomes and have identified 32 independent mutations that define at least nine different genes. On PTZ, ten of these alleles have full body convulsions while the remaining 22 have anterior convulsions. By complementation testing, we have confirmed new alleles of *unc-43, unc-25, unc-47, unc-49* and *unc-10 (rim-1).*

Finding *unc-10* was a particularly interesting and surprising result since we would not have expected a gene conferring a general synaptic defect to have convulsions. As a follow up, we have assayed additional mutants known to have synaptic defects and several genes involved specifically in endocytosis also have anterior convulsions. Teasing apart the role of these genes in the control of excitation promises to be challenging since mutations in them have pleiotropic effects on the worm. Recent studies have implicated many components of the endocytotic machinery to be required for the proper localization of several neurotransmitter receptors such as GLR-1 and UNC-49. These findings suggest an attractive model for a link between anterior convulsions and mislocalized and misregulated receptors. These experiments expand our use of *C. elegans* as a new model for mechanisms underlying simultaneous excitation of neuronal networks. Similar types of neuronal synchrony may cause human epilepsies, since CaMKIIa and GAD65 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.

76. Genetic and biochemical approaches to study modulation of neurotransmitter release.

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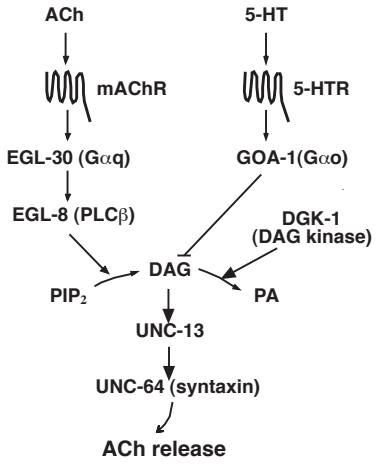
My lab is investigating the mechanisms by which certain neuromodulators cause widespread changes in synaptic strength which are believed to underlie changes in global behavioral states. For example, serotonin has been implicated in several aspects of mood and behavior, including depression, eating disorders, alcohol consumption, and aggression. To investigate neuromodulation we have carried out a genetic analysis of inhibition and facilitation of neurotransmission in an *in vivo* system, the *C.elegans* neuromuscular junction (NMJ). *C.elegans* changes it's rate of locomotion in response to a variety of stimuli, such as nutritional state and response to touch. In addition locomotion rates can be altered by the addition of exogenous neuromodulators, for example, addition of exogenous serotonin causes a pronounced decrease in locomotion. A screen for mutants defective in their response to serotonin has defined two competing G protein pathways that facilitate or inhibit release of acetylcholine (ACh) at the NMJ and thus alter rates of locomotion [1-4]. These two pathways, containing the trimeric G-protein alpha subunits *goa-1* Gαo and *egl-30* Gαq, antagonistically control levels of the second messenger DAG and this in turn regulates the abundance of the DAG binding protein UNC-13 at synaptic release sites. Since UNC-13 interacts with syntaxin, an essential component of the synaptic vesicle release machinery, these experiments define a genetic pathway that leads from G-protein coupled receptors to the general synaptic release machinery.



We are analyzing the biochemical pathways controlled by Gαo which lead to a reduction in DAG levels, in particular the role of a DAG kinase, DGK-1, which was identified in the screen for serotonin resistant mutants. Current projects that will be discussed are a screen for suppressors of a *dgk-1(gf)* phenotype, suppressors of constitutively high levels of ACh release caused by the serotonin antagonist methiothepin, identification of DGK-1 interactors by yeast 2 hybrid and co-immunoprecipitation, and analysis of another 4 DAG kinases in the *C.elegans* genome.

1. Nurrish et al., (1999) Neuron:24 231-242
2. Lackner et al., (1999) Neuron:24 335-346
3. Hajdu-Cronin et al., (1999) Genes Dev 13: 1780-1793
4. Miller et al., (1999) Neuron 24: 323-333

77. Gonadal and hormonal signals that regulate life span in *C. elegans*

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Life span of *C. elegans* is influenced by the Insulin-like signaling pathway and by signals from the gonad. The nuclear hormone receptor (NHR) DAF-12 plays a role in both of these pathways since mutants enhance the longevity of *daf-2(e1370)* (the insulin-like receptor) and abolish the life span extension of germline laser ablated animals. *daf-9* acts downstream of insulin-like inputs but upstream of *daf-12*. It encodes a cytochrome P450 suggesting a function in the production of a ligand for DAF-12. A sterol may be the *daf-9* substrate and *daf-12* ligand because cholesterol deprivation phenocopies mutant defects.
The molecular identities of *daf-12* and *daf-9* indicate an endocrine mechanism regulating life span in *C. elegans.*

We investigated the role of *daf-9* in the gonadal signaling pathway affecting life span. We performed laser ablations of somatic gonad and germline precursors in both a null mutant and a hypomorphic allele of *daf-9*. Germline ablation did not lengthen life span and somatic gonad ablation did not further shorten life span. We conclude that *daf-9*(+) is necessary for life span extension in the gonadal pathway. In hermaphrodites, *daf-9* is expressed in one pair of head sensory neurons, the hypodermis and the spermatheca. In males, *daf-9* is also expressed in the head and the hypodermis but not in the somatic gonad. Possibly, the spermathecal expression in hermaphrodites defines a life extending signal from the somatic gonad. If so, then males, lacking the expression in the gonad would also lack this gonad signal and would not live long upon germ line ablation.
We therefore measured the life span of hermaphrodites and males kept solitary in liquid culture. We find that control males lived longer than control hermaphrodites, as previously described by Gems and Riddle (2000). However, after germline ablation, hermaphrodite median and maximum lifespan increased dramatically (by 100% and 165% respectively) whereas for males median lifespan did not increase and maximum lifespan increased only modestly (20%). These results suggest that under the experimental conditions used, signals from the gonad are sex specific. Further investigation is needed to show if *daf-9* is indeed involved in the sex specificity of the signal.

We also want to identify more components in the putative endocrine pathway. We are currently characterising the mutant *daf(k114)*, which displays gonadal and Daf-c phenotypes similar to *daf-9*. By epistasis analysis we showed that, like *daf-9*, *k114* acts downstream or parallel to TGF-ß and Insulin-like signaling but upstream of *daf-12*. *k114* mutant worms are also cholesterol sensitive. We are now fine mapping *k114* by SNP mapping.

78. DNA damage transcriptional responses in *C.elegans*

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Maintenance of genome stability requires an appropriate response to genotoxic stresses such as ionizing radiation, oxygen radicals and monofunctional alkylating agents. This response is based on complex biochemical signaling networks that activate numerous processes that promote cell cycle arrest and damage repair, and hence, cellular survival – or, when damage is excessive, programmed cell death which eliminates the potentially hazardous cells [1].
We wish to identify new genes in *C.elegans* that participate in DNA damage responses, using DNA microarray technology to detect genome-wide transcriptional responses to DNA damage (X-rays and ENU treatment), in wild-type and in known checkpoint mutants, such as *hus-1* and the *C.elegans* p53 gene *cep-1*. Using quantitative real-time RT-PCR (Q-RT PCR), we test the candidate genes identified in our microarray experiments in different mutant backgrounds to decide on the physiological relevance of the transcriptional response to the checkpoint function.

As is the case in mammals, DNA damage induces germ cell apoptosis [2]. The proapoptotic BH3 domaincontaining protein, EGL-1, is important but not essential for this process (death is reduced but not abolished in *egl-1(n3082)* (lf) mutants). Using northern analysis and Q-RT PCR, we found that *egl-1* mRNA levels are upregulated in wild-type worms after ionizing radiation. Interestingly, this increase is dependent on HUS-1 and CEP-1. Combining this evidence and the fact that death is completely absent in *cep1(gk138)* mutants, we suggest that, CEP-1 induces transcription of EGL-1 and at least one other death activator following DNA damage, which together synergistically induce germ cell apoptosis.
Because important biological pathways are often evolutionarily conserved, and because mutations in these pathways are likely to alter the sensitivity of tumor cells to DNA damaging agents, this work might increase the understanding of how and why tumors can develop resistance to radio- and chemotherapy.

1. Rich T., Allen R.L., Wyllie A.H. 2000. Defying death after DNA damage. Nature **407**: 777-83
2. Gartner A., Milstein S., Ahmed S., Hodgkin J., Hengartner M.O. 2000. A conserved checkpoint pathway
mediates DNA damage-induced apoptosis and cell cycle arrest in *C.elegans*. Mol. Cell **5**: 435-43

79. MEI-3, a novel kinesin-like protein is essential for female meiotic spindle assembly

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During meiosis two major events, the setup of the spindle and the segregation of chromosomes, must be exactly coordinated to ensure generation of haploid gametes. Microtubule-based motor proteins of the kinesin and dynein superfamily are involved in these processes, both during mitosis and meiosis.
We have initialized the functional characterization of *mei-3*, encoding a novel kinesin-like protein in the nematode *C. elegans*. The amino-terminal motor domain of MEI-3 is most similar (60%) to members of the Klp-2 subfamily, which are involved in centrosome separation and spindle maintenance during mitosis. In contrast, MEI-3 seems dispensable for centrosomal spindle assembly during male meiosis and mitosis but is essential for acentrosomal female meiosis. Depletion of MEI-3 activity by RNA mediated interference causes a completely disrupted meiotic spindle with disorganized microtubules projecting into the cytoplasm. As a consequence, correct alignment and segregation of chromosomes fail, leading to the generation of aneuploid embryos. The same phenotype becomes obvious, when MEI-3 is depleted in *emb-27(g48)* mutants, which normally arrest in meiotic metaphase I. To determine the subcellular localization of MEI-3, we raised antibodies against the carboxyterminal part of the protein. MEI-3 expression starts in the germline precourser cells Z2 and Z3. The staining persists throughout gonad development, getting stronger in the cytoplasm of mature oocytes in the proximal part of the gonad. After nuclear envelope breakdown (diakinesis), MEI-3 colocalizes with MTs, which have formed around the bivalent chromosomes. Although amino-terminal KLPs are proposed to be plus-end directed motors, MEI-3 is mainly localized at the spindle poles of the female meiotic spindle during prometa-, meta- and anaphase.
We propose that MEI-3 function is at least essential for the initial steps of meiotic spindle formation, starting from diakinesis to metaphase I.

80. Development of a bio-monitoring screening system based on the xenobiotically induced gene expression of *Caenorhabditis elegans* - The Celegans Toxchip

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From an ecotoxicological point of view it is essential at which concentration a contaminant becomes a pollutant, implying a toxic effect. At high environmental concentrations reproduction and mortality are used to define toxicity. But what about lower concentrated contaminants? In that case no significant effect could be found using classical ecotoxicological test systems. This presentation shows the development of a bio-monitoring screening system based on the xenobiotically induced gene expression of *Caenorhabditis elegans* - The Celegans Toxchip. Such a system should be more sensitive than classical ecotoxicological tests.

Taking advantage of the *C. elegans* whole genome DNA microarray created in Stuart Kim's lab (Stanford University, USA), around 60 genes inducible by different xenobiotics (e.g. the herbicide Atrazin, ß-Naphtoflavone, Clofibrate, the endocrine active substance Diethylstilbestrol and Fluoranthen) were selected for the Celegans Toxchip. It contains genes of the following families: Cytochrome P450, UDP-Glucoronosyltransferase, Glutathione-S-transferase, Heat shock-protein, Carboxylesterase and more.

cDNA fragments from the selected genes were spotted on a low density DNA array and hybridized against mRNA isolated from *C. elegans* liquid cultures incubated with different xenobiotics. With the DNA array technique a distinct gene expression pattern can be obtained in response to the compound used for the treatment. It allows the high throughput necessary for an ecotoxicological test system. This system of xenobiotically induced gene expression of *C. elegans* is an excellent new tool in a complex bio-monitoring screening system.

81. Mutational analysis of *tbb-2*, a beta-tubulin required for proper P_0 spindle orientation

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In a screen for embryonic lethal temperature sensitive mutants, or 362 was identified which shows defects in centration and rotation of the first mitotic spindle. or362 fails to complement a gene previously known as *rot-2* (t1623), which was identified in a screen for non-conditional maternal effect mutants (Gonczy *et al.* 1999). Using snip-snp mapping, we were able to map and subsequently identify or362 and t1623 as having missense lesions in the GTP-binding and assembly domains, respectively, of one of two embryonically expressed β-tubulin genes known as *tbb-2*.

After fertilization in *C. elegans* one-cell stage embryos, the maternal pronucleus migrates from the anterior of the zygote towards the posterior where it meets with the paternal pronucleus. The pronuclei then move back towards the center of the embryos as the centrosome pronuclear complex rotates 90 degrees such that the first mitotic spindle sets up along the longitudinal A-P axis of the one-cell stage embryo called P_0.

In *tbb-2* (or362ts) mutants, the P_0 spindle fails to rotate and remains orientated transversely in the posterior of the embryo. Furthermore, in *tbb-2* (or362ts) embryos, the maternal pronucleus frequently fails to migrate posteriorly. In these embyos, the sperm pronucleus-associated centrosomes nevertheless form a transversely-oriented spindle. Immunofluorescent analysis using antibodies specific to TBB-2 shows that this protein is present at greatly reduced levels within the P_0 spindle in *tbb-2* (or362ts) embryos. Additionally, we raised antibodies to the other embryonically expressed β-tubulin , known as tbb-1, to examine how its protein can function in a *tbb-2* background. Here, we see a less robust spindle apparatus, which in combination with reduced levels of TBB-2, presumably leads to spindle instability. This may account for the spindle orientation defects observed.

We also obtained a deletion of tbb-2 from the International *C. elegans* gene knockout consortium, gk129, that takes out approximately 1/3 of the 5' coding region. No TBB-2 staining is detected in *tbb-2* (gk129) embryos. While the deletion strain shows some brood lethality and spindle defects, it is homozygous viable. Interestingly, the recessive loss of function missense alleles have more of an impact on microtubule-dependent processes than a putative null deletion strain. Current experiments are underway to examine levels of TBB-1 and TBB-2 in both *tbb-2* (or362ts) and *tbb-2* (gk129) mutant embryos to further understand how the two β- tubulin proteins function to control microtubule dynamics in the early *C. elegans* 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).

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

82. *rad-51* gene in meiosis and in soma

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We have analyzed in detail the effects of *rad-51* RNAi in several genetic backgrounds in order to dissect the pathways in which *rad-51* is involved in *C. elegans* in the germline and to establish the role of this gene in the somatic cells.
We have verified that the high levels of embryonic lethality are due to maternal effect. We have explored the effect of *rad-51* RNAi in mutants lacking fundamental components of the recombination machinery and postulated roles for such protein and for RAD-51 during meiosis. Silencing of the *rad-51* gene also causes a reduction in fecundity, which is suppressed by mutation in the DNA damage checkpoint gene *rad-5*, but not in the cell death effector gene *ced-3*. Finally, RAD-51 depletion is also seen to affect the *soma*, resulting in hypersensitivity to ionizing radiation in late embryogenesis.

83. A *glr-2* channel pore domain mutant with phenotypic effects only in the presence of the wild type gene

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Ion permeability and single channel conductance are determined primarily by the M2 pore forming domain of ionotropic glutamate receptors. In mammals the codon for a conserved glutamine(Q) residue in M2 of the GluR-B subunit is edited so that it encodes arginine(R), limiting the conductance and permeability of AMPA channels containing it. In *C. elegans* no such editing has been found for the four *glr* genes containing this conserved domain with the Q residue, *glr-1*(C06E1), *glr-2* (B0280.12), *glr*(ZC196.7) and *glr*(K10D3.1).

We utilized site directed mutagenesis to generate a *glr-2* gene that encodes an R in place of Q. The GFP-labelled mutant transgene expressed normally in the animal, and caused no apparent phenotype in the *glr-2* knock-out background, VM207(*ak10*). Unlike the case with the wild type GFP-labelled transgene, no phenotypic rescue of the *glr-2* knock-out was observed. However, in the N2 wild-type background (wt), we observed a low penetrance lethality accompanied by Unc and Clr phenotypes. These phenotypes were also evident after integration of the transgenes. Single-worm PCR allowed us to estimate the copy number of the transgenes as at least 5 fold above that of the endogenous gene, whose expression in mass transgenic populations could not be detected by RT-PCR over that of the R mutant transgene.

For further analysis, we began injections of different R/Q transgene ratios directly into VM207. Pure wild type (Q) or mutant (R) transgenes generated no phenotype, while a one to one ratio of Q to R resulted in a low penetrance phenotype similar to that seen with the R transgene in the wt background. Strikingly, injection of a three to one ratio of R to Q resulted additionally in a low recovery of lines, with a profound arrest phenotype in many animals. For instance, only 5 of 44 primary transgenics from one set of injections matured to egg-laying adulthood. RT-PCR is being utilized currently to correlate phenotype with expression.

To determine whether the phenotypes might be due to alterations of synaptic function and not to an artifactual effect, for instance, on early development, we injected the three to one R/Q transgene mix into wt worms and into a strain carrying a mutation in a putative vesicular glutamate transporter, *eat-4*. In the wt background the strongest phenotypes, including developmental arrest and lethality, were evident, while phenotypes in the *eat-4* mutant worms were entirely suppressed (5/5 lines). This suggests that putative GLR-2(R/Q) -containing channels exert their effects in a way that is dependent on actual glutamatergic synaptic transmission.

84. Heat Shock Factor in *Caenorhabditis elegans* and in parasitic nematodes

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Filarial parasites of the genus Brugia live in mammals (including humans) and are transmitted between hosts by the bite of a blood-feeding mosquito vector. The infective form for the mosquito is the L1 or microfilariae (Mf) a life cycle stage that is highly adapted for life in the bloodstream of the mammal. Mf are developmentally arrested and undergo no further development until ingested by a mosquito. The link between the progression of the developmental cycle and the transition between hosts implies that the Mf has a mechanism by which it can sense its changing environment. Results in other systems have shown that heat shock factor (HSF) can act as a cellular thermometer directly monitoring temperature and oxidative state. As temperature is one of the major differences between the mosquito and mammalian hosts we are interested in investigating the role of HSF in developmental progression. As the tools for functional genomics do not exist for Brugia, we are using C. elegans as a model system in which to define the role of HSF in a nematode. ACeDB identifies a single HSF-like gene with a highly conserved DNA binding domain. Using an RNAi feeding vector containing fragments of different lengths of *C.elegans* HSF, we have defined a number of phenotypes. The penetrance of these phenotypes increases with the size of the RNAi fragment and higher growth temperatures. RNAi treated worms have defects in thermotolerance, lifespan, fertility and egg-laying. In addition treated worms are significantly smaller and have a scrawny appearance. In an hsp-16/GFP reporter background, RNAi abolished GFP expression in the intestine but not in the pharynx or nerve ring. The decrease in HSP-16 levels has been confirmed by immunoblotting. Using a yolk protein/GFP reporter, flourescence in the oocytes is significantly reduced in RNAi treated worms. These results may relate to defects in gut function in RNAi treated worms, and this is consistent with a decreased gut function as defined by feeding assays. Ongoing studies are focused on determining the spatial and temporal expression pattern of *hsf* throughout development. Our aims are to determine the pathways in which HSF is active under normal developmental conditions and to identify down-stream targets of HSF.

85. A transcription factor and a hsp70 homologue required for cuticle synthesis

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C. elegans hypodermal cells synthesise five distinct cuticles through the nematode life cycle: the first one within the egg prior to hatch; the remainder at the end of each larval stage prior to moulting. Previous work has shown that mutations in genes involved in assembly of the cuticle can cause a spectrum of effects from alterations in the animal shape to lethality after elongation of the embryo. The severity of phenotype will depend on the severity of cuticle synthesis defects. Accordingly, we have isolated by different approaches two embryonic lethal mutants that complete the elongation process but appear to partially fail in assembling the first nematode cuticle.

Our lab has performed forward genetic screens to identify recessive mutants with cuticle synthesis failure phenotypes. We have been characterising one mutant allele, named *ijØ15*, generated from this screen. Homozygous embryos for *ijØ15* express *dpy-7*_GFP and *col-12*_GFP constructs and are able to secrete certain components of the cuticle to some extent. We have cloned the gene of the allele identified by *ijØ15* and show it to be a member of the stress 70 protein chaperone family. It contains significant homology to a previously described human and rat hsp70, *stch*, which is targeted to the ER by a signal peptide. HSP70 proteins are highly conserved ATPase molecules that participate in diverse protein processing events including folding, translocation across membranes, and multimer assembly/disassembly, however, there is no direct evidence of their implication in collagen folding. The possible role of this gene in collagen biosynthesis in *C. elegans* is currently being researched.

From un-related research in our lab, we observed that inhibition of the expression of a predicted gene encoding a bZIP transcription factor by RNAi results in a combination of phenotypes indicative of an abnormal cuticle. We have shown that this transcription factor is expressed in the hypodermis of embryos and later stage animals. Two recessive mutations have been identified in this gene: one nonsense generating a truncated product; the other misense. Null mutants seem to express collagen genes but are not surrounded by an intact cuticle as detected by means of immunofluorescence experiments and scanning electron microscopy. These findings suggest our transcription factor is not required for transcription of all collagen genes, however is required for either secretion or assembly of a normal cuticle.

86. The unique forms of the prolyl 4-hydroxylase complex found in *C. elegans* are essential for development due to their cuticular collagen modifying activity

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Unique forms of the prolyl 4-hydroxylase (P4-H) complex modify the collagen rich cuticular ECM of *C. elegans*. ER localised P4-H enzyme complexes hydroxylate proline residues within the Gly-X-Y repeat regions of procollagen molecules. The nematode cuticle acts as an exoskeleton and is required for maintenance of worm body morphology. Mutations in collagens forming this cuticle, and the enzymes that process and modify procollagen, cause lethality and severe alterations to body shape as illustrated by the *sqt-3* and *bli-4* mutant phenotypes.

C. elegans P4-H complexes are encoded by three genes. *phy-1* and *phy-2*, which constitute the enzymatically active alpha-subunits, and *pdi-2*, that encodes the beta-subunits. PDI-2 has an ER retention motif and keeps complexes in a soluble, active state within the ER. Expression pattern studies reveal that these three enzymes are expressed in the hypodermis in a temporal pattern mirroring that of their cyclically expressed collagen substrates. RNAi and mutant analysis demonstrates that the complexes are essential for development and body shape maintenance. These P4-H mutants are also shown to be defective in collagen formation/deposition and in formation of specific cuticular structures.

Biochemical evidence indicates that PHY-1, PHY-2 and PDI-2 combine to form three unique forms of P4-H complexes. The predominant form is a mixed alpha-subunit tetramer formed from PHY-1/PHY-2/$(PDI\text{-}2)_2$. Two less abundant dimers can also form. No mixed alpha subunit tetramer or dimer has been described in any other species to date. Formation of the PHY-1/PDI-2 and PHY-2/PDI-2 dimers becomes upregulated in *phy-2* null and *phy-1* null backgrounds respectively and can partially compensate for loss of other froms of the complex. Chemical inhibition of P4-H complexes in *C. elegans* and characterisation of parasitic nematode P4-H enzymes will also be discussed.

87. Functional conservation and specificity of GATA transcription factors.

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The GATA transcription factors are a gene family of zinc finger DNA binding proteins, present in a wide range of organisms, which are involved in the regulation of differentiation and tissue specific gene expression. There is little sequence conservation between different GATA transcription factors except for the DNA binding domain. We are studying functional conservation of GATA factors both within the nematode Phyla and between nematodes and vertebrates. We are also aiming to identify functional domains involved in determining tissue specificity.

C.elegans GATA factors are capable of activating programs of tissue specific differentiation when forced expressed in early *C.elegans* embryos and show remarkable specificity of function in these assays. Endodermal GATA factors such as *elt-2, end-1* and *end-3* activate a program of endodermal differentiation and hypodermal GATA factors such as *elt-1* and *elt-3* activate hypodermal differentiation. We have investigated the effect of ectopically expressing the vertebrate GATA factors (mouse GATA-1 to –6) by placing their cDNAs under the control of the heat shock promoter. None of the mouse GATA genes appear to arrest development when force expressed in early embryos. Furthermore, although GATA-4, 5, and 6 are expressed in the vertebrate endoderm, they do not appear to be capable of activating endodermal gene expression in *C.elegans* embryos. We have also isolated a GATA factor from the parasitic nematode *Haemonchus contortus* which appears to be a close homologue of *elt-2*. Its expression pattern in the parasite, as determined by immunostaining with specific antisera, appears to be identical to that of the *C.elegans elt-2* gene. Ectopic expression of this gene in early *C.elegans* embryos, in contrast to the vertebrate genes, appears to both arrest development and activate a number of gut specific markers.

We are also examining the multiple functions of the hypodermal GATA factor *elt-1* during *C.elegans* development. *elt-1* is both necessary and sufficient for the specification of hypodermal cell fates in early embryos but appears to have additional roles later in development. A chromosomally integrated elt-1::GFP reporter is expressed in hypodermal cell precursors (consistent with the antibody staining of Page et al 1997). From the comma stage onwards, GFP expression is confined to the lateral seam cells. Post-embryonically, there is continued expression in seam cells and additional expression in the ventral nerve chord, many cell bodies of the retro-pharyngeal ganglion and in the vulval muscles. We have performed RNAi experiments by feeding and by inducible expression of dsRNA under the control of the heat shock promoter in order to investigate *elt-1* function after its early essential role. In addition to a phenotype similar to that seen in the genetic null (elt-1 (zu180), we see lumpy/dumpy, unc and protruding vulva phenotypes. The lpy/dpy phenotype is not simply a weaker version of the null, i.e. a loss of hypodermal cells. The hypodermal organisation of these worms is disrupted with a specific loss of seam cells and a redistribution or possibly an increase in the number of dorsal/ventral hypodermal cells. We also see effects on gene expression in the dorsal/ventral hypodermis with a down-regulation of *elt-3* suggesting that *elt-3* may be directly regulated by *elt-1*.

88. FEM-3 is a cytoplasmic protein that is expressed in sperm and spermatocytes

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The switch from spermatogenesis to oogenesis in the hermaphrodite is dependent on post-transcriptional repression of the *fem-3* mRNA. *fem-3* is necessary for the transient production of sperm in the hermaphrodite. In adults that produce oocytes, *fem-3* is repressed through several *trans*-acting factors, including six *mog* genes, *mep-1*, *fbf* and *nos-3*. FBF binds to a *cis*-acting regulatory region in the *fem-3* 3'UTR that is named PME (point mutation element). The PME consists of at least five nucleotides and is necessary for repression of the *fem-3* messenger in that dominant gain-of-function mutations that disrupt the PME cause masculinized germlines in otherwise female adults.
While *trans*-acting repressors of *fem-3* are being characterized, the fate of the *fem-3* mRNA is still unclear since it is unknown whether it is regulated at the level of its stabilization, its transport or its translatability. In addition, the expression pattern of *fem-3* is largely unknown. In order to understand the expression and regulation of *fem-3*, we have raised antibodies against the FEM-3 protein and find that it is expressed in the cytoplasm of mature sperm and sperm precursors. This finding is in accordance with *fem-3* being required for spermatogenesis. To address the mechanism through which *fem-3* is repressed, we have investigated the presence of the *fem-3* messenger by *in situ* hybridization and by quantifying the relative amounts of wild-type and gain-of-function mutated *fem-3* mRNAs in masculinized animals.

89. The *C. elegans* Heterochromatin Protein 1 homologue HPL-2 acts in germline and vulval development

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Proteins of the highly conserved heterochromatin protein 1 (HP1) family have been found to function in the dynamic organization of nuclear architecture and in gene regulation throughout the eukaryotic kingdom. In addition to being key players in heterochromatin-mediated gene silencing, HP1 proteins may also contribute to the transcriptional repression of euchromatic genes via the recruitment to specific promoters. To investigate the role played by these different activities in specific developmental pathways, we identified HP1 homologues in the genome of *C. elegans* and used RNA-mediated interference (RNAi) to study their function. We have show that one of the homologues, HPL-2, is required for the formation of a functional germline and for the development of the vulva by acting in an Rb-related pathway. Our results suggest that by acting as repressors of gene expression, HP1 proteins may fulfill specific functions in both somatic and germline differentiation processes throughout development.
The HP1 chromo shadow domain is thought to act as a protein interaction module able to mediated the assembly of macromolecular complexes; the specificity of action of chromo domain proteins could then be generated by interaction of this domain with specific partners. In order to identify HPL-2 interacting proteins, a two-hybrid screen is under way. Results from this screen will be presented.

90. A *C. elegans* model of neuronal dysfunction in Huntington's Disease: Expanded polyglutamines cause severe neuronal phenotypes without cell death.

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Proteins with expanded polyglutamine repeats cause Huntington's Disease (HD) and several other neurodegenerative disorders. HD is a dominant neurodegenerative disorder caused by polyglutamine expansion in the protein huntingtin. HD pathogenesis appears to involve the production of a mutated amino terminal fragment, cytoplasmic and nuclear aggregates, and abnormal activity of huntingtin interactor proteins essential to neuronal survival. Before cell death, neuronal dysfunction may be an important stage of HD pathogenesis. The number of proteins and genetic pathways implicated in HD is large and is growing. Simple model organisms may provide a convenient setting in which to manipulate and decipher polyglutamine mediated neuronal toxicity. We have used *Caenorhabditis elegans* to investigate the effects of expressing normal and mutant N-terminal huntingtin fused to fluorescent marker proteins in a portion of the nematode nervous system responsible for touch reception. Mutated huntingtin (128 Glns) produced a loss of touch response at the tail at high penetrance. Similar perinuclear deposits and faint nuclear accumulation of fusion proteins was observed for normal and expanded polyglutamine containing proteins. In contrast, significant deposits and morphological abnormalities in the touch receptor PLM cell axons were observed in animals expressing 128 glutamines. Neuronal cell death was not detected. These animals indicate that significant neuronal dysfunction can occur without cell death and may be induced by expanded polyglutamines and may correlate with axonal insult and not cell body aggregates.

C. elegans amenability to genetic analyses and pharmacological screens may uncover aspects of polyglutamine toxicity difficult to manipulate in vivo by other means. Our model provides a system suitable in suppressor screens of polyglutamine dependent neuronal dysfunction. We have performed screens for genetic and pharmacological suppressors from which we have obtained candidate targets.

91. The Ubiquitin-like Nedd8 protein modification pathway regulates microtubule and microfilament function in the early *C. elegans* embryo.

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NEDD8 is a small polypeptide closely related to ubiquitin. Like ubiquitin, NEDD8 is conjugated to other proteins through E1 activating and E2 conjugating enzymes. In contrast to ubiquitination, conjugation of NEDD8 to its only known targets, the cullin proteins, does not target them for degredation. Rather, cullins are part of SCF ubiquitin-ligases. Conjugation of NEDD8 to the cullin subunit stabilizes these complexes, which then mediate the ubiquitination of a subset of ubiquitin targets, leading to their degredation.

We identified a temperature-sensitive allele of the *C. elegans* UBA3 subunit of the E1 activating enzyme for NEDD8, which we named *rfl-1 (ruffle-1)*, based on its early embryonic phenotype. While in other systems the Nedd8 pathway has mainly been implicated in regulating cell cycle progression, *C. elegans* embryos lacking RFL-1 function show defects in microfilament and microtubule-related processes as well as interphase delays. Most interestingly, the Nedd8 pathway seems to be required to negatively regulate microfilament contractility: *rfl-1(-)* embryos initiate ectopic cleavage furrows during cytokinesis and also show extensive cortical contractility during pronuclear migration. Additionally, rotation of the first mitotic spindle is often delayed or absent in *rfl-1(-)* embryos and about 50% of the embryos exhibit a late cytokinesis defect. Using immunocytochemistry and tubulin::GFP labeling of the mitotic spindle, we were able to determine that *rfl-1(-)* embryos have unstable mitotic spindles: astral microtubules are shorter and the central spindle is displaced towards one pole of the mitotic spindle in about 50% of the embryos. This central spindle mislocalization always co-incites with the cytokinesis defect, indicating that the cytokinesis failure in *rfl-1* mutants is due to microtubule instabilities, rather than reflecting a direct role for the Nedd8 pathway in the completion of cytokinesis.

Similar spindle defects have been previously observed in mutant embryos that mislocalize the microtubule severing complex katanin, consisting of the two proteins MEI-1 and MEI-2, to the mitotic spindle. We were able to show that *rfl-1* mutants also ectopically localize MEI-1 to the mitotic spindle, suggesting that the microtubule severing activity of katanin is accountable for the observed spindle defects. Moreover, *mei-1(RNAi)* was able to rescue the spindle orientation and cytokinesis defects observed in *rfl-1* mutants, but failed to rescue some aspects of the ectopic cortical activity and the cell cycle delay. Finally, we were able to show by RNAi that all other previously identified pathway components, including the *C. elegans* cullin *cul-3*, exhibit the same embryonic defects than *rfl-1*, indicating that the pathway is intact in the early embryo.

As inactivation of *mei-1* only partially rescues the phenotype associated with the *rfl-1* mutation, we propose that the Nedd8 pathway serves multiple purposes in the early embryo. Aside from MEI-1/MEI-2, we also expect proteins that are required for negative regulation of cortical contractility and for cell cycle progression to be regulated in a Nedd8 dependent manner.

92. bHLH transcription factors are involved in specifying the cell-death fate of the NSM sister cells

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In the developing *C. elegans* embryo, the NSMs differentiate into pharyngeal, serotonergic neurosecretory motoneurons; their sisters, however, die shortly after they are born as a result of programmed cell death. The gene *ces-1*, which codes for a Snail-like transcription factor, plays a major role in this specific cell-death event. In *ces-1* gain-of-function (gf) mutants the NSM sister cells fail to die but instead develop into NSM-like cells also producing serotonin (1). The function of CES-1 in cell-death regulation might be conserved, since its human homolog, SLUG, is involved in the cell-death specification of B cells (2). Genetically, *ces-1* acts as a negative regulator of the cell-death activator gene *egl-1*. *egl-1* is the first gene of the central cell-death pathway and codes for a BH3-only protein, which is transcriptionally regulated in the NSMs.
Our data indicate that, in the surviving NSMs, CES-1 binds to Snail-binding sites in a conserved region of the *egl-1* locus that is necessary for this specific cell-death event (Thellmann, M. et al., European *C. elegans* Meeting, abstract). We postulate that CES-1 thereby blocks a NSM-specific transcriptional activator of *egl-1*. In the NSM sister cells, CES-1 might be present at lower levels, which would allow the NSM-specific activator to induce *egl-1* transcription, resulting in the death of these cells.
Using two different approaches, we plan to identify the activator or activators of *egl-1* transcription which are blocked by CES-1 in the NSMs.
It has been shown in other organisms that Snail-like proteins can antagonize the action of basic helix-loop-helix (bHLH) transcription factors (in particular the action of heterodimers of Daughterless and Achaete-Scute-like proteins) by direct competition for DNA binding to Snail-binding sites/E-boxes (3,4,5). Using existing mutants and RNAi, we therefore tested wether bHLH proteins are involved in specifying the death of the NSM sister cells.
We found that a weak loss-of-function (lf) mutation in the only *C. elegans* Daughterless-like gene *hlh-2*, *hlh-2(bx108)*, leads to the survival of the NSM sister cells with a low frequency. Furthermore, *hlh-3(RNAi)* causes the NSM sister cells to survive at a low frequency in wild-type animals and enhances the *hlh-2(bx108)* phenotype. We were able to show that a HLH-2/HLH-3 heterodimer can bind to the Snail-binding sites of the *egl-1* locus *in vitro*. These data and additional *in vivo* evidence (Thellmann, M. et al., European *C. elegans* Meeting, abstract) suggest that an HLH-2/HLH-3 heterodimer acts as an activator of *egl-1* transcription in the NSM sister cells.
In addition, we are performing a genetic screen for mutations that enhance the weak NSM sister cell survival phenotype of *hlh-2(bx108)* mutants in order to identify other factors that might act with HLH-2 and HLH-3 to activate *egl-1* transcription.

(1) Ellis, R. E. and H. R. Horvitz (1991) Development **112**(2): 591-603.
(2) Inukai, T.,et al. (1999) Mol Cell **4**(3): 343-52.
(3) Fuse, N. et al. (1994) Genes Dev **8**(19): 2270-81.
(4) Fuse, N. et al. (1999) Dev Genes Evol **209**(10): 573-80.
(5) Kataoka, H. al. (2000) Nucleic Acids Res **28**(2): 626-33.

93. Differing susceptibilities to systemic RNAi within the *Caenorhabditis* clade

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Although certain features of the mechanism responsible for the RNAi response appear evolutionarily conserved (e.g. Dicer), the phenomenon of «spreading» exhibited by *C. elegans*, whereby the worm produces a systemic response to the localized introduction of dsRNA, is more species-specific. Delivery of dsRNA into *C. elegans* by microinjection (Fire et al., Nature 391: 806), soaking (Tabara et al., Science 282: 430), or feeding (Timmons & Fire, Nature 395: 854) can lead to the systemic depletion of targeted mRNAs, with the exception of a few resistant cell types (e.g. most neurons). The presence of this systemic response lead to the hypothesis that an uptake mechanism must exist that functions to transport the dsRNA or a related RNA product from one cell or tissue to another (e.g. from the gut to the germ line and other tissues). Indeed Winston *et al.* have just recently identified a putative transmembrane protein SID-1 (Science 295: 2456) that appears to function as an integral component of just such a transport mechanism. Although a similar spreading phenomenon has been documented in plants, systemic RNAi does not appear to be a common feature in other animals examined so far, such as Drosophila. In experiments designed initially to identify conditions that would result in «cross-interference» (the targeting of a mRNA by a dsRNA containing sequence mismatches), we noticed that a strain of *C. briggsae* was RNAi-resistant via soaking but not by injection. This lead to our testing of a dozen strains within the *Caenorhabditis* clade for susceptibility to RNAi by different delivery methods (injection, soaking, and feeding). Our results indicate that some strains are capable of a systemic RNAi response if dsRNA is delivered via microinjection but not if delivered by soaking and/or feeding. Thus, these results suggest that a separate or additional uptake mechanism may be required to transport the initial dsRNA across epithelial boundaries and that further transport, possibly of processed dsRNA (e.g. siRNAs) and involving SID-1, represents a second transport mechanism. Some strains may be deficient in the first mechanism but not the second. Our results also suggest that the many nematode species outside the *Caenorhabditis* clade that have been labeled RNAi-resistant may, like the majority of *C. elegans* neurons, simply lack the transport machinery needed for a systemic RNAi response, but may still be capable of cell autonomous RNAi.

94. Exploring the role of sterol-sensing domain proteins in *C. elegans*

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Sterols play important roles in determining the biophysical properties of eukaryotic membranes and also serve as precursors in the synthesis of steroid hormones, lipoproteins and bile acids in mammals. We are interested in understanding how sterols have the potential to affect *C.elegans* development. In particular we are studying a family of proteins that we have named *ptr* (for patched-related), which are also present in vertebrates. The *ptr* genes encode proteins with sequence and topological similarities to Patched, a receptor for Hedgehog in *Drosophila* and vertebrates. The PTC and PTR proteins are 12-pass membrane proteins that carry a sterol sensing domain (SSD). The SSD consists of about 180 amino acids that form 5 consecutive membrane spanning domains. This motif was initially identified in HMG-CoA reductase and SCAP, two proteins involved in cholesterol homeostasis in mammals. Subsequently, additional proteins such as Disp/CHE-14, NPC-1,-2, PTC and PTR (PTC-related proteins) have been shown to carry SSDs. SSD proteins appear to play various roles in the transport of lipids, sterols or sterol-modified proteins and in cholesterol homeostasis.

To gain rapid insights into the roles of PTR family members, we have surveyed the 24 *ptr* genes in *C. elegans* using RNAi. We have found that the *ptr* genes are involved in a number of diverse processes ranging from cytokinesis to molting. Here we present an analysis of the gene *ptr-4*. We have cloned a full-length cDNA corresponding to *ptr-4* to verify that it encodes a predicted membrane protein that carries an SSD with sequence similarity to PTC. *ptr-4(RNAi)* worms show multiple defects in motility, molting, osmoregulation and male tail morphogenesis. Many of these mutant phenocopies have a late developmental onset. We have characterised these defects using a variety of fluorescent compounds and GFP reporters. Taken together our studies indicate that the absence of *ptr-4* activity may lead to developmental abnormalities by affecting exocytosis and endocytosis. In addition, several of the mutant phenocopies generated by *ptr-4(RNAi)* resemble those found in animals carrying mutations in genes implicated in sterol regulation or utilisation. We hypothesise that PTR-4 gene is a critical component in multiple processes that might involve lipid or protein transport in *C. elegans*.

95. The Leucine-Rich Repeats of LAP proteins mediate basolateral localisation in epithelial cells.

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Recent studies have identified LAP proteins as a new protein family containing LRR (Leucine Rich Repeats) and PDZ (PSD95/ Dlg/ ZO-1) motifs. LAP proteins described so far in mammals (hScribble, Erbin, Densin-180 and Lano), *Drosophila* (Scribble) and *C. elegans* (LET-413) present 16 LRRs of 23 amino-acids in amino-terminus and either 0, 1 or 4 PDZ domains in carboxy-terminus. A signature of the family are two LAP Specific Domains (LAPSD) of 39 and 24 residues, respectively lying immediately downstream of the LRR and separated from the PDZ domains by a non conserved region. LAP proteins, except Densin-180 which is neuron-specific, are mainly expressed in epithelial cells where they are localised at the basolateral membrane (LET-413 and Erbin) or associated with subapical junctions (Scribble). Analysis of *Scribble* and *let-413* mutants in *Drosophila* and *C. elegans*, respectively, clearly demonstrate their role in the establishment and maintenance of epithelial cell polarity. We are currently investigating the pathway in which LAP molecules are involved.

To clearly establish the role of the different domains involved in the basolateral localisation and the function of LAP proteins, we have chosen an *in vivo* approach using *C. elegans* as a model system. LET-413::GFP constructs carrying a series of deletions have been generated and tested for their localisation and rescuing activity. Deletions in the LRR domains of LET-413 lead to a cytoplasmic localisation of the protein. Moreover, a single substitution in one LRR of a conserved Proline residue results in the complete loss of membrane addressing. Conversely, deletions affecting either the LAPSDs or the PDZ domain do not affect the basolateral localisation. LET-413 basolateral localisation is crucial for its function as desmontrated by the fact that none of the mutations leading to cytoplasmic localisation is able to rescue the phenotype of a let-413 null mutant. Surprisingly, deletion of the PDZ domain does not affect the function of LET-413 during embryogenesis.

The same analysis have been performed in mammalian cells for Erbin, a human homologue of LET-413. GFP::Erbin fusion proteins were expressed in Hela cells and their subcellar localisation was observed. This study reveals that the LRR domain of Erbin is necessary for the targeting to the basolateral membranes.

These data indicate that: 1) the LRR, but not the PDZ, domains of LAP proteins are absolutely essential for the basolateral localisation 2) the basolateral addressing of the LAP proteins in epithelial cells, is a prerequisite for their function.

We will present our current model for the adressing of LAP protein to the basolateral membrane by the binding of the LRR domain to a membrane associated ligand.

96. Identification and characterization of the *pvl-1* gene, a member of the PDGF/VEGF family, in C.elegans

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Vascular Endothelial Growth factors (VEGFs) and Platelet-Derived Growth factors (PDGFs) have been recently classified as members of the cysteine-knot growth factor superfamily. Biologically active PDGF and VEGF molecules are secreted either as homodimeric or heterodimeric glycoproteins. PDGFs are potent stimulators of mesenchymal cell proliferation and migration and are essential for vessel wall stabilization. VEGFs induce differentation, proliferation and migration of endothelial cells, in the processes of vasculogenesis and angiogenesis. The molecular mechanisms of action of PDGFs and VEGFs are still poorly understood. We searched the genomic *C.elegans* sequence and identified one possible candidate gene (named *pvl-1*) for a *C.elegans* member of the PDGF/VEGF family. A full-length cDNA from the *C.elegans* collection (Y.Kohara) has been sequenced and shows high percent of identity and similarity with members of PDGF/VEGF family. Our predicted sequence can be aligned best with all members of the PDGF/VEGF family that have a C-terminal 70 amino acid extension and that bind to heparin. Reporter experiments with the presumptive regulatory region of this gene indicate that expression may be confined to a subset of body wall muscle cells in adult and larval stages. We have isolated a putative null allele of *pvl-1* (*ev763*), but we haven`t found any phenotype yet. Considering that *pvl-1* may produce a secreted protein which could act in an autocrine or in paracrine manner, this deletion mutant could have defects in different cell types. We are now checking neuron migrations, using the unc-*129::GFP* (DA, DB, DD, VD neurons) and the *mec-7::GFP* reporters (touch neuron cells). We will continue our experiments to completely define the phenotype of *pvl-1* mutant using other markers. Moreover, we have produced a recombinant pvl-1-His fusion protein in human cells. The purified protein can be tested for its ability to bind the mammalian VEGF/PDGF receptors as well as the *C. elegans* putative receptors (see description in Dalpe et al., WBG 16(5):43, 2001). The functional role of *pvl-1* in the induction of cell-migration as well as its potential angiogenic ability can be assayed in in vitro and in vivo tests.

97. Early cell migrations in the *Caenorhabditis elegans* embryo

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Nematodes were considered to be important paradigms for determinate development, where fates are assigned autonomously by descent and cells are placed by cell cleavages. After it is now generally accepted, that many inductions occur during early embryogenesis of *C. elegans*, it was now also shown that cells in the worm embryo are not placed by cell cleavages but by cell migrations into their terminal positions. Thus the worm is also suited to study the mechanisms of cell migration in the early embryo. It appears that the *C. elegans* embryo is highly regulative not only in cell fate determination but also in positioning cells during pattern formation.
Initial experiments showed that the fate of a cell determines its position in the terminal embryo. If the fate of a cell is altered by a manipulation it redirects its movement and migrates to the same position a cell with this specific fate occupies in the normal embryo. To uncover the mechanisms by which cells find their position in the early embryo, we tested, whether a global signal, guiding posts or specific local cell-cell interactions, corresponding to the normal neighbourhood, guide cells to their positions. Surprisingly neither of the mechanisms alone or any redundant combinations appear to be involved in navigating cells. Removing the eggshell showed, that it does not appear to supply any essential information to guide migrations. Also altering the neighbourhood relations of cells and the combination of cell fates to configurations, which normally never occur in normal embryos, does not affect the sorting by migrations. These manipulations are achieved in either mutant and/or laser ablated embryos. We also combined cells from different embryos in an *in vitro* culture system under a 4D-microscope. We must conclude that a general sorting mechanism exists in the early embryo, which guides cells using information provided by the direct surrounding. Since cells orient themselves properly in any cellular environment, the system appears to be extremely robust. A model for such a general guiding mechanism is discussed.

98. The HOX gene *mab-5* has a conserved function in vulva formation in *C. elegans* and *P. pacificus*.

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To study the evolution of cellular mechanisms involved in vulva formation we compare vulval morphogenesis in two nematode species. One is *Caenorhabditis elegans* (*Cel*; *Rhabditidae)* [1], the other is a satellite model nematode *Pristionchus pacificus* (*Ppa*; *Diplogastridae*) [2]. Here we describe vulva formation in *Ppa* and compare it to what is known for *Cel*. To expand the evolutionary perspective of vulva formation we have characterized and compared HOX gene *mab-5* mutants in both species. To study vulva formation we follow the rearrangement of marked adherens junctions of epithelial cells in vulva primordia using confocal microscopy and 3D computer reconstruction.

The vulva in *Cel-wt* is a hollow organ formed initially by 22 cells that are arranged into a pyramid of seven ring-shaped cells [1]. In *Ppa-wt* eight vulval rings are formed from 20 cells and the same general cellular mechanisms (apical extensions rearrangement, migration to the midline) as in *Cel* are involved. In spite of this similarities there are differences such as different division/migration and migration/fusion rates.

Ppa-mab-5 mutants were previously described as animals generating a posterior pseudo vulva, which is a result of a cell fate transformation and divisions of P8.p cell, that fuses to the hypodermis in wt [3]. We have found that the phenotypes of *Ppa-mab-5* mutants can be grouped into five distinct categories (90% penetrance). Our hypothesis is that the reason to this diversity is the existence of more than one vulval organizer [4]. In 15% of studied hermaphrodites we found unfused P11.p/P12.p cells at the posterior.

To our surprise when we analyzed *Cel-mab-5* mutants we found that 3% of the hermaphrodites express the same bivulvae phenotypes as in *Ppa-mab-5*. This phenotype was not reported previously. As well we found that in 50% of cases there are unfused Pn.p cells at the most posterior area of *Cel-mab-5* mutants. Our findings in *mab-5* mutants of both species suggest that there may be a cellular pathway that involves MAB-5 for vulva induction.

[1] Sharma-Kishore et al. (1999), [2] Sommer et al. (1998), [3] Jungblut and Sommer (2001), [4] Shemer et al. (2000).

99. Regulation by nuclear hormone receptor CHR3 (NHR-25, NF1F4) meets with multiple regulatory pathways on a transcription cofactor, SKIP

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CHR3 (*nhr-23*, NF1F4), the homologue of *Drosophila* DHR3 and mammalian ROR/RZR/RevErbA nuclear hormone receptors, is important for proper epidermal development and molting in the nematode *C. elegans*. Disruption of CHR3 function by RNA mediated interference (RNAi) leads to developmental changes including developmental arrest, incomplete molting and a Dumpy phenotype. CHR3 expression cycles during larval development and disruption of physiological peaks of CHR3 expression triggers molting defects and developmental arrest in all four molts.

We have searched for cofactors which may be involved in CHR3 regulation by RNA interference directed to inhibit proteins predicted in *C. elegans* database as potential homologues of cofactors of nuclear hormone receptors in other species.

RNA interference directed to inhibit a *C. elegans* homologue of SKIP triggered a phenotype almost identical to the CHR3 loss of function. SKIP is a transcription cofactor present in all eukaryots, identified as a cofactor in activation and /or repression of transcription in Notch, Vitamin D receptor and TGF-beta regulation.

Our data show that SKIP is required for Polymerase II dependent transcription of several developmentally regulated genes visualized by expression from GFP containing fusion genes and indicate the functional connection of CHR3 regulatory cascade with multiple regulatory pathways.

100. Studies on the anthelmintic effects of inhibitors of the glycosphingolipid biosynthesis and choline metabolism

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Phosphorylcholine (PC) is a widespread antigenic eptitope of pathogens like parasitic nematodes, which are characterized by the presence of PC-substituted glycosphingolipids and proteins (1). There is evidence that such PC-substituted molecules express multiple immunological effects by influencing signal transduction pathways in B- and T-lymphocytes (2). *Caenorhabditis elegans,* a free-living nematode expressing analogous PC-substituted antigens (3), represents a suitable model for biosynthetic studies on this epitope due due to ist easy handling *in vitro* and fully sequenced genome. Cultivation of *C. elegans* in a chemically defined medium in the presence of various inhibitors of glycosphingolipid biosynthesis and choline metabolism displayed pronounced effects on the development of the worms and fertility. Such inhibitors might, therefore, be regarded as potential candidates for the development of anthelmintics.

1. Lochnit G *et al* (2000) *Biol Chem* **381:** 839-47
2. Harnett W *et al* (2000) *Mod Asp Immunobiol* **1:** 40-42
3. Gerdt S *et al* (1999) Eur J Biochem **266**:952-963

101. Genetic analysis of the Rap1-pathway in C. elegans

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The family of Ras-like GTPases functions in diverse signal transduction routes in which they transmit signals received by cell surface receptors to downstream effector molecules. Despite a high level of homology and conservation during evolution, the function of most Ras-like GTPases other than Ras remains poorly defined. This also holds for Rap1, for which functions have been suggested as diverse as antagonizing Ras, regulation of secretion and activation of integrins (1). In vertebrates, Rap1 is activated by several extra-cellular stimuli via at least three different second messengers, namely calcium, cAMP and diacyl-glycerol. Currently, four types of Rap-specific guanine nucleotide exchange factors have been reported and a single homologue for each of these GEFs can be found in the *C. elegans* genome. In addition, two Rap1 homologues (*rrp-1* and *rrp-2*) and a single Rap2 homologue are present. Our goal is define the function of Rap1 by mutating all of these genes and by investigating the consequences of over-expression of activated or dominant negative versions of their gene products.

PXF-1 (T14G10.2) is a Rap-specific GEF, which is homologous to the mammalian PDZ-GEF. *pxf-1* encodes at least three isoforms, which share a common catalytic domain. Green fluorescent protein (GFP) reporter construct demonstrate that *pxf-1* is expressed in the hypodermis, gut, various neurons and somatic cells of the distal gonad. Particularly striking is the oscillating expression of the downstream promoter in the pharynx during the four molting stages L1 to L4. Worms homozygous for a mutation in *pxf-1*, that deletes part of the PDZ-domain and the complete catalytic domain, hatch normally but develop slower and move uncoordinated. They have severe problems in removing the old cuticle during molting. In addition, vesicles are frequently formed underneath the cuticle, indicating a defect in cell-matrix or cell-cell interaction. Most animals die before adulthood, but those that reach this stage are largely sterile. We suspect that the molting phenotype results from malfunctioning of the seam cells, which loose expression of the SCM marker during larval stages and form discontinuous alae.
The *pxf-1* mutant phenotype is different from that of its putative downstream targets *rrp-2* and *rap-2*: worms mutant for either one or both of these GTPases seem fully viable (2). In collaboration with Dr. J. Culotti we are currently trying to isolate a *rrp-1* mutant, which may be the crucial PXF-1 target or function in a redundant fashion with the other Rap-like GTPases.

1. Bos, J.L., de Rooij, J. and Reedquist, K.A. Nat. Rev. Mol. Cell. Biol. 2001 (5) 29-77
2. A rap-2 mutant was kindly provided by the Moerman lab (Gene knock out consortium)

102. Positive selection of mutants with increased resistance and longevity.

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We developed selective conditions for long-lived mutants by subjecting the first larval stage to thermal stress. At the moment, we have isolated a total of 64 thermotolerant mutants, 50 of them exhibited increased longevity. All the known classes of longevity mutants affected in the dauer signaling pathway were found (Unc, Dyf and Daf-c). 7 of them fail to complement *daf-2*, 5 *age-1*, and 22 *unc-31* all of them are known genes involved in the regulation of longevity. We also isolated 2 long-lived alleles of *dyf-2* and one of *unc-13*, those genes were previously identified by other reasons and not related to longevity before. Thirteen additional mutations identified at least four new genes. We call this new mutant class Liv mutants (Long lIved and viable after thermal stress).
One of these mutants is *liv-6*. This mutant resembles the *age-1* reference allele, with Daf-c phenotype at 27ºC, long-lived and similar levels of thermotolerance. *age-1* encodes a phosphatidylinositol 3 kinase (PI3K). *liv-6* was mapped to the left arm of chromosome I, in the region where the only PI3K adapter subunit worm homologue is located. Sequencing of this gene in the mutant strain and rescue experiments demonstrated that *liv-6* encodes for the PI3K adapter subunit.

103. Functional characterisation of mammalian syndecan homologue in the *C.elegans* nervous system

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The syndecans are a family of transmembrane heparan sulphate proteoglycans (HSPGs). There are four distinct vertebrate syndecans, one or several of which are expressed in all adherent cells. Syndecan homologues have also been cloned from *Drosophila*, *Xenopus*, and *Siona savignyi*.
Studies on the vertebrate syndecans using mice models have revealed important biological functions for them. These range from mediation of cell adhesion to defence against pathogens, regulation of neuronal plasticity and response to physiological stimuli. In several cases, studies using syndecan null or mutant mice have, however, revealed very subtle and sometimes unexpected phenotypes, suggesting that other members of the syndecan family may compensate for the loss of one member of the family.
The genome of *C.elegans* contains a single homologue of the mammalian syndecans thus providing an excellent model to study syndecan function *in vivo*. We have cloned and sequenced the *C.elegans* syndecan (F57C7.3) cDNA and genomic DNA and shown that the predicted genomic structure is correct. Northern analysis reveals a single mRNA for *C.elegans* syndecan. Whole mount immunohistochemistry studies together with translational and transcriptional GFP expression reveal that syndecan expression is restricted to neuronal cells in *C.elegans*. Syndecan localises specifically to a set of neuronal cell bodies and their processes in the nerve ring and to a set of neurons in the tail region. The specific identity of these neurons is currently under study. RNAi of syndecan causes uncoordinated movement of animals, especially backward movement is affected. The posterior parts of the animals coil when they try to reverse. Syndecan RNAi also causes defects in the development of the gonad and vulva leading to defects in egg laying. The specific nature of these defects is currently under study.

104. Sensory neuron Ca^{++} imaging 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 and 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 as well as looking at 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 a novel calcium sensor called cameleon (Kerr et al. 2000). Camaleons are genetically-encoded fluorescent indicators for Ca^{++} in which a shortwavelength 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). We decided to use this approach and we started by investigating the activity of the sensory cells. Using a cell specific promoter, we have generated lines expressing camaleon in the ASH sensory neuron (*osm-10*::YC2.3) and the Ca^{++} influx of this neuron has been imaged after stimulation with different chemical repellents including high osmotic strength, copper ions, SDS and quinine. As complementary strategy, we are producing cell cultures of the same transgenic strain to isolate in vitro ASH sensory neuron expressing camaleon and compare its responses to the selected repellents.
The outcome of these experiments will be presented.

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.

105. Investigating the functional role of candidate neuropeptide activated GPCRs in *C. elegans* using RNAi.

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Neuropeptides are a diverse family of signalling molecules, which play a critical role in neurotransmission in both vertebrates and invertebrates. In the nematode *Caenorhabditis elegans* several distinct families of neuropeptides have been identified including the ubiquitous FMRFamide related peptides (FaRPs) whose members are characterized by containing the COOH-terminal RF-amide (Arg-Phe-NH_2) motif. 22 FMRFamide-like peptide (flp) genes have been identified in *C. elegans*, which encode over 50 possible FaRPs. More recently 32 non FMRFa-like peptide genes have been identified encoding 151 further putative neuropeptides, including several peptides showing homology to the invertebrate neuropeptides buccalin and myomodulin. The majority of neuropeptides are thought to mediate their effects through G-protein coupled receptors (GPCRs). Peptide activated GPCRs have been identified in invertebrates and vertebrates. However, despite the wealth of information regarding the identity of specific neuropeptides and their physiological actions in *C. elegans* and other nematodes, the role and identity of the receptors mediating these events is unknown. To address this we set out to identify and characterize the physiological role of neuropeptide activated GPCRs using reverse genetics.

We identified approximately 70 candidate neuropeptide activated GPCRs in *C. elegans* based on their comparison to known peptide activated GPCRs. To assess the functional role of these GPCRs we used RNA-mediated genetic interference (RNAi). RNAi was induced by feeding L4 stage worms with bacteria engineered to express double stranded RNA corresponding to short regions of target gene RNA. Animals were phenotyped 72-96h later using a variety of standard behavioural assays.

The results from this initial screening identified several receptors having important roles concerning locomotion, egg-laying and viability. In addition, we are employing reverse pharmacological methods to identify the respective ligands for these receptors as part of a long term strategy to investigate the identity and function of specific ligand::receptor pairs in *C. elegans*.

Supported by the BBSRC.

106. Maternal EMB-29 is required during early development

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The mutant *emb-29* was originally isolated several years ago and many laboratories have identified numerous alleles. The most characterised alleles are temperature sensitive which give rise to defects in the timing of several cell divisions in early embryogenesis[1]. The cells finally stop dividing at approximately the 150-cell stage when they are thought to be arrested in the G2 phase of the cell cycle[2]. Several attempts by different laboratories have been made to clone *emb-29*. A likely candidate is the cell cycle regulator gene *cdc-25.2*. Homologues of this gene in other organisms have been found to control the transition from G2 to M phase. Unfortunately, *cdc-25.2* (RNAi) gives larval lethality instead of the predicted embryonic lethality. Furthermore, two overlapping cosmids that do not contain *cdc-25.2* are able to rescue *emb-29*. None of these cosmids contain obvious cell cycle regulators. It is not until the expression of two *cdc-25* homologues (*cdc-25.2* and *cdc-25.3*) was disrupted simultaneously by RNAi that the embryos appeared more Emb-29-like. Interestingly, if the expression of another homologue, c*dc-25.1*, is disrupted in an *emb-29* mutant, no cell divisions were ever observed resulting in a single-cell embryo phenotype. This single-cell phenotype is similar to when all the known *cdc-25* genes that are expressed in the hermaphrodite are disrupted at the same time, but with some subtle differences. For example, meiosis never occurred in the triple *cdc-25* (RNAi) treated embryos or in the *emb-29* embryos treated with *cdc-25.1* (RNAi). In the triple *cdc-25* (RNAi) treated embryos the maternal chromatin began to unravel from a tightly congressed metaphase-like state and migrated to the centre as the embryos grew older; tubulin also slowly disassociated from the meiotic spindle and took on a interphasic appearance. Although the condensed maternal chromatin and meiotic spindle was slowly lost in the *emb-29* embryos treated with *cdc-25.1* (RNAi), centrosomes continued to duplicate and multiple nuclei accumulated despite the absence of cell divisions.

1 Denich *et al*. 1984 Rouxís Arch. Dev. Biol. **193**, 164-179
2 Hecht & Berg-Zabelshansky 1987. J. Cell. Sci. **87**, 305-314

107. Investigating the COP9 signalosome using *C. elegans* and *S. pombe*

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The COP9 signalosome is a 450 kDa conserved nuclear protein complex that has been found in plants, mammals, insects and yeast. The complex is composed of eight distinct subunits, designated CSN1 to CSN8, and structurally resembles the lid of the 26S proteasome. The complex was first identified in *Arabidopsis thialana* through the isolation of mutants that mimic light-induced seedling development when grown in the dark. These mutants were known as constitutive photomorphogenesis (COP) mutants, the majority of which were found to correspond to subunits within this complex. In *Drosophila*, the homologous complex is essential for embryo development. In *S. pombe* several subunits of the complex were found to be important for S-phase progression. Despite this, the exact function of the complex remains largely unknown.

Signalosome subunits interact with cullins, core components of SCF ubiquitin ligases which function in ubiquitin-dependent protein degradation mediated by the 26S proteasome. Although phosphorlyation is one major determinant of SCF's activity, covalent attachment of the peptide NEDD8 to the cullin subunit is often required. The COP9 signalosome is believed to have a denedylation activity, as mutant *S. pombe* cells accumulate neddylated cullins. The complex and its corresponding subunits also interact with a wide range of proteins including c-Jun, p105, IκB, $p27^{Kip1}$, a number of nuclear hormone receptors and the cytoplasmic domain of the integrin LFA-1. These observations suggest that the signalosome regulates multiple pathways via the protein degradation machinery.

We have identified homologues for seven *csn* subunits in *C. elegans*, and have systematically disrupted the expression of these subunits using RNAi administered by both feeding and injection. The resulting phenotypes were pleiotropic and included sterility, slow growth, larval arrest and lethality. Most notable was *csn-7* which showed 100% penetrance and exhibited lethality and sterility. *csn-3* and one *csn-2* homologue displayed no phenotype. The RNAi-induced sterility observed for all *csn* subunits was due to a germline proliferation defect. Corresponding work in *S. pombe* has shown that *csn1* and *csn2* mutants are sensitive to DNA damage and exhibit slow DNA replication. Consequently, we are investigating the sensitivity of these RNAi-treated nematodes to DNA damage. We are also generating a *C. elegans* deletion library in the hope of isolating knockouts mutants. Future plans include investigating the inferred deneddylation activity, over expressing CSN subunits, performing suppressor screens and monitoring *in situ* expression patterns. We also plan to make use of *csn* gene knockouts recently available in *S. pombe* to further characterize the signalosomeís activity in yeast.

108. Characterization of Neuropeptide Receptors in *Caenorhabditis elegans.*

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Neuropeptides are classical neurotransmitters that also act as neuromodulators. The family of peptides most studied in *C. elegans* is the FMRFamide-like peptides encoded by *flp* genes. Little is known of their function although it is thought they have a role in locomotion, egg laying and pharyngeal pumping. The aim of this project is to characterize neuropeptide signalling and identify their receptors.

It is believed that neuropeptides act through G protein coupled receptors (GPCR). Sequencing of the *C. elegans* genome has revealed 140 non-olfactory GPCRs, approximately half of which are likely to encode for receptors that have a neuropeptide ligand. To increase the confidence level that the genes are indeed neuropeptide receptors, an extensive database analysis was conducted using Edinburgh Biocomputer Systems. Using the dsRNAi approach, a number of candidate GPCRs were screened for neuropeptide related phenotypes such as locomotion, egg laying and lethality. The initial screen revealed various abnormalities. Of the genes screened, 10 showed possible egg laying defects. Characterization of these genes is currently underway in order to identify their role in the egg laying pathway.

109. Identification in *Caenorhabditis elegans* of cross-reacting carbohydrate epitopes of *Schistosoma mansoni* immunogens by immunocytological staining.

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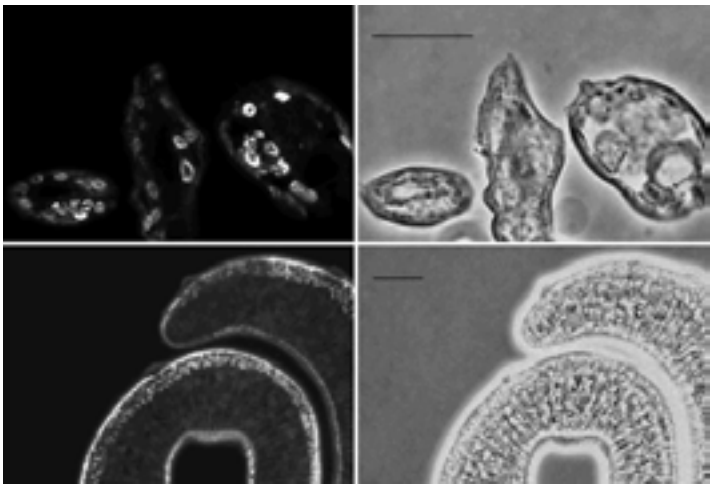
Schistosomiasis is a major human parasitic worm infection in sub-Saharan Africa and parts of South America. We have studied the immune response in mice experimentally infected by *Schistosoma mansoni*, and identified some cross-reacting carbohydrate epitopes recognized by sera from infected individuals. Using monoclonal and polyclonal antibodies as reference, we could identify one such immunogenic epitope as Gal(beta1-3)GalNAc-O. This epitope is also expressed in carcinomas due to incomplete glycosylation and known to be an onco-fetal antigen.

We here describe the reactivity of monoclonal antibodies (MoAbs) against the Gal(beta1-3)GalNAc-O epitope with *Caenorhabditis elegans* using immunofluorescence to localize cells in frozen and paraffin sections. Antibodies were generated by immunization with keyhole limpet hemocyanin (KLH) and asialoglycophorin (ASG) two proteins known to express the Gal(beta1-3)GalNAc-O epitope. These antibodies reacted with fibrillar intercellular somatic and tegumental material and with excretory ducts of *S. mansoni*.

The results show that the *C. elegans*-cross-reactive Gal(beta1-3)GalNAc-O epitope is expressed at the surface of scattered individual somatic cells and groups of cells (which remain to be identified). The reactivity of MoAb 1B8 on sections of C. elegans (upper set) and S. mansoni is seen in *Figure*) We also show that some so far unidentified KLH-cross reactive carbohydrate epitopes are expressed in *C. elegans* gut epithelium and at the surface of muscle cells.



110. The human and *C.elegans* p66 NuRD chromatin remodelling components: transcriptional repression and SynMuv activities

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We are currently using the *C. elegans* vulva as a model to study chromatin remodelling activity. An important chromatin remodelling complex for vulva specification is NuRD. Studies in mammalian cells has shown that the NuRD complex contains ATP-dependant nucleosomal remodelling activity and histone deacetylase activity and it is thought to be involved in transcriptional repression. Vulval development requires positive and negative signals: three of the six vulval precursor cells (VPCs) are positively induced to have a vulval fate by Ras signalling, and an antagonising activity from the SynMuv (synthetic multiple vulvae) genes prevents vulval cell determination of the remaining three VPCs. There are two groups of SynMuv genes, SynMuvA and SynMuvB, which act redundantly to repress vulval development.
Simultaneous inactivation of one gene in each class results in ectopic vulval development through inappropriate Ras activation. Inactivation by RNAi of the NuRD complex components in a SynMuvA or SynMuvB mutated genetic background has been shown to cause a Muv phenotype. The NuRD components that have been tested so far appear to be part of either the SynMuvA or the SynMuvB pathway and in some cases both. One component of the NuRD complex, EGL-27, is a SynMuvA gene and related to human MTA1 (metastasis-associated gene 1). To try identifying EGL-27 interacting partners, a two-hybrid screen was undertaken and the *C.elegans* homologue of the NuRD component p66 was found. This gene has a predicted GATAlike zinc-finger at its C-terminal end. RNAi by injection results in lethality, so to analyse vulva development, RNAi by feeding was used. We found that p66 inactivation in wild type animals results in a weak Muv phenotype (3%) and that this phenotype is strongly enhanced (65%) in a SynMuvB background. We have also tested the transcriptional activity of two human p66 homologues by transfection assay in human 293T kidney cells and have found that both efficiently repress transcription when fused to GAL4 DNA binding domain. Testing of *C.elegans* p66 is in progress. Therefore, p66 is a new SynMuvA gene and its human counterparts can repress transcription, supporting the idea that NuRD is involved in repression of vulval development genes. Finally, because the vulva is a good paradigm for studying interplay between the Ras-signalling pathway and signals antagonising its effects, we are using our nearly comprehensive RNAi library to screen for new SynMuv genes.

111. A role for embryonic polarity components in the development of the *Caenorhabditis elegans* vulva

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The incomplete penetrance of RNAi by feeding allows the identification of hypomorphic phenotypes for genes whose mutants are embryonic lethal. In the course of a large scale RNAi screen in our lab[1] it was observed that a number of loci implicated in embryonic polarity also appear to play a role in vulval development. Specifically, RNAi of CDC-42, PAR-3, PAR-6 and PKC-3 results in a Multivulval (Muv) phenotype in a proportion of the "survivors" which manage to complete embryogenesis.

An EGF (LIN-3) signal from the gonadal anchor cell (AC) induces a vulval fate in three of the overlying vulval precursor cells (VPCs) through the Ras signalling pathway. Classical Muv phenotypes arise as a result of the induction of more than three VPCs by hyperactivity of this pathway or the disruption of an inhibitory pathway (SynMuv A and B pathways). In addition, Muv phenotypes are observed in certain reduction of function mutants of the EGF receptor (LET-23) which is polarised basally in the VPCs and may play a role in limiting the range of LIN-3 signalling as well as transducing it.

PAR-3, PAR-6 and PKC-3 form a complex which is localised to the anterior cortex of the embryo and is required for the generation of wild type asymmetries in the first mitotic division. CDC-42 appears to positively regulate this complex through interaction with PAR-6. All four proteins are conserved in flies and mammals where they have been shown to play roles in epithelial polarity and junction formation. As the VPCs which give rise to the vulva are part of a polarised epithelium, it is tempting to speculate that this conserved complex is required for the polarity and/or junctional coherence of this tissue. If this is the case then disruption of these genes could lead to a Muv phenotype by various means, including mis-localisation of LET-23 (and hence increased range of LIN-3).

We have shown that RNAi of these genes does indeed cause hyperinduction of the VPCs and that junctions are disrupted to some extent. We are currently investigating the cause of these defects, their dependence on the members of the Ras signalling pathway and their effect on the polarity of the VPCs.

[1] Fraser et al, Nature 2000, 408: 325-330; Kamath et al, in preparation

112. Identification of genes implicated in ubiquinone biosynthesis in *C. elegans*

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Aging is a complex term to define all the physiological changes accumulated with time that lead organisms to death. Many genes have been related with the control of aging in *C. elegans*. These include different classes as *daf, age,* or *clk* genes. At the biochemical level it has been reported that aging induces oxidative damage and vice versa. Antioxidants have been proved to modulate aging rate in some organisms such as worms, flies and mammals, probably by preventing oxidative damage. Ubiquinone (coenzyme Q) is an ubiquitous lipid antioxidant found in all aerobic organisms studied so far, and the balance between its endogenous content and the dietary uptake modulate life span.

We have identified by RNAi silencing, eight genes involved in ubiquinone (Q_9) biosynthesis in the nematode *C. elegans*. These genes are homologues to those described previously in *S. cerevisiae* and include *clk-1*, previously implicated in this biosynthetic pathway, and Y57G11C.11/ *coq*-3 whose deletion in *C. elegans* is lethal and leads to sterility.

Silenced animals show lower levels of both Q_9 and Q_8 provided by dietary *E. coli* strains. *C. elegans* also contained rhodoquinone (RQ_9) and the interference on some of these genes also affects its concentration. Unlike clk-1 mutants, none of the interfered animals accumulated the Q-biosynthetic intermediate demethoxy-Q_9 (DMQ_9), movement or pharyngeal pumping, were not significantly affected. Q_9 levels reverted after diet restitution based on non-interfering *E. coli* strains. Worms silenced in one of these genes showed an extended life span compared with non-interfered animals, but either movement or pharyngeal pumping was not significantly affected.

These results suggest that proteins encoded by genes C24A11.9, F57B9.4, Y57G11C.11, T03F1.2, ZK652.9, K07B1.2, ZC395.2 (*clk-1*) and C35D10.4, are involved in Q_9 biosynthesis in *C. elegans*.

113. Dissecting the complexities of *C. elegans* DAF-2 insulin/IGF like receptor signalling pathway

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An insulin/IGF-like signalling pathway in *C. elegans* plays a role in early development, the dauer/non-dauer decision, fertility and life span (1). Components of this pathway identified by genetic analysis include DAF-2, a homologue of the vertebrate insulin and IGF-I receptors, AGE-1, a subunit of phosphoinositide 3-kinase (PI3K), DAF-18 (PTEN) phosphatase, PDK-1(phosphoinositide-dependent kinase), AKT-1 and AKT-2 (protein kinase Bs) and DAF-16, a forkhead transcription factor. Mutations in *daf-2* or in *age-1* cause dauer larva arrest, and can double adult life (2-4). Although this signal transduction pathway between *daf-2* and *daf-16* has been well defined, several findings suggest the existence of a second *daf-2-daf-16* pathway. For example, phenotypic analysis of 15 *daf-2* mutants, which fall into two distinct classes, 1 and 2, suggested that the gene has two functional components, *daf-2A* and *daf-2B* (4). These potentially correspond to different signalling pathways emanating from *daf-2* into the cell. In addition, a gain-of-function allele of *akt-1*(5), and the hypomorphic allele *daf-18(e1375*), fully suppress the dauer constitutive (Daf-c) phenotype of several severe *age-1* alleles yet only weakly suppress the relatively weak allele, *daf-2(e1370*).
To investigate and characterise the putative second *daf-2* pathway, we have carried out interaction studies between several class 1 and class 2 *daf-2* alleles and various components of the PI3K and other signalling pathways. Results of these interaction studies together with a revised model of *daf-2* signalling will be presented.

(1) Guarente, L., Kenyon, C. *Nature* 408: 255 (2000). (2) Kimura, K.D. *et al. Science* 277: 942 (1997). (3) Morris, J.Z. *et al. Nature* 382: 536 (1996). (4) Gems, D. *et al., Genetics* 150: 129 (1998). (5) Paradis, S., Ruvkun, G. *Genes Dev*. 12: 2488 (1998).

114. The EH network in *C.elegans*

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The EH domain is an evolutionary conserved protein-protein interaction domain present in proteins from yeast to mammals. A number of its cellular ligands, together with its binding specificity, the NPF tripeptide, have been identified and demonstrated to define a complex network of protein interaction in eukaryotic cell. Many of the EH-containing and EH-binding proteins display characteristics of endocytic accessory proteins, suggesting a role for the EH network in regulation of various step in endocytic processes and, more in general, in protein sorting within the cell. Since endocytosis is a mechanism by which cells interact with the environment, interesting aspects of this process differ between unicellular and multicellular organisms.
Our final goal is to define the functions of EH-containing proteins and their binding partners in a multicellular organism contest.
In *C.elegans,* two EH-containing proteins have been identified and characterized:
EHS-1 (Salcini et al. Nature Cell Biology, Vol.3, August, 2001) and RME-1 (Grant et al. Nature Cell Biology, Vol. 3, June, 2001) homologues of Eps15 and EHD-1, respectively.
The first involved in synaptic-vesicle recycling, the second in yolk uptake in oocytes.
Screening of *C.elegans* data bank revealed at least two other EH-containing proteins: Y39B6A.G, with no homology except in the EH domain, and Y116A8C.36, most likely the homologue of the Drosophila DAP160, a dynamin-binding protein localized in synapses.
It is our interest to characterize the binding proprieties of EH-containing proteins, to create a map of the EH-NPF network and investigate the roles of these proteins in *C.elegans*. To this end, EH domains of the four *C.elegans* proteins will be used as baits in two-hybrid screen to clone interacting proteins. *In silico* interactors with NPF repeats, such Epsin protein, a known binding partner of Eps15 in mammals, or Amphiphysin protein, involved in mammals in endocytic processes, will be also analyzed.

115. A multifaceted approach to elucidate the regulation of ciliogenesis in *C. elegans*

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Cilia are evolutionarily conserved subcellular organelles functioning in cell motility, movement of extracellular fluids, sensory perception (e.g. smell) and determination of left-right asymmetry. While a great deal is known about the structure, function and motility of cilia, very little is known about the molecular mechanisms that regulate ciliogenesis in a cell-type specific and developmental manner. How do cilia become functional? How can they do what they do?

The gene *daf-19* was shown to be essential for sensory neuron ciliogenesis in *C. elegans*.
It encodes a member of the RFX family of transcription factors. By acting through its target site in promoter regions, the x-box, DAF-19 appears to be the main regulator of a number of effector genes involved in sensory cilium formation and function, including *che-2*, *osm-1*, *osm-5*, *osm-6* and *odr-4*.

Our major aim is to define the complete set of DAF-19 target genes coding for cilia components and determine their structural/functional roles in cilia formation. This is based on ongoing genome-wide screens in *C. elegans*, *C. briggsae* and *Drosophila* for genes containing consensus DAF-19 or RFX-type binding sites in their regulatory regions. Candidate genes (*xbx* genes) are being validated in *C. elegans* by examining their expression in *daf-19* mutants. Their role in cilia is being addressed by using RNA interference, by generating deletion mutants and by interaction screens using already known cilia components. The first gene identified and analyzed with this approach, *xbx-1*, shows similar mutant phenotypes to previously identified DAF-19 target genes (e.g. *osm-6*). Additional aims are to perform structure-function analysis of DAF-19 and to use genetic screens to identify other factors that cooperate with DAF-19 in ciliated sensory neuron differentiation. To further characterize the set of genes that comprise the cilium formation module we are (in collaboration with computer scientists) searching through promoter sequences of selected subsets of cilium specific and *xbx* genes for other conserved promoter elements using *C. elegans* and various other (including human) genome information.

Elucidation of the conserved regulatory pathways controlling ciliogenesis will advance our basic knowledge on a highly conserved organelle (“after all, worms smell and taste just like humans”) and will contribute to our understanding of human pathologies implicating cilia.

116. *Ten-1* is involved in sperm development in *C. elegans.*

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The ten-m gene was identified by Baumgartner et al. [1994] in *Drosophila*. It is the first pair rule gene that is not a transcription factor but a cell surface protein. In addition to the early embryonic expression it is also expressed in the developing nervous system of the fly. Members of the Ten-m protein family were also identified in vertebrates and have been named “teneurins” [Minet et al., 1999]. Vertebrate teneurins are highly expressed in the nervous system. They are type II transmembrane proteins. The Nterminal, intracellular part is followed by a single transmembrane domain. The extracellular domain is around 2500 aa and contains EGF-like repeats. The *C. elegans* ten-1 is located on chromosome III and is mapped to the cosmid R13F6. By 5’-RACE analysis we identified an alternative transcription start located 8 kb upstream of the predicted one. Both ten-1 forms are expressed early in *C. elegans* morphogenesis. In post-embryonic development the protein from the upstream promoter is expressed in the developing somatic gonad, the pharynx and a subset of neurons. The protein is also expressed in the germ line, both in developing oocytes and sperm. The protein from the downstream promoter in adult worms has a broad neuronal expression. RNAi aimed against ten-1 transcripts results in a low penetrance phenotype affecting worm morphogenesis in various stages of development. To further analyze the function of teneurin in *C. elegans* we screened a mutant library for deletions in the *ten-1* gene. No mutant carrying a deletion of the downstream transcription start that would result in a complete null could be rescued. However, a mutant carrying a deletion of the upstream transcription start including the four first exons was recovered. We conclude that this results in a null mutation of the longer form of ten-1. Heterozygote worms show variable embryonic and larval lethality. Adult worms show defects in gonad migration. Worms have little or no sperm. Sperm is often pushed to the uterus by ovulating oocytes. Ovulation is defective resulting in ruptured oocytes and abnormally small embryos.
Endomitotic oocytes form in the uterus. Worms are constipated. No -/- embryos develop suggesting that Ten-1 is involved in oocyte and/or sperm development. +/- males are fertile and have +/- progeny suggesting that at least in males Ten-1- sperm develop. This phenotype is reminiscent of *spe-8, spe-12, spe-27* and *spe-29* mutants that are defective in spermatid maturation. We are testing the hypothesis of Ten-1 involvement in spermatozoon development.

117. The Mi-2 chromatin-remodelling proteins LET-418 and CHD-3 act negatively on the Hox LIN-39 expression levels and permit correct vulval precursor cell fate determination in *C.elegans*

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The *C.elegans* Mi-2 chromatin remodelling proteins LET-418 and CHD-3 possess both shared and unique functions during vulval cell fate determination. One of these include the involvement of LET-418 and CHD-3 in the proper execution of the 2° cell fate of the vulval precursor cells P5.p and P7.p, a process that depends on Lin-12/Notch signalling (von Zelewsky, T. *et al.,* 2000; see accompanying abstract from Zhang, Y. and Müller, F.).
In *let-418*;*chd-3* double mutants, 30% of the P5.p and 40 % of the P7.p descendants are detached from the hypodermal syncitium, a feature typical for the 1° sublineage, suggesting that P5.p and P7.p adopted a 1°-like or a 1°/2° hybrid cell fate (von Zelewsky, T. *et al.*, 2000). We have looked at the expression pattern of *lin-39* in P5.p to P7.p in the *let-418*; *chd-3* mutant background. In wt animals, LIN-39 levels are high in P6.p and lower in P5.p/P7.p. Interestingly, we found that in *let-418; chd-3* double mutants, LIN-39 expression was significantly increased in P5.p and P7.p, and reached a level similar to the one of the 1° vulval fate found in P6.p of wt animals. Furthermore, we observed that *lin-39(lf)* mutations suppress the *let-418* Evl phenotype, suggesting that LET-418 acts upstream or in parrallel of LIN-39. Moreover, absence of LET-418 in a *lin-39* sensitized mutant background increased the frequency of worms with an everted vulva and decreased the frequency of vulvaless worms. Altogether, these results are consistent with the hypothesis that a Mi-2/NuRD complex could have a direct or indirect regulatory effect on LIN-39 expression.

von Zelewsky, T. *et al.*, 2000, *Development* 127, 5277-5287

118. Analysis of early *ceh-13* expression and anterior embryonic patterning

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The *C. elegans labial/Hox1* type gene *ceh-13*, in contrast to some other members of the *C. elegans* Hox cluster, is required for viability, in particular for the proper organization of anterior structures during embryogenesis[1]. Since spatiotemporal *ceh-13* expression appears to be controlled at the level of transcription[1,2] we have undertaken a deletional and mutational promoter analysis[3]. In order to complement these earlier studies we have cloned and preliminarily sequenced the *ceh-13* ortholog from *C. remanei* (*Cr-ceh-13*) and compared its sequence with *Ce-ceh-13* and with *ceh-13* from *C. briggsae (Cb-ceh-13*) that has recently been sequenced by the *C. briggsae* sequencing consortium[4]. For the moment we are concentrating our promoter analysis on a 740 bp element that acts as an early enhancer (enh740)[3]. Within this fragment we have identified two short elements of about 70 bp each that appear to be important for the proper function of enh740. One of them contains a highly conserved sequence element. Currently we are performing a yeast one-hybrid analysis with these two regions as baits. The results will be presented.

In a more general approach to learn more about anterior patterning we performed a genetic screen for mutations whose phenotypes resemble the one of *ceh-13(sw1)*. We are in the process of characterizing and mapping three mutations (*sw2, sw3,* and *sw4*) that define three different genes.
sw2 mutants arrest as larvae and display a disorganized anterior. *sw2* maps to the left arm of the X.
sw3 is an embryonic or early larval lethal that maps to the right arm of V. The animals that manage to hatch die within a short time and are variably, but always severely, disorganized.
sw4 is an about 50% penetrant larval lethal and maps to the cluster on V. 100% of the early larvae display a clearly enlarged anterior. Nevertheless, many of them grow up to become Dpy looking older larvae and fertile adults that lack the anterior part of the pharynx.

[1)] Brunschwig et al. (1999) Development 126:1537-1546
[2)] Wittmann et al. (1997) Development 124:4193-41200
[3)] Streit et al. (2002) Dev. Biol. 242:96-108
[4)]Gautron et al. (2001) WBG 17:62

119. Screens to identify positive and negative regulators of neurotransmission

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The drug Aldicarb inhibits acetylcholine-esterases preventing the removal of the neurotransmitter Acetylcholine (ACh) from the synaptic cleft. The build up of ACh results in hypercontraction of muscles, paralysis and eventual death of animals. Mutants unable to release ACh are resistant to Aldicarb and screens for Aldicarb resistance, performed by many groups, have greatly increased our understanding of the essential components of the synaptic release cycle. However, less is known about the signalling pathways that impinge upon the synaptic release cycle to alter rates of ACh release. We are interested in defining the regulators of the synaptic release cycle using 2 genetic approaches based on our (and others) previous work demonstrating that serotonin inhibits ACh release in a diacylglycerol kinase DGK-1 dependent manner[1-4]. Activation of DGK-1 causes the secondary messenger diacylglycerol to be phosphorylated to form phosphatidic acid and this is believed to cause the loss of the DAG binding protein UNC-13 from release sites. UNC-13 is essential for neurotransmitter release [5] and it's enrichment at release sites correlates with an increase in neurotransmitter release. In the first screen we have slowed ACh release by over-expressing DGK-1 [dgk-1(xs)] resulting in reduced rates of locomotion. We hope to identify either positive regulators of DGK-1 or negative regulators of the synaptic release cycle as a whole by identifying suppressors of the reduced locomotion phenotype. We have presently screened 3906 haploid genomes and have identified 8 candidates of which 4 are strong suppressors of DGK-1 over-expression. We are currently mapping these suppressors using single nucleotide polymorphisms (SNIP-SNPs) and have associated 2 of these suppressors to the left arm of chromosome 1. In the second screen we have increased the rates of ACh release by the addition of the serotonin antagonist methiothepin which results in hypersensitivity to Aldicarb. We are identifying animals that are unable to sustain high levels of ACh release caused by methiothepin but which are normal for ACh release in the absence of methiothepin. We have screened 1330 haploid genomes and have identified 9 suppressors one of which has been mapped to the left arm of X by SNIP-SNPs. These mutants are candidates for positive regulators of ACh release.

1. Nurrish et al., (1999) Neuron:24 231-242
2. Lackner et al., (1999) Neuron:24 335-346
3. Hajdu-Cronin et al., (1999) Genes Dev 13: 1780-1793
4. Miller et al., (1999) Neuron 24: 323-333
5. Richmond et al., (1999) Nat.Neurosci 2: 959-964

120. Identifying regulators of DGK-1

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We (and others) have previously described two competing G-protein pathways acting in motor neurons to either facilitate or inhibit release of the neurotransmitter Acetylcholine (ACh) at neuromuscular junctions (NMJs) [1-4]. Inhibition of release requires a diacylglycerol kinase (DGK-1) which phosphorylates DAG to Phosphatididic Acid. Activation of DGK-1 is believed to lower levels of DAG causing the loss of the DAG binding protein UNC-13 from release sites. UNC-13 is essential for neurotransmitter release [5] and it's enrichment at release sites correlates with an increase in neurotransmitter release.

The diacylglycerol kinases form a highly conserved family of proteins each possessing different protein-protein and protein-lipid interaction domains in addition to the kinase domain which defines the family. Evidence from mammalian DAG kinases suggests that they can be regulated by changes in localization and/or kinase activity [6] but the signaling pathways involved have not been identified. To understand DGK-1 regulation we are taking 4 approaches. Firstly we are conducting structure function studies to determine which DGK-1 domains are vital for inhibition of ACh release. Secondly we are using a yeast two hybrid screen to test for protein-protein interactions with DGK-1. Thirdly we have generated a rescuing MYC tagged GFP::DGK-1 construct. The myc-DGK-1 protein can be immunoprecipitated from adult animals and possesses DAG kinase activity. We are currently testing whether other proteins are coimmunoprecipitated with DGK-1. Finally using the GFP-DGK-1 construct we are testing whether DGK-1 sub-cellular localization is regulated.

In addition to DGK-1 there are another 4 DAG kinases in the *C.elegans* genome, each of which has a highly conserved homolog in vertebrates: F46h6.2, F42a9.1, F54g8.2, and K06a1.6. A deletion in F46h6.2 (*dgk-2*) has been identified by the knockout consortium and it appears to have no obvious defects. Full length cDNAs for each of the DAG kinase genes have been isolated and at least 1 gene (F42a9.1) is alternatively spliced. Full length GFP fusions to the DAG kinases generated by F46h6.2 and F54g8.2 have been made and we hope to report on their expression at the meeting. Fusions to the other 2 genes are being constructed.

1. Nurrish et al.,(1999) Neuron:24 231-242
2. Lackner et al.,(1999) Neuron:24 335-346
3. Hajdu-Cronin et al.,(1999) Genes Dev 13: 1780-1793
4. Miller et al.,(1999) Neuron 24: 323-33
5. Richmond et al., (1999) Nat.Neurosci 2: 959-964
6. Blitterswijk et al., (2000) Cellular Signalling 12: 595-605

121. An investigation into the mechanism of action of the novel anthelmintic emodepside using *A.suum*

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Resistance of parasitic nematodes to existing anthelmintics has encouraged the search for novel compounds. A new compound, emodepside, a 24 membered cyclic depsipeptide, acts as a potent, broad-spectrum anthelmintic. It causes fast onset paralysis of nematodes, favouring the view that it is neuropharmalogically active. The aim of this project is to define and characterise the site of action of emodepside.

The action of emodepside on the body wall muscle of the nematode *Ascaris suum* was investigated. It was shown that emodepside relaxes the muscle in a concentration-dependent and irreversible manner. Dorsal muscle strips (DMS) were bathed in artificial perienteric fluid (APF). The DMS was contracted using acetylcholine (ACh; 30µM, 30secs). The amplitude of this contraction after pretreatment of the DMS with emodepside (10µM) was expressed as a percentage of the control contraction (i.e. without emodepside pretreatment). Emodepside reduced the contraction by 39% ± 4 (n=9). Emodepside was also shown to relax the DMS following prolonged contraction with ACh. The DMS was contracted with ACh (30µM, 10mins). A slow relaxation of the DMS was then observed over a period of 10mins. Addition of 10µM emodepside at the point of maximum contraction, caused a significantly ($P<0.005$) faster relaxation of the DMS, 9.0% min^{-1} ± 0.3 (n=5), compared to control (6.1% min^{-1} ± 0.6, n=5).

The action of emodepside was similar to that of an inhibitory neuropeptide PF2 (SADPNFLRFamide), and differed from that of the inhibitory neurotransmitter GABA. Application of PF2 (1µM) at the maximum point of ACh contraction caused a faster relaxation of the DMS (9.2%min^{-1} ± 0.4 n=6) compared to ACh control (7.4% min^{-1} ± 0.3, n=6), which was similar to emodepside (9% min^{-1} ± 0.4, n=6). In contrast to the slow relaxation of the muscle observed with emodepside and PF2, the response to GABA was rapid and returned to base line tension.

To elucidate whether emodepside acts pre-or post-synaptically at the neuromuscular junction, the effect of emodepside was investigated on a denervated muscle strip preparation. Application of emodepside (10µM) to the denervated muscle strip caused no reduction in ACh induced contraction (n=7). These data suggest that emodepside may inhibit nematode motility by triggering the release of an inhibitory neuropeptide at the neuromuscular junction.

Emodepside therefore acts pre-synaptically to cause a relaxation of worm muscle. Emodepside action is similar to that of the inhibitory peptide PF2 and differs from that of GABA. Currently I am determining how emodepside acts pre-synaptically to cause release of an inhibitory peptide. To this end the effect of emodepside on *c.elegans* will be investigated, to give an insight into the molecular mechanisms of emodepside action.

122. *dyf-8* encodes a type I trans-membrane receptor with a zona-pellucida domain and may be a worm homologue of endoglin.

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TGF-like signaling cascades have been implicated both in the control of body size and the regulation of dauer stage entry and exit in C. elegans. In the dauer pathway, it is thought that a soluble signaling molecule of the TGF/BMP family (DAF-7) activates a DAF-1/DAF-4 (TGF type I/TGF type II) complex (Gunther et al, 2000). This activation leads to SMAD signaling.
In some systems, a TGF type III receptor molecule has also been implicated in SMAD signaling. This type III receptor molecule is thought to be incapable of direct signaling in the absence of a Type I/Type II complex. The type III-receptor binds the soluble ligand with a very high affinity and hands the ligand off to the Type I/Type II complex, thus increasing the sensitivity SMAD signaling. No known type III receptor partners have been isolated in *C. elegans* screens for body size or dauer formation defects.
Endoglin is a type III TGF-receptor protein found in some epithelial tissues. In humans, endoglin mutations are the causative agent of a disease called Hereditary Hemorrhagic Telangiectasia type I (HHT I). Patients present with bleeding lesions around the soft mucous tissue that arise due to weakness in vascular endothelium. This, and other evidence suggests that the type III co-receptor is required for the stabilisation of endothelial juxtapositions. Endoglin, and the related TGF Type III co-receptor, Beta-glycan, each have a weak signature for a zona pellucida domain.
One notable hypodermal (epithelial) juxtaposition in the worm is made by the sheath cell and the socket cell in worm sensory organs, the amphid being the most well-studied of these. The structure of the amphid organ can quickly be assayed by soaking worms in the lipophillic dye, DiI. The penetrating amphid sensory neurons quickly backfill in wild type animals, but in the absence of an intact amphid organ, these neurons fail to fill. Dyf-filling (dyf) defective screens have yielded several mutants (Starich et al, 1995). We recently cloned one of these, *dyf-8*, using our SNP mapping methods (Wicks et al 2001). Although quite divergent at the level of primary sequence, the molecule possesses the modularity of an endoglin homologue, with a signal sequence, a zona pellucida domain, a trans-membrane domain and a short intracellular tail with no kinase domains. A clustal alignment of the ZP domains, including all of the TGF receptor subtypes from worm mouse and human show that *dyf-8* clusters weakly with the endoglin family. The mutant phenotype is full rescued by re-introduction of the cosmid containing the gene.

We generated a nearly full length translational fusion of the genomic sequence to GFP to determine the expression pattern of *dyf-8*. Transgenic worms express the fusion product in the egg after 350 min of development until hatching, in the sheath and socket endothelium/hypodermis and in the adult hermaphrodite, but not male, spermatheca. These lines also demonstrated a variable semi-dominant lethality at hatching. Worms often extruded material or even whole cells from the hypodermal pores formed in the cuticle. The degree of lethality was correlated with the levels of expression. It is our belief that the lethality arises as a consequence of the highly-expressed GFP tagged construct binding all the available soluble ligand but being unable to interact properly with the TGF type I/Type II complex, and hence completely abolishing SMAD signaling in the sensory organ.

123 *C.elegans dlg-1* cooperates with the catenin-cadherin system under hierarchical control of *let-413* and *crb-1*

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The intestine of *C.elegans* forms a simple tube made up of 20 cells. It provides an excellent system to study the process of epithelial development in a nematode embryo. We previously described the complete pattern of the zonula adherens in the gut and identified DLG-1, the homologue of the *Drosophila* tumour-suppressor gene *discs-large*, as an essential component for correct formation of this intercellular junction. Here we have extended our analysis to dissect the genetic network involved in the establishment of the apical junction, the maintenance of cell adhesion and the formation of the apical membrane domain in the *C.elegans* gut. The dependence of DLG-1 localization on the catenin-cadherin system, *ajm-1*, *let-413* and *crb-1* was investigated in detail. We further analyzed characteristics of intestinal cell adhesion after inactivation of *dlg-1* and the catenin-cadherin system. Finally the formation of the apical membrane domain in the intestine was judged under various conditions where the formation of the zonula adherens was disturbed. The correct spatial and temporal localization of DLG-1 in the embryonic gut mostly depends on *let-413* but also on *ajm-1*. After supplementary inactivation of the catenin-cadherin system the DLG-1 pattern is refurbished. We could attribute this rescue activity to *crb-1*, the homologue of the *Drosophila* gene *crumbs* in *C.elegans*. Tissue integrity of the gut epithelium is guaranteed by cooperation of *dlg-1* and the catenin-cadherin system. Both systems and *let-413* are all dispensable for correct apical distribution of atypical protein kinase C. A model presents cooperative gene interactions with regard to correct DLG-1 localization and cell adhesion in the embryonic gut epithelium of *C.elegans*.

124. Inositol 1,4,5-trisphosphate (*itr-1*) receptor function in males

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The second messenger inositol 1,4,5-trisphosphate (IP_3) regulates intracellular calcium signals through IP_3 receptors (IP_3Rs). This pathway is central to a wide range of cellular processes. However the functions of IP_3R mediated signalling in the biology of whole animals have been rather poorly understood. Recent studies in *C. elegans* have identified a range of functions in the development, behaviour and physiology of hermaphrodites. IP_3Rs in *C. elegans* are encoded by a single gene, *itr-1*. It has been shown that *itr-1* participates in the control of ovulation[1] and defecation2. In addition we have shown that IP_3-mediated signalling is important in multiple events during embryogenesis and in the regulation of pharyngeal pumping in response to food[3]. To develop a fuller understanding of the range of functions of *itr-1* in *C. elegans* we investigated its roles in the biology of males. *Itr-1* has three promoters (pA, pB, pC), each of which directs expression in a different and specific set of cells in hermaphrodites[4,5]. Promoter GFP fusions were crossed into a *him-8* background and the expression pattern for each promoter was determined. The three promoters gave different expression patterns, which included male specific structures. RNAi by feeding has been used to characterise the function of *itr-1* in hermaphrodites[3]. We carried out RNAi on *itr-1* in males and demonstrated that IP_3R function in pharyngeal pumping and defecation are conserved. We also observed a severe reduction in male fertility in *itr-1* RNAi males. Male mating behaviour was examined, turning, vulva location and spicule insertion were scored. RNAi males show an increase in number of "sloppy" turns. We also examined sperm transfer and discovered that RNAi males transfer less sperm than wild type males. However fertility assays suggest that these alterations are insufficient to account for the observed decrease in offspring. Thus in *itr-1* knock-down animals the ability for sperm to fertilise may also be reduced.

1. Clandinin *et al.* (1998) *Cell* **92**, 523-533
2. Dal Santo *et al.* (1999) *Cell* **98**, 757-767
3. Walker *et al*. (2002) *Mol. Biol. Cell* **13**, in press
4. Baylis *et al.* (1999) *J. Mol Biol* **294**, 467-476
5. Gower *et al.* (2001) *J. Mol Biol* **306**, 145-157

125. Regulation of inositol 1,4,5-trisphosphate receptor gene (*itr-1*) expression by *pha-4*

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Inositol 1,4,5-trisphosphate receptors (IP_3Rs) play a central role in the organisation of calcium signals by regulating intracellular calcium flux. IP_3Rs in *C. elegans* are encoded by a single gene, *itr-1* (also called *lfe-1*[1] and *dec-4*[2]). We have shown previously that the *itr-1* gene has three putative transcriptional start sites[3]. Sequence comparisons to the *C. briggsae itr-1* gene supported the hypothesis that *itr-1* has three promoters (pA, pB and pC), each of which gives rise to an alternative mRNA and hence unique protein. By using transgenic GFP reporter constructs we showed that these promoters direct cell-specific expression of *itr-1* variants and further that this results in adjacent cells in the same tissue containing different IP_3R isoforms[4].

Promoter A directs expression in the pharyngeal terminal bulb, the rectal epithelial cells and vulval hypodermal cells. Comparison of pA from *Ceitr-1* and *Cbitr-1* revealed several conserved regulatory elements. Subsequent deletion analysis identified a 100bp region (C1-C2) that is essential for terminal bulb and rectal epithelial cell expression. The C1-C2 region contains putative binding sites for forkhead and nkx 2.5 transcription factors. Using a yeast one-hybrid system we have screened a number of known members of these families and identified PHA-4 as a regulator of C1-C2 activity[5,6]. Electrophoretic mobility shift assays reveal that PHA-4 binds more strongly to a site within C1-C2 than to a previously defined PHA-4 binding site from *myo-2*[7]. By deleting sites in the pA::GFP construct we have demonstrated that *pha-4* is essential for the expression of *itr-1* in the terminal bulb but not in the rectal epithelial cells.

1. Clandinin *et al.* (1998) *Cell* **92**, 523-533
2. Dal Santo *et al.* (1999) *Cell* **98**, 757-767
3. Baylis *et al.* (1999) *J Mol Biol* **294**, 467-476
4. Gower *et al.* (2001) *J Mol Biol* **306**, 145-157
5. Mango *et al.* (1994) *Development* **120**, 3019-3031
6. Kalb *et al.* (1998) *Development* **125**, 2171-2180
7. Okkema and Fire (1994) *Development* **120**, 2175-2186

126. Mutations in glutamate-gated chloride channel genes affect *C. elegans* locomotion and fecundity

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The glutamate-gated chloride channels (GluCl) are important in human and veterinary medicine as the sites of action of the avermectin/milbemycin group of anthelmintics and insecticides, the best known of which is ivermectin. Ivermectin causes paralysis of worms, suggesting that the GluCl are likely to play a role in locomotion. In *C. elegans*, five genes (*avr-14* &*-15* and *glc-1*, *-2* & *-3*) encode GluCl subunits and at least some of these are widely expressed in the nervous system. We have raised antibodies against the products of all of these genes and are using these to study the distribution of the subunits. So far, we have confirmed that AVR-14B is present in neurones of the motor nervous system. We examined the movement of *avr-14* and *avr-15* mutants and observed a 50% reduction in the duration of forward movement compared to wild type worms. The *avr-14/avr-15* double mutant had an increased reduction compared to either of the single mutants. RNA interference experiments with *avr-14* or *avr-15* on wild-type worms also reduced the duration of forward movements to a similar extent, confirming that we can use this method to study the effect of reducing expression of genes for which no mutant alleles are yet available. In contrast RNAi with *glc-2*, which we have reported to be only expressed in pharyngeal muscle, had no effect on locomotion (Table 1). These effects on the duration of forward movements are the opposite to those obtained with mutants in genes encoding the excitatory glutamate receptors, such as NMR-1, that are expressed on the command interneurones. This raises the possibility that the excitatory and inhibitory glutamate receptors act in an antagonistic fashion to regulate locomotion and we are currently trying to determine if *nmr-1* and the GluCl genes are expressed on any of the same neurones.

wild-type	*avr-14*	*avr-15*	*avr-14/15*	avr-14 RNAi	avr-15 RNAi	glc-2 RNAi
23.7 ± 2.8	12.6 ± 3.3	12.3 ± 1.1	8.3 ± 1.1	12.5 ± 0.8	10.6 ± 0.8	19.5 ± 3.0

Table 1. Effect of GluCl mutations and RNAi on the duration of forward movements (secs).

Ivermectin also reduces the fecundity of some parasitic nematodes when used therapeutically, so we examined the effects of GluCl mutations and RNAi on egg production. The number of eggs laid by single worms was reduced by about 60% in the *avr-14* and *avr-15* mutants and by about 30% in wild-type worms receiving *glc-2* RNAi treatment. Since there is no direct evidence for expression of GluCl in reproductive muscle or any innervating neurones, the basis of this reduced fecundity is unclear.

127. Xenobiotically induced cytochrome P450 gene expression in *Caenorhabditis elegans*.

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Cytochromes P450 (CYP) are a super family of heme containing NADPH dependent monooxygenases. These enzymes play important roles in the biotransformation of many drugs, carcinogens, steroid hormones and environmental chemicals. CYP gene expression can be induced by the presence of these compounds in the environment. At high concentrations many of the inducers produce a wide range of biological effects on animals and other organisms, e.g. mortality or reproductive failure.

The nematode *Caenorhabditis elegans* possesses the remarkable number of 80 different cytochrome P450 genes. The functions of the encoding proteins are almost unknown. In order to study xenobiotically induced gene expression in *C. elegans* liquid cultures were exposed to different well-known xenobiotic inducers. The mRNA expression was detected by two different DNA arrays and semi-quantitative RT-PCR. In addition, using concentration-effect experiments, a classical reproduction test was compared with CYP gene expression screens.

β-naphthoflavone, PCB52, Lansoprazol and Fluoranthene were the most active CYP inducers. They mainly induced almost all CYP35 isoforms. The reproduction test with nine different xenobiotics revealed Tributylzinn, Endosulfan and Fluoranthene as most toxic substances. The threshold of the semi-quantitative RT-PCR experiments showed a significant higher sensitivity than the reproduction tests. Obviously, the xenobiotic inducible gene expression of *C. elegans* is a very sensitive tool to reveal defense mechanisms against potential toxic substances and can be used to develop a biomonitoring system.

Interestingly, the CYP35 isoforms promoter regions contain xenobiotic response elements (XRE) similar to mammalian CYP1 forms. A transgenic *C. elegans* line expressing GFP under control of the CYP35A2 promoter was constructed. With this tool it was possible to follow the GFP production *in vivo* depending on the added inducer. In the control worms a slight but distinct label was visible at the anterior and posterior part of the intestine. In β-naphthoflavone treated worms the total gut emitted a very strong GFP fluorescence.

128. Dna polymorphism in *Pristionchus pacificus*

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To understand the evolution of developmental process at the microevolutionary level, it is important to study the biogeography and the evolutionary alterations of the developmental processes in detail. To acuminate this, we are working on the satellite model *Pristionchus pacificus.* The genus *Pristionchus* has a worldwide distribution and different strains of *Pristionchus pacificus* exist from Washington (PS 1843), Hawaii (JU 138), Ontario (AF 8130), Poland (RS 106) and the wild type strain from California (PS 312). Previous AFLP studies showed a high degree of polymorphisms between the strains from Washington, Hawaii and California. BAC end sequencing and SSCP analysis of BAC end fragments between the strains from California and Washington confirmed these original observations. To obtain a better picture of the distribution of polymorphisms between the strains of *P. pacificus*, we have chosen a random 5 Kb region on Chromosome III that contains no open reading frame. Our results showed a high degree of polymorphism (3.46%) in both Washington and Hawaii when compared to the laboratory strain California. There were ~ 0.7% transitions, ~ 0.5% transversions, ~ 0.3% insertions and ~ 0.1% deletions. Most of the polymorphisms observed were single base pair events except a few which were up to 5 bp . Further studies are underway to extend this study, comparing the *ceh-20* and *ceh-40* genes in these strains and to eventually include the different species' of *Pristionchus* namely *P. maupasi* and *P. lheritieri.*

129. Four Classes of Vulvaless Mutants in *Pristionchus pacificus*

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We are studying the evolution of cell fate specification, using vulva development as a model system. Comparative analysis between *C. elegans* and *Pristionchus pacificus* revealed that the specification of the vulva is highly diverse at the cellular level between these two nematodes. Vulva formation in *P. pacificus*, for instance, depends on continuous gonadal induction by multiple cells, whereas in *C. elegans* the single anchor cell is sufficient for inducing the vulva. Mutagenesis experiments in *P. pacificus* identified four classes of vulvaless mutants. Besides generation and induction vulvaless mutants, two classes of mutants show defects restricted to the 2° and 1° cell fate, respectively. Two strong induction vulvaless mutants were isolated, namely *vul-1* and *vul-2*. Cell lineage analysis indicates that *vul-1* mutant animals can be defective in the specification at P (5-7).p. In some animals, only one vulval precursor cell undergoes vulva differentiation, adopting a 1° or 2° cell fate depending on its position. Mutations in *vul-2* affect P (5, 7).p more severely than P6.p. Linkage analyses using genetic and molecular markers revealed that these two mutants are on *P. pacificus* chromosome IV. Fine mapping of *vul-1* reveals that this mutation is closely linked to the SNP marker S181. Positional cloning is currently in progress.

130. Genetic and molecular analysis of the vulval patterning mutants *ped-7* and *ped-17* in *Pristionchus pacificus*

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We compare vulva development between *Caenorhaditis elegans* and *Pristionchus pacificus* to study the evolution of developmental processes. Cell fate specification during vulva development differs in these two nematodes. In *C. elegans*, P8.p is a member of the vulva equivalence group and adopts a 3° fate under wild-type conditions. In contrast, several experiments indicate that P8.p in *P. pacificus* represents a novel cell type. First, P8.p is unable to respond to an inductive signal to form vulval tissue, but it can respond to lateral signal from the neighboring 1° cell P6.p. Second, P8.p provides a lateral inhibitory signal that prevents P5.p and P7.p, but not P6.p, from adopting a 1° fate. Third, P8.p provides a negative signal that inhibits gonad-independent vulva formation of P(5-7).p. To study P8.p specification, we screened for mutants with P8.p specification defects and identified three mutants in which P8.p behaves like a normal VPC. In one of these mutants, namely *ped-17*, the nucleus of P8.p has an abnormal size. Cell ablation experiments in *ped-17* revealed that P8.p is able to respond to inductive signal from the gonad to form vulval tissue. Also, P8.p in this mutant cannot provide the lateral inhibitory signal. After P6.p ablation in these animals, either P5.p or P7.p can adopt a 1° fate. In addition, P8.p loses the function of providing negative signal in *ped-17* mutant animals since gonad-independent vulva formation occurs. In another mutant, *ped-7*, P7.p migrates towards P8.p and forms an ectopic invagination. Genetic analysis revealed that *ped-7* represents a multivulva mutation. We have mapped both mutants to the genetic linkage map of *P. pacificus*. *ped-7* maps to chromosome V and is located between the markers S130 and S13. *ped-17* is on chromosome II and is located between S111 and S24. Positional cloning of *ped-7* and *ped-17* is currently ongoing.

131. Genetic screen for suppressors of *ced10(t1875)* utilizing the loss of a *ced-10::gfp* transgenic array

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Programmed Cell Death (Apoptosis) is crucial for elimination of excess and/or damaged cells both during development and germline proliferation. Part of the process of elimination is the engulfment/phagocytosis of the apoptotic cell. Phagocytosis in *C. elegans* occurs via two partially redundant pathways. One pathway involves *C. elegans* CED-1 and CED-7 proteins that are the homologues of the mammalian *S*cavenger *R*eceptor from *E*ndothelial *C*ells (SREC) and *A*TP *B*inding *C*assette (ABC-1) respectively. The other pathway involves CED-2, CED-5, CED-12 and CED-10. Which are homologous to mammalian CRKII, DOCK 180, ELMO and Rac-1 respectively.

ced-10 seems to occupy an important position relatively downstream of this second cascade, as overexpression of CED-10 rescues the engulfment defect seen in *ced-2*, *ced-5* and *ced-12* mutants. CED-10 is the *C. elegans* member of the Rho/Rac family of GTPases that function in many signaling pathways and developmental processes. In *C. elegans, ced-10* mutants are defective in Distal Tip Cell (DTC) migration, neuronal guidance and cell corpse engulfment following apoptosis. The function/s of CED-10 in all these different processes is still not fully understood.

We have recently isolated a point mutant in *ced-10, ced10(t1875)* that is believed to be a null mutation. Worms homozygous for the *ced10(t1875)* allele are not viable. In order to better comprehend the function/s of CED-10, we will attempt to isolate extragenic suppressors of *ced-10(t1875).*

132. A screen for suppressors of ced-6(n1813), a mutant defective in engulfment of apoptotic cell corpses.

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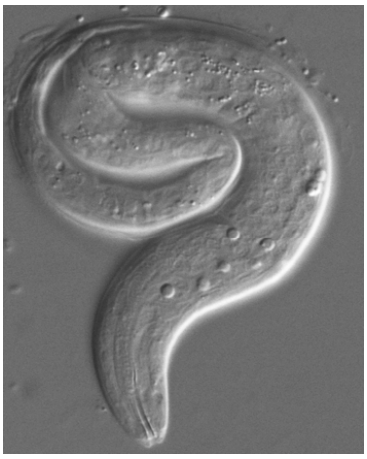
Programmed cell death (apoptosis) plays a crucial role in animal development as well as in the elimination of damaged cells. The pathway underlying this process is evolutionarily conserved from worms to humans. An important step in apoptosis is the removal of dying cells from the organism via phagocytosis. In *C. elegans*, removal of dying cells proceeds via two partially redundant pathways.

The traditional Nomarski screen for worms that have persistent cell corpses has resulted in the identification of seven genes that promote engulfment via two partially redundant pathways. The first pathway is composed of two proteins at the cell membrane, CED-1/SREC and CED-7/ABC1, which may function in the recognition of the dying cell, and CED-6, a putative adapter protein. The second pathway is comprised of CED-2/CrkII, CED-5/Dock180, CED-10/Rac1, and CED-12/ELMO, thought to be involved in reorganization of actin filaments, which is required to extend the engulfing cell's membrane around the dying cell. There still exist, however, large gaps in both pathways. In order to isolate novel mutations involved in engulfment, we have performed a screen in which we suppress *ced-6(n1813)* mutant worms to attempt to isolate mutations that function downstream of *ced-6* in engulfment. We have isolated 15 mutations and are currently characterizing them.



ced-6(n1813)

133. Identification of a new mutant defective for DNA damage responses in the *C. elegans* germ line.

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The inability to efficiently repair or remove cells with damaged genomes has been linked to several human cancers. DNA damage induces multiple biological responses that insure the integrity of the genome. Checkpoint genes that sense the damaged DNA and transduce signals to the cell cycle, repair and apoptotic machinery mediate these responses. In *C. elegans*, DNA damage induces cell cycle arrest and apoptosis in the mitotic and meoitic germ cells, respectively. The core apoptotic machinery is required for proper activation of DNA damage induced apoptosis. Furthermore, mutations in several genes have identified a conserved checkpoint pathway that mediates germ cell cycle arrest and apoptosis in response to genotoxic stress[1]. These include members of the yeast 'rad' family, *hus-1* (E.R. Hofmann and M.O. Hengartner, unpublished) and *mrt-2*, as well as *rad-5*, the *S. cerevisiea tel2*$^{+}$ homologue[2,3].
In order to identify new genes involved in DNA damage responses in *C. elegans*, we employed a genetic screen for mutants defective in ionizing radiation induced cell cycle arrest. In an ongoing screen, we have identified one mutant, *op259*, which shows multiple defects for DNA damage responses. In addition to a cell cycle arrest defect, *op259* is also defective for DNA damage induced apoptosis. Furthermore, *op259* has high embryonic lethality, is sterile at 25°C and has a weak Him phenotype. *op259* is also slow growing and has abnormally small germ nuclei. Mapping experiments have linked *op259* to chromosome I. Thus, *C. elegans* is an excellent genetic tool for identifying new genes involved in DNA damage responses.

1. Gartner, A., Milstein, S., Ahmed, S., Hodgkin, J. & Hengartner, M. O. *Mol Cell* **5**, 435-43. (2000).
2. Ahmed, S. & Hodgkin, J. *Nature* **403**, 159-64. (2000).
3. Ahmed, S., Alpi, A., Hengartner, M. O. & Gartner, A. *Curr Biol* **11**, 1934-44. (2001).

134. Genome-wide RNAi screen for genes that control germ cell apoptosis in *C. elegans*

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Programmed cell death (apoptosis) is an important component of animal development and homeostasis. Apoptosis is an efficient way to physiologically eliminate cells in multicellular organisms and it is a major regulatory feature of embryonic development, establishment of immune self-tolerance and regulation of tissue homeostasis in mammals. Changes in the levels of apoptosis have been implicated in the pathology of many diseases, including cancer, autoimmune diseases, stroke, neuro-degenerative disorders, myocardial infraction and AIDS.
Each of the key *C.elegans* cell death genes has one or several mammalian homologues, suggesting that the apoptotic machinery identified in *C.elegans* is evolutionarily conserved. While work over the last few years has shed light on key players, proteins controlling the apoptotic machinery and the effector mechanisms that mediate cell death, little is known about the regulatory signals by which a cell controls its suicide machine. We propose that these upstream regulatory pathways are also conserved, such that an analysis of these pathways in *C. elegans* will, indirectly, also shed light on how apoptosis is regulated in humans. Almost 50% of predicted *C. elegans* genes have a significant human match including many genes implicated in human disease and functional analysis of the *C.elegans* genome has shed light on many conserved biological processes and molecular pathways.
Germ cells can undergo apoptosis in response to a physiological pathway that is used to limit germ cell numbers during oogenesis as well as a checkpoint pathway that activates the apoptotic machinery in response to DNA damage. To understand the molecular basis of germ cell apoptosis we are currently using “genome-wide RNA-mediated interference (RNAi)” to systematically screen the *C.elegans* genome for genes that participate in the regulation of germ cell death. Germ line apoptosis is determined by acridine orange staining/fluorescent microscopy and confirmed by Nomarski microscopy. Using *C. elegans* as a model for genes that participate in the regulation of germ cell death will enhance our understanding of the genetic basis of the apoptotic machinery. Furthermore, it will enable us to elucidate specific genetic responses associated with the global changes that occur during apoptosis.

135. A direct interaction between *C. elegans* IP3 receptors and a LIN-15B homologue that functions in gonadogenesis, control of defecation and modulation of pharyngeal pumping rate

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Inositol 1,4,5 triphosphate receptors (IP_3Rs) are ligand-gated Ca^{2+} channels that control Ca^{2+} release from intracellular stores. They are central to a wide range of cellular responses. IP_3Rs in *C. elegans* are encoded by a single gene, *itr-1* and are widely expressed[1,2,3]. Signalling through IP_3 and IP_3Rs is important in ovulation[4], control of the defecation cycle[2,5], modulation of pharyngeal pumping rate[3] and embryogenesis[3]. To further elucidate the molecular basis of the diversity of IP_3R function, we used a yeast two-hybrid screen to search for novel interactions between ITR-1 and other proteins. We have identified an interaction between ITR-1 and F44C4.4, a previously uncharacterised protein with strong homology to LIN-15B[6]. These form part of a large family of *C. elegans* genes for which homologues have not been identified in other organisms. Knockdown of F44C4.4 expression by RNAi results in a sterile, *evl* phenotype, as a consequence of severe disruption of gonadogenesis. F44C4.4 animals are also deficient in several ITR-1 mediated processes, namely, upregulation of pharyngeal pumping in response to food, and control of the defecation cycle. We will discuss the cellular basis of these functions, and their significance with respect to ITR-1 mediated signalling events.

1. Baylis *et al*. (1999) *J. Mol. Biol.* **294**, 467-476
2. Dal Santo *et al*. (1999) *Cell* **98**, 757-767
3. Gower *et al.* (2001) *J. Mol. Biol.* **306**, 145-157
4. Clandinin *et al*. (1998) *Cell* **92**, 523-533
5. Walker *et al.* (2002) *Mol. Biol. Cell* **13**, In press
6. Huang *et al*. (1994) *Mol. Biol. Cell* **5**, 395-411

136. Characterisation of a *C. elegans* calexcitin.

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Calexcitins are small (20 kDa) EF-hand, Ca^{2+}-binding proteins, with a carboxyl-terminal GTP binding domain that shows homology to the ARF family of small G proteins[1]. Calexcitin was first implicated in associative learning on the basis of its phosphorylation following training protocols in the mollusc *Hermissenda*[2]. Upon phosphorylation by protein kinase C, calexcitin moves to the plasma membrane and inactivates voltagedependent K+ channels[3]. Calexcitin is also known to interact with and activate ryanodine receptors, whose upregulation is also implicated in associative learning[4]. The *C. elegans* genome contains two predicted calexcitin genes. We describe the characterisation of F56D1.6, which shares over 50% amino acid similarity with known calexcitins, as well as the predicted EF-hand structures and GTP binding motifs. Knockdown of F56D1.6 expression by RNAi results in a significant decrease in pharyngeal pumping, and the failure of serotonin to restore pumping rate to wild type levels suggests that F56D1.6 is implicated in pharyngeal muscle function. In addition, we will describe the function of F56D1.6 in both the muscle contractions and timing of defecation, and in associative learning.

1. Nelson, T.J. *et al*. (1996) *Proc. Natl. Acad. Sci. USA* **93**, 13808-13813.
2. Neary, J.T. *et al*. (1981) *Nature* **293**, 658-660.
3. Alkon, D.L. *et al.* (1998) *Trends Neurosci*. **21**, 529-537.
4. Cavallaro, S. *et al.* (1997) *Proc. Natl. Acad. Sci. USA* **94**, 9669-9673.

137. Transient disruption of IP_3 receptor function in *C. elegans*.

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Inositol 1,4,5 trisphosphate (IP_3) controls Ca^{2+} release from intracellular stores via IP_3 receptors (IP_3Rs) and is central to a wide range of cellular responses, but its specific functions in animals remain poorly understood. ITR-1, the *C. elegans* IP_3R is widely expressed[1,2,3] and known to participate in control of ovulation[4] and defecation[2]. To determine the functional significance of IP_3 signalling more fully, we have disrupted IP_3 signalling in whole, live animals using two transient approaches: inducible, high level expression of the IP_3 binding domain of ITR-1 and RNA interference by feeding[5]. While confirming the roles of IP_3 signalling in regulation of defecation and in ovulation, this approach identifies important, previously unidentified functions. Firstly, the up-regulation of pharyngeal pumping in response to food is dependant on IP_3 signalling. RNAi studies and analysis of *itr-1* mutants show that this process is also IP_3R dependant. Secondly, the tissue specific expression of the dominantnegative construct enabled us to circumvent the sterility associated with loss of IP_3 signalling through the IP_3R and thus determine that IP_3-mediated signalling is required for multiple steps in embryogenesis[6].

Using information gained from the dissection of the *itr-1* promoter we have disrupted IP_3 signalling in a cell-specific manner[3]. This approach demonstrates that, for example, expression in the terminal bulb is sufficient to disrupt the up-regulation of pharyngeal pumping in response to food.

1. Baylis *et al*. (1999) *J. Mol. Biol*. **294**, 467-476
2. Dal Santo *et al*. (1999) *Cell* **98**, 757-767
3. Gower *et al.* (2001) *J. Mol. Biol*. **306**, 145-157
4. Clandinin *et al*. (1998) *Cell* **92**, 523-533
5. Timmons & Fire (1998) *Nature* **395**, 854
6. Walker *et al*. (2002) *Mol. Biol. Cell*. In press

138. Functional analysis of the *C. elegans* centaurin ß (CNT-1) protein

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Members of the centaurin protein family are characterised by containing both a pleckstrin homology (PH) domain and an Arf GTPase activating protein (ArfGAP) domain. PH domains often mediate protein-lipid interactions. ArfGAP proteins stimulate inactivation of members of the Arf family of small G-proteins. Centaurins are widely conserved in mammals and are divided into four groups, a, b, g and d. We identified two centaurin genes in *C. elegans*, one centaurin b gene (*cnt-1*) and one centaurin g gene (*cnt-2*). Analysis of the *cnt-1* gene revealed that it undergoes alternative splicing to produce 2 mRNAs and thus 2 proteins, one with a substantial N-terminal extension. The pattern of expression of *cnt-1* was determined using transgenic GFP reporter gene constructs. *Cnt-1* is expressed in the distal tip cells of the gonad, the pharynx, the excretory cell, neurons of the head and nerve ring, the spermatheca and epithelial cells of the anus. We are currently investigating the function of CNT-1 in *C. elegans*.

It has been shown that the PH domains of certain centaurins can mediate interactions with the phosphoinositides phosphatidylinositol 4,5-bisphosphate (PIP_2) and phosphatidylinositol 3,4,5-trisphosphate (PIP_3). The latter is the second messenger product of Class I phosphoinositide 3-kinases. We therefore tested the ability of CNT-1 to bind to and respond to these lipids. Dot blot assays of lipid binding suggest that the CNT-1 PH domain binds both PIP_2 and PIP_3. We then expressed proteins containing defined regions of CNT-1 fused to GFP in transfected COS-7 cells. A fusion protein of the CNT-1 PH domain and GFP translocated from the cytosol to the membrane in response to the production of PIP_3. However further analysis revealed that a CNT-1:GFP fusion that does not contain the PH domain also translocates in the same circumstances. These results suggest that translocation of CNT-1 in response to PIP_3 may be a complex process, possibly involving direct and indirect effects of PIP_3.

139. TRPm channel function in the defecation rhythm of *Caenorhabditis elegans*

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Intracellular calcium signals regulate an amazingly diverse range of important processes in animals, and yet do so with great specificity. Calcium is unusual amongst second messengers since its levels cannot be regulated by synthesis or degradation. Thus the proteins that regulate calcium flux, in particular channels, are central to the structure and function of calcium signals. Physiological studies have led to the identification of a wide range of plasma membrane calcium fluxes for which the molecular basis is unknown. More recently molecular and genomic analysis has led to the identification of a large family of cation channels related to the *Drosophila* TRP protein. This family is divided into several subgroups. Little is known about the mechanisms that regulate these channels or of their roles in animal biology. One such family is the TRPm channels (also known as LTRP channels). These proteins are found in mammals, *Drosophila* and *C. elegans*. *C. elegans* contains 3 such proteins, GON-2, C05C12.3 and F54D1.5. Mutations in *gon-2* disrupt gonad development.

In order to elucidate the functions of TRPm channels in animals and to dissect the mechanisms by which they are regulated we are investigating C05C12.3 and F54D1.5. To determine the pattern of expression of these genes, we used transgenic GFP reporter gene constructs and antibody staining. These results show that all three genes, *gon-2*, C05C12.3 and F54D1.5, are expressed in the intestine and as well as at other sites. In order to further characterise the function of these proteins in the intestine, RNAi was used to specifically decrease the expression of the three genes. We found that reducing expression of each gene alone had no significant effect on intestinal function but when *gon-2* and C05C12.3 were knocked-down in combination the periodicity of the defecation cycle severely was disrupted. This is a process that is known to be dependent on both calcium and IP_3 signalling. We therefore hypothesise that TRPm channels are part of a calcium signalling network that regulates the defecation rhythm generator.

We are currently analysing the function of these proteins in more detail.

140. Variation in parasite resistance between natural isolates of *Caenorhabditis elegans*

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Although the nematode *Caenorhabditis elegans* is one of the main model organisms in biological research, only little is known about its natural ecology. It is a free-living soil nematode, which inhabits potentially parasite-rich environments. Parasites represent one of the most powerful selective forces due to their ability for rapid adaptation. Therefore, they should play an important role in *C. elegans* evolution. Since their diversity is likely to vary across both space and time, there should also be spatial and temporal differences in the *C. elegans* response. Therefore, I decided to test the importance of parasites on *C. elegans* evolution by an assessment of natural variation in parasite resistance, using different strains of the soil bacterium *Bacillus thuringiensis*. The current study provides first evidence for the existence of such natural variation and thus represents a promising starting point for future evolutionary analysis of host-parasite interactions, including assessment of the evolution of host resistance, immunocompetence and parasite virulence.

141. *C. elegans* whole genome microarray: An update

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We have produced a *C. elegans* microarray consisting of exon-rich genomic fragments, corresponding to approximately 17,500 genes. These products have been spotted onto poly-L-lysine coated slides. Each microarray contains various controls to assess 1) the uniformity of hybridisation 2) the quality of the cDNA and 3) the specificity of signal detection. Currently we are investigating the performance of these controls and the reproducibility of our arrays.

To manage the microarray data we are using the software program GeneSpring™ (Silicon Genetics). To build a *C. elegans* microarray database within GeneSpring™, information about each gene product on the array was extracted from a variety of public databases using Perl scripts. For example, Pfam domain annotations have been included to provide functional information; approximately 50% of the predicted *C. elegans* proteins carry one or more Pfam domains.

Updating the microarray database is a continuous process. We have found that many of the gene predictions have changed from the time the primers were designed to the time the amplified gene products were arrayed. In addition, the latest version of WormPep reveals that ~900 gene sequences (confirmed by EST data) are not currently on the *C. elegans* array. Attempts are being made to include these sequences on future versions of the array. The full genome microarrays will soon be available as a scientific resource primarily for members of the UK research community. Please contact Joanne (jms@sanger.ac.uk) or Patty (pek@sanger.ac.uk) if you interested in using the *C. elegans* microarrays, or contact Jeena (jr4@sanger.ac.uk) if you are interested in obtaining the annotated database.

142. Novel drug targets: the identification and characterisation of candidate transmembrane proteins

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The availability of the entire genome sequences of the nematode *Caenorhabditis elegans* and man has enabled the identification of numerous homologous genes between the two organisms. Using bioinformatics, worm orthologs of novel transmembrane proteins in the human genome have been identified. In an *in silico* study Remm and Sonnhammer (Remm and Sonnhammer, 2000) identified 174 putative worm-human orthologs of transmembrane proteins of both known and unknown function. We are particularly interested in novel candidate transmembrane proteins that show neuronal expression. Such proteins are commonly used as primary drug targets for various diseases. Establishing their function may therefore lead to the development of drugs with new mechanisms of action. In addition, we may also be able to identify novel neuropsychiatric disease genes. The *in silico* predictions of putative transmembrane worm orthologs are now being followed by functional studies of the novel genes in *C. elegans*. In an initial phase, the function is studied by dsRNA-mediated interference (RNAi) by feeding. Of an initial sample of 18 genes, 11% have shown a phenotype by RNAi. To identify neuronally expressed genes we are constructing transgenic lines, where GFP expression is under the control of a putative promoter for the novel genes. Additional experiments, like isolation of the real mutant, *in situ* hybridisation, immunostaining etc, will provide further insight into the novel genes. Genes that show neuronal expression are to be further pursued in mammals to further confirm their orthology.

Remm, M. and Sonnhammer, E. (2000) Classification of transmembrane protein families in the *Caenorhabditis elegans* genome and identification of human orthologs. *Genome Res* **10**: 1679-1689.

143. Specificity of G-protein mediated signaling in odorant detection by *C. elegans*

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The olfactory system of *C. elegans* is ideally suited for studying specificity of G-protein coupled signal transduction. *C. elegans* can detect more than 60 odorants, using only two pairs of sensory neurons, AWA and AWC. This is possible due to the expression of several G-protein coupled odorant receptors per neuron. Furthermore, six Ga-subunits are expressed in AWA and AWC, which probably contribute to the ability to detect different odorants and discriminate between many of them. These Ga-subunits are GPA-2, -3, -5, -6, -13 and ODR-3.

We studied the involvement of the Ga-subunits in odorant detection by determining the effect of different combinations of loss-of-function alleles on chemotaxis. For each odorant tested, we found that the main signaling pathway involves ODR-3. ODR-3 alone is sufficient for wild-type chemotaxis. Furthermore, GPA-3 is redundant to ODR-3 and also sufficient for chemotaxis. GPA-5 inhibits signaling, but this is dependent on the odorant and concentration that is used. Both GPA-2 and GPA-6, although less clear, can stimulate and inhibit detection, again depending on the odorant and concentration. Finally, detection of some odorants involves GPA-13.

To understand how GPA-5 inhibits signaling, we performed a forward genetic screen to isolate genes involved in its signaling pathway. Overexpression of GPA-5 (5XS) leads to defective diacetyl chemotaxis. Therefore, we mutagenized 5XS-mutants with EMS and screened their offspring for normal diacetyl chemotaxis. In this way we expect to pick up genes involved in GPA-5 mediated signaling. To increase the efficacy of our screen, NH_4Ac was used as a counterattractant. Thus far, we have isolated at least 8 independent mutants. These are currently being tested for chemotaxis to odorants and salts. We will identify the genes involved using SNP-mapping.

144. 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, while the target recombination site for the FLP recombinase is the *FRT* site. A sequence flanked by a *loxP* site at the 5' end and by a *FRT* site at the 3' end is called 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 c*re* and *flp* coding sequences, each under the control of the *C. elegans 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

145. Annotation of *C.elegans* protein sequences in SWISS-PROT.

Eleanor Whitfield and Rolf Apweiler.

SWISS-PROT is a curated, non-redundant protein sequence database that provides a high level of annotation and integration with other databases. It is accompanied by TrEMBL (Translation from EMBL Nucleotide Sequence Database), a computerannotated database which was created due to the increasing amount of data from genome projects. TrEMBL contains translations of all coding sequences present in the EMBL Nucleotide Sequence Database which are not yet integrated into SWISSPROT.

C. elegans is one of a number of model organisms chosen in SWISS-PROT. Therefore we aim to have as complete a collection of *C. elegans* sequences as possible, to add new sequences and updates to existing entries as quickly as possible and to provide cross-references from both SWISS-PROT and TrEMBL entries to WormPep.

The genome has been sequenced at two centres; the Wellcome Trust Sanger Institute, Cambridge, UK and the Genome Sequencing Center at Washington University, St. Louis, U.S.A. All translations are available in SWISS-PROT and TrEMBL, the total number of entries being 21,274.

There are currently 2218 *C. elegans* SWISS-PROT entries (Release 40) and TrEMBL has 19056 entries (Release 20). For a list of entries in SWISS-PROT please see http://expasy.ch/cgi-bin/lists?celegans.txtl and for a table of the cosmids, the chromosome they are on, and all associated TrEMBL and SWISS-PROT entries please see http://www.ebi.ac.uk/~eleanor/Celecosmid-ext.html

A non-redundant, regularly updated, complete *C. elegans* proteome set of SWISSPROT + TrEMBL entries is available at http://www.ebi.ac.uk/proteome/

146. Identification of factors specifically involved in the regulation of physiological germ cell death

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In the germ line of *C. elegans* hermaphrodites, programmed cell death (PCD) is the fate of approximately 50% of all germ cells. This physiological germ cell death requires the same central cell death machinery (consisting of *ced-9, ced-4*, and *ced-3*) as somatic cell death during development and DNA-damage induced non-physiological germ cell death. The factors leading to the activation of the central cell death machinery in 50% of all germ cells, however, appear to be different. Somatic cell death, as well as non-physiological germ cell death, are dependent on the BH3-only cell death activators EGL-1 and CED-13 (see N. Wittenburg *et al*., EWM 2002 abstract), which induce PCD by antagonizing the function of the anti-apoptotic Bcl-2-like CED-9. In contrast, loss-offunction mutations in either *egl-1* or *ced-13* do not affect physiological germ cell death. Therefore, it is likely that factors other than BH3-only proteins negatively regulate the function of CED-9 to induce germ cell death. We try to elucidate how physiological germ cell death is activated in the germ line by using several approaches.

We performed clonal F2 screens for mutants with reduced germ cell death. So far about 6,000 haploid genomes have been screened and 11 mutants were isolated. Two of these mutants also have reduced somatic cell death and were subsequently shown to carry lossof-function mutations in *ced-3* and *ced-4*. The other nine mutations specifically block PCD in the germ line. Complementation analysis showed that five of these nine candidates might define one complementation group.

Another cause of non-physiological germ cell death, besides DNA damage, is the infection of *C. elegans* with *Salmonella typhimurium* (Aballay and Ausubel, 2001). We wondered whether the level of so called “physiological” germ cell death could be due to the exposure of the animals to *E. coli* (also gram negative, potentially pathogenic). We therefore raised animals in different bacteria-free/axenic media. When we looked at the progeny of animals that were raised in commonly used liquid Hb medium, we found that a large proportion of the animals formed dauer larvae. This could be due to a high concentration of pheromone, but we think it was due to the lack of nutrients, which did not allow proper development into adults. This is supported by the observation that L4 larvae that were transferred into liquid Hb medium had small underdeveloped gonads later on as adults, indicating a reduction in germ cell proliferation, similar to what is found in starved animals.

We also raised animals on plates seeded with a drop of milk. When we transferred L4 larvae onto these plates, we found that the adults had fully developed gonads. The level of germ cell death of engulfment defective *ced-6(n2095)* animals raised on either milk or on *E. coli* was the same, indicating that the cultivation of *C. elegans* on *E. coli* does not induce germ cell death.

We also studied the effect of various mutations and environmental stress factors on the level of germ cell death. Taken together, our results so far indicate that the physiological death of 50% of all germ cells is in fact due to a germ line intrinsic program rather than being influenced by environmental factors.

147. Identification of an AAA ATPase as an interacting partner of *C.elegans* DLG-1

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We have performed a yeast 2-hybrid screen for direct binding partners of *C.elegans* MAGUK protein DLG-1 [1], the homologue of *Drosophila* tumor suppressor gene *discs large*. By screening 2.3 x 10^6 transformants, with PDZ domains 1-3, we have isolated 32 cDNA clones, corresponding to 21 different genes. At present, putative interacting partners are analyzed using RNA mediated interference (RNAi). Among these clones, six cDNAs were found to encode fragments of a *C.elegans* AAA ATPase (ATPases associated with a variety of cellular activities). A typical PDZ-binding motif (ETAV) is found at the carboxy terminus. AAA ATPases play important roles in a variety of cellular activities including proteolysis, protein folding, membrane trafficking, cytoskeletal regulation, DNA replication and intracellular motility [2-4]. Depletion of the protein, using RNAi, results in viable offspring that show developmental arrest during early larval stages.
Staining of RNAi embryos and larvae with a-DLG-1 and MH27 antibodies revealed no mislocalization, so far. We are currently analyzing the functional basis of the larval-arrest phenotype and further validate the interaction with DLG-1. To determine the localization of the AAA ATPase, we expect to raise antibodies. Preliminary results will be presented.

1. Bossinger O, Klebes A, Segbert C, Theres C, Knust E: Zonula adherens formation in Caenorhabditis elegans requires dlg-1, the homologue of the Drosophila gene discs large. *Dev Biol* 2001, 230:29-42.
2. Vale RD: AAA proteins. Lords Of the ring. *J Cell Biol* 2000, 150:F13-20.
3. Dalal S, Hanson PI: Membrane traffic: what drives the AAA motor? *Cell* 2001, 104:5-8.
4. Ogura T, Wilkinson AJ: AAA+ superfamily ATPases: common structure—diverse function. *Genes Cells* 2001, 6:575-597.

148. Multi-parametric & Dual Color Fluorescent Analysis and Flow Sorting of C. elegans

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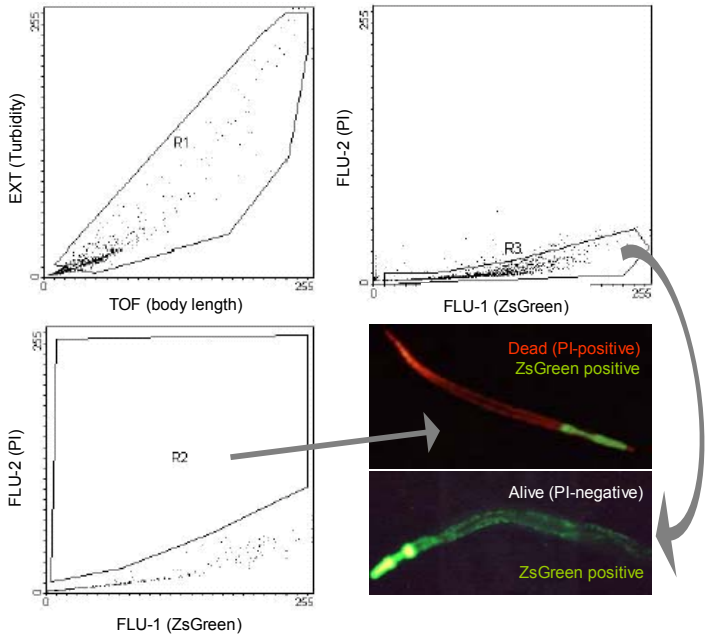
Technology. The COPAS *BIOSORT* is a high speed flow sorter that analyzes and sorts *C. elegans*. The system is equipped with two lasers. A 633 nm excitation diode laser analyzes two physical parameters of the organism: time of flight (TOF, a measure of the length of an organism) and extinction (EXT, a measurement of body opacity). TOF and EXT allow sorting of pure embryos, a larval stage or adults from a wild type population as well as a number of mutants with affected body length or opacity of internal structures.

The 488/514 nm multi-line argon laser is used to measure the expression levels of fluorescent proteins like GFP, YFP, DsRed, ZsGreen, ZsYellow or the binding of fluorophores like Sytox™-green, propidium iodide (PI) or phycoerythrin (PE)-tagged lectins to *C. elegans* (sub-) populations. Single color applications such as live-from-dead (PI, Sytox green) or male-from-hermaphrodites animals (wheat germ agglutinin-PE) have been developed as well as protocols to isolate animals with predefined levels of expression of a fluorescent protein for e.g. high speed genetic/enhancer/suppressor screens or bulk isolates for micro-array experiments. Here we exemplify how the three photon multiplyer tubes (PMT's) of the COPAS *BIOSORT* allow multi parametric plus dual color sort setting of gatings for TOF/EXT ***plus*** two fluorescent signals.

Isolation of viable-ZsGreen positive animals.



A mixed *C. elegans* population of wild type (N2) and a strain expressing ZsGreen (Clontech) in the pharyngeal muscle (UBI68) was stained with PI (Molecular Probes) to label dead nematodes. The R1 gating was set to collect all animals and excludes debris. Fluorescent emissions FLU-1 (498-522 nm) corresponds to ZsGreen and FLU-2 (575-595 nm) to PI. The scales of the dot plots are linear. Gates R2 and R3 reflect the 'dead' PI-positive and 'live'& ZsGreen positive populations respectively. In order to evaluate spectral overlap between the filter sets of the two FLU PMT's, negative and single color population controls are necessary to calibrate instrument settings. Therefore, the purity of sorted PI & ZsGreen 'double positives' (R2) and 'ZsGreen only' populations (R3) were confirmed using microscopy (see micrographs). The results indicated that spectral overlap between ZsGreen and PI is negligable so each population (living versus dead) can be clearly separated from each other in one sort run.

Conclusion. Dual color gatings enable in one COPAS-*BIOSORT* run, to isolate e.g. a given life stage (TOF) that is positive for a fluorescent protein (FLU-1) from animals that label with the 'dead' marker PI (FLU-2). Multi-parametric dual color applications will facilitate faster bulk isolation of 'pure viable' populations for e.g. micro-array, biochemical or proteomics experiments.

149. Enhanced One-step Nematode Recognition on micrographs of Living *C. elegans* Cultures in 384-well Plates using Linear Scale Space Mathematics

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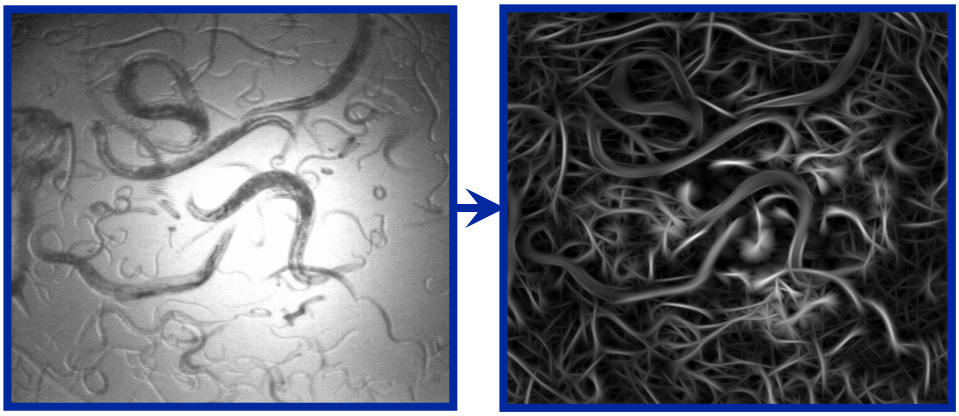
Introduction. Meniscus effects, culture-dependent changes in *E. coli* concentration and superimposed ('crossing') nematodes in *C. elegans* liquid micro-cultures in 384-well plates leads to poor nematode recognition by conventional image analysis algorithms. Scale space theory provides a powerful framework for ***one-step*** feature extraction in 'high-complexity' microscopy images[1]. Differential geometry is applied in image analysis by convolving the image with Gaussian derivatives of the appropriate scale (σ) for the objects of interest. Applying these principles to microscopy of *C. elegans* allows for rapid development of image analysis algorithms very well suited for robust high volume image analysis, such as in drug screening.

Applying scale space to C. elegans microscopy. Feature detectors can be constructed based on differential invariants, which are relatively insensitive to changes in illumination condition and signal-to- noise ratio, which is an important feature in microscopy (see micrograph).

$$Lxx + Lyy - \frac{1}{2}\sqrt{(Lxx - Lyy)^2 + 4Lxy^2} > 0$$

For example a detector for ridges is shown below, the scale of the Gaussian kernel (σ) is 1.0 (equation). L denotes the image itself, Lx and Lxx the first and second Gaussian derivative in the x direction of the image. This filter adequately detects elongated structures, in microscopic images of living wild type *C. elegans* in 384-well plates in liquid *E. coli* culture in a wide spectrum of background conditions. Including directionality into the algorithm makes it even more sensitive to curled-elongated structures as *C. elegans*, the filter response is optimized to the underlying structure[2] (see image derived after filtering below).



Conclusion. Scale space mathematics can robustly convert grayscale images of viable *C. elegans* microcultures in 384-well plates with varying population density, *E. coli* concentrations, background intensity one-step into binary images for downstream image analysis of population composition.

References

1. Koenderink, 1984, Biol. Cybern.: **50**, 363
2. Seinstra *et al.,* 2001, *In*: Eds.Sakellariou, *et al.,* '**Euro-Par 2001 Parallel Processing**', pp 653-662. Springer-Verlag (LNCS 2150)

150. Identifying transcriptional targets of the DAF-12 nuclear hormone receptor

Axel Bethke and Adam Antebi

The *daf-12* gene encodes a nuclear hormone receptor that integrates inputs from insulin/IGF, TGF-β and cGMP signalling pathways and influences dauer formation, the heterochronic circuit and life span. Therefore, the set of *daf-12* target genes should be highly interesting.
Shostak and Yamamoto (IWM, 2001) defined several putative DAF-12 DNA target sequences in the *lit-1* promotor by *in vitro* and *in vivo* experiments. We are searching for additional physiological candidate genes in identified signalling pathways. Surprisingly, one is *daf-9*, a cytochrome P450 related to vertebrate steroidogenic hydroxylases. DAF-9 is thought to metabolize a DAF-12 hormone and acts by genetic epistasis experiments upstream of DAF-12. In contrast to this, we find that hypodermal *daf-9::gfp* expression does not occur in *daf-12* null mutants, suggesting feedback regulation by DAF-12 (See abstract by Gerisch et al). Another candidate target is *let-7*, a heterochronic gene coding for a small temporal RNA (stRNA) that regulates the larval to adult switch. Slack and Johnson (IWM, 2001) showed that a *let-7::gfp* construct is temporally upregulated by the L4 stage in the same cell type as *daf-12::gfp*. In addition, *daf-12* alleles with delayed heterochronic phenotypes, such as *rh61*, also result in delayed or inhibited *let-7* expression (personal com. Slack et al). To investigate whether these candidates are directly regulated by DAF-12 *in vivo*, we are beginning chromatin immunoprecipitation (X-ChIP) experiments to define DNA regions to which the nuclear receptor binds. We will also dovetail these experiments with gel mobility shift assays using protein from nuclear extracts to narrow down the region of receptor binding and to identify corresponding response elements.

151. Proteome analysis reveals distinct molecular differences in developmental stages of wild type *Caenorhabditis elegans*

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Caenorhabditis elegans is a well-known model organism of complex molecular processes such as programmed cell death of mammalian cells because of evolutionary conserved pathways. Our interests is to investigate normal development in wild type worms by proteome analyses to characterize stage-specific proteins and lay the ground for further comparisons and mutation studies. Up to now only proteome maps of populations with mixed stages have been constructed [1-3].

Wild-type *C. elegans* (Bristol, N2) was grown at 20ºC on either agar plates or liquid medium using water-soluble cholesterol and its onthogenesis was synchronized as described [4]. 2-D gelelectrophoresis of L1, L2, L3, L4 and adult samples were carried out independently in 17x18 cm gels using IPG strips ranging from pH 4 to 7. After staining with colloidal Coomassie Brillant Blue approximately 900 spots were detected on the gels with 0.8 mg protein content by computer assisted image analysis. The triplicate gel images were processed to normalize maps of developmental stages. The differences in the expression profiles were detected and spots on the gels were excised. From the 96 proteins that were analyzed and identified by MALDI-TOF MS up to now, about 12 spots were found reproducibly different in larva and adult stages. For some of them the function is still unknown and they are described only as hypothetical proteins in the database. For example, the amount of protein CE16999 and CE25224 increased during the onthogenesis suggesting their physiological importance. We also detected changes of other proteins with known function. For example, the amount of Cu-Zn superoxide dismutase and arginin kinase decreased during the development from L1s to adults. By contrast, we observed the increase in the amount of the Ca^{2+}-binding protein CE12368.

Our data clearly show changes in the expression patterns of abundant proteins during the onthogenesis of the nematode. Proteome maps obtained by synchronization reflect stage-dependent molecular differences and they are well suited for comparisons of proteome maps of strains with different genetic background.

[1] Bini, L., Heid, H., Liberatori, S., Geier, G., Pallini, V., and Zwilling, R. (1997) *Electrophoresis 18*, 557-562.
[2] Kaji, H., Tsuji, T., Mawuenyega, K.G., Wakamiya, A., Taoka, M., and Isobe, T. (2000) *Electrophoresis 21*, 1755-1765.
[3] Schrimpf, S.P., Langen, H., Gomes, A.V., and Wahlestedt, C. (2001) *Electrophoresis 22*, 1224-1232.
[4] Chen, W., Lim, H.H., and Lim, L. (1993) *J. Biol. Chem. 268*, 320-324.

152. A new screen to identify negative regulators of Egfr signalling in *C. elegans*.

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Negative regulation acts to limit the magnitude and duration of signalling and thus forms part of a threshold setting mechanism that governs a cell's sensitivity to signalling. Negative regulation also prevents pathway noise from evoking a response and acts to attenuate signalling once activated. Thus, negative regulation is an essential feature of signalling that allows for the precise control of responses. In this poster I will present a new screen I have performed to identify negative regulators of Egfr signalling in *C. elegans*.

The Egfr is utilised for at least 5 inductive signalling events during *C. elegans* development, all of which activate the highly conserved Ras/MAP kinase signalling pathway. Previous screens for negative regulators of Egfr signalling in *C. elegans* have used the requirement for Egfr signalling to induce the hermaphrodite vulva as their basis. The Egfr/Ras/Map kinase signalling pathway also provides an essential function early in development, which is believed to be in the induction of the excretory duct cell. SEM-5/Grb2 is composed of two SH3 domains flanking a central SH2 domain that serves as an adaptor protein to recruit SOS-1 to the membrane to activate LET-60/Ras. The two SH3 domains of SEM-5/Grb2 are potentially redundant in that they can both bind Sos. However, *sem-5* (*n1619*), a mutation that disrupts the N-terminal SH3 domain, is very severe whereas *sem-5* (n2019), a mutation that disrupts the C-terminal SH3 domain, is almost silent. Indeed, the biochemical interaction between Sos and the N-terminal SH3 domains of Grb2 is much stronger than that with the C-terminal SH3 domain. Together, this indicates that though both SH3 domains can mediate signalling through Sos, it is the N-terminal SH3 domain that carries most of the activity and the C-terminal SH3 domain probably has additional functions. Though the N-terminal SH3 domain mutation *sem-5* (*n1619*) does not eliminate activity, it is severe enough to cause complete lethality. Available evidence suggests that this mutant is not defective in negative signalling and is very sensitive to additional mutations affecting negative regulators. Thus, I have screened for suppressors of *sem-5* (*n1619*). This screen has several advantages over previous screens. Firstly, mutations affecting the localisation of Egfr are not expected to be recovered from this screen. Secondly, vulval screens for enhancers of a known negative regulator are inefficient because the increased signalling screened for results in partially penetrant hyperinduced vulval differentiation and embryonic lethality phenotypes. Both of these difficulties are overcome in the new screen. Thirdly, since non-suppressed animals are inviable, a very large (and saturating) screen can be performed.

Animals homozygous for *sem-5* (*n1619*) are weakly viable in the F1 due to maternal rescue. No animals survive into the F2. This creates a technical difficulty as the screen requires F2 suppressed animals to be distinguished from F1 viable homozygotes. The solution I have devised is to make a selection for *sem-5* (*n1619*) homozygote animals and look for suppressors (viable animals) in the next generation. I have constructed the following strain: *daf-8/szT1; sem-5 (n1619)/szT1*. The translocation *szT1* is a recriprocal translocation between chromosomes I and X, which balances both *daf-8* and *sem-5*. It is possible to chemically select for *daf-8* homozygous animals as they form constitutive dauers at 25°C. From a selection of approximately 2000 F1 *daf-8; sem-5 (n1619)* animals, 24 had viable progeny and had lost *szT1*. Preliminary analysis of these 24 suppressors will be presented.

153. Functional analysis of a collection of maternal effect mutants of *C.elegans* through 4D-microscopy.

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We have previously identified 389 maternal-effect embryonic lethal muta-tions on Chr. IV and V. They define 156 different genes. 48 of those genes are represented with more than one allele. In a first step, the mutants were classified according to the terminal phenotypes. Then, we explored the phenotypic spectrum by analysing as many genes as possible. The analysis has been carried out using a new digital 4D microscope recording system developed in the Schnabel Lab. The mutants were grouped by phenotypic criteria:

Morphogenesis: Embryos mutant for *t1777* or *t1831* both show a normal lineage, and regions are correctly formed at the premorphogenetic stage, but fail to undergo normal morphogenesis. Embryos from mothers homozygous for the mutation *t2160* have a "small" pharynx cluster without any lineage defect. The 3D model at the premorphogenetic stage looks WT. The gene may thus have a function in the morphogenesis of the pharynx.
Metabolism: *t2129* embryos accumulate a lot of bubbles during development. We did not find a lineage defect. However cells death are not engulfed.
Cell death: *t1875* is a new maternal effect lethal allele of *ced-10*. This Rac protein is also involved in cell migrations during embryogensis.
Cell migration: *t1903* embryos are unable to define the normal regions in the embryo. All the regions spread out along the anterior-posterior axis of the embryo, even those normally form clusters in the anterior of the embryo (ABala, ABara and ABalp); but also the fates of the AB blastomeres are aberrant. In a *t1855* embryo two descendants of the ABpla lineage migrate in a few minutes over the dorsal side of the embryo to join the ABpra descendants.
Cell fate: Three *t1724* embryos showed aberrant C and D lineages. In addition the cleavage directions of the E descendants are turned by 90°, but the intestine differentiated properly. Thus the gene appears to be involved in the specification of posterior lineages. A *t1930* embryo showes a left-right transformation of the ABa lineage that can be explained by the loss of the MS induction. *t2121* is a new *gad-1* allele. In a *t1881* embryo the size of the pharynx cluster is reduced since descendants of ABara do not contribute normally to the pharynx.

Because of their genetic position many of these genes may define new players in interesting pathways. We have initiated the cloning of the genes corresponding to *t1903* (cell migration) and *t1724* (C and D lineage defect). *t1903* was rescued with the cosmids F08B4 and H23L24. *hst-1,* a Heparan sulfate sulfotransferase, seems to be the best candidate to explane the fate transformation (since heparan sulfate acts as coreceptor in the wnt pathway) and the putative migration defect. After the three factor crosses with the Hawai strain and the SNP mapping, *t1724* must be place in the position 8.8 in the Ch.IV. At present, we are inyecting the cosmids to rescue these mutation.

154. Characterization and identification of genes involved in transposon silencing.

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The transposon Tc1 is active in somatic cells of all *C. elegans* isolates. However, Tc1 activity in the germ line is restricted to certain strains: Tc1 is active in the germ line of Bergerac BO, but silenced in Bristol N2. Mori *et al.* showed that multiple genetic loci are involved in regulation of germ-line transposition (Mori *et al.*, 1988).
In an EMS screen for activation of Tc1 in the germ line of Bristol N2, 43 mutants were isolated that fall into two classes: mutants that are resistant to *pos-1* RNAi, such as *mut-7(pk204)*(Ketting *et al.* 1999) and *mut-14(pk738)*(Tijsterman *et al.* 2002), and mutants that are wild type for *pos-1* RNAi. The latter class consists of 20 mutants. These mutants are sensitive to RNAi directed against germline-expressed genes (*pos-1, rba-2*), as well as somatically-expressed genes (*unc-22*). Furthermore, one of the mutants, *mut-11 (pk724)*, was tested for *gld-1* cosuppression, and found to be sensitive. Similarly, two previously isolated Tc1 mutators, *mut-4(st700)* and *mut-6(st702)* are completely sensitive to both RNAi (Tabara *et al.*, 1999; our data) and cosuppression (Ketting and Plasterk, 2000).
RNAi-sensitive mutators do share some additional phenotypes with *mut-7(pk204)*. Both RNAi-resistant and RNAi-sensitive mutators are thermo-sensitive sterile. DAPI-staining of the gonads several mutants shows a reduction of gonad size and the presence of univalents in diakinesis. Like *mut-7*, most RNAi-sensitive mutators show a high incidence of males. Classical genetic mapping of the Tc1-excision phenotype has placed the *mut-13(pk766)* mutation in the center of chromosome I.
As an alternative approach to rapidly identify genes involved in transposon silencing in *C. elegans*, we are performing genome-wide screens using the RNAi feeding library to inactivate each *C. elegans* gene (in collaboration with Julie Ahringer, Cambridge, UK). We identified three putative mutator genes on chromosome I by scoring for Tc1 excision after feeding of *unc-22::Tc1* worms.

Ketting, R. F., Haverkamp, T. H., van Luenen, H. G. & Plasterk, R. H. (1999) Mut-7 of C. elegans, required for transposon silencing and RNA interference, is a homolog of Werner syndrome helicase and RNaseD. *Cell* **99,** 133-41.
Mori, I., Moerman, D. G. & Waterston, R. H. (1988) Analysis of a mutator activity necessary for germ-line transposition and excision of Tc1 transposable elements in *Caenorhabditis elegans*. *Genetics* **120,** 397-407.
Tabara, H., Sarkissian, M., Kelly, W. G., Fleenor, J., Grishok, A., Timmons, L., Fire, A. &
Mello, C. (1999) The rde-1 gene, RNA interference, and transposon silencing in C elegans. *Cell* **99,** 123-132.
Tijsterman, M., Ketting, R. F., Okihara, K. L., Sijen, T. & Plasterk, R. H. (2002) RNA helicase MUT-14-dependent gene silencing triggered in C. elegans by short antisense RNAs. *Science* **295,** 694-7.

155. Homologous gene targeting in *C. elegans*.

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In several model organisms including mouse, yeast and *E. coli*, homologous recombination is a method of choice for gene targeting and alteration. A few anecdotal cases of integration via homologous recombination after microinjection has been reported for *C. elegans*, however a low frequency of homologous recombination (~3% of integration events, 1) makes use of the method impractical in the worm. The limiting factor is production of hundreds of integrated lines, only a few of which could be a result of homologous recombination. Recently, a robust method to produce single and low-copy integrated lines by microparticle bombardment has been developed (2), and its application for homologous disruption gave one successful event (Shai Shaham, personal communication at 13th International *C. elegans* Meeting). We have explored the potential of this method for scaling up to produce amounts of integrated lines required to overcome the current numbers problem in homologous recombination technique.

The PDS-1000/He particle delivery system (Bio-Rad) with hepta adapter has been used to bombard 10-cm plates full of worms, and unc-119 gene has been used as a selection marker for transformation (2). This setup allowed us to routinely produce 10-18 independent transformants per bombardment, approximately half of which proved to be integrated lines. Next we made an "ends-in" construct to target the unc-22 gene. The construct contains 6-kb region of homology to unc-22 gene and a unique restriction site approximately in the middle of the region, allowing linearisation of the plasmid prior to bombardment. Using this construct, 400 independent transformants have been obtained in 28 bombardments. Progeny of the transformed worms has been analyzed, and 3 independent lines appeared to have unc-22 phenotype. PCR analysis (using construct-specific and unc-22 flank-specific primers) confirmed insertion of our construct in the homologous region, rather than an accidental non-homologous disruption of the unc-22 gene. We are currently also disrupting other genes, and preliminary results will be presented. Thus we provide evidence that ballistic transformation of worms could be a good approach to put homologous recombination into a powerful yet incomplete "*C. elegans* toolkit".

1. Broverman et al. 1993. Proc. Natl. Acad. Sci. USA, **90**, 4359-4363.
2. Praitis et al. 2001. Genetics, **157**, 1217-1226.

156. Genes involved in genome stability in *C. elegans*

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The stability of genetic information is essential for the survival of an organism and its offspring. Multiple DNA protection and repair systems have evolved to ensure genome stability. DNA damage is the cause of many human cancers. All human cancers show some form of DNA instability such as base substitutions and frameshifts, as well as large deletions and translocations.
To date, the search for genes that ensure genome stability has only involved unicellular organisms such as bacteria and yeast. However, C. elegans is an excellent model organism to identify new genome stability genes because it is multicellular but nevertheless available in large numbers required for mutation analysis.

Several approaches are taken to identify *C. elegans* genes that ensure genome stability. To monitor somatic repeat instability we constructed transgenic worms carrying a high copy array of a LacZ reporter fusion downstream of a DNA repeat that puts the reporter gene out of frame. Constructs that acquire a frameshift will express the reporter gene. In a genome wide RNAi screen several genes involved in DNA stability were identified.

A second type of DNA damage under investigation are gross chromosomal rearrangements (GCRs), such as deletions and translocations. GCRs are thought to be the result of errors in double strand break repair. Stretches of microhomology are often found around wrongly repaired breakpoints. This homology dependent double strand break repair is used in our GCR-reporter worm strain using a high copy array of an interrupted LacZ gene with stretches of microhomology on its flanks.

As a third approach we are investigating the use of the *unc-93(e1500)* reversion assay in a genome wide RNAi screen. Animals homozygous for the *unc-93(e1500)* mutation are uncoordinated and egg-laying defective, while complete loss of function of the unc-93 gene has no strong visible phenotype. Loss-of-function mutations in four other genes (*sup-9, sup-10, sup-11* and *sup-18*) revert the *unc-93(e1500)* phenotype, thereby increasing the chance of reversion. The *unc-93(e1500)* reversion assay provides the possibility to score genes involved in germline genome stability, regardless of the type of DNA damage.

157. DYSTROPHIN AND ASSOCIATED PROTEINS IN *C. elegans*

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Dystrophin is the product of the gene mutated in Duchenne muscular dystrophy, a neuromuscular disease leading to muscle necrosis. The function of the dystrophin protein is not known. In mammals, dystrophin is located under the muscle plasma membrane, and is associated with a protein complex (DGC) spanning the membrane. The *C. elegans* genome contains a dystrophin homologue named *dys-1*. Our goal is to understand dystrophin function in *C. elegans*.
Loss-of-function mutations in the *dys-1* gene do not alter the muscle structure, but make animals hyperactive and slightly hypercontracted. In a forward genetics approach, we isolated additional mutations with *dys-1*-like phenotypes. Mapping and complementation analysis revealed that these mutations correspond to 4 additional genes. Previously, we cloned two of them: *dyb-1* (Gieseler et al., 2001) and *dyc-1* (Gieseler et al. 2000 and abstract by Gieseler et al.).
dyb-1 is the *C. elegans* homologue of dystrobrevins, a family of proteins belonging to the dystrophin complex and known to bind dystrophin directly through coiled-coil domains present on each protein. *dyc-1* has no known homologue.
We now report the identification of two additional dystrophin-related genes:
dys-4, which encodes a 300 amino acid protein and has no known homologue.
dys-5, which corresponds to *slo-1*, a calcium dependent K+ channel (Wang et al. 2001).
Since *C. elegans* possesses a dystrophin-like and a dystrobrevin gene, we investigated whether other homologues of the DGC members known in mammals could also be found in the *C. elegans* genome. Conserved homologues were found for dystroglycan, delta/gamma-sarcoglycan and syntrophin. Divergent but related proteins were found for alpha- and beta-sarcoglycans. No sarcospan counterpart was found. The expression of the conserved homologues was inactivated using the RNAi technique. Phenotypes similar to that of *dys-1* were obtained, both in the wild-type background and in combination with other mutations. These results strongly suggest that a protein complex comprising functional analogies with the DGC exists in *C. elegans*.

Wang et al. (2001) Neuron 32: 867-881.
Gieseler et al. (2001)J Mol Biol.307 :107-17.
Gieseler et al. (2000) Current biology 10:1092-7

158. DYC-1 a dystrophin related protein of *C. elegans*

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The nematode *Caenorhabditis elegans* has a homologue of dystrophin (*dys-1*), the protein mutated in patients suffering from Duchenne muscular dystrophy. In a forward genetics approach (see poster of Grisoni et al.), we isolated mutations in other genes with *dys-1*-like phenotypes. One of these genes corresponds to the *C. elegans* dystrobrevin gene *(dyb-1)*, which encodes a protein that binds dystrophin directly through coiled-coil domains. The forward genetics approach also made it possible to isolate a novel gene functionally related to dystrophin, *dyc-1*. The DYC-1 protein is not homologous to any known mammalian proteins, but comprises two regions of similarity to CAPON, a nNOS-binding protein. Like DYS-1 and DYB-1, DYC-1 contains a putative coiled-coil domain. A search for protein motifs with the Prosite program reveals a high number of putative PKA- and PKC-dependent phosphorylation sites in DYC-1.
Our data indicate that dyc-1 has muscular and neuronal isoforms. The muscular isoform is expressed in head muscles, body wall muscles and vulva muscles. A gfp fusion protein with the neuronal isoform stains approximately 20 neurones in the animal. Staining is these neurones appear as dots along the neuronal processes. The nature of these dots has not been identified.
Loss-of-function mutations in the *dys-1* or in the *dyc-1* gene do not alter the muscle structure, but make animals hyperactive and slightly hypercontracted. However, the combination of a *dys-1* null mutation or a *dyc-1* null mutation with a weak mutation of *hlh-1,* the *C. elegans MyoD* homologue, results in a time-dependent disorganisation of musculature. The overexpression of *dyc-1* in a *dys-1; hlh-1* genetic background partially suppresses the muscle phenotype. This observation, as well as the similarity of *dys-1* and *dyc-1* loss-of-function phenotypes suggests that both genes participate in the same physiological function (Gieseler et al., 2000).
Using the yeast two-hybrid system, we identified a putative partner of DYC-1: F42G4.3. This protein, which has at least two isoforms, has no known homologues in other species. A preliminary analysis using GFP-reporter gene expression shows that F42G4.3 is expressed in muscles and neurones in a pattern resembling that of dyc-1 and dyb-1.

Gieseler et al. (2000) Current biology 10:1092-7

159. A novel LIM domain protein (Y105E8A.6) that was isolated as an interactor protein of RNF-5, is localized to muscular focal adhesions

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Search for proteins in *C.elegans* that share homology within the RING domain of MDM2/BRCA1/Cbl led to the identification of RNF-5, a 26 kDa RING finger protein with C-terminal tail that may acquire membrane anchoring properties. RNF-5 was found to be important for the growth and development of *C. elegans* and exhibits intrinsic E3 ligase activity (Broday et al., submitted). Yeast 2-hybrid screen identified the *C. elegans* LIM protein Y105E8A.6 as a partner of RNF-5. As the first step to study the functional importance of this interaction, we characterized the pattern of expression of the LIM interactor using transgenic animals harboring extrachromosomal arrays of GFP fusion constructs. We demonstrate that this novel LIM domain protein is expressed in body wall muscles at dense bodies and along the M lines. In the vulval muscles the protein is also localized at sites of muscle attachment to the hypodermis. Expression was detected in the anal depressor muscle and in two arms that extend from muscle cells to the nerve ring. In addition to the expression in dense bodies and M-lines, the fusion LIM::GFP is localized to the nuclei of muscle cells and to the cell cytoplasm. These observations suggest a role for this LIM domain protein in focal adhesion assembly or maintenance.
In order to follow the *in-vivo* interaction between RNF-5 and its interactor LIM protein, and their putative role in the regulation of the integrity of the basement membrane connecting the myofibrils and the hypodermis, we are currently performing over-expression experiments of RNF-5 under the heat shock promoter and rnf-5(RNAi) analysis, both are carried out on the background of the LIM::GFP expressing animals.

160. Systematic analysis of cell specific enhancers in *C. elegans*

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How the cell-specific gene expression is precisely controlled?

To understand mechanism of the transcriptional regulation, we have taken a simple strategy. We have been trying to extract consensus regulatory sequence by comparing many upstream sequences, which share spatial and temporal expression pattern in a certain cell.

There are a large number of GFP reporters that are known to show cell-specific expression in particular set of cells i n *C. elegans*. One can accordingly expect that the same transcription machinery might regulate the genes, which are expressed in a certain cell. We check the expression patterns of a series of deletion promoter::GFP reporters in transgenic worms, and identify minimal enhancer < /span>$B!H(Jcore$B!I(J for the cell-specific expression. Not all, but some cases, such a core should share the common (or similar) sequence, and most probably it would be the binding site for upstream transcription factor.

We are currently collecting and analyzing head neuron-specific promoters. Thus far, we are narrowing down 7 promoters for the expression in the thermosensing neuron AFD; for example, 30 bp sequence of *gcy-8* (guanylyl cyclase), 120 bp sequence of *nhr-38* (nuclear hormone receptor), etc. Interestingly, in these sequences we have found binding consensus for the transcription factor OTX1, which has important roles for head development in fly and vertebrate. Eventually, it has been revealed that the *ttx-1* (OTX1 homolog of *C. elegans*) regulates both *gcy-8* and *nhr-38* expression in AFD (Satterlee et al. 2001). Systematic promoter analysis also demonstrated that some deletion cause ectopic expression in additional neurons. This observation suggests negative regulatory mechanism seems to participate in cell specific ation. Further analysis will be reported at the meeting.

Ref.: J. S. Satterlee et al. Neuron 31: 943-956 (2001)

161. Novel downstream targets of G-protein α-subunits in *C. elegans*

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Heterotrimeric G-proteins transduce signals from various serpentine transmembrane receptors to intracellular effectors. Several G-proteins have been identified in *C. elegans*: 21 Gα-subunits, including one homologue of each of the four mammalian classes Gs, Gi/o, Gq and G12, two Gβ-subunits and at least two Gγ-subunits. To identify novel downstream targets of G-protein signaling pathways, we are following two different approaches: second-site suppressors screens of G-protein mutants and two-hybrid interaction screens.

Animals that lack both *gpb-2* (Gβ5) and *goa-1* (Goα) function, arrest during larval development, whereas single *gpb-2* and *goa-1* mutants are viable. This synthetic larval lethal phenotype is rescued when EGL-30 (Gqα) activity is reduced. While this result suggests that EGL-30 activity causes the *gpb-2 goa-1* double mutant synthetic lethality, no suppression is observed when EGL-8 (a potential downstream effector of EGL-30) activity is reduced. Thus, we expected to find as yet unidentified mediators of EGL-30 signaling in a screen for suppressors of the synthetic lethal phenotype of *gpb-2 goa-1* double mutants. So far, we have isolated 47 suppressors of this synthetic lethality, and found that most suppressors are alleles of *egl-30*. At present, we are mapping the remaining suppressors that are likely to disrupt novel downstream effectors of EGL-30.

Secondly, we screened for components in G12-mediated signaling. Overexpression of activated GPA-12 (G12α/G13α) results in larvae that arrest their growth at an early stage. We found that mutations in *tpa-1*, encoding two isoforms (TPA-1A and TPA-1B) of a protein kinase Cθ subunit, completely suppress the activated GPA-12 induced growth arrest. The results suggest that activated GPA-12 induces a developmental growth arrest through a protein kinase-signaling pathway.

Finally, we performed two-hybrid interaction screens using all *C. elegans* Gα- subunits. As a result, we identified 11 different proteins that interact with 4 different G-proteins. Interestingly, we found two novel interactions between G-proteins and nuclear receptors: GPA-7 interacts with NHR-22, and GPA-13 interacts with a novel nuclear receptor. Because *nhr-22* mutant animals do not show any obvious phenotypes, or modulate *gpa-7* gain- or loss-of-function mutant phenotypes, we examined whether there is *in vivo* evidence for an interaction using membrane-targeted GFP reporter constructs. We co-expressed GFP-tagged GPA-7 and NHR-22 tagged with a membrane localization signal in muscle cells. In the absence of NHR-22, GPA-7 is localized in the nucleus and cytoplasm. However, in the presence of membrane-targeted NHR-22, GPA-7 re-localizes to the membrane. Taken together, these results show that GPA-7 and NHR-22 do interact both *in vitro* and *in vivo*.

162. Genome analysis of the effect of the host immune response on *Strongyloides ratti*.

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The establishment, survival and fecundity of nematodes is dramatically affected by the host immune response. However the molecular and biochemical nature of these effects have yet to be fully explored. We propose to exploit the nematode genome sequencing projects and EST data to analyse these phenomena. To this end we have generated a number of cDNA libraries from different stages of *Strongyloides ratti* including those from parasitic adults which had been exposed to different degrees of immune pressure. To date 7,793 ESTs have been sequenced and the cluster analysis of these clones will be presented. In order to generate microarrays all unique clones are being amplifed by PCR and arrayed. These microarrays will be differentially probed with cDNA isolated from populations of worms from varying immune pressures. We aim to identify gene sets whose expression levels vary as a result of changes in the host immune response and as such may be the basis of the immune dependant changes in the fitness of nematode infections.

163. The role of protein turnover in caloric restriction and aging

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Elucidation of the basic molecular mechanisms underlying the progressive decline in cellular function that accompanies aging and eventually leads to senescence will have an immediate impact on the design of novel interventions that could reduce or delay age-related deterioration in humans. Protein synthesis and degradation are the two essential interlinked cellular processes responsible for maintaining a functional protein content in every cell.

A role of protein turnover in aging and life-extending protocols is suggested by, 1) studies implicating aberrant protein modification as a major factor in aging, 2) the strong correlation between diminished protein turnover and aging, and 3) lowered generation of protein modifiers such as oxidation products, and increased protein turnover, under conditions of caloric restriction. Although a decrease in protein turnover is associated with senescent decline, and caloric restriction that confers longevity increases protein turnover, a direct molecular link between aging and regulation of protein turnover has not been established. We exploit the experimental strengths of the *Caenorhabditis elegans* model system in an effort to identify the specific biochemical steps underlying alterations of protein turnover during aging and under caloric restriction.

Our working hypothesis is that the delicate balance between detrimental protein modification and protein turnover that exists early in life is tipped in favor of deleterious protein modification during late stages of life. The rate at which a protein pool is refreshed at any given point in time is determined by the rate of protein synthesis and protein degradation at that particular point. Protein turnover cannot keep up with ever increasing accumulation of damaged proteins during aging. Increased protein turnover might consequently be one of the major causes of lifespan extension under caloric restriction or in long-lived mutants, by facilitating the maintenance of a fresher pool of proteins with less accumulated damage.

Our preliminary worked suggests a direct link between protein synthesis and longevity. Experimentally increasing protein turnover can extend lifespan in *C. elegans*, while a decrease in protein turnover has the opposite effect on longevity. We have generated nematode strains with increased protein turnover rates that have a lengthened lifespan and also strains with decreased protein synthesis rates that have a shortened lifespan. Moreover, we show that a long-lived nematode mutant shows a significant increase in protein synthesis rates. Our data provide the first causal molecular evidence linking lowered protein synthesis/degradation rates with senescent decline.

164. Functional analysis of the ACR-13 nicotinic acetylcholine receptor subunit

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Nicotinic acetylcholine receptors (nAChRs) mediate fast cholinergic synaptic transmission at nerve-nerve and nerve-muscle junctions of both nematodes and vertebrates via a transient increase in cation permeability. The gene family of *C. elegans* nAChRs is extensive and diverse, with 27 members identified and several additional strong candidates (1). Subunits are designated α or non-α; α-subunits are defined by the presence of a pair of cysteines in loop-C of the ACh binding site. Each AChR molecule contains five subunits. Different combinations of subunits in different cell types and at different stages of development result in the functional diversity observed for nAChR subtypes. However, there are many subunits for which a specific role is yet to be determined. ACR-13 (**ac**etylcholine **r**eceptor) is a member of the *C. elegans* nAChR ACR-8-like subunit subfamily. ACR-13 is an α-subunit with an unusual histidine (H) residue at the –1' position in the M2 (second transmembrane, channel lining) region. Molecular modelling of this E-to-H modification has predicted a profound effect on channel function with the calculated electrostatic potential energy suggesting that it may be a candidate anion channel subunit (2). We have shown that the mutant *lev-8* is a null mutation of the gene encoding ACR-13. Hitherto, *lev-8* was known only as a levamisole-resistance gene with a phenotype of weak resistance to the anthelmintic drug, levamisole (3-5). Detailed studies of ACR-13 (LEV-8) and a mutant of ACR-12, another member of the ACR-8-like subfamily, are in progress.

1. Mongan N. P. et al. (2002) *Protein Science* **11**: 1162-1171
2. Mongan N. P. et al. (1998) *Receptors and Channels* **6**:213-228
3. Lewis J. A. et al. (1980) *Genetics* **95**: 905-928
4. Lewis J. A. et al. (1987) *J. Neuroscience* **7**: 3059-3071
5. Fleming J. T. et al. (1997) *J. Neuroscience* **17**: 5843-5857

165. The function and interactions of the *C. elegans* orthologue of the SMN gene: *CeSMN*

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Spinal muscular atrophy (SMA) is a common human autosomal recessive neuromuscular disorder characterized by a progressive degeneration of the spinal motor neurons. The SMA gene, designated survival motor neurone (*SMN*) gene, has been cloned, and lies on a complex duplicated region of human chromosome 5q13 (1). The *SMN* gene encodes a protein of 294 amino acids, which is involved in a number of biological processes and interactions (2). The mechanism by which reduced SMN protein levels result in loss of motor neurons in SMA is unknown.

The *SMN* gene is evolutionarily conserved. The *C. elegans* orthologue of the *SMN* gene (*CeSMN*) has been isolated and characterized (3). The *CeSMN* gene consists of five exons and encodes a protein of 207 amino acids. The CeSMN protein is maternally transmitted and has significant homology to human SMN, with an overall amino acid similarity of 36% (3). Homology is particularly high in functionally important regions, including regions important in RNA and protein binding. CeSMN over-expression or disruption by RNA interference results in locomotion defects and developmental defects, including embryonic lethality and sterility (3).

A *CeSMN* mutant (ok355) has been obtained from the Oklahoma Medical Research Foundation, containing a deletion of most of the *CeSMN* gene. We have generated an outcrossed heterozygous line of ok355, and maintained this line by use of the hT2 (I;III) balancer chromosome (provided by Ann Rose, Vancouver). Using this line, we are studying mutants homozygous for the deletion. The homozygous mutant exhibits locomotion and developmental defects, similar to those seen by RNA interference of *CeSMN*. For example, mutants have developmental arrest at approximately L4 larval stage, display an un-coordinated (unc) phenotype and have a marked reduction in pharyngeal pumping. This mutant will be used in the deployment of suppressor screens, to identify novel genes functionally linked to *CeSMN*.

In addition, we have identified a number of *C. elegans* homologues of human proteins that are known to interact with SMN, which could represent potential CeSMN binding partners. SMN-interacting protein sequences were used to search the predicted *C. elegans* proteins found in Wormpep, by BLASTp (4). The best *C. elegans* match for each protein was identified, with most of these proteins having a significant match ($p>1.0 \times 10^{-5}$). Two predicted proteins from this subset have been selected for further characterization, the homologues of human proteins SIP1 and FUSEbp (termed CeSIP and CeFBP respectively). Initial studies show that gene silencing of *CeSIP* by RNA interference results in embryonic lethality, whereas silencing of *CeFBP* has no observable effect.

1. Lefebvre, S., et al. (1995) *Cell*, **80**, 155-65.
2. MacKenzie, A. E., and Gendron, N. H. (2001) *Nat Struct Biol*, **8**, 13-5.
3. Miguel-Aliaga, I., et al. (1999) *Hum Mol Genet*, **8**, 2133-43.
4. Altschul, S. F., et al. (1990) *J Mol Biol*, **215**, 403-10.

166. The _-catulin gene *ctn-1* is alternatively spliced and encodes a component of muscle tissue.

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During our studies of the cadherin-catenin adhesion system in *C. elegans*, we (and others) noticed a locus that encoded an _-catenin-like protein in addition to the previously characterized *hmp-1* _-catenin. We present evidence that this _-catenin-like molecule belongs to a distinct conserved family of proteins, the _-catulins, and we have termed this gene *ctn-1* (_-catulin-1). The _-catulins are distinct from the _-catenins due to the lack of a central domain of approximately 150 amino acids corresponding to the "adhesion modulatory domain" of _-catenins (1). Single _-catulin loci have been identified in the human and mouse genomes, and there is also an _-catulin locus in *Drosophila*, but little is known of their functions.

Information from cDNA sequences suggests the existence of at least two alternative *ctn-1* splice isoforms in *C. elegans*, the shorter isoform resulting in an alternative C-terminus predicted to truncate the protein in the actin-binding VH3 (vinculin homology 3) region. The *ctn-1* locus is highly conserved in *C. briggsae*, which also appears to encode two main *ctn-1* isoforms.

Expression data from human _-catulin demonstrates a near-ubiquitous expression pattern in most tissues tested (except neural tissues) (1), but a *C. elegans ctn-1* promoter::GFP fusion directed expression exclusively throughout body wall, vulval and anal muscle tissue. We have also constructed GFP fusions to the C-termini of both main *ctn-1* isoforms, which displayed similar expression patterns.

We have attempted to disrupt the functions of *ctn-1* in *C. elegans* using RNAi, but we have been unable to detect any obvious differences between wild-type and *ctn-1(RNAi)* animals. Injection or feeding of *ctn-1* dsRNA also fails to completely remove GFP fluorescence in animals containing GFP fusions to either of the *ctn-1* isoforms, suggesting that *ctn-1* may be intractable to standard RNAi approaches. We are currently attempting to isolate a *ctn-1* deletion allele.

Reference: 1) Janssens *et al.* (1999) *Biochim. Biophys. Acta* **1447**:341-347.

167. A comparison of the action of ivermectin and emodepside on *C.elegans*

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The cyclodepsipeptide, emodepside, is a novel broad spectrum anthelmintic with a distinct mode of action. Here we report preliminary studies comparing the commercially available broad spectrum anthelmintic ivermectin with emodepside. The free-living nematode *Caenorhabditis elegans* was used to identify the effective concentration window of emodepside inhibitory action on the growth and development of *C.elegans*. This information will subsequently be utilized in forward genetic strategies to identify emodepside resistant phenotypes, with the aim of identifying genetic determinants of emodepside action.

The action of ivermectin and emodepside on the growth and locomotion of a synchronized population of *C.elegans* (N2 Bristol strain) was determined. Eggs were isolated from gravid adult hermaphrodite worms by the alkaline hypochlorite method (Lewis, Fleming, 1995) resuspended in M9 buffer (composition in g/liter Na_2HPO_4 6, KH_2PO_4 3, NaCl 5, $MgSO_4.7H_2O$ 0.25). Approximately 100 eggs were placed on agar plates containing OP50-*E.coli* and either emodepside (100pM – 950nM), ivermectin (100pm-9.5nM) or vehicle control (1% ethanol). The plates were incubated for 5 days at 20˚C and the developmental progress of the worms, through larval stages, to fertile adulthood, monitored. Locomotion was quantified by counting body bends/min.

Ivermectin inhibits the development of larval stages to fertile adults at concentrations as low as 2.25nM. There exists a concentration dependent inhibition of larval development to adulthood up to 9.5nM, at which point 90% of juvenile worms are arrested at L1 stage (n=2). Emodepside has no observable effect on larval stages of *C.elegans* at any of the concentrations tested (n=4). However, in mature fertile adults there was a concentration-dependent effect on locomotion. The threshold for this effect was observed at 4.5nM (13 ± 2 compared to control 25 ± 1, body bends/min, n=4), and at 90nM locomotion was dramatically decreased to 1.3 ± 0.3 body bends/min. It was observed that above 180nM emodepside growth to adulthood was retarded, on average by 24 hrs, when compared with worms on lower emodepside concentration and control plates. Furthermore, at 950nM hermaphrodite adults were arrested before egg laying could occur (n=2).

In conclusion, these data suggest that emodepside has a distinctly different anthelmintic mechanism to ivermectin, in that it interacts with a target that is expressed, or functionally important, in the adult stage only. Furthermore, this target is involved in motor control.

Kiran Amliwala is a BBSRC-CASE student.

1. Dents, J. A., M. W. Davis, and L. Avery. avr-15 encodes a chloride channel subunit that mediates inhibitory glutamatergic neurotransmission and ivermectin sensitivity in Caenorhabditis elegans. *EMBO J.* 16: 5867-5879, 1997.
2. Zahner, H., A. Taubert, A. Harder, and G. Samson-Himmelstjerna. Effects of Bay 44-4400, a new cyclodepsipeptide, on developing stages of filariae (Acanthocheilonema viteae, Brugia malayi, Litomosoides sigmodontis) in the rodent Mastomys coucha. *Acta Trop.* 80: 19-28, 2001.

168. The nicotinic acetylcholine receptor family of *Caenorhabditis elegans*

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Nicotinic acetylcholine receptors (nAChRs) are prototypical members of the ligand-gated ion channel super-family. They are pentameric membrane proteins, which mediate the fast actions of the neurotransmitter acetylcholine (ACh) at the neuro-muscular junction and in the nervous system. To date, 49 putative nAChR subunits (23 α and 26 non-α) have been identified in *C. elegans*, which is by far the largest nAChR family yet observed. Why nematodes have such a diverse nAChR family (mammals appear to possess 9 α and 7 non-α subunits) has yet to be fully resolved. Previously, the *C. elegans* nAChR subunits have been subdivided into five major groups (1, 2). Recent analysis of the *C. elegans* genome has identified an additional DEG-3-like α subunit candidate and two more potential ACR-16-like non-α subunits. [A *deg-3* gain of function mutation in the channel-lining region results in neural degeneration (3)]. In addition, 20 "outlier" subunits have been identified including six non-α subunits on the T01H10 cosmid. To gain further understanding of this diverse nAChR family, GFP constructs are used to determine expression patterns and gene knockdown is studied by dsRNAi. Studies with GFP constructs of several nAChR subunits have already shown striking differences in spatial and temporal expression patterns. Electrophysiological studies have implicated nAChRs in regulating pharynx function. Using intracellular microelectrode recordings from the pharynx of wild type worms, deploarizing responses to nicotine and ACh were observed. The nicotinic responses were blocked by d-tubocurarine.

1. Mongan N. P. et al. (1998). *Receptors and Channels* **6**:213-228.
2. Mongan N. P. et al. (2002). *Protein Science* (in press).
3. Treinin M. et al (2001). Mol Cell Neuroscience **3**: 589-99

169. Stage-specific effects of the cholinergic anthelmintic drugs, levamisole, morantel and pyrantel on *Caenorhabditis elegans*

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Nicotinic acetylcholine receptors (nAChRs) are pentameric membrane proteins, which mediate fast synaptic transmission at neuro-muscular junctions and in the nervous system. Recent analysis of the *C. elegans* genome has revealed the largest and most diverse nAChR family to date, with nearly fifty putative subunits identified (1). Why nematodes should have such an extensive nAChR family when vertebrates have less than twenty subunits is unknown. Several anthelmintic drugs target nAChRs. As part of our studies aimed at understanding the roles of the diverse members of the nematode nAChR family, the effect of the drugs levamisole, morantel and pyrantel, on different stages of worm development were tested. The drugs were found to have stage-specific effects. This was particularly evident for levamisole: L1 worms were more sensitive to levamisole at concentrations up to 0.03 mM, in comparison to other stages. However, at higher levamisole concentrations L1 worms were still motile whilst more developed worms were completely immobilised. Earlier genetic studies showed that the drugs target a particular subset of nAChRs (2) and the present studies indicate that expression and function of nAChR subunits may vary during development. Understanding the sensitivity of various stages of nematodes to cholinergic ligands may be important in providing insights into the actions of novel anthelmintic drugs.

1. Mongan, N. P. et al. (2002). *Protein Science* **11**:1162-1171
2. Fleming, J. T. et al. (1997). *J Neuroscience* **15**:5843-5857

170. Towards a two-component based enhancer trap system in *C. elegans*: a progress report

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In Drosophila, P-element based enhancer trap screens have proven invaluable tools in both forward and reverse genetic approaches (Brand and Perrimon, 1993). Engineered P-elements that contain the coding sequence of the yeast transcriptional activator Gal4 are allowed to randomly insert into the genome. When a P-element has inserted in or close to a gene (depending on the configuration used), this will result in Gal4 expression. Importantly, because Gal4 is now under the control of promoter and enhancer sequences of the gene the element has inserted in, Gal4 will be expressed in an identical spatial and temporal pattern. Transposon based enhancer trap screens can be used to identify genes that are expressed in a cell or tissue of interest. However, once a collection of specific Gal4 expression patterns has been established, the system can also be used as a tool to drive the expression of other genes in specific cells or tissues. Thus, by using a minimal promoter containing Gal4 binding sites (Gal4-UAS), cDNAs of genes (including toxins to ablate a specific cell or tissue) can be expressed in the same pattern as Gal4 itself.

Two major bottle necks have so far prevented the development of gene and enhancer trap systems in C. elegans. First, attempts to generate a Gal4 based two-component system had not been successful. Second, no transposon system was available to insert engineered transposable elements from a transgene into the germline of C. elegans. We have solved part of the first problem. We found that a Gal4/VP16 fusion protein can activate the expression of a transgene containing Gal4 binding sites (UAS) and a minimal pes-10 promoter. Thus, expression of Gal4/VP16 from a heat-shock promoter activates the expression of a UAS::pes-10::gfp reporter construct on a separate transgene. Furthermore, expression of Gal4/VP16 from a myo-2 or myo-3 promoter induces the expected tissue specific expression of GFP from the UAS-pes-10::gfp transgene. Using the heat-shock and myo-2 promoters, GFP is expressed at high levels. We are currently testing low copy number Gal4/VP16 expressing transgenes to test the efficiency of this two-component system. The second bottle neck is a transposon system that can randomly insert the Gal4/VP16 coding sequence (about 800 bp) into the genome. Although the Mos1 transposon has been shown to jump efficiently in the C. elegans germline (Besserau et al. 2001), Mos1 shows a low tolerance for inserts. Therefore, we plan to test alternative transposon systems such as Sleeping Beauty and Minos, which have been shown to jump efficiently with larger inserts. If this is successful, we will construct SB or Minos elements containing a 5' splice site or a minimal promoter and the Gal4/VP16 coding sequence. Insertions in or close to genes will be identified using the UAS::pes-10::gfp transgene. If successful, we will document these expression patterns and cryopreserve strains which contain interesting enhancer trap insertions. We will make this information and the strains available as a resource.

Brand AH, Perrimon N. (1993) Development 118:401-15.
Besserau et al. (2001) Nature 413:70-4.

171. Y74C10AM.1 and W09D6.6 are the *Caenorhabditis. elegans* orthologues of the human genes ABC7 and ABC6, respectively

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Mitochondrial iron homeostasis is abnormal in a number of human diseases associated with ataxia, Friedreich's ataxia (FRDA) and X-linked sideroblastic anemia with ataxia (XLSA/A). Mitochondrial iron is accumulated matrix in both disorders. XLSA/A is characterised by mild hypochromic, microcytic anemia with bone marrow sideroblastosis and nonprogressive cerebellar ataxia. It is caused by mutations in a gene encoding a mitochondrial ATP-binding cassette transporter protein, ABC7, located at human chromosome Xq13. ABC7 is the human counterpart of *S. cerevisiae* protein Atm1p. Atm1p plays a role in the export of iron/sulfur clusters from yeast mitochondria, where they are generated, to the cytoplasm. We are interested to investigate the pathogenesis of human disorders involved in mitochondrial iron homeostasis using *C. elegans* as an animal model. A first step is to demonstrate that an orthologue of the human candidate gene is present in *C. elegans*.

Y74C10AM.1 is a *C. elegans* annotated gene with a size of 2,671 bp on chromosome I. The predicted protein has 320 amino acids. Y74C10AM.1 protein shows high homology with the central region of human ABC7. We postulated that Y74C10AM.1 is a part of a bigger gene. To confirm this hypothesis we performed RT-PCR and RACE experiments. Primers we designed based on genomic homology between ABC7 and flanking regions of Y74C10AM.1. cDNA ends were obtained by 5'RACE using SL1 and 3'RACE. We further obtained a full length mRNA of 2,504 bp with 8 exons and two polyadenilation sites. The gene is spread almost 13.4 kb on the genome. It encodes a predicted protein of 703 amino acids, which displays an identity of 52% and a similarity of 73% (CLUSTAW) with ABC7. The new Y74C10AM.1 shows an alternative splicing between exon 4 and exon 6 with a premature stop codon at 158. Y74C10AM.1 is *trans*-pliced to both spliced leaders SL1 and SL2 sequences, being the stronger signal with SL1. A gene, Y74C10AL.2, is mapped 119 bp downstream Y74C10AM.1. We postulate that Y74C10AM.1 may be the first 5' gene of an operon that consists of at least two genes.

A second ABC transporter, Mt-ABC3, involved in mitochondrial iron homeostasis has been reported in *S. cerevisiae*. W09D6.6 is a *C. elegans* annotated gene with a size of 8,404 pb on chromosome III. According to the databases, W09D6.6 has 10 exons. By RT-PCR we have characterised two additional exons. Thus, the size of the W09D6.6 mRNA is 2,571 bp with 12 exons and encodes a predicted protein of 801 aa. The protein displays an identity of 46% and a similarity of 67% (CLUSTAW) with ABC6. W09D6.6 is *trans*-spliced using SL2 preponderantly, but we found an amplified band with SL1 as well. Data from the genomic region suggest that W09D6.6 may be the fourth 5' gene of an operon.

172. Characterisation of the *Caenorhabditis elegans* orthologue of the Friedreich's ataxia gene

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Friedreich's ataxia is an autosomal recessive neurodegenerative disease caused by loss of function mutations in the *FRDA* gene. The most frequent mutation in the expansion of a GAA trinucleotide repeat in the first intron of the gene. Deficiency of frataxin, the product of the *FRDA* gene, results in ataxic movements, sensory axonal neuropathy, cardiomyopathy, skeletal deformities, and diabetes mellitus or glucose intolerance. This protein has been localized in the inner membrane of mitochondria. Lack of yeast frataxin, Yfh1p, produces an increase in mitochondrial iron, increased sensitivity to oxidative stress, and respiration deficiency. Some other experiments, in yeasts and mammalian cells point towards the possibility that the protein is implicated in ATP production or in assembling sulfur-iron cluster proteins. Our aim is to establish a *C. elegans* model to study the function of the frataxin and the pathophysiology of the disease.

As a first step in the study of the frataxin we wanted to demonstrate that the F59G1.7 gene, which shows homology with the *FRDA* gene, described in ACeDB is really the orthologue of the frataxin gene. To address this point we have isolated the cDNA of F59G1.7 and we have analyzed the region were it is located. We conclude that the gene is located in a putative operon, that consist in between 6 and 8 genes. By the other hand, we focused own work to elucidate how similar is the expression of the *C. elegans* frataxin with the human frataxin by doing *gfp* gene construct expression. We found that the gene is at least expressed in gut, pharynx, and some neurons in the head and along the body, in adults. By immunostaining analysis with a polyclonal antibody against human frataxin we have observed expression during the embryonic development. We have also performed genetic analysis by generating transient knock-down mutants of F59G1.7. We did RNAi experiments to get frataxin mutant worms by several methods: RNAi feeding, dsRNA injection, and by making stable lines of worms that express dsRNA of the frataxin gene. Worms of the F1 generation show an specific phenotype: *egl* (egg laying) phenotype, thin and short worms, slow and arritmic pharynx pumping, and a decrease in the rhythm of defecation.

173. Homology modelling of nicotinic acetylcholine receptors in *Caenorhabditis elegans*

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Nicotinic acetylcholine receptors (nAChRs) are members of the ligand-gated ion channel superfamily of receptors. The nAChR molecules are pentameric trans-membrane proteins that mediate fast cholinergic synaptic transmission. Each subunit has an N-terminal extracellular domain (the ligand-binding domain, LBD) of approximately 200 amino acids containing residues that bind the natural ligand, the neurotransmitter acetylcholine (ACh). The *C. elegans* family of nAChR subunits is the largest currently known (1). Although to date there is no X-ray structure for any nAChR molecule, recently a crystal structure has been obtained for a soluble ACh-binding protein (AChBP) isolated from another invertebrate, the mollusc *Lymnaea stagnalis* (2). The AChBP molecule is a homologue of the LBD region of nAChRs (2). Here, we present homology models for members of the *C. elegans* ACR-16 subfamily of nAChR subunits. Members of this subfamily show homology to both the AChBP and α7-like, homomer-forming subunits of vertebrates. Loops that appear to protrude into the lumen formed by the extracellular portions of the 5 ACR-16 polypeptides are described. These loops (formed by the residues SVDTNFDS in ACR-16) are not present in the AChBP and are less pronounced in chicken and human α7 subunits. A similar loop is present in ACR-11 but not all *C. elegans* ACR-16-like subunits possess such loops. The location of lumen loops close to the predicted ACh binding region points to a possible functional role. As the ACR-16 subunit can be heterologously expressed, the role of these loops is amenable to investigation using mutagenesis and functional expression studies.

1. Mongan, N. P. et al. (2002). *Protein Science* **11**:1162-1171.
2. Brejc, K. et al. (2001). *Nature* **411**: 269-276.

174. Cloning of a novel regulator of the LET-23 EGFR/ LET-60 RAS/ MPK-1 MAPK signaling pathway.

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The EGFR/RAS/MAPK pathway is used reiteratively to control cell differentiation during *C. elegans* development. We are focusing on the role of this pathway during vulval development. In this process, the gonadal anchor cell (AC) secretes the inductive signal LIN-3 EGF to specify the vulval cell fates. The six equivalent Vulval Precursor Cells (VPCs) express the LET-23 EGFR that binds LIN-3 EGF on the basolateral surface of the VPCs. P6.p, the VPC most proximal to the AC, receives most of the inductive signal and adopts the 1° fate. In turn, P6.p sends a lateral signal to its neighbours P5.p and P7.p to activate the LIN-12 NOTCH signalling pathway. The lateral signal prevents P5.p and P7.p from adopting the 1° fate and specifies the 2° fate. The three remaining VPCs (P3.p, P4.p and P8.p) that do not receive enough inductive or lateral signals adopt the 3° fate, they divide once and fuse with the hypodermis. The three induced VPCs (P5-7.p) then divide to generate 22 vulval cells that form a functional vulva.
To clone new regulators of the EGFR/RAS/MAPK pathway we performed a genetic screen in a *gap-1(lf)* sensitised background. The *gap-1(lf)* mutation slightly activates the EGFR/RAS/MAPK pathway but not enough to cause a visible phenotype. When a second mutation that further increases the activity of the EGFR/RAS/MAPK pathway is generated in the *gap-1(lf)* background, a multivulva (Muv) phenotype may be observed. From this screen, we isolated the *zh8* mutation that appears to increase EGFR/RAS/MAPK pathway activity. *zh8* suppresses the vulvaless (Vul) phenotype of *lin-3* and *lin-25* reduction-of-function mutations and increases the penetrance of the Muv phenotype of *lin-10(lf); gap-1(lf)* double mutants and of *gaIs36[HS-mpk-1, mek-2(gf)]* animals. Furthermore, the *zh8* mutation appears to bypass the lateral inhibition of the 1° fate by LIN-12 NOTCH, since adjacent VPCs can adopt the 1° fate in *zh8; gap-1(lf)* or *zh8; gaIs36[HS-mpk-1, mek-2(gf)]* animals.
zh8 was mapped between the cosmids C52A11 and M106 using SNPs and a point mutation was found in the T19E10.1/*let-21* gene. *let-21* is the *C. elegans* ortholog of the *Drosophila pebble* gene and the mouse *ect2* proto-oncogene. Pebble and Ect2 are guanine nucleotide exchange factors (GNEFs) for the Rho family of small GTPases, and they play an important role in the assembly of the actomyosin contractile ring essential for cytokinesis (Prokopenko et al. 1999, Genes & Devel. 13:2301; Tatsumoto et al. 1999, J. Cell Biol. 147: 921). The *zh8* mutation changes a conserved amino acid in the second of the two BRCT domains in the N-terminal portion of LET-21, leaving the putative GNEF domain intact. Feeding RNAi against T19E10.1 suppressed the Muv phenotype of *let-21(zh8); gap-1(lf)* animals (the vulval induction index was reduced from 4.5 to 3.1), suggesting that *let-21(zh8)* could be a gain-of-function mutation. Consistent with this idea, *let-21* loss-of-function mutations cause a pachytene arrest phenotype in the germ cells, similar to mutations that inactivate RAS/MAPK signaling (Ohmachi et al., 2000 Midwest meeting abstract 85). To test the hypothesis of a gain-of-function mutation, we are performing rescue experiments examining if a *let-21(zh8)* genomic DNA fragment is able to rescue the *let-21(lf)* phenotype and cause a Muv phenotype in a *gap-1(lf)* background.

175. The mammalian FGFs belonging to the 9, 16 and 20 subfamily but neither *C. elegans egl-17* nor *Drosophila Bnl* genes can functionaly replace the *C. elegans let-756/FGF* gene.

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Complete nucleotide sequences are available for different animal species such as worm, fly and humans and more and more information are coming from chordate non vertebrate genomes, particularly amphioxus and ciona. Such information make possible to compare various gene families in different species. We are interested in the *FGF* gene family, which comprises 22 members in mammals but only few in invertebrates i.e. one member in *Drosophila, Bnl*, and two in *C. elegans*, *let-756* and *egl-17*. Information given by phylogenetic analysis allows to establish a classification of vertebrate FGFs in various subfamilies. However, the analysis does not show direct phylogenetic relationships between invertebrate and mammalian FGFs and we could not include the *Drosophila* and worm FGFs in any of the defined subfamilies. . The purpose of the present study was to assess which human subfamily was functionally closer to LET-756.
The let-756 loss-of-function s2887 allele is lethal at larval stages (Roubin et al., 1999). By testing the rescue of let-756 mutant with human-worm chimeras we present evidence that LET-756 is functionally closer to the FGF9/16/20 subfamily since only sequences from these 3 FGFs can lead to the mutant rescue. The basis for this rescue, which was not expected from the result of the phylogenetic analysis, lies in the particular structure of the FGF9/16/20 subfamily.

176. Somatic cell fusion and fertility in *C. elegans:* the *eff-1* connection

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From fertilization throughout development cell fusion is an essential process in multicellular organisms. Recently, we isolated and characterized *eff-1*, a gene encoding type-I membrane proteins that is essential for all epithelial cell fusion events in *C. elegans* [1].
To investigate whether *eff-1* has a function in the fertilization process we looked for fertility defects in *eff-1*(*hy21)* and *eff-1*(*oj55)*. We also generated and tested strains carrying *mnDf105* deficiency over a mutated *eff-1* gene and preformed RNAi. We have found in all these experiments a significant percent of sterility and reduced number of progeny. In the *eff-1* strongest allele *hy21ts* there was 12.4% sterility at the restrictive temperature (25°C) compared to 1% at the permissive temperature (15°C). In the strain carrying *eff-1(hy21)* over *mnDf105* deficiency we found 25%, 9.6% and 29% sterility at 15°C, 20°C and 25°C respectively.
To study the reduced function of *eff-1,* we used RNAi experiments by injecting N2 and *eff-1(hy21)* worms with 800bp dsRNA complementary to *eff-1* cDNA. We found 28% sterility in *eff-1(hy21)* injected animals at 15°C (the permissive temperature) and F1s of either N2 or *eff-1* injected animals showed 9% to 40% sterility. In addition to the increase in sterility and a reduction in the number of progeny (for non sterile worms) there was an increase in the number of worms exhibiting other morphological defects associated with the *eff-1(hy21ts)* mutant (e.g., Dpy, Unc and Egl).
Two alternative hypotheses can explain the fertility problems in *eff-1* worms: 1) Somatic cell fusion failure in vulva, uterus and hypodermis cause low fertility. And 2) EFF-1 activity is directly required for egg-sperm fusion. In order to distinguish between these hypotheses, we crossed sterile hermaphrodites to N2 males. These crosses resulted in a restoration of the fertility, suggesting a putative role for EFF-1 in egg-sperm fusion and/or spermatogenesis.
Our results show correlation between worms with reduced *eff-1* activity and fertility problems. The results of the genetic crosses may imply that indeed some fertility defects are a result of sperm defects. The above results suggest that our strongest *eff-1* allele (*hy21ts)* is not a null. We are doing non-complementation screens in order to find new *eff-1* alleles. New alleles of *eff-1* will help us to better understand the role of cell fusion in the development of *C. elegans* and in fertility.

[1] Mohler et al.(2002) *Dev. Cell* 2:355-362

177. New players in RNA interference: Implication of *rde-1* homologues.

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In a variety of organisms, double stranded RNA (dsRNA) has been shown to induce gene silencing phenomenon called RNA interference (RNAi), in which small RNAs derived from the dsRNA trigger destruction of homologous cellular RNAs. In previous genetic screens, we have indentified several *rde* (*R*NA interference-*de*ficient) mutants that define genetic loci required for RNAi in *Caenorhabditis elegans* [Tabara *et al*, Cell **99**: 123-132 (1999)]. Among these, the *rde-1* gene encodes a member of a functionally novel but highly conserved eukaryotic gene family implicated in gene silencing in various organisms including *C. elegans*, fungi, plant and *Drosophila*. To date, we have identified 27 homologues of *rde-1* in *C. elegans* genome. In order to address if these *rde-1* homologues could also play a role in RNA interference, we have injected dsRNAs targeting all the 27 *rde-1* homologues. The gene-silencing of four subclass of genes affects either the somatic or germline-specific RNAi. Interestingly, two homologues of *rde-1*, *alg-1* and *alg-2*, previously described to be required for the processing and the activity of *lin-4* and *let-7* in development [Grishok *et al*, Cell **106**: 23-34 (2001)], interact with small temporal RNAs (i.e. LIN-4 and LET-7) *in vivo*. We are currently investigating the cellular proteins interacting specifically with *rde-1* homologues *in vivo*.

178. *C. elegans* as a model organism for lysosomal storage disorders

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Lysosomal storage disorders are devastating diseases. In most cases, multiple organs of the patients are affected, including the brain.
Many enzyme-encoding genes involved in lysosomal storage disorders have been identified by functional complementation of cell lines. Positional cloning has been used to identify additional genes without known function. One possibility to elucidate the function of these genes is to characterize model organisms carrying mutations in the homologous genes. Due to its well-characterized nervous system, completely known genome sequence and amenability to large genetic screens, C. elegans seems to be a logical choice.
To exploit these advantages, we are using C. elegans as a model for juvenile neuronal ceroid lipofuscinosis, a disorder caused by defects in the putative integral lysosomal membrane protein CLN3. The worm has 3 homologues (cln-3.1, cln-3.2, and cln-3.3) of the human gene. Single deletion mutants do not have an obvious phenotype. To find out which mutant would represent the best model, the expression of promoter-GFP fusion constructs has been determined. The cln-3.1::GFP fusion is mainly expressed in the gut, whereas cln-3.3::GFP expression is detected in the intestinal muscle. In addition, we decided to compare our data with those about other lysosomal genes of the worm.

179. The *lin-26* gene is regulated by tissue-specific cis-elements

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Lin-26, which codes for a zinc-finger transcription factor, is expressed and required to maintain all epithelial cell fates of the ectoderm and mesoderm (the somatic gonad), and can also induce the expression of epithelial markers when overexpressed. Since the *C. elegans* lineage is invariant, one issue was to determine whether the complex *lin-26* expression pattern is established mainly through lineage-specific or tissue-specific promoter elements. To dissect the *lin-26* promoter, we used four parallel approaches: 1) A sequence comparison between *C. elegans* and *C. briggsae lin-26* homologs identified seven conserved regions spread over 11 kb. 2) We tested the enhancer activities of conserved sequences by cloning promoter fragments in front of a minimal promoter linked to GFP. 3)We examined whether these elements were required for expression of a *lin-26::gfp* construct and 4) for the rescue of a null allele. In this way we found several promoter elements, which interestingly become activated from 3' to 5' during embryogenesis:

Three redundant elements control expression in the hypodermis. Two non-overlapping promoter fragments of 1.3 and 2.1 kb can drive GFP expression in the major hypodermis. When both are deleted from the full length promoter of about 11 kb, GFP is still expressed in the hypodermis, implying the existence of a third element which we could not pin down to a small sequence. These redundancies were confirmed by rescue experiments. The expression of a 4 kb element, that comprises the 1.3 and 2.1 kb elements, requires the activity of *elt-1* since in the *elt-1(zu180)* null mutant the number of GFP positive cells is dramatically reduced. The regulation could be direct since three conserved GATA sites are found in that 4 kb element.

A support cell and minor hypodermal cell element (559 bp), which leads to GFP expression in about 20 cells in the head (mostly support cells and few minor hypodermal cells), 6 cells in the tail (PHsh, hyp8,9,10) and also in the excretory cell. We know by dye filling experiments that this element is required for the formation of functional chemosensory organs, composed of support cells and sensory neurons. These expressions are region specific since they can be separated into an anterior and posterior elements. So far, we know that the posterior Hox genes *nob-1* and *php-3* are not involved in the posterior expression of *lin-26.*

The 559 bp element, in both orientations, also acts synergistically with highly conserved sequences, which have no apparent enhancer activity on their own, to give new expression patterns in the rectal cells (B,Y,U,F,K,K') and in the P cells.

A somatic gonad element (444 bp), which drives GFP in the somatic gonad precursor cells Z1 and Z4, and later in the uterus. When this element is deleted, animals are sterile and show gonad defects.

A rep cell element (3.5 kb), only defined by its enhancer properties, which turns on GFP in the rectal rep cells. The expression of *lin-26* in these cells is not essential for viability.

Since we did not identify elements that could control expression in C-derived or AB-derived cells but rather elements active in cells related by function, we conclude that the *lin-26* regulation is controlled by redundant elements acting in a tissue-dependent manner rather than by lineage-dependent mechanisms. This study will provide useful GFP markers and drivers to the worm community.

180. Olfactive learning and memory in nematodes

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We already suggested that olfactory imprinting (or odor memory) could exist in nematodes (Remy J.J., EWM 2000). Further experiments confirmed now that adults worms can be sensitized to odorant molecules that were present during a short period of their early development. For a number of *C. elegans* strains (N2, TR403, CB4705), as for *Osheius* (CEW1) we were able to define a critical period of early development during which worms can learn and memorize short odor inputs.
Chemotactic assays performed at the adult stage showed that an early exposure to beta-citronellol or to benzaldehyde, both sensed by the chemosensory neurone AWC, does sensitize worms to these attractants, although sensitizations are restricted to the conditioning concentrations of odorants. As it has been reported for temperature memory, odor memorisation in nematodes is associated with the presence of food. Although starving prevents learning, exogenous serotonine can compensate starvation during the learning phase, and preliminary pharmacological analysis of this experience dependent behaviour identified the octopamine agonist synephrin as an enhancer of olfactive memory. The physiological significance of this behaviour is not known, but one think of fast environmental adaptative processes.
It is known that the thermosensory AFD and chemosensory neurones responsible for attraction to volatil attractants, AWA and AWC, cosynapse the same interneurone AIY.
Using a candidate gene approach, we found that the two alleles *ot-22* and *ks-5* of the thermotaxis mutant *ttx-3*, a LIM homeobox exclusively expressed in AIY (1)), although not affected in their chemotaxis behaviour, unable to perform olfactory learning and memory. Sel-12 mutants (2) , as well as the null mutant for the neuronal calcium sensor NCS-1 (3) both displaying AIY morphological and functional defects, presented olfactory memory impairment. Alltogether, these observations provide new experimental support to the idea of cointegration and memory of thermo and chemosensory inputs in *C. elegans* (Pierce-Shimomura, IWM 2001), through common involvment of the postsynaptic AIY interneuron.
Memory mechanisms at the cellular and molecular levels are not well understood : they can involve the cAMP/CREB pathway, synaptic strength modulations, or establishment of new synapses. Our current view for explaining odorant memory in *C. elegans*, as it is specific for odorant concentrations, is that it must (at least) rely on odorant specific olfactory receptors expression levels -transcriptional control in the different chemosensory neurones- or/and at the level of efficiency of their individual coupling to the transducing partners. Specific experiments, including microarray approaches, have now to be carried out to verify this hypothesis.

1) Hobert O et al., Neuron 1997, 19:345-357
2) Levitan D and Greenwald I. Nature. 1995 Sep 28;377(6547):351-4.
3) Gomez M et al., Neuron 2001, 30: 241-248

181. *feh-1* and *apl-1*, the orthologues of Fe65 and Alzheimer's β-amyloid precursor APP control a common pathway involved in the regulation of the pharyngeal activity in *C. elegans*

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The main histopathological feature of Alzheimer's disease is the occurrence of the senile plaques in the brain. These are made of aggregated forms of the amyloid peptide (Aβ), derived from the proteolytic processing of a membrane precursor, APP. The cytosolic domain of APP contains the YENPTY sequence, which is the site of interaction for various PTB domain-containing adaptors. These proteins, interacting simultaneously with APP and other ligands, generate macromolecular complexes, which may underlie their biological functions. Emerging evidence indicates that such complexes may constitute a scaffolding site for kinases such as Abl and Jnk. Among the APP ligands, the mammalian Fe65 proteins share a common modular organisation, made of a WW domain and two PTB domains. Their PTB2 modules are engaged in the interaction with the cytodomains of APP and related proteins APLP1 and APLP2. A functional significance of Fe65/APP interaction may reside in the cytosolic retention of Fe65 by APP. Gamma-secretase cleavage of APP, one of the events which determines release of Aβ, results in the nuclear translocation of Fe65. In the nucleus, likely through its interaction with other proteins, including the transcriptional factor CP2/LSF and the histone acetyltransferase Tip60, Fe65 is involved in transcriptional regulation. The highly redundant system of Fe65 and APP multigenic families renders the mammalian models hard to dissect by reverse genetics. In C.*elegans*, we have identified a gene, *feh-1* (Fe65 homolog-1), which encodes a protein structurally and functionally related to mammalian Fe65s, that is capable of binding APL-1, encoded by the nematode orthologue of APP. The unicity of both *feh-1* and *apl-1* in the nematode genome prompted us to analyze the *C. elegans* system. *feh*-1 is expressed in the nervous system and in the pharynx. We generated a null allele of *feh-1* (*gb561*): the homozygous mutants arrest as embryos or as L1 larvae. No pharyngeal pumping can be detected in L1s, which do not develop further, before dying, suggesting that they cannot feed. Surprisingly, the heterozygous worms *(gb561/+)* have an increased pharyngeal pumping. Using RNAi by injection, as well as RNAi by feeding, we have observed that strong reductions in FEH-1 levels determine pharyngeal arrest, which results in the lethal phenotypes, while milder reductions are associated to viability and increased pharyngeal activity. Interestingly, *apl-1* RNAi similarly determines an increased pharyngeal contraction rate. APL-1 reduction in the *gb561/+* background unexpectedly restores the normal contraction rate of the pharynx. Taken together, such data suggest that FEH-1 and APL-1 act in a common pathway, regulating feeding behaviour. The pharyngeal activity is finely tuned by the stoichiometry of the complex between the two proteins. In order to gain information about the molecular events involving FEH-1 and APL-1 in pharyngeal activities, we are exploring potential interactions between these and other genes, which are involved in these phenomena. These results will be useful for the understanding of the basic biology of Fe65-APP interaction and of the molecular phenomena regulated by this evolutionarily conserved system of interacting proteins.

182. Screening for paternal-effect mutations involved in the establishment of polarity in the early *C. elegans* embryo.

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Whereas the eggs of many species have a highly polarised structure, in *C. elegans*, the oocyte appears to have no predetermined polarity. Polarisation of the anteroposterior (AP) axis occurs soon after fertilisation, the posterior pole being defined by the position of sperm entry. The establishment of polarity triggers a cytoplasmic reorganisation and the asymmetric localisation of several PAR proteins to distinct cortical domains. Eventually the first cell division results in two unequally-sized blastomeres which have different developmental potential. Although many factors have been described, the nature of the cue provided by the sperm is unknown. More generally, the parternal contribution to early development remains poorly understood. To date, only one paternal-effect gene has been reported, the *spe-11* gene. We asked which parternally required genes play a role in early development, particularly in the establishment of polarity.

We first attempted to look for those genes using a RNAi approach. We injected double-stranded RNA into males which were subsequently mated to *fog-2* females. Using *spe-11* RNA as a positive control, we could never phenocopy the male *spe-11(hc77)* null phenotype. Based on this experiment, RNAi may not be an efficient technique to inactivate genes in the sperm. Therefore we undertook a forward genetic approach. Given that mutagenised males mate poorly, we sought for maternal non strict mutations (in other words, maternal effect mutations that can be rescued by crossing to wild type males). The mutations found were then retested for a real paternal requirement (by crossing mutant males to *fog-2* females). To facilitate the ease of analysis of homozygous animals, this screen aims to identify temperature-sensitive mutations. In a pilot study of 2272 genomes, we found 85 maternal-effect mutations out of which 8 were found to be non strict (*dd1* to *dd8*). Out of those, one was found to be paternal, but did not have a role in polarity. A description of these alleles will be presented.

183. Double strand break repair induced by transposon excision

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Double-strand breaks in DNA are repaired in three ways : (1) nonhomologous end joining, (2) illegitimate recombination, or (3) gene conversion. We are studying the repair of double strand breaks in the nematode by inducing excision of a *Mos1* transposon. *ox171* is a *Mos1* insertion near the 3' end of *unc-5*. The *Mos1* transposase is expressed under the control of a heat shock promoter. When the transposase was induced by heatshock in *unc-5(ox171)* homozygotes, there were 0 revertants among 23,500 chromosomes. Moreover, when we looked directly for *Mos1* excision in these progeny 0 of 85 chromosomes had excised the transposon. These data suggest that either excision occurs very infrequently or that excision occurs frequently but the break is efficiently repaired by copying the *Mos1* transposon back into the original site by gene conversion.

To determine whether efficient gene conversion is taking place we placed the *Mos1* insertion in trans to wild-type sequences. *ev447* is a deletion of the 5' end of *unc-5* and the adjacent gene *fem-1*. Animals of the genotype *unc-5(ox171)* / *ev447* are uncoordinated because there is no functional copy of *unc-5* but the *Mos1* transposon is in trans to wild-type DNA. When the transposase was induced in the *unc-5(ox171)* / *ev447* heterozygotes, 4% of the *Mos1*-bearing chromosomes reverted to the wild type. These data indicate that the transposon excises frequently and that the break is repaired from information on the homologous chromosome. In this experiment, the double strand break is repaired to the wild-type sequence to generate a complete *unc-5(+)* locus. In the previous experiment using an *ox171* homozytgote, the *Mos1* transposon is copied back into the broken chromosome and no revertants are observed.

It is likely that such events occur in premeiotic cells. No conversion events are detected in progeny laid in the first 24 hours after heat shock. Thus, it is likely that only nuclei in the distal arm of the gonad, that is mitotic nuclei, can experience repair from the homolog. The revertants are usually simple gene conversion events, that is, the chromosome is repaired without recombination. However, 7% of the revertants are associated with exchange of flanking markers.

Disrupting the pairing of the chromosomes disrupts the process of repair. For example, the *unc-5(ox171)* mutation can be balanced by the translocation *nT1*. This translocation reduces recombination in this region of chromosome IV and hence it is presumed to disrupt chromosome pairing. Only 3 wild-type chromosomes were recovered from 833 *ox171* chromosomes (0.4%) when in trans to *nT1*. What happens to double strand breaks in these animals ? Nonhomologous endjoining events were not observed – 0 of 171 *unc-5* chromosomes had lost *Mos1*. There are two other possible outcomes : either gene conversion from the sister strand restores the transposon insertion or nuclei containing double strand breaks are eliminated by cell death. Gene conversion can also be disrupted by deficiencies. Specifically, when *unc-5(ox171)* was heterozygous with the deficiency *nDf41*, 0 wild-type chromosomes were observed from 259 chromosomes after expression of the transposase. Thus, the process of repair depends on a paired homolog.

184. Patch-clamp study of the cationic currents in the pharyngeal muscle cells of the adult nematode *C.elegans*

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Continuing contractions and extensive intercellular coupling prevent reliable whole-cell current recording from the enzymatically cleaned muscle cells of the *C.elegans* pharynx. However, confirming data previously obtained with intracellular recording with sharp microelectrodes (Pemberton et al., 2002), it has been shown that sodium omission from the Dent's physiological saline blocks inward currents in response to depolarisations from the holding potential of –120 mV to – 40 mV both in wild type worms and *egl 19* (n582). In outside-out patches tested with Cs-internal solution and Dent's saline and held at –80 mV channels with very low opening probability were observed. Amplitude of the unitary currents in these patches was reduced in $0Na^+$ Dent's saline but not in $0Ca^{2+}$ solution. Currents were further reduced and then abolished in $0Na^+$ $0Ca^{2+}$ solution. Exposure of the patches to the Dent's saline with 10-times reduced concentration of Na^+ caused a shift in reversal potential smaller that expected in accordance with the Nernst equation. Channels can be blocked by gadolinium ions and their mean opening time increased by veratridine. In inside out patches tested in symmetrical 150 mM Na^+ solution containing no Ca^{2+} and K^+ ions opening probability of the recorded channels at –80 mV was 3 times higher than in outside-out patches but was reduced to the level observed in outside-out patches when 3 mM Ca^{2+} were added to the solution contacting the external surface of the membrane patches. Taken together these data suggest the presence of some sort of sodium permeable channels in pharyngeal muscle cells of the adult worms.

185. Resistance to the nicotinic agonist DMPP defines a new class of genes.

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Acetylcholine is the most prominent excitatory neurotransmitter in the *C.elegans* nervous system. Cholinergic transmission is mediated by two classes of receptors: muscarinic G-protein coupled receptors and nicotinic ligand-gated ion channels. 42 putative genes encoding nicotinic receptor subunits have been detected in the *C.elegans* genome. However, the function of most of these genes remains unknown. To identify new components of nicotinic transmission in *C.elegans*, we have undertaken a screen for mutants resistant to the nicotinic agonist DMPP (dimethyl phenyl piperazinium). In vertebrates, DMPP has a broad spectrum and activates several types of nicotinic receptors. In *C. elegans*, electrophysiological recording has demonstrated the presence of two acetylcholine receptors at the neuromuscular junctions. One receptor is sensitive to levamisole and one receptor is insensitive to levamisole, a nematode-specific cholinergic agonist. DMPP preferentially activates the levamisole insensitive receptor (Janet RICHMOND, personal communication).

When adult worms are transferred on DMPP plates, they become uncoordinated and slightly hypercontracted but they survive and lay eggs. However, worms cultivated on DMPP develop to the early L2 larval stage and then arrest. The DMPP target is distinct from the levamisole receptor since levamisole-resistant mutants do not grow on DMPP. To test whether it was possible to generate DMPP resistant mutants, we performed an EMS pilot screen. Mutagenized P0s were allowed to lay eggs on standard plates and adult F1s were transferred on DMPP. We screened for F2 individuals that reached adulthood on DMPP. Of 3,700 mutagenized haploid genomes, we identified 7 DMPP resistant mutants. Interestingly, one of these mutants was an *unc-22* mutant. *unc-22* encodes the muscle protein twichin and *unc-22* mutants are known to be resistant to nicotine.

In order to rapidly identify DMPP resistant genes, we used *Mos1*-mediated mutagenesis (see Bessereau *et al.*, EWM 2002). Mutagenic insertions are localized in the genome using inversePCR. Of 8,000 mutagenized F1s, we isolated 6 DMPP-resistant mutants. One of them was a twicher and we identified a *Mos1* insertion in an exon of the *unc-22* gene. The second mutant that we analyzed contained an insertion in a gene encoding a putative cation transporter of the P-type ATPase family. We will confirm whether this mutation is responsible for DMPP resistance by rescue experiments. The expression pattern of this gene will be characterized using GFP. The identification of other DMPP resistance genes is in progress.

186. Characterisation of two EF-1a homologues: translational elongation factors SelB and *cgp-1*.

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Selenoproteins are usually involved in redox reactions. Most eukaryotic selenoproteins are believed to be necessary for the protection of the cell against oxygen radicals.

Selenocysteine is incorporated into proteins by a mechanism that requires the specialised elongation factor SelB. SelB binds selenocysteyl-tRNA^{Sec} and directs insertion of selenocysteine at in-frame UGA codons in all organisms that synthesise selenoproteins. In *Archaea* and *Eukarya* this codon is specified as a selenocysteine codon by an RNA secondary structure located in the 3' untranslated region of the mRNA. The *C. elegans* gene C47b2.7 is predicted to be a homologue of the translation elongation factor SelB of the archaeon *Methanococcus jannaschii*. In *C.elegans* the SelB homologue is located on the right end of chromosome I and shows approximately 50% similarity to *M. jannaschii* SelB.

By chemical mutagenesis, we have created a *C. elegans* deletion library from which we are currently isolating SelB mutants. Preliminary experiments with RNA interference techniques indicate that selenoprotein synthesis might not be essential for *C. elegans* development. The SelB deletion strains will be studied as hetero- and homozygotes and will be challenged with oxygen radical producing conditions.

Like SelB, the *cgp-1* gene product has the highly conserved GTPase and ribosome binding domains typical of EF-1a homologues. The aminoacids that are known, from the EF-TU*GTP*tRNA crystal structure, to be in contact with the tRNA are highly conserved. In addition to these domains, *cgp-1* also contains an extra domain located at the N-terminus. This domain shows no similarity to other proteins of known function.

We have isolated a *cgp-1* deletion strain, and will now procede to characterize it phenotypically. We are also working on GFP reporter constructs to determine the expression patterns of *cgp-1*.

187. A novel conserved rna-binding domain protein, Rbd-1, is essential and important for ribosome biogenesis

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Synthesis of the ribosomal subunits from pre-rRNA requires a large number of trans-acting proteins and snoRNPs to execute base modifications, RNA-cleavages and structural rearrangements. We have characterized a novel protein, RBD-1 (RNA Binding-Domain-1), that is involved in ribosome biogenesis. This protein contains six consensus RNA-binding domains and is conserved as to sequence, domain organization and cellular location from yeast to human. In the dipteran *Chironomus tentans*, RBD-1 (Ct-RBD-1), binds pre-rRNA *in vitro* and anti-Ct-RBD-1 antibodies repress pre-rRNA processing *in vivo*. Ct-RBD-1 is mainly located in the nucleolus in an RNA polymerase I transcription dependent manner, but is also present in discrete foci in the interchromatin and the cytoplasm. In cytoplasmic extracts, 20-30% of Ct-RBD-1 is associated with ribosomes and, preferentially with the 40S ribosomal subunit. cDNA clone yk417f6 (kind gift of Yuji Kohara) spans the whole open reading frame of *C. elegans rbd-1*. We have performed RNAi experiments using this cDNA and shown that this gene is essential in *Caenorhabditis elegans*. Animals arrest mainly at the L1 larval stage. Our data suggest that RBD-1 plays a role in structurally coordinating pre-rRNA during ribosome biogenesis and that this function is conserved in all eukaryotes.

188. Microtubule-dependent processes in the one-cell embryo of *C. elegans*.

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We are interested in how cells regulate microtubule (MT) dynamics and how this regulation contributes to the proper positioning of intracellular structures in *C. elegans*. We approach this problem from two directions. In one case, we use RNAi to identify *C. elegans* genes required for pronuclear migration, positioning of the first spindle and anaphase spindle movement (all MT-dependent processes). In the other approach, we examine the worm homologues of yeast, Xenopus and human proteins implicated in MT function. This systematic method allows the classification of the various phenotypes and defines the range of MT defects possible by loss-of-function analysis.

Our analysis has already yielded novel insights into the mechanisms by which MTs may be regulated. For instance, one gene that affects MT-dependent processes in the early embryo is a member of the phosducin-like proteins (PhLPs). In retinal cells, phosducin has been shown to bind to the G-protein βγ subunit in a light-dependent manner, thereby attenuating G-protein signaling. In *C. elegans*, Gα and Gβγ have been implicated in regulating spindle position and orientation, possibly by directly affecting MT behaviour (Gotta and Ahringer, 2001). The CePhLP(RNAi) causes a dramatic shortening of MTs in the early embryo. However, the defects appear much more severe than any of the G proteins' RNAi phenotypes, either alone or in combination. This suggests that PhLP in *C. elegans* may act on MTs independently of G-protein signaling. Antibodies to PhLP indicate cytoplasmic location of the protein; in addition, a GFP::PhLP strain is currently under construction to verify the immunostaining result.

While *C. elegans* is a good system for gene discovery, it is difficult to study the dynamic properties of MTs. *Xenopus* is an ideal system to study the biochemistry of MT dynamics because of the ease at which cytoplasm can be extracted from synchronized eggs. Therefore, we cloned a Xenopus PhLP homologue (XPhLP1) from an oocyte cDNA library and generated antibodies that detect a single band of appropriate size via Western blot analysis. This reagent will be used for Xenopus extract immuno-depletions to study the mechanism by which MT length is regulated by PhLP.

We are currently developing various assays to help distinguish known modulators of MT dynamics in *C. elegans*. With more comprehensive tools for analysis, we can better classify these genes with respect to their function *in vivo*. This work is supported by a HFSP Fellowship to M. Srayko.

189. Identification of novel downstream effectors of Rap1 and Rap2.

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Rap1 and Rap2 belong to the Ras superfamily of small GTPases that cycle between an inactive GDP-bound state and an active GTP-bound state. In their active form these small GTPases can bind to certain downstream effectors and thereby exert their biological effects. The region within downstream effectors that mediates Rap1/2 binding is structurally conserved and referred to as the Ras binding domain (RBD) or Ras associating domain (RA).

To identify novel downstream effectors of Rap1 and Rap 2, we have performed yeast two hybrid screens with activated versions of both the *C. elegans* Rap1 homologue RRP-1 (C27B7.8/*rrp-1*) and the Rap2 homologue RAP-2 (C25D7.7/*rap-2*) using a *C.elegans* library.
The RRP-1 screen yielded 4 groups of positive clones. Two groups were found to encode homologues of the previously identified interactors F28B4.2/RalGDS, a guanine nucleotide exchange factor for the small GTPase Ral and Y37E3/AF-6, an adherence junction protein. Two groups of clones encoded novel putative RRP-1 interactors: 1. W05B10.4, predicted to encode an RA-domain only protein and 2. T14G10.2/*pxf-1*, an exchange factor for RRP-1 and RAP-2.
A screen using an active version of RAP-2 led to the identification of four groups of positive clones. Again, one group was found to encode F28B4.2/RalGDS, a known interaction partner of RAP-2. Novel putative RAP-2 interactors identified: 1. F53A2.3, encoding a predicted protein with no detectable domains; 2. T14G10.2/*pxf-1* and 3. T20G5.1/Clathrin heavy chain. Of particular interest to us is the putative RAP-2-Clathrin heavy chain interaction, since, at least in yeast, our clathrin clone does not interact with RRP-1 and thus seems specific for RAP-2. Moreover, this interaction is GTP-dependent and therefore may be involved in RAP-2 specific signaling.

Currently we are trying to confirm these interactions by a combined biochemical and genetic approach.

190. Update on *warthog* genes

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Cell-cell communication via the *hedgehog* signaling pathway is used widely during embryonic development to polarize tissues, to help establish borders and to induce different cell types. The *C. elegans* genome encodes only some molecules of this pathway and lacks a bonafide homolog of the *hedgehog* signaling molecule. Some of the *C. elegans warthog* and *groundhog* genes, however, encode the HINT/hog domain also found in Hedgehog. This domain cleaves off the signaling domain and anchors it to cholesterol. The putative signaling part of the *C. elegans* genes is markedly different from that of Hedgehog, but probably has a common evolutionary origin. Presently, the most parsimonious hypothesis is that the *warthog* and *groundhog* genes evolved from an ancestral hedgehog gene in the nematode lineage.

To investigate the function of a *warthog* gene we isolated a deletion mutant in the *warthog* gene *wrt-6*. Homozygous mutants are viable and fertile but dye-filling defective in amphids and phasmids, which is rescued by a full-length *wrt-6::gfp* transgene. We looked for ultrastructural defects in amphids of wrt-6(*ch5)* by electron microscopy and found that ciliated endings of single neurons would run along the cuticle instead of reaching through the socket cell channel to contact the outside. The ultrastructural defects are not strong enough to fully explain the dye-filling defect.

We also tested osmosensation and chemosensation by popluation assays. The chemosensory tests revealed little or no difference between N2 and *wrt-6* animals. However, we found reduced osmosensation in *wrt-6* worms. This defect can be rescued by the full-length *wrt-6::gfp* transgene.

We are investigating a possible relationship of *wrt-6* with *che-14*, a *C. elegans dispatched* homolog. *Drosophila dispatched* is required for the secretion of Hedgehog protein. *che-14* is necessary for apical protein sorting in epithelial cells and has a similar phenotype. The rescueing *wrt-6::gfp* is localized to the apical side (tip) of amphid and phasmid socket cells. Preliminary results suggest that in the *che-14* mutant background, this localization is lost in some animals.

191. Genetic analysis of Ras-like GTPases in C. elegans

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GTPases from the Ras-like family, including Ras, Ral, Rap1, Rap2 and R-Ras, function as molecular switches in various signal transduction pathways. They have been highly conserved during evolution, but for most of these proteins their exact function has remained elusive. We have started a systematic approach to study all Ras-like GTPases in *C. elegans*.

Expression patterns of Ras-like GTPase were studied by introduction of green fluorescent protein (GFP) reporter constructs into worms. Here we report the results of these studies for the Rap1 homologue *rrp-1* (C27B7.8), the related *rap-2* (C25D7.7) and the R-Ras homologue *ras-1* (C44C11.1). *rrp-1* is expressed in various neurons in the head and tail, the rectal epithelial cells and body muscle. In addition, expression was seen in the hypodermis and somatic cells of the gonad. *rap-2* is also expressed in various neurons and shows high expression in the pharynx.
RNA interference studies are performed to get a first impression of the function of the Ras-like GTPases. Of the various GTPases tested so far, only for the RalA homologue *ral-1* (Y53G8AR.3) a clear phenotype was seen: about 20% of the embryos died during an early stage.
A third approach is to make transgenic animals over-expressing wild type, constitutively active or dominant negative versions of the various GTPases under the control of a heat shock promoter. Results will be presented for the *rrp-1* and the *ral-1* constructs.

192. Genetic interactions between *lin-25* and *C. elegans* Mediator components

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Induction of vulval fates in the *C. elegans* hermaphrodite is mediated by a conserved RTK/Ras/MAP kinase signalling pathway. *lin-25* and *sur-2* are two genes that have been shown to function genetically downstream of this pathway. Previous work has shown that *lin-25* and *sur-2* very likely function in the same process in the cell during vulva induction (1, 2, 3). In addition, we have shown that mutations in *lin-25* and *sur-2* also affect a number of other cell fate specification events in the worm that are known to require Ras/MAP kinase mediated signalling (4). LIN-25 is a 130 kD protein of unknown biochemical function. During vulval development it is expressed first in all of the six vulva precursor cells but later becomes restricted to the descendants of the induced cells. A human homologue of *C. elegans* SUR-2 has been identified and found to associate with a large multiprotein complex called Mediator. In yeast and human cells Mediator functions in transcriptional regulation by mediating signals from different transcriptional regulators to the basal transcripton machinery. Since Mediator itself is thought to be involved in all regulated transcription, we have investigated whether mutations in *lin-25* and *sur-2* affect other signalling pathways besides Ras signalling, such as TGF-β, FGF and LIN-12 (Notch). Our results to date suggest that neither *lin-25* nor *sur-2* are involved in these other signalling pathways. Spcifically mutations in *lin-25* or *sur-2* do not cause defects similar to those caused by mutations in genes encoding components of these other pathways. Furthermore, *lin-25* or *sur-2* mutations do not enhance phenotypes caused by hypomorphic mutations in such genes.
Together these observations suggest a model in which LIN-25 and SUR-2 might be required specifically for the recruitment of Mediator to promotors activated by Ras and MAP kinase. In *C. elegans* around ten homologues of yeast and human Mediator proteins have been identified. We are currently investigating possible interactions between *lin*-25 and these homologues. Preliminary results suggest that there are genetic interactions between *lin-25* and certain *C. elegans* Mediator genes. We are also performing immunoprecipitation experiments to investigate biochemically if there are physical interactions between LIN-25 and *C. elegans* Mediator proteins.

(1) Sing, N. and Han, M. (1995). *Genes& Dev.*9, 2251-2265.
(2) Tuck, S. and Greenwald, I. (1995). *Genes& Dev.*9, 341-357.
(3) Nilsson, L., Li, X., Tiensuu, T., Auty, R., Greenwald, I. and Tuck, S. (1998). *Development* 125:4809-4819.
(4) Nilsson, L., Tiensuu, T. and Tuck, S. (2000). *Genetics* 156:1083-1096.

193. The *C. elegans* Mi-2 orthologs LET-418 and CHD-3 are together required for the LIN-12/ Notch signaling pathway

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The Mi-2 protein is the central component of the recently isolated human nucleosome remodelling and histone deacetylase (NuRD) complex. The two *C. elegans* Mi-2 orthologs, LET-418 and CHD-3, play essentialand partially redundant roles during development. The two proteins have both shared and unique functions during vulval cell fate determination. Based on the following observations we conclude that LET-418 and CHD-3 are involved in LIN-12/ Notch signaling:

1). In wild-type hermaphrodites the VPC nearest to the anchor cell, P6.p, adopts a 1° fate, while the adjacent P5.p and P7.p take over a 2° fate. It was shown that the 2° fate depends on a LIN-12/ Notch mediated lateral signal produced by P6.p. In *let-418;chd-3* double mutants, 30% of the P5.ps and 40% of the P7.ps adopt a 1°-like or a 1°/2° hybrid cell fate, a phenotype similar to that observed in lin-12(0) mutants.

2). *lin-12(gf)* causes the constitutive activation of the LIN-12/ Notch signaling pathway resulting in the ectopic execution of the 2° fate in all VPCs. We found that *let-418* and *chd-3* double mutations were able to suppress partially the 2° fate in P3.p, P4.p and P8.p in *lin-12(gf)* mutants so that these cells adopted a normal 3° fate.

3). During *C. elegans* development, lateral LIN-12/ Notch inhibitory signaling among two developmentally equivalent bipotential cells causes one to adopt the ventral uterine (VU) precursor cell fate while the other becomes the anchor cell (AC). In the absence of *lin-12* activity both cells become ACs. In agreement with the previous findings we observed that > 20% of the *let-418; chd-3* double mutants had two or more ACs.

In summary, our data show that *let-418*;*chd-3* double mutations mimic *lin-12(0)* mutations and suggest that LET-418 and CHD-3 together are required for LIN-12 Notch signaling.

194. *Cln-3.3*, one of the *C. elegans* homologues of the human CLN3 gene involved in neuronal ceroid lipofuscinosis, is expressed in the intestinal muscle

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C. elegans has three genes of which the proteins are homologous to the human Cln3 protein. Mutations in the human CLN3 gene are the cause for a neurodegenerative disease called juvenile neuronal ceroid lipofuscinosis, j-NCL or Batten's disease. This devastating lysosomal storage illness is part of a group of common hereditary neurodegenerative disorders. The first symptom of the NCL's is progressive visual failure, and this group of diseases is also characterised by the accumulation of lipopigments in the lysosomes of neurons, and other cells. We focus on the juvenile form of the disease, since it is the most frequent form of NCL, and we set out to use *C. elegans* as a model organism to investigate this complicated disorder.
The spatial and temporal expression patterns of the *C. elegans cln-3* genes are being analysed by cloning the putative promoter regions in GFP expression vectors. Transgenic nematodes carrying the *cln-3.1*-GFP fusion construct show fluorescence suggesting *cln-3.1* gene expression in the intestine. The absence of fluorescence in transgenic nematodes containing a construct consisting of the intergenic region upstream of *cln-3.3* fused to the GFP gene may indicate the gene is part of an operon. The promoter region of the gene ZC190.2 upstream of *cln-3.3* was fused to GFP and resulted in fluorescence signals, implying expression in head and tail neurons. Faint fluorescent signals in transgenic nematodes containing the entire *cln-3.3* operon with the GFP gene fused to the *cln-3.3* gene suggest *cln-3.3* gene expression in the intestinal muscle. These results seem contradictory, but may be explained by the presence of additional regulatory sequences in the latter construct.
Currently we are examining the *cln-3* deletion mutants, and we expect that the determination of the spatial and temporal expression pattern of the *cln-3* genes will be helpful in the characterization.

195. *zyg-11* and *cul-2* are required for meiotic cell cycle progression and AP polarity in one cell stage *C.elegans* embryos

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We are interested in further exploring how polarity is set up along the antero-posterior (AP) axis of one-cell stage *C.elegans* embryos. A sperm component, which most likely correspond to astral microtubules nucleated from the sperm-inherited centrosome, initially confers posterior character to one side of the embryo. Downstream of the sperm component, a set of maternal genes (*pars, pkc-2, cdc-42*) is required for establishing AP polarity cues. In response to these polarity cues, the mitotic spindle is displaced towards the posterior and factors such as PIE-1 or P-granules are segregated towards the posterior of one-cell stage embryos. Consequently, the first division results in two daughter cells that differ in size and fate.

We have become interested in the *zyg-11* locus because *zyg-11 (mn40)* mutant embryos have defective first divisions and misegregation of P-granules, suggesting that AP polarity cues are aberrant (Kemphues et al; Dev Biol, 1986). In addition, *zyg-11* hypomorphic mutant embryos have a delay in meiosis II cell cycle progression. ZYG-11 belongs to an evolutionary conserved family of proteins with no assigned function (Carter et al; MGG, 1990).

We initiated a study of *zyg-11* to better characterize the meiotic defect and begin addressing whether the meiosis II delay is responsible for the polarity defects. We utilized *in utero* time-lapse fluorescent microscopy of GFP-histone and GFP-tubulin expressing worms to characterize the kinetics of cell cycle progression in *zyg-11(RNAi)* embryos. We found that the first deviation from the wt is a striking delay at meiosis II. Instead of the normal 9 minutes, *zyg-11(RNAi)* embryos spend 33 minutes in metaphase of meiosis II. Thus, *zyg-11* is required for proper metaphase to anaphase transition at meiosis II. We next examined the distribution of PAR-2 and P-Granules in *zyg-11(RNAi)* embryos and found them to be affected starting at the prolonged meiosis II. PAR-2 localized to the anterior cortex and P-granules were enriched in the anterior cytoplasm in 58% of embryos examined. Therefore, *zyg-11* appears to be required upstream of the *par* genes to set up proper AP polarity.

Interestingly, using time lapse fluorescent microscopy with GFP-histone and GFP-tubulin transgenic animals, we found that inactivation of the cullin *cul-2* by RNAi results in a meiotic phenotype indistinguishable from that of *zyg-11(RNAi)* embryos. Moreover we found that PAR-2 and P-granules are distributed abnormally as in *zyg-11(RNAi)* embryos. These results taken together suggest that a persisting spindle at meiosis II is sufficient to generate inverted polarity much like the situation described with an arrested meiosis I spindle (Wallenfang et al; Nature, 2000). However, *zyg-11* and *cul-2* may be both independently required for progression through meiosis II and establishment of AP polarity.

We sought to determine the distribution of ZYG-11 by generating transgenic animals expressing an integrated *zyg-11::gfp* transgene. We found that ZYG-11::GFP is cytoplasmic at meiosis and disappears before the first interphase, consistent with temperature shift experiments showing that *zyg-11* is required in a short time period after fertilization (Kemphues et al; Dev Biol, 1986).

We are in the process of investigating the link between *zyg-11* and *cul-2* and plan to present data pertaining to their mechanism of action in the context of meiotic cell cycle progression and setting up of AP polarity.

196. *C. elegans* cut superclass genes, *ceh-44*, *ceh-32* and *ceh-37*

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The cut superclass of homeobox genes has been divided into three classes: CUX, ONECUT and SATB. Given the various completed genomes, we have now made a comprehensive survey. We find that there are only two cut domain containing genes in *Drosophila*, one CUX and one ONECUT type. *Caenorhabditis elegans* has undergone an expansion of the ONECUT subclass genes and has a gene cluster with three ONECUT class genes (*ceh-21*, *ceh-39* and *ceh-41*), one of which has lost the cut domain. Two of these genes contain a conserved sequence motif, termed OCAM, that also occurs in another gene in *C. elegans*; this motif seems to be nematode specific. A recently uncovered *C. elegans* CUX gene has sequence conservation in its amino-terminus with vertebrate CUX proteins. This gene is the bona fide orthologue of *Drosophila* cut, and has been named *ceh-44* (WBG 17(1): 63). Further, the 5' end of this gene containing the conserved region can undergo alternative splicing to give rise to a protein with a different carboxy-terminus lacking the cut- and homeodomain. This protein is conserved in its entirety with vertebrate genes, termed CASP - which are also alternative splice products of the CUX genes - and with plant and fungal genes. The highly divergent SATB genes share a conserved amino-terminal domain, COMPASS, with the *Drosophila defective proventriculus* gene and a *C. elegans* ORF. These two "COMPASS" family genes encode two highly divergent homeodomains, may be homologues of the SATB genes and thus probably belong to the cut superclass as well.

We have performed a deletion screen for a series of homeobox genes and have identified a deletion in *ceh-44*. The primers were chosen to remove conserved cut and/or homeo-domains. The mutation is now being back-crossed and we hope to present first results at the meeting. In collaboration with Natalie Pujol, we plan to further study this gene.

From the genome knock-out consortium we have received deletions in two other homeobox genes, *ceh-32* (a sine oculis homeobox gene, see Dozier et al. 2001, Dev. Biol., 236, 289-303) and *ceh-37*. The *ceh-32* deletion is being back-crossed, and preliminary analysis shows that it is lethal. *ceh-37* has been back-crossed, but shows no lethal phenotype and sequencing of the deletion shows that the deletion is 3' to the homeobox, and thus may not be a null mutation.

197. A functional analysis of the *extradenticle* ortholog *ceh-40*

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HOX proteins are a conserved set of transcription factors that pattern the body axis during animal development. To regulate both, their own transcriptional activity and that of their target genes, the HOX proteins often require additional cofactors such as TALE homeodomain proteins EXD/PBX and HTH/MEIS/PREP. CEH-20, a *C. elegans* ortholog of EXD/PBX, has been shown to function together with CEH-13, LIN-39 and MAB-5. *Ceh-13(lf)* mutants exhibit an incomplete embryonic lethalithy, whereas putative null alleles of *ceh-20* confer larval but not embryonic lethality. To investigate whether this phenomenon reflects a functional redundancy with another EXD ortholog, we performed a genetic analysis of CEH-40, the closest paralog of CEH-20.

To determine the genomic structure of *ceh-40*, we identified the corresponding cDNA by RT-PCR amplification and subsequent sequencing, predicting a 329-amino acid protein. CEH-20 and CEH-40 show 42.6 % overall amino acid identity. Zygotic expression of a CEH-40::GFP fusion protein can be first detected at the ~50-cell stage and appears throughout embryonic development. During the larval stages, CEH-40::GFP is strongly expressed in the ventral nerve cord, a pattern that is very similar to that of CEH-20 (Bürglin pers. com.). Consistent with its expression profile, inactivation of *ceh-40* by RNAi causes an Egl phenotype. However, animals coinjected with *ceh-20* and *ceh-40* dsRNA exhibit synthetic embryonic lethality and early larval arrest, a phenotype, that was not observed in *ceh-20* or *ceh-40* single mutants. This suggests that the two genes interact genetically.

To test whether these cofactors can bind to a HOX protein essential for embryogenesis, we performed band shift experiments with the anterior paralog CEH-13. Interestingly, preliminary results show that CEH-20 forms a complex with LABIAL and HOXB1 but not with CEH-13. Currently we are testing the role of CEH-40 in forming a functional EXD/HOX complex.

198. Phenotypic Plasticity In Nematodes.

Luke Tain

Body size is determined by two cellular processes, cell number and cell size. In nematodes cell size is associated with changes in somatic ploidy. Many organisms such as Drosophila can regulate their body size independent of cell number, and often do in response to environmental cues, such as nutrition. In C.elegans, I show that the TGF-b signalling pathway, known to strongly influence body size and somatic polyploidy is necessary for phenotypic plasticity in a low food environment. The detection of low food levels is transmitted from the external environment to the internal growth mechanisms by the ciliated nerve endings within the amphidal pores. I also put forward a possible negative regulator of the TGF-b DBL-1 signalling pathway and a role for the neurotransmitter serotonin in the transmission of the low food signal.

199. Functional Analysis of Acyl-Binding Proteins in *C. elegans*

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Fatty acids and long-chain acyl-CoA esters (LCA-CoA) serve as essential substrates for lipid synthesis, fatty acid elongation and energy production. In bacteria as well as in higher organisms, fatty acids and fatty acid derivatives are increasingly being recognized as important physiological regulators. The LCA-CoA molecules have been found to regulate several cellular functions and enzymes including Ca2+ release from sarcoplasmic reticulum, the *E.coli* FadR transcription factor, acetyl-CoA carboxylase, protein kinases and vesicular trafficking. The many important functions of LCA-CoAs in lipid homeostasis and signaling require that thioesterases, acyl-CoA transporters and acyl-CoA pool formers tightly regulate the intracellular acyl-CoA concentration.
Acyl-CoA Binding Protein (ACBP) is a small cytosolic protein made up of 86 to 92 amino acids and has a molecular weight of approximately 10 kDa. The basal isoform is a four-helix bundle protein which binds saturated and unsaturated acyl-CoA esters with high specificity and affinity, K_D = 1-15 nM. The affinity towards acyl-CoA esters strongly depends upon the chain length, with a preference for acyl-CoA esters with 14-22 carbon atoms in the acyl chain.
The ACBP protein family is characterized by a high degree of conservation and is found in all eukaryotic species examined. These species range from yeast and plants to birds, fish and mammals. The ACBP isoforms are generally grouped into four different types: type 1 is the general basic form expressed in all tissues in all examined eukaryotes incl. *C. elegans*, type 2 is a testes-specific form (tACBP) called endozopine-like protein (ELP), which only is expressed in bovine, mouse, and rat spermatozoa. Type 3 is a brain specific isoform, which has been identified in duck and frog brain. Finally, type 4 consists of a group of proteins which all harbor an ACBP sub-domain.
Besides the basal isoform *C. elegans* contain 7 putative open reading frames encoding a protein either solely an ACBP protein or a protein harboring an ACBP domain. The proteins vary from 86 to 372 amino acids. The latter ORF encodes an ankyrin repeats and a BolA DNA binding site apart from the ACBP domain.
We have taken advantage of the nematode *C. elegans* to study the function of the ACBP family of proteins in a multicellular organism. Currently we are investigating the expression patterns and spatial occurrence of the ACBP proteins in *C. elegans.* Furthermore, we have undertaken a reverse genetic approach to determine the role of the 266 amino acid ACBP domain protein. Using RNAi interference we are currently studying the phenotype of animals depleted for this isoform. The worms show a temperature dependent migration defect and we are in the process of characterizing this phenotype in more detail.

200. Functional analysis of the micro RNA genes of *C. elegans*

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The heterochronic genes *lin-4* and *let-7* encode unusually small (21-22nt) non-protein coding regulatory RNAs[1,2]. Strains carrying a mutation in either of these genes display retarded development, with some cell lineages having an altered temporal pattern of cell division and differentiation. *lin-4* and *let-7* inhibit translation of target genes that when mutated lead to an opposing phenotype, precocious development and early expression of some cell lineages.
Recently, molecular and bioinformatic approaches identified many genes encoding small RNAs in *C. elegans*, *Drosophila* and mammalian cell lines[3,4,5,6]. All of these genes encode 21-25nt RNAs derived from longer transcripts that contain partially double-stranded RNAs. These small RNAs, termed microRNAs (miRNAs, *mirs*), define a large, new class of genes.
We have identified over 100 miRNAs in *C. elegans* to date, a subset a subset of which is conserved in *Drosophila* and mammals. To understand the biological functions of miRNAs, we decided to generate mutations in the majority of these genes. Using a library of *C. elegans* mutants and automated liquid handling, we are screening for deletion strains. In parallel, we are seeking to establish the temporal and spatial expression patterns of these genes.

[1]Lee RC, Feinbaum RL, Ambros V. (1993). The *C. elegans* heterochronic gene *lin-4* encodes small RNAs with antisense complementarity to *lin-14*. Cell *75*, 843-54.
[2]Reinhart BJ, Slack FJ, Basson M, Pasquinelli AE, Bettinger JC, Rougvie AE, Horvitz HR, Ruvkun G. (2000). The 21-nucleotide *let-7* RNA regulates developmental timing in *Caenorhabditis elegans*. Nature *403*, 901-6.
[3]Lagos-Quintana M, Rauhut R, Lendeckel W, Tuschl T. (2001). Identification of novel genes coding for small expressed RNAs. Science *294*, 853-8.
[4]Lau NC, Lim LP, Weinstein EG, Bartel DP. (2001). An abundant class of tiny RNAs with probable regulatory roles in *Caenorhabditis elegans*. Science *294*, 858-62.
[5]Lee RC, Ambros V. (2001). An extensive class of small RNAs in Caenorhabditis elegans. Science. *294*, 862-4.
[6]Mourelatos Z, Dostie J, Paushkin S, Sharma A, Charroux B, Abel L, Rappsilber J, Mann M, Dreyfuss G. (2002). miRNPs: a novel class of ribonucleoproteins containing numerous microRNAs. Genes Dev. *16*, 720-8.

201. CUT-1-like proteins involved in the formation of the alae of L1 and dauer larvae

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The *C.elegans* cuticle is an an extracellular matrix that is important to determine and maintain the normal body shape of the nematode.
We are interested in understanding how the alae, specialized structures of the nematode’s cuticle that run along the lateral lines of L1s, dauers and adults, are formed and in discovering the genes involved in this process.
We have investigated the role in alae formation of three members of the CUT-1 like proteins family and we have demonstrated that CUT-3 is necessary for the alae of L1s, CUT-1 for the alae of dauers and the CUT-5 partecipates in both these processes. The proteins may act as substrates for cross-linking reactions that compact the internal layer of the cuticle along the two sides of the body. The alae result from the wrinkling of the external layer over the internal one. Our attention is now focused on documenting the mechanism of the process and on identifying the other players involved.
We have used combinations of RNA interference and overexpression of proteins to ascertain the effect of varying the amount of the CUT-1-like proteins on alae formation. The results indicate that proper alae formation requires a fine regulation of the amount of each protein produced and that quantitative variations lead to abnormalities of alae and body shape.

202. The *C. elegans F47F2.1* gene encodes a cyclic AMP-dependent protein kinase (PK-A) catalytic subunit

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PK-A mediates all known effects of cyclic AMP on cellular activity in eukaryotes. The holoenzyme is an inactive tetramer of two regulatory (R) subunits and two catalytic (C) subunits. Following binding of cyclic AMP to the R subunits, dissociation of active C-subunits occurs. In mammals, α, β and γ isoforms of C-subunit, encoded by different genes, have been identified.

We [Tabish, M., Clegg, R. A., Rees, H. H. & Fisher, M. J. (1999) 'Organization and alternative splicing of the *Caenorhabditis elegans* cyclic AMP-dependent protein kinase (PK-A) catalytic subunit gene (*kin-1*)' Biochem. J. **339,** 209-216] have searched the *C. elegans* sequencing project database for sequences with similarities to murine PK-A Cα-subunit sequence. This search identified the *kin-1* gene on chromosome 1 and the *F47F2.1* gene on the X chromosome. The F47F2.1 polypeptide shows 45% identity with the murine PK-A Cα-subunit sequence, including all the 12 key conserved residues identified within the eukaryotic protein kinase superfamily. RT-PCR and Northern blotting experiments confirmed the presence of extremely abundant mature transcripts derived from *F47F2.1* in *C. elegans*. Here we report on the expression and properties of the F47F2.1 polypeptide in *C. elegans* and a similar polypeptide in *C. briggsae*.

203. *C.elegans* genes coding for proteins involved in the biosynthetic pathway and regulation of carnitine.

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We are interested in studyng how organisms control and regulate lipid metabolism as it represents a complex process whose alterations affect the function of various tissue and organs, and the physiology and behavior of the entire organism; for this reasons *C. elegans* is an attractive animal model. β-oxidation of fatty acids, which occurs in mitochondria, play a central role in lipid metabolism and carnitine is a key molecule required for the transfer of fatty acids across the mitochondrial membrane.
In humans two genes involved in carnitine biosynthesis have been identified. One, TMLH, maps in Xq28 region, and one BBOX1, maps in the 11p14 region. The gene TMLH codes for ε-*N*-trimethyllysine hydroxylase and the gene BBOX1 code for γ-butyrobetaine hydroxylase, which catalise, respectively, the first and the last of the four enzymatic reactions of the carnitine biosynthetic pathway. ε-*N*-trimethylysine hydroxylase catalyzes the reaction of hydroxylation of ε-*N*-trimethylysine and is the only mitochondrial enzyme in the pathway; γ-butyrobetaine hydroxylase catalyzes the reaction of hydroxylation of γ-butyrobetaine to carnitine. To gain further insight into the role in vivo of these enzymes we identified and characterized the *Caenorhabditis elegans* (CeBBOX) orthologues of human ε-*N*-trimethyllysine hydroxylase and γ-butyrobetaine hydroxylase. We have named them *gbh-1* and *gbh-2* and they appear to be orthologues of the human genes hBBOX1 and hTMLH, respectively.
We show that in the nematode, a GBH-2::GFP fusion is most prevalently expressed in the intestinal cells, with a weaker GFP-signal detectable in head and body muscles as well as in some head neurons. Expression begins very early in embryogenesis, at the stage of 4 - 8 intestinal cells and does not appear to change substantially during development. We also found that this gene has a mitochondrial localization. A comparable expression pattern was observed in adult worm for the *gbh-1* gene while the expression in the embryo is more generalized and not restricted to the gut.
We have used RNA interference to reduce the expression of *gbh-1* and *gbh-2* and to study the phenotype generated by this transient knock out. In both cases, the interferred worms show the presence of bubble-shaped fatty acid accumulation in the pseudocaelomic cavity, twisted gonads, and reduced fertility. On the basis of this observation we hypothesize that RNA interfered worms under-utilize lipids as energy sources and accumulate fatty acids. The reduced fertility and the abnormal gonad morphogenesis of *gbh-1* and *gbh-2* interfered worms are probably secondary to the alteration in fatty acid metabolism. The gonad migration phenotype, which appears to be due to an anticipation of the dorsal turn is stronger and more penetrant in *gbh-2* over-expressing worms.
Furthermore we want to isolate mutants in both genes using chemical mutagenesis and PCR screening.

204. Analysis of the G protein coupled receptors family to identify candidates chemosensory receptors

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C. elegans detects and avoids many water soluble substances that are toxic or related to toxic compounds. Many of these substances also taste bitter to humans, suggesting a conserved response to toxicity and an evolutionary relationship between avoidance and bitter taste. We would like to unravel the cellular and molecular mechanisms involved in the response to water soluble repellent and identify the molecular receptors for repellents.
In mammals bitter sensation is mediated by the T2Rs, a family of G protein-coupled taste receptors (1,2). These receptors are co-expressed with the taste-specific G protein, gustducin in a subset of the sensory cells of the tongue.
In *C. elegans* the identification of genes for the primary chemosensory receptors has proved more difficult than expected. It is possible that these genes are, in *C. elegans*, partly or largely redundant. Thus, although many genetic screens for mutants unable to respond to chemicals have been conducted they have generally not led to the identification of the receptors. The only important exception has been, so far, the identification of *odr-10*, the receptor for the volatile attractant diacetyl.
Also the reverse genetic approach to identifying these genes does not appear to be trivial in *C. elegans*. The nematode genome contains a very large family of 7-TM receptors, many of which are thought to function as chemoreceptors, however sequence comparison fails to identify the classes of genes present in other species. For instance, although *C. elegans* senses denatonium as a repellent, no close homologs of the T2Rs, the family of mammalian genes to which the receptor for the bitter substance denatonium belongs, can be recognized.
The *C. elegans* sensory neuron ASH, ADL and ASK have been identified as having a role in the detection of repellents. (3, 4, and our unpublished data). The two phasmid neurons PHA and PHB also detect repellents and appear to have a modulating role in the avoidance response (4). These 'avoidance neurons' should express the receptors for repellents. We have thus started to analyse the expression pattern of genes for G protein-coupled receptors (GPCR) to identify those that are expressed in the avoidance sensory neurons. We follow two criteria to choose, among the over 650 genes for GPCR, which ones to begin to analyse. One criterion is to study GPCRs located on the physical map of the genome very close to genes known to specifically affect avoidance. One group, chosen on the basis of this criterion, is a cluster of five genes located within 22Kb of *qui-1*, a gene we have identified in a screen for quinine non avoider mutants. The second criterion is to choose GPCR genes from the gene expression landscape map (5). Also in this case we have looked for GPCR whose expression profile is closely related to that of genes known to be involved in avoidance. The results of the preliminary analyses conducted so far will be presented.

1- Adler et al. (2000) Cell 100:693-702.
2- Chandrashekar et al. (2000) Cell 100:703-11.
3- Bargmann CI, Thomas JH, Horvitz HR (1990) Cold Spring Harb Symp Quant Biol 55:529-38.
4- Hilliard M.A, Bargamann, C.I. and Bazzicalupo, P. (2002). Current Biology 12: 1-20.
5- Kim K.S. et al. (2001) Science, 293: 2087-2092.

205. Functional conservation between the human and nematode KAL proteins

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The molecular mechanisms through which cells and neuronal growth cones migrate, adhere to the substrate and find their targets are varied, appear to often work in redundant pathways, and appear to be conserved in evolution. Although many molecules involved in these processes have already been identified and studied, many other still need to be discovered, especially the components of the extra cellular matrix (ECM) that play a role in the final steps of morphogenesis. We have focused our study on *kal-1*, the *C. elegans* homolog of the X-linked Kallmann syndrome gene. Kallmann syndrome is an inherited disorder defined by the association of anosmia and hypogonadism, owing to impaired targeting and migration of olfactory axons and gonadotropin-releasing hormone secreting neurons. The gene responsible for the X-linked form of Kallmann syndrome, *KAL-1*, encodes a secreted protein which has been proposed to be involved in some aspects of olfactory axon guidance.
We have identified the *C. elegans* homolog, *kal-1*, and have documented its function in the nematode studying its expression pattern and using loss of function and overexpression mutants. We have shown that *kal-1* is part of a mechanism by which neurons influence migration and adhesion of epidermal cells undergoing morphogenesis during ventral enclosure and male tail formation. We also have shown that *kal-1* affects neurite outgrowth in vivo by modulating branching (Rugarli et al., 2002). This last finding has been recently confirmed by work on organotypic and primary cell cultures of rodents (Soussi-Yanicostas et al., 2002). Our findings add a new player to the set of molecules, which appear to underlie both morphogenesis and axonal/neuronal navigation in vertebrates and invertebrates.
We show that human *KAL-1* cDNA can compensate for the loss of worm *kal-1* and that overexpression of worm or human *KAL-1* cDNAs in the nematode results in the same sets of phenotypes. These data indicate that, despite the fact that on a residue to residue basis the identity between the human and the nematode protein is only 22% and that overall similarity is about 35%, there is significant functional conservation between these proteins. In addition these results definitely establish *C. elegans* as a powerful organism in which to investigate KAL function *in vivo*.

Rugarli EI, Di Schiavi E, Hilliard MA, Arbucci S, Ghezzi C, Facciolli A, Coppola G, Ballabio A, Bazzicalupo P. "The Kallmann syndrome gene homolog in C. elegans is involved in epidermal morphogenesis and neurite branching" **Development**. 2002 Mar; 129(5):1283-94.

Soussi-Yanicostas N., de Castro F., Julliard A. K., Perfettini I., Chedotal A. and Petit C. "Anosmin-1, Defective in the X-Linked Form of Kallmann Syndrome, Promotes Axonal Branch Formation from Olfactory Bulb Output Neurons" **Cell** 2002 April; 109: 217–28.

206. *cow-1* is required to spatially restrict contraction of the actin cytoskeleton in the early embryo.

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The actin cytoskeleton is essential for a number of processes of the early embryo including anterior cortical ruffling and pseudocleavage following fertilization. In order to identify gene products that regulate the actin cytoskeleton during early embryogenesis we performed a genetic screen for temperature-sensitive, embryonic lethal mutations with defects in actin dependent processes. In this screen we identified one allele (*or295*) of a gene we called *cow-1* (contractile waves) The most prominent defect in this mutant allele is waves of extenuated contractile activity that takes place throughout the first cell divisions. In *cow-1* mutant embryos the restriction of cortical ruffling to the anterior of the one-cell zygote is lost; excessive contractions are prominent throughout the embryo and become more prominent through later cell divisions. Interestingly, analyses of Nomarski movies indicate no defects in cytokinesis and embryonic polarity. These cortical waves of contraction require an intact actin cytoskeleton and associated motor protein activity; reduction of a *C. elegans* profilin (*pfn-1), cyk-1* or *mlc-4* gene activity by *RNAi* silences the ectopic cortical contractions. To determine the role of *cow-1* in the regulation of the actin cytoskeleton we have begun staining mutant embryos for protein localization of actin, the associated motor proteins, *NMY-2* and *MLC-4*, and *CYK-1*. In order to determine the molecular identity of *cow-1*, we have mapped it to a 0.5 map unit interval on LGV. We are currently pursuing cosmid rescue while getting more map data.

207. The human PTEN tumor suppressor regulates diapause and longevity in *Caenorhabditis elegans.*

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The tumor suppressor gene *PTEN* (also called *MMAC1* or *TEP1*) is somatically mutated in a variety of cancers. In addition, germline mutations of *PTEN* are responsible for two dominantly inherited cancer syndromes called Cowden disease and Bannayan-Zonana syndrome. *PTEN* encodes a protein which displays both a dual-specificity protein phosphatase and a lipid phosphatase activities. PTEN antagonizes phosphatidylinositol-3 kinase (PI3K) by catalyzing the hydrolysis of the phosphate on position D3 of phosphatidylinositol (3, 4, 5) triphosphate (PIP3). We and others have recently shown that DAF-18, the orthologue of PTEN in *C. elegans*, is a component of the insulin-like signalling pathway that controls the entry into dauer and adult longevity. Daf-18 mutants are dauer defective and have a shorter lifespan than wild type worms.
Although PTEN and DAF-18 proteins share the highest homology within the catalytic domain, they do not exhibit a marked similarity outside this region. Notably, the C-terminus of PTEN, which mediates interaction with PDZ domain proteins and regulates the protein stability, is not conserved in DAF-18. We now report that PTEN can substitute to DAF-18 in *C.elegans* and we are using the nematode to uncover the mode of regulation of PTEN.

We have isolated a genomic fragment that contains the putative *daf-18* promoter and used this sequence to drive the expression of *daf-18* or *PTEN* cDNAs in double mutants *daf-18 (mg198) ; daf-2(e1370).* In these conditions, either *daf-18* or *PTEN* rescued the dauer and lifespan phenotypes due to the *daf-18 (mg 198)* null allele. Furthermore, a PTEN mutant (G129E) deficient in the lipid phosphatase activity but retaining the protein phosphatase activity failed to restore the Daf-2 phenotype, thus indicating that the level of the lipid second messenger PIP3 regulates longevity and the entry into the dauer diapause state. We are pursuing this analysis and the characterization of several PTEN mutants that affect distinct regulatory modules of the protein.

The *daf-18* promoter drives the expression of the green fluorscent protein (GFP) in different tissues of *C.elegans*. The GFP is strongly expressed in a few head neurons. Also, the GFP is expressed at a lower level, in the intestine, the hypodermal cells and in the ventral and dorsal nerve cords. To determine in which cells DAF-18 and PTEN acts to regulate dauer and longevity, we are currently using tissue specific promoters that drive expression of either *daf-18* or *PTEN* cDNAs and analyzing the phenotype of transgenic animals.

208. Cytological characterization of DNA damage response pathways in *C. elegans*

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DNA double strand breaks (DSB) occur normally during meiosis and are induced by ionizing irradiation. In *C. elegans* DNA damage response pathways can be monitored in the germ line and result in the activation of cell cycle arrest in mitotic germ cell nuclei and trigger apoptosis in meiotic pachytene nuclei. We aim at a cytological description of meiotic, repair and checkpoint proteins in the *C. elegans* germ line and choose Rad-51p cytology as a starting point.
Rad51p is a homologue of the bacterial RecA protein and represents a key player in homologous DNA repair pathway catalyzing DNA strand exchange. The *rad-51* homologue in *C.elegans* has been cloned and phenotypically described (1,2). *rad-51* (*RNAi)* shows increased apoptotic response indicating an accumulation of unrepaired DSB. We are therefore using a Rad51p antibody as a "cytological marker" for monitoring Rad51p during DNA repair and during meiosis. We have shown that Rad51p foci accumulate in germ line cells upon irradiation, in addition we could show that Rad51p foci occur naturally in meiotic germ line cells. We will present the cytology of Rad51p in mitotic and meiotic germ cells in various DNA repair, checkpoint and meiotic mutations.
In addition, to describe novel genes involved in the processing of double strand breaks, we performed a genetic screen for radiation sensitive and/or meiotic recombination defective mutations. Preliminary results will be presented.

1. Gartner et. al. (2000). A conserved checkpoint pathway mediates DNA damage-induced apoptosis and cell cycle arrest in *C.elegans.* Mol. Cell, Vol. 5, 435-443.
2. Rinaldo et al. (2002). Roles for *C. elegans rad-51* in meiosis and in resistance to ionizing radiation during development. Genetics, Vol. 160, 471-9.

209. *ccf-1*, a putative deadenylase encoding gene, is necessary for meiotic progression.

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The BTG genes encode a family of proteins with two domains conserved between members and also during evolution. An antiproliferative function has been demonstrated for three members of this family. The unique *C. elegans* homologue of the BTG genes is *fog-3*, involved in the differentiation of germ cells into sperm.
Yeast two hybrid analysis identified the protein CAF1 (CCR4 associated factor 1) as a protein interacting with BTG1 and 2. CAF1, initially characterized in yeast, is associated to CCR4 in a large transcriptional complex with deadenylase activity.

Animals and their progeny in which the *C. elegans* CAF1 homologue, *ccf-1*, is inactivated by RNAi display several phenotypes :
- oogenesis in P0 animals is abnormal from entry of germ cells into prophase of the first meiotic division, and about 50% of germ cells do not develop beyond this stage (partial sterility).
- developing embryos display several defects during the first successive divisions ; most embryos arrest before reaching the 50 cell stage.
- F1 animals that reach adulthood are sterile because of arrest of their germ cells in prophase of the first meiotic division.

ccf-1 seems thus involved in meiotic progression and germ cell differentiation. CCF-1 by its putative deadenylase activity could control mRNA translation of genes involved in these pathways. Such a translation control by regulation of the poly(A) tail has already been demonstrated for *tra-2*.

To determine *ccf-1* expression pattern a *ccf-1 : :gfp* fusion and a Northern analysis are in progress. An antibody directed against a CCF-1 peptide is also being produced.

Several genes have already been identified in meiotic progression pathways and available antibodies represent markers to further characterize the *ccf-1*(RNAi) phenotype.

Results obtained will be presented and discussed during the meeting.

210. Functional characterisation of the *Caenorhabditis elegans* homologue of mammalian puromycin-sensitive aminopeptidase that has reduced sensitivity to puromycin

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Mammals possess both membrane-associated and cytosolic forms of the puromycin- sensitive aminopeptidase (PSA; EC 3.4.11.14). Increasing evidence suggests a role in the central nervous system for the membrane-associated isoform. The functional role(s) of the cytosolic isoform is however less clear, though recent work with a *Drosophila* mutant indicates a role in spermatogenesis.

The genome of the nematode *Caenorhabditis elegans* encodes one aminopeptidase, F49E8.3, that is homologous to PSA, and sequence analysis predicts it to be cytosolic. We have determined the spatio/temporal expression pattern of the *C. elegans* PSA by using the promoter region of *F49E8.3* to control expression in the nematode of a second exon translational fusion of F49E8.3 to the green fluorescent protein. Cytosolic fluorescence was observed in the intestinal cells of larval and adult stage nematodes.

Recombinant F49E8.3, fused at the N-terminus to a His-tag, was expressed and purified from *Escherichia coli*. Biochemical analysis showed that F49E8.3 hydrolysed the N-terminal amino acid from peptide substrates. Favoured substrates had positively charged or small neutral amino acids in the N-terminal position. Peptide hydrolysis was inhibited by the chelating agent 1,10-phenanthroline (IC_{50}, 155 μM) and by the aminopeptidase inhibitors actinonin (IC_{50}, 0.1 μM), amastatin (IC_{50}, 0.2 μM) and leuhistin (IC_{50}, 0.6 μM). Interestingly, the enzyme was ~ 100-fold less sensitive to puromycin (IC_{50}, 100 μM) than other PSA homologues. Divalent metal ions inhibited F49E8.3 in a dose-dependent manner. Following inactivation of the enzyme with 1,10-phenanthroline, activity was recovered with Zn^{2+}, Co^{2+} and Ni^{2+}.

We propose that the *C. elegans F49E8.3* gene encodes a functional aminopeptidase that clusters phylogenetically with the PSA family, despite its lack of sensitivity to puromycin, and that it is most likely to function in the processing of peptides absorbed from the lumen of the intestine.

Research funded by the BBSRC and MRC.

211. An RNAi screen for axon guidance genes

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The complete genome sequence of *C. elegans* has opened up opportunities for genome wide screens, identifying genes involved in various developmental processes. We are interested in understanding the molecular basis for axon guidance and formation of stereotypic neural networks in the nematode. Recent discovery of RNA interference (RNAi) in *C. elegans* and other organisms has profoundly impacted the genome wide screens designed to identify genes involved in different biological processes (1). For example, a library of ~2500 *E. coli* strains expressing dsRNA, representing 87% of ORFs on chromosome I, has been generated and used to feed worms in order to identify the genes required for early development (2). We have combined the simplicity and tractability of RNAi with transgenic animals expressing GFP in a set of interneurons and motor neurons. A worm strain expressing GFP in these neurons, projecting their axons along anterio-posterior and dorso-ventral axes, was fed with ~700 *E. coli* clones expressing dsRNA corresponding to ORFs on chromosome I. Several RNAi experiments gave rise to axon guidance along with other developmental defects not specifically related to axon growth or guidance. However, based on bioinformatics data these genes are expected to be involved in basic metabolic processes or are structural components of the cell (e.g., DNA/RNA metabolism, ribosomal genes, innexins, etc.). The failure to identify specific axon guidance phenotypes could be due to the refractory nature of neurons to RNAi in *C. elegans*. To address the problem of neuronal resistance to RNAi, we performed a standard mutagenesis screen in order to identify mutants that allow efficient RNAi in neurons. We used a worm strain that moderately expressed GFP in all neurons for mutagenesis. This strain did not show any decrease in neuronal GFP even after feeding GFP dsRNA expressing bacteria for many generations. The EMS-mutagenized F2 animals of the strain were then fed with GFP dsRNA expressed in *E. coli*. Mutants were isolated showing complete loss of GFP in neurons after feeding GFP dsRNA bacteria for one generation. We tested the feeding RNAi efficiency of these mutants with known neurally expressed genes involved in axon morphology. Feeding these mutants with dsRNA of known genes phenocopies the defects seen in the mutants. The characterization and application of these mutants for future screens is in progress.

1. Fire A, et al. (1998); Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. *Nature,* 391(6669):806-11.
2. Fraser AG, et al. (2000); Functional genomic analysis of C. elegans chromosome I by systematic RNA interference. *Nature,* 408(6810):325-30.

212. Axon guidance in the ventral cord of *C. elegans*

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The ventral cord is the major longitudinal axon tract in *C. elegans* containing essential components of the motor circuit. It consists of two bundles running on either side of the ventral midline. Most axons enter the ventral cord after exiting the nerve ring in the head of the animal. Essentially all axons cross over to the right side near the retrovesicular ganglion leading to a highly asymmetrical ventral cord. Motorneuron axons originating from cell bodies at the ventral midline also exclusively grow in the right axon tract. The arrangement of processes within the cord is highly invariant implying that there is a precise navigational system guiding axons not just to the cord but also within the cord. The order of outgrowth of axons in the ventral cord during embryonic development has been studied by Richard Durbin[1], who also initiated a first analysis on the pioneering function of certain early outgrowing axons. A number of genes are now known to affect guidance of axons in the ventral cord.
The importance of particular guidance cues and pioneers for the navigation of individual classes of axons in the ventral cord can now be studied more systematically, since it is possible to visualize all major groups of ventral cord axons at the light microscopic level with GFP markers. To analyse pioneer-follower relationships several strains were made which express color variants of GFP in different groups of axons. The importance of pioneers like AVG was tested in laser ablation experiments. It was found that interneurons (glr-1 expressing) and D-type motorneurons are highly dependant on the pioneer AVG, whereas DA/DB motorneurons are almost not affected in the absence of AVG. Navigational mistakes are made individually. Errors of early outgrowing axons (DD) typically do not lead to mistakes of later outgrowing axons. Order of outgrowth apparently does not always reflect a pioneer-follower relationship. Similar observations were made in the absence of guidance cues like UNC-6. AVG and UNC-6 appear to be the main sources of navigational information for D-type motorneuron axons, but not so much for DA/DB motorneuron or interneuron axons indicating that different classes of neurons with axons in the ventral cord use different navigational cues. This analysis is currently extended to include other guidance cues and to test for redundancy among early outgrowing axons for the guidance of later outgrowing ones.

1. R.M. Durbin, thesis, University of Cambridge, Cambridge, UK (1987).

213. Searching for target genes of the nuclear receptor NHR-25 in *C. elegans*

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Of the enormous 270 nuclear hormone receptors predicted in *C. elegans,* only a handful are conserved and of known function. The *nhr-25* gene encodes a nuclear receptor orthologous to transcription factors FTZ-F1 in insects and SF-1 in mammals. Both FTZ-F1 and SF-1 are connected with steroid signaling. FTZ-F1 is induced by the ecdysone signaling cascade and its function is required for metamorphosis, cuticle synthesis and molting. SF-1 null mutant mice die early in postnatal development and lack adrenal glands and gonads (Luo *et al.* 1994). Loss of *nhr-25* function results in defects in embryogenesis, gonad and vulva development and molting (Asahina *et al.* 2000, Gissendanner *et al.* 2000). To further analyze the role of *nhr-25* in *C. elegans* development and the mechanism of its action, we are currently searching for target genes of NHR-25.

We reason that enzymes involved in sterol metabolism could be among NHR-25 target genes, because (1) SF-1 activates steroidogenic cytochrome p450 genes (CYP), and (2) molting in *C. elegans* requires both dietary sterol and NHR-25. Although no steroid hormone has been found in *C. elegans* as yet, cholesterol starvation can induce gonadal and molting defects (Yochem *et al.* 1999) similar to those of *nhr-25* RNAi. We explore the possibility that some p450 gene is regulated by NHR-25 in *C. elegans*. We selected several candidates from the ~80 *C. elegans* CYP genes based on two criteria: (1) similarity to insect or mammalian steroidogenic CYP genes, and (2) the presence of FTZ-F1/SF-1 binding sites in their promoter regions. We prepare GFP fusion constructs using these CYP promoter regions and test their activity in wild type versus *nhr-25* RNAi backgrounds. One of our candidate genes turned out to be *daf-9* (Gerish *et al.* 2001, Jia *et al.* 2002), whose expression patterns and some of the mutant phenotypes correspond well to those of *nhr-25* RNAi.

Asahina *et al.* (2000). Genes Cells 5, 711-723
Gerish *et al.* (2001). Dev Cell 1, 841-851
Gissendanner *et al.* (2000) Dev. Biol. 221, 259-272
Jia *et al.* (2002). Development 129, 221-231
Luo *et al.* (1994). Cell 77, 481-490
Yochem *et al.* (1999). Development 126, 597-606

214. Functional analysis of IgCAMs expressed in the nervous system

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Signaling molecules and receptors important for axon guidance and fasciculation are likely to be found among extracellular and cell surface molecules. The complete sequence of the *C. elegans* genome allows the identification of all members of known receptor and adhesion families. One of the largest families of adhesion molecules with a role in nervous system development is the immunoglobulin-superfamily (IgCAM-SF). IgCAM family members can be grouped according to their modular organisation. 28 genes encode transmembrane or GPI-anchored proteins with extracellular IG modules. We are focusing on a core of 17 IgCAMs. Five of them consist of IG-modules only, the remaining twelve have one or more fibronectin III (F3) modules in addition to IG-modules. For 15 of them no function is known.
To identify IgCAMs with a putative role in axonal outgrowth we started to analyze expression patterns for these genes. Transgenic animals were generated expressing promoter-GFP fusion constructs for individual IgCAMs. Expression typically is dynamic and not confined to a single tissue. Some IgCAMs (e.g. C26G2.1) show predominantly non-neuronal expression. Many others are expressed mainly in particular subsets of neurons (see also Supplementary Material to Aurelio O. et al. (2002); *Science*, 295: 686-690). Five IgCAMS (C53B7.1, F41D9.3, K02E10.8, K09E2.4, Y42H9B.2) are expressed in motorneurons or interneurons with axons in the ventral cord. We are currently isolating deletion alleles in these genes from our deletion library to begin a functional analysis.

215. *Ast-1* , a novel gene important for fasciculation of ventral cord axons

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One of the major problems of developmental neurobiology is how axons build their specific pattern of synaptic connections, which is crucial for the function of every nervous system. The mechanisms through which axons respond to guidance cues are not well understood. In the final stage of axon guidance the selection of synaptic partners within the target area takes place. Since synapses in *C. elegans* are made with neighbouring axons in an axon bundle, the correct fasciculation of axons even within a bundle is crucial for the correct wiring of the nervous system. To study the molecular basis of this recognition mechanism between axons, a screen for mutants exhibiting fasciculation defects was designed. Animals that express GFP under the control of the *glr-1* (glutamate receptor) promoter were used to label interneuron axons that are part of the motor circuit. In wildtype animals GFP labelled axons form a tightly fasciculated subbundle within the right ventral cord. After mutagenesis with EMS F2 progeny were scored under the fluorescence microscope for defects in fasciculation. This direct screen for axonal outgrowth defects is very sensitive for obtaining mutants with minor defects that do not lead to gross behavioural defects.

One novel gene identified in these screens is currently studied in more detail. *ast-1* mutants are characterized by defasciculated axons in the ventral cord, where axons cross from the right to the left bundle. An additional defect in several animals is misguidance of one single interneuron axon, which doesn´t reach the ventral cord, but runs in lateral position instead. The penetrance of these defects is about 45%. There are no obvious defects in the outgrowth of other axons as judged by the correct position of axons in animals expressing GFP markers in other sets of neurons. Non-neuronal tissues and cell migrations appear normal. *ast-1* mutants don´t show any severe movement phenotype and display only subtle changes in exploratory behaviour.
The ventral midline crossing defects are also seen for example in mutants in genes of the *slt-1/sax-3* or *unc-6* pathways. Using double mutant analysis we are currently testing whether *ast-1* is acting in one of these pathways affecting axon guidance in the ventral cord.

Two alleles of *ast-1* called *rh300* and *hd1* were isolated independently. Their phenotypes are very similar and the mutants do not complement. *ast-1* maps to chromosome II four map units left of *dpy-10* near *vab-1*. *ast-1(hd1)* complements *vab-1(dx31)*. Fine mapping was based on single nucleotide polymorphisms that can be detected by RFLP. The gene locus has been narrowed to a 250 kb region enclosed by cosmids F56D3 and K07E1. The YAC Y102C2 is covering this region and rescues the *ast-1* phenotype.

216. Expression and function of ftz-f1/nhr-25 in *Caenorhabditis elegans*

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FTZ-F1 is one of the ancient nuclear receptors and its ortholog is termed NHR-25 in *C. elegans*. The important roles of this molecule were identified genetically in mice (differentiation of primary steroidogenic glands and synthesis of steroid hormones) and in *Drosophila melanogaster* (embryonic segmentation, larval molting, metamorphosis).
nhr-25/ftz-f1 mRNA expression was detected by *in situ* hybridization throughout embryonic development. Zygotic expression was seen particularly in the gut and epidermal precursor cells. Epidermal expression persisted until L2 stage, when the developing gonadal cells took over the expression. Adult gonads were loaded with the *nhr-25* transcript (Asahina *et al*. 2000). Activities of transgenic *nhr-25::gfp* constructs mirrored the embryonic and the larval epidermal mRNA pattern. Two *nhr-25::gfp* constructs showed different expression profiles, indicating elements for stage specific regulation of *nhr-25*.
We have previously isolated an *nhr-25* deletion mutant (*jm2389*) which is recessive embryonic lethal at the 1.5-fold stage (the time of cuticle synthesis and elongation of the embryo). To address a possible NHR-25/FTZ-F1 role in postembryonic stages, we performed RNA interference by injection. RNAi confirmed the embryonic lethality and in addition showed molting defects at L1 and L2 (Asahina *et al*. 2000; Gissendanner and Sluder, 2000), and gonadal defects from L3 to adult stage. The gonad was typically tumorous, misshapen and undifferentiated. These worms were vulvaless and sterile as adults, and showed abnormal cuticle.
To resolve temporal requirements for *nhr-25*, we applied RNAi also via feeding and soaking of larval stages. The prolonged exposure to dsRNA resulted in pronounced late phenotypes (Vul) and newly observed L4 to adult molting defects. These results suggest that NHR-25/FTZ-F1 acts in a stage specific manner in different tissues. Finally, we wanted to exclude the possibility of non-specific RNAi effects due to the conservation of the zinc finger DNA binding domain (DBD). We found no difference whether RNAi was performed with dsRNA including or lacking the DBD region.
A polyclonal antibody was raised against recombinant bacterial NHR-25. It recognized a single band on immunoblots of worm extracts. Immunocytochemistry performed on gonads showed striking NHR-25 expression in the nuclei of the adult somatic gonadal cells, namely the distal tip cells (DTC) and the sheath cells. These cells are known to be important for the gonadal guidance and the differentiation of the germline, both processes dependent on NHR-25.
NHR-25 expression and loss-of-function phenotypes are in a good correlation and suggest various important roles of NHR-25 throughout the worm development.

[1] Asahina et al. (2000) Genes Cells 5: 711-723
[2] Gissendanner and Sluder (2000) Dev Biol. 221: 259-272

217. Expression and characterisation of the *C. elegans* NEP-like gene T25B6.2.

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Post-secretory processing and metabolism of neuropeptide or peptide hormones occurs predominantly through the action of peptidases that are attached to the plasma membrane. General features of membrane peptidases, or ectoenzymes, are that their active sites face toward the extracellular surface. Many of these are zinc metallopeptidases, which play key roles in the processing and metabolism of a diverse range of peptides. Neprilysin (NEP: EC 3.4.24.11) is a unique membrane endopeptidase anchored in the lipid bilayer by a short hydrophobic anchor at the N-terminus, and extracellular active site. They have the potential to participate in a range of cardiovascular functions, and re implicated in the control of hypertension, pain perception and cartilage and bone metabolism. We are using *C. elegans* as a model organism to explore the function of NEP-like genes in the organism, so that we may elucidate the function of NEPs in higher organisms. Spatio/temporal GFP and *Lac Z* reporter data indicates that T25B6.2 (a homologue to human NEP) is highly predominantly expressed in the intestine in all postembryonic stages. Our primary aim is to biochemically analyse the recombinant protein to establish its potential substrates. Previous attempts to generate heterologous recombinant protein in bacterial, yeast (*Pichia pastoris*) and mammalian (CHO) systems have proved unsuccessful. Currently, we are attempting to ectopically express native T25B6.2 by transforming *C. elegans* with genomic DNA tagged with two epitopes (Flag/His or C-Myc/His) to allow detection and purification. A trial run using GFP (green fluorescent protein) containing these tags has been very successful. In the experiments, GFP protein expression was controlled using either the heat shock promoter (hsp 16-41) or a strong constitutive promoter (*unc-54*). The entire coding region was placed downstream of the promoter and appropriate tags (Flag/His or C-Myc/His) were introduced at the C-terminus by PCR. High expression levels were seen with both promoters and western blot analysis confirmed detection was successful using both Flag and His tags. Purification via a Ni-NTA column was also successful as the GFP protein could be purified from whole worm extracts to apparent homogeneity. Having established this expression and purification technique with GFP, we are currently analysing T25B6.2 and other *C. elegans* zinc metallopeptidases.

218. The *RAD27*/FEN1 homolog is essential during early development in *Caenorhabditis elegans*

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The *RAD27* gene of Saccharomyces cerevisiae encodes a 5'-3' flap exo/endonuclease, which plays role in Okazaki fragment maturation during DNA replication. rad27 Delta mutants are viable in yeast, although temperature sensitive. They also exhibit increased sensitivity to alkylating agents, enhanced spontaneous recombination, and repetitive DNA instability. Biochemical and genetical analyses indicate that Okazaki fragment processing can be assured by other enzymatic activities or by alternative pathways such as homologous recombination. It has been demonstrated, making use of a synthetic lethality assay, that all the genes of the recombinational repair pathway are required for the survival of rad27 Delta strains. Recombination as well as other pathways can therefore bypass the replication defects that arise in the absence of the Rad27 protein.

RNA interference of the *C. elegans* ORF Y47G6A.8, potentially coding for the homolog of the yeast Rad27 protein, leads to embryonic lethality. We demonstrated that such lethal phenotype is due to zygotic effect and not to maternal effect. However, we also investigated the time of appearance of such lethal phenotype and postulated potential genetic interactions with maternally expressed genes. Inactivated mothers also show reduced fertility.

219. The *C.elegans* Forkhead gene F26B1.7.

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The forkhead family of transcription factors is a large family which was first identified as a homology between the rat genes *HNF3* α and β and *fork head (fkh)* in *Drosophila.* The highly conserved region of 100 amino acids necessary for DNA binding characterizes Forkhead genes.
Additional family members have been identified in fungi and animals. They have proved to be involved in many different processes. For example early development, tumuorogenisis and cellcycle regulation.
The nomenclature was revised in 2000 and Forkhead proteins are named Fox as root symbol and a letter after that for subfamily and number within the subfamily.

In *C.elegans* 18 forkhead genes can be identified within the sequenced genome. Several interesting mutants have been studied by other groups *e.g.* pha-4, daf-16, unc-130, lin-31 and pes-1.

We are studying the gene F26B1.7. This is the only Fox F homologue in *C.elegans*. FoxF genes have been identified as important factors for mesoderm development in both mouse and *Drosophila*. By analyzing the mutant and different GFP markers we will present data of the function of this gene in *C.elegans*.

220. Identifying ligands for NPR-1

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Social and solitary feeding behavior in wild isolates of *C. elegans* are associated with two isoforms of the putative neuropeptide receptor NPR-1[1]. Wild isolates that feed in groups encode NPR-1 215F, while wild isolates that feed alone encode NPR-1 215V. Three other nematode species, *C. briggsae*, *C. remanei* and CB5161, all encode NPR-1 215F, suggesting that NPR-1 215V, which is genetically dominant over NPR-1 215F, arose in *C. elegans* by a gain-of-function change. To explore how the two NPR-1 receptor isoforms differ in their biological activity we identified ligands for NPR-1. We screened *Xenopus* oocytes expressing the NPR-1 215F receptor with 33 synthetic neuropeptides predicted from the *C. elegans* genome[2]. A FMRFamide related peptide encoded by the *flp-21* gene, GLGPRPLRFamide, but not other tested ligands, activated NPR-1 215F at nanomolar concentrations. To elucidate how the NPR-1 215F and NPR-1 215V forms of the receptor differ, we compared their signalling properties in *Xenopus* oocytes. We find that the genetically dominant NPR-1 215V form of the receptor is 50% activated at a 10-fold lower concentration of FLP-21 than NPR-1 215F. Preliminary results suggest that over-expression of *flp-21* induces solitary feeding in wild social *C.elegans* strains, but not in *npr-1(null)* mutants, suggesting that FLP-21 is the endogenous ligand for this receptor. The role of FLP-21 as a neurotransmitter in the regulation of social feeding behaviour will be further investigated using electrophysiological techniques.

[1]de Bono M., and Bargmann, C. (1998). Natural variation in a neuropeptide Y receptor homolog modifies social behavior and food response in *C. elegans*. Cell 94: 679-689;
[2]Li, C., Kyuhyung, K., and Nelson, L. (1999). FMRFamide-related neuropeptide gene family in *Caenorhabditis elegans*, Brain Research 848: 26-34.

221. Embryonic development of the free-living, marine nematode *Pellioditis marina*

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Until now only the embryonic cell lineage of the model organism *Caenorhabditis elegans* has been described (Sulston et al., 1983). The embryonic cell lineage of the free-living nematode *Pellioditis marina* has been traced from zygote up until the initiation of muscle contraction by means of 4D-microscopy, marking the second detailed description of the embryonic development of a nematode. *P. marina* is a close relative of *C. elegans*, but has adapted to a marine, brackish environment. The overall lineage resembles strongly on that of *C. elegans*, with a few small differences. The developmental tempo of the early embryogenesis (until division of E cell) is more then two times slower than *C. elegans*. But the primordial germline cell P4 is already present at the 15-cell stage (in *C. elegans* at the 24-cell stage). At the stage of muscle contraction (when most cells are established), *P. marina* has as many cells as *C. elegans* (571 cells) but less cell deaths (67 and 106 respectively). Tissue conservation varies from highly conserved to highly variable. The intestine, the primordial gonad and the body muscles are highly conserved in the two species, while the pharynx, the epidermis and the nervous system have a more variable configuration. The systematic position of *Pellioditis* remains unsolved, whether *Caenorhabditis* or *Rhabditis* is the closest relative. The early embryogenesis and the developmental timing are comparable with that of other *Rhabditis* species, while the overall cell lineage is almost identical with that of *C. elegans*. The latter is a strong argument to place *P. marina* close to *C. elegans* in the classification. In more primitive nematodes (like *Halicephalobus* sp.), sublineages form identical cells, which migrate to their exact location. *C. elegans* has adjusted these lineages to avoid these migrations (Borgonie et al., 2000). This could explain the 'chaotic' fate topology in the *C. elegans* cell lineage. *P. marina* falls in between: it has already adjusted the Caa-lineage to form two nerve cells, but still has migrations that are avoided in *C. elegans*.

Borgonie, G., Jacobsen, K. & Coomans, A. (2000). Embryonic lineage evolution in nematodes. *Nematology* **2**, 65-69.
Sulston, J. E., Schierenberg, E., White, J. G. & Thomson, J.N. (1983). The embryonic cell lineage of the nematode *Caenorhabditis elegans*. *Dev. Biol.* **100**: 64-119.

222. Suppressors of social feeding behaviour

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npr-1 encodes a putative G-protein coupled receptor similar to neuropeptide Y receptors. Social or solitary feeding behaviour in wild isolates of *C. elegans* is associated with a single residue change in this receptor[1]. Null mutants of *npr-1* exhibit strong social feeding behaviour, suggesting that this gene acts to suppress social feeding. I am characterizing and mapping mutations that disrupt social feeding to delineate molecular pathways and neuronal networks that regulate this behaviour.

Two screens that were previously carried out identified about 50 mutations that convert *npr-1(null)* social feeders into solitary feeders (see Abstract by Cheung and de Bono). In an attempt to reach saturation, and provide more alleles of these suppressors, we carried out a third screen that identified a further 100 mutants that prevent aggregation of *npr-1* mutant animals.
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Using snip-SNP mapping I have mapped representative alleles from each of seven complementation groups that suppress social behaviour to within 500 kb. All suppressors show wild-type mating behaviour and dye-filling and are not uncoordinated. One of the suppressors, represented by the allele *db44*, fails to complement *tax-6*, which encodes the *C. elegans* calcineurin A subunit[2]. Non-complementation experiments and map positions suggest that the other suppressors are mutated in previously uncharacterised genes. I am currently carrying out transgenic rescue experiments for *db47*, for which there is a promising candidate gene.

[1]de Bono and Bargmann, 1998. Natural variation in a neuropeptide Y receptor homolog modifies social behavior and food response in *C. elegans*. Cell, 94:679-89.
[2]Kuhara A, Inada H, Katsura I, Mori I., 2002. Negative regulation and gain control of sensory neurons by the *C. elegans* calcineurin TAX-6. Neuron, 33:751-63.

223. Essential roles for four cytoplasmic intermediate filament proteins in *Caenorhabditis elegans* development

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The structural proteins of the cytoplasmic intermediate filaments (IF) arise in the nematode *C. elegans* from eight previously reported genes and an additional three genes now identified in the complete genome. Using double stranded RNA interference (RNAi) for all 11 *C. elegans* genes encoding cytoplasmic IF proteins we observe phenotypes for the five genes A1, A2, A3, B1 and C2. These range from embryonic lethality (B1) and embryonic/larval lethality (A3) to larval lethality (A1 and A2) and to a mild dumpy phenotype of adults (C2). Phenotypes A2 and A3 involve displaced body muscles and paralysis. They probably arise by reduction of hypodermal IF which participate in the transmission of force from the muscle cells to the cuticle. The B1 phenotype has multiple morphogenetic defects while the A1 phenotype arrested at the L1 stage. Thus, at least four IF genes are essential for *C. elegans* development. Their RNAi phenotypes describe the first lethal defects due to silencing of single IF genes. In contrast to *C. elegans* no IF genes have been identified in the complete *Drosophila* genome, posing the question of how *Drosophila* can compensate for the lack of these proteins which are essential in mammals and *C. elegans*. We speculate that the lack of IF proteins in *Drosophila* can be viewed as cytoskeletal alteration in which, for instance, stable microtubules, often arranged as bundles, substitute for cytoplasmic IF.

224. Characterization of *M. nematophilum* resistant mutants *bus-3* and *bus-5*

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Microbacterium nematophilum was recently identified as a pathogen of *C. elegans* (*1*). These bacteria adhere to the post-anal region of the worm, cause a swelling of the tail, or Deformed anal region (Dar), and constipation. Worms carrying mutations that conferred resistance to *M. nematophilum* were termed *bus* for *b*acterially *u*nswollen. *bus-3* and *bus-5* are two of 13 loci identified in an EMS screen for resistant loci. Alleles of *bus-3* or *bus-5* were not identified in screens relying on endogenous Tc1 transposon mutagenesis, suggesting that these genes may be essential.

bus-3 has been mapped to LGI and was originally defined by two alleles, *e2696* and *e2695*. Worms homozygous for either allele are Unc on OP50 and 100% Bus when grown on *M. nematophilum*. However, complementation crosses between these mutants resulted in 60% Dar *e2696/e2695* progeny, the remaining *e2696/e2695* progeny were weak Dar and Bus. This result suggests that *e2696* and *e2695* may have atypical allelic interactions or they may define different *bus* loci and exhibit nonallelic interactions. We are mapping both mutations using snipSNP and deficiencies.

bus-5 has been mapped to LGXL. This locus is defined by seven alleles, *e2686, e2685, e2688, e2701, e2704, e2794* and *e2699*. Worms homozygous for any of these alleles are Unc backwards, Con, and Squirmy. In addition, all are Egl except worms homozygous for the strongest allele *e2686*, which is semi-sterile. Nonetheless, worms homozygous for any of the alleles are 100% Bus when grown on *M. nematophilum*. The 'Squirmy' phenotype refers to a loss-of-traction that is apparent when the mutant worms are not on the bacterial lawn. This phenotype has been noted for some of the *srf* mutants, in particular, *srf-2, srf-3* and *srf-5*. These mutants have altered displays of lectin-binding antigens on their cuticles (*2*). These mutants are also Bus (*1*). Lectin staining of *bus-5(e2688)* demonstrated that surface antigens are altered in mutant larval worms compared with wild-type (Reindert Nijland pers. comm.). Thus, *bus-5* may play a role in determining cuticle properties of the worms that are required for bacterial pathogenesis to progress.

To date none of the EMS derived mutations that confer resistance to infection by *M. nematophilum* have been cloned. By understanding what these genes are, we should be able to get a handle on what is required for pathogenesis by *M. nematophilum* and perhaps other Gram-positive bacteria. Further characterization and mapping data for *bus-3* and *bus-5* will be presented.

1. J. Hodgkin, P. E. Kuwabara, B. Corneliussen, *Current Biology* **10**, 1615-1618 (2000).
2. C. D. Link, *et al.*, *Genetics* **131**, 867-881 (1992).

225. Molecular cloning of *C. elegans bus*-6 using snip/SNP mapping

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Microbacterium nematophilum is a novel bacterial pathogen of *C elegans* (Hodgkin *et al*, 2000). This pathogen adheres to the rectum and post-anal region of the worm causing swelling of the underlying tissues, severe constipation and slow growth rates. Affected worms are described as having a Dar (Deformed Anal Region) phenotype. To define the molecular events involved in this host-pathogen interaction, we have used the mutator strain, *mut-7*, and EMS mutagenesis to screen for mutant worms that are resistant to infection. These worms exhibit a Bus (Bacterially UnSwollen) phenotype.

In order to identify the *bus* loci we initially used transposon insertion display (TID) to try to clone the *bus* mutants isolated in the *mut-7* screen. However, TID analysis of 14 alleles of 8 *bus* loci failed to identify any of the *bus* genes. We are now using conventional mapping techniques and are reducing our intervals using snip/SNP mapping (Wicks *et al*., 2001). In this poster we will present data on our progress in the mapping of one of the *bus* mutants, *bus-6*.

bus-6 adult worms show no gross changes in surface lectin staining (Reindert Nijland) but have slight changes in larvae, similar to *bus-1* worms. *Microbacterium nematophilum* do not adhere to *bus-6* worms, suggesting that *bus-6* may encode a protein that facilitates attachment of the bacteria to the cuticle.

bus-6 has been mapped to LGV, between the *dpy-11* and *unc-42* markers. We will describe our progress in narrowing this interval using both deficiencies and snip/SNPs.

Hodgkin, J. Kuwabara, P. E. Corneliussen, B. *Current Biology* 2000 10 ;1615-1618 A novel bacterial pathogen, *Microbacterium nematophilum*, induces morphological change in the nematode *C. elegans.*

Wicks, S. R. Yeh, R. T. Gish W.R. Waterston, R. H. Plasterk, R. H.A. *Nature Genetics* 2001 28:160-164.
Rapid gene mapping in Caenorhabditis elegans using a high density polymorphism map.

226. *C. elegans – M. nematophilum* interaction: Genetic and molecular characterization of the gene *bus-1*.

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The interaction between *C. elegans* and *Microbacterium nematophilum* will enhance our understanding of the basic mechanisms of the nematode response to a specific bacterial infection.

To this end, we have isolated numerous *C. elegans* mutants that are resistant to the infection by *M. nematophilum* using both EMS and transposon-induced mutagenesis approaches. Bus mutants (*B*acterially *U*n-*S*wollen) have been selected on the basis of improved growth and tail morphology; an absence of swelling in the presence of the pathogen indicates resistance (13th International *C. elegans* Meeting abstracts 319, 606, 608).

Here we describe the genetic and molecular characterisation of the *bus-1* gene in *C. elegans*. We have focused on this gene because it was frequently mutated in both screens. Of 106 mutants identified, a total of 23 *bus-1* alleles have been isolated (17/70 and 6/36 were transposon and EMS-induced, respectively), suggesting that *bus-1* may play an important role in this interaction.

Although mutations in *bus-1* result in total penetrance of the Bus trait, a discrete set of defects leading to phenotypes such as Egl, Con, extrusion of male copulatory spicules and body shape deformities has also been observed. All *bus-1* alleles are recessive to wild type and behave genetically as though they reduce or eliminate gene activity.

Extensive attempts to detect Tc1 insertions in the transposon-induced alleles have been unsuccessful suggesting that these mutations might have been caused by hit-and-run events. Therefore we pursued an alternative approach by conventional positional cloning.

bus-1 maps to LGV between *dpy-11* and *unc-42*. Further refinement of its genetic position by means of deficiency tests and snip-SNPs place *bus-1* within the overlapping region of *mDf1* and *sDf71*, corresponding to a 0.2 map unit interval and between cosmids F25G6 and R03H4.

Rescue experiments by microinjection DNA from cosmids within this region are underway and progress will be reported.

227. Cloning and characterization of *srf*-3

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The nematode cuticle is a complex collagenous structure which is important for maintenance of morphology and locomotion. The cuticle consists of at least two distinct classes of proteins: the collagens and the surface associated proteins. Mutations in collagens very often result in animals with altered morphology e.g. dpy or rol phenotype. Mutations affecting cuticle structure have been extensively studied in *Caenorhabditis elegans* and various dpy, rol and sqt mutations have been described in molecular detail.

Given that the exoskeleton serves as a barrier to the environment, it provides a target for attack by natural enemies. Consequently nematodes have developed surface defenses against predators, which includes nematode trapping funghi or as in the case of parasitic nematodes the host immune system. *C. elegans* mutants with wild type gross morphology but changed surface binding of antisera raised against the adult cuticle and ectopic lectin binding have been isolated and are thus described as surface phenotypes, or *srf*. To date none of the *srf* loci have been identified at the molecular level.

Here we describe the cloning and characterization of *srf*-3. It was cloned by cosmid rescue using the resistance of *srf*-3 animals to infection with *Microbacterium nematophilum* (Hodgkin et al. 2000), a bacterial pathogen recently reported to infect *C. elegans* causing a deformed anal region (*dar*) phenotype.

228. Characterisation of the Multiubiquitin Chain Assembly Factor (E4) in *Caenorhabditis elegans*

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Proteins modified by multiubiquitin chains are the preferred substrates of the proteasome. Ubiquitination involves a ubiquitin-activating enzyme, E1, a ubiquitin-conjugating enzyme, E2, and often a substrate-specific ubiquitin-protein ligase, E3. In *Saccharomyces cerevisiae* it was shown that efficient multiubiquitination needed for proteasomal targeting of a model substrate requires an additional conjugation factor, named E4. This protein, previously known as UFD2 in yeast, binds to the ubiquitin moieties of preformed conjugates and catalyzes ubiquitin chain assembly in conjunction with E1, E2, and E3. Intriguingly, E4 defines a novel protein family that includes homologs in human, *Dictyostelium*, fission yeast and one homolog in *C. elegans*.
We are interested in the function of the E4 enzyme in multicellular organisms and therefore studying the function of the *C. elegans* homolog of UFD2, T05H10.5. First we recombinantly expressed and purified T05H10.5. Using an *in vitro* ubiquitination assay, we were able to prove the binding of T05H10.5 to a ubiquitinated model substrate and its multiubiquitination, as it was previously shown for yeast UFD2.
Furthermore we analysed the expression pattern of *T05H10.5* in transgenic animals using GFP fusion proteins driven by the endogenous promoter. Interestingly, T05H10.5::GFP is expressed in all neurons of the worm, in some muscle cells of the pharynx and somatic muscle cells and is localised predominantly in the nucleus. Comparing early embryos and different developmental stages of the animals indicates that there might be a stage specific redistribution of T05H10.5 from the cytosol to the nucleus. Currently we are testing candidate interaction partners that might be involved in this regulated localisation by two-hybrid and RNAi analysis.
In order to continue the analysis of the function of this E4 enzyme, we generated a deletion mutant of the entire corresponding gene and which we are currently phenotyping.

229. Characterization of *mau-8* mutants

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mau-8 mutants genes are defined by maternal effect uncoordinated genes. *mau-8* mutants have a number of behavioural and morphological abnormalities: they are lethargic, present a discontinuous ratchet-like movement when moving backward and they show defects in the defecation cycle. These phenotypes are not due to defects in neuronal development since the overall structure of the nervous system appears normal.
We first concentrated on looking at double mutants to see if a certain class gives a synthetic lethal phenotype. Our preliminaty results suggest that unc-116 strongly interacts genetically with *mau-8*. Unc-116 encodes a kinesin heavy chain involved in intracellular transport of organelles such as secretory granules and mitochondria. Since mitochondrial transport is normal in musles and mecanosensory neurons of *mau-8* mutants, we concentrated in the study of the transport of secretory granules and lysosomes. We will show results about the distribution of proteins involved in vesicles trafficking in *mau-8*.
In parallel, we started the analysis of the ventral nerve cord of *mau-8* by electron microscopy to look for potential structural abnormalities of *mau-8* neuromuscular junctions. Preliminary results show abnormal membranous whorls in some axons. These whorls are identical to those found in mutants such as *mec-4* known to induce necrosis-like neuronal death.

mau-8 is localized between unc-26 and dpy-4 on chromosome IV. We have positionned mau-8 to Y62E10. Progress in the molecular characterization of mau-8 will be presented.

230. *mab-9/Tbx20* orthologues and the generation of morphological diversity.

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mab-9 (male-abnormal) is essential for patterning of the *C. elegans* hindgut and for the correct functioning of the nervous system. In *mab-9* mutants, two male-specific blast cells B and F, which normally give rise to important copulatory structures of the male tail, adopt the lineage fates of their anterior neighbours, Y and U. This cell fate transformation results in gross abnormality of the male tail. *mab-9* hermaphrodites are also abnormal as the B and F cells are structural cells in hermaphrodites and are important in development of the rectum. *mab-9* mutants of both sexes are also weakly backwards Unc.

mab-9 encodes a T-box protein and is most closely related to the *Tbx20* sub-family of T-box transcriptional regulators. *Tbx20* orthologues have been identified in a diverse range of metazoan phyla and include human *TBX20*, mouse *Tbx20* and *Drosophila H15*. In vertebrates and *Drosophila Tbx20* orthologues are expressed during early heart development, suggesting an important role for *Tbx20* in specification of the heart. Thus despite a common evolutionary origin, *Tbx20* orthologues appear to have acquired diverse functions. This provides an interesting comparative system in which to examine the molecular basis of morphological diversity. To which extent are *Tbx20* genetic pathways, gene expression and gene function between nematodes and chordates conserved? Trans-species rescue experiments are in progress to investigate whether vertebrate *Tbx20* can completely or partially functionally substitute for *mab-9 in vivo*. One other interesting line of enquiry is the possibility that body structures specified by *Tbx20* orthologues may share some kind of phylogenetic relationship.

A few other *C. elegans* T-box genes have orthologues in other species. We are extending our trans-species analysis of T-box gene expression and function with some of these genes in order to examine other potential phylogenetic connections.

231. WormBase: a web-accessible database for *C. elegans* biology

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WormBase (http://www.wormbase.org) is the central web-accessible repository of *C. elegans* genomic, genetic and biological data. The main contents are:

· the physical map and genome sequence data from the *C. elegans* Sequencing Consortium with 21 352 annotated gene structures (including splice variants)
· the genetic map curated by the *Caenorhabditis* Genetics Centre
· WormPep, a collection of *C. elegans* proteins based on ESTs and cDNA sequences, homology with *C. briggsae* genomic sequence, protein homology, and other relevant data
· WormRNA, a collection of non-coding *C. elegans* genes
· additional information from detailed literature curation
· large-scale datasets e.g. RNAi experiments, SNP data, transgenes, gene expression, regulation and function data, additional sequence features, cell function, cell lineage information, diagrams of cells, etc.

We intend to provide an online demonstration of WormBase during the meeting in order to introduce it to new users and help existing users with any questions that they may have.

We rely on and want to further encourage users not only to inform us of errors in information or software performance but also to give feedback on content and presentation. Comments and data can be forwarded to curators via web-based forms or email (wormbase-help@wormbase.org). WormBase exists to serve the *C. elegans* and broader biomedical community, and we thank our many contributors and collaborators.

232. *Caenorhabditis elegans* as a model for infantile neuronal ceroid lipofuscinosis (INCL)

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Infantile neuronal ceroid lipofuscinosis (INCL) is the most severe subtype of the NCLs, a group of inherited neurodegenerative disorders that occur with a frequency of 1:12 500 births. Onset is between 6 and 18 months and is characterised by the accumulation of an autofluorescent storage material in lysosomes of all tissue and subsequent neuronal death. Mutation of palmitoyl protein thioesterase-1 (*PPT1*), a lysosomal enzyme, has been shown to be responsible for disease onset but the mechanism of pathogenesis and reason for the selective death of neuronal tissue is unknown.

The completely mapped nervous system and fully sequenced genome of *Caenorhabditis elegans* make it an ideal model for the further study of this and other genes underlying human neurological disorders. By studying this gene thoroughly in *C. elegans* it is hoped that some clues as to the pathways involved and the pathogenic mechanisms of neurodegeneration in INCL will be determined.

BLAST searching has found one homologue to *CLN1*, *CePpt-1*, which exhibits 75% similarity and 54% identity to the human protein. We have shown it to be expressed at the mRNA level and have determined the gene structure by RT-PCR and subsequent sequencing of amplicons. Protein prediction programmes suggest that, like the human protein, *CePPT-1* is soluble. RNAi of *CePpt-1* in non-neuronal cells, using both feeding and microinjection techniques, was found to produce a reproductive phenotype. Further phenotypic analysis, including electron microscopy is underway. To study the neuronal phenotype an inverted repeat construct has been made and four strains containing different deletion alleles have been obtained from the Knockout Consortium[1]. Due to the nature of the human disorder a neuronal phenotype is expected. Finally, information on developmental expression patterns has been obtained through the Kim Lab microarray resource[2]. These suggest *CePpt-1* expression in hermaphrodites is highest in eggs and adults, but that expression in adult males is higher than in adult hermaphrodites. Additional expression analysis through immunolocalization, using antibodies produced via a GST construct is planned.

1) Deletion mutations used in this work were provided by the *C. elegans* Reverse Genetics Core Facility at the University of British Columbia, which is funded by the Canadian Institute for Health Research, Genome Canada and genome BC.
2) Jiang, M., Ryu, J., Kiraly, M., Duke, K., Reinke V., and Kim, S. K. Genome-wide analysis of developmental and sex-regulated gene expression profiles in *C. elegans*. PNAS 98, 218-223, 2001.

Acknowledgements:
Dr George Joshua, London School of Hygiene and Tropical Medicine; Patty Kuwabara, Sanger Institute; Mounira Chebah, UCL; Rebecca Allen, UCL.
This work was supported by the Medical Research Council in the UK and the Batten Disease Support and Research Association and Children's Brain Diseases Foundation in the USA.

233. Genetic and molecular characterisation of two new dumpy genes: *dpy-31* and *dpy-32*

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In *C. elegans* there are many genes that have mutant non-lethal phenotypes affecting body size and shape. Mutations in *dpy (d*um*py)* genes cause a short and fat body phenotype. To date, more than 30 *dpy* genes have been defined. Many *dpy* genes have been already cloned and have been shown to be involved in cuticle formation or function. Some genetically identified loci encode collagen genes, such as *dpy-2*, *dpy-7*, *dpy-10* and *dpy-13*. Others, such as *dpy-18* and *dpy-11*, encode enzymes responsible for posttranslational modification or assembly of collagen molecules. At least one *dpy* gene, *dpy-20,* encodes a previously unknown type of protein, suggesting that there may be other classes of cuticle components still to be discovered and characterised mutationally. Here we describe two new *dpy* genes: *dpy-31* and *dpy-32.*

dpy-31 (*e2770*) originally arose as a spontaneous mutation in a *mrt-2* screen. *dpy-31* mutant worms show a severe dumpy phenotype and are temperature sensitive (ts): at the permissive temperature (15°C) *dpy-31* worms exhibit ~50% lethality and at the restrictive temperature (25°C) the strain is completely inviable.
The ts lethality permits strong selection for revertants, and several have been isolated. Some of these strains show a dumpy phenotype but are not ts (*e2810*); others are complete revertants to wild-type (*e2808*). All appear to be due to mutations tightly linked to *e2770*.
The easy isolation of revertant strains by EMS mutagenesis may suggest that *dpy-31* is a gain-of-function (gf) mutation. However, *dpy-31* does not behave genetically as a gf mutation. It is recessive and a complementation test of the revertant (*e2808*) with the original *e2770* strain showed that reversion to wild-type complements the dumpy mutation.
dpy-31 was mapped to the center of LGIII. Using snip-SNP mapping and three-factor crosses we placed the mutation at –0.81 on LGIII between *unc-36* and *sma-3*. Unaccountably, injection of 9 cosmids spanning ~300 Kb of genomic DNA of this region has failed to rescue the mutant phenotype. Sequencing of a candidate gene, *col-8,* revealed no alterations in the mutant. We are currently trying to rescue heterozygous double mutants carrying a balancer on LGIII [*dpy-31* (*e2770*) *sma-3* (*e491*)/ *eT1* (*e873*)].

The other mutant we have been characterising is *dpy-32* (*np29*), which was generated in a *Mos1* screen. Preliminary data indicate that the *Mos1* transposon has inserted into the second exon of C26D10.5, thus inactivating this gene that encodes for a phospholipase type A2. We demonstrated that the *dpy* phenotype is rescued in *np29* worms carrying cosmid C26D10 on an extrachromosomal array. Further progress will be reported.

234. Comparative and experimental embryogenesis of Plectidae (Nematoda)

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Recent studies challenge the traditional phylogenetic position of the nematode family "Plectidae". While morphological criteria led to their placement in the subclass Adenophorea, molecular data suggest a closer affiliation with Secernentea. As embryogenesis appears to differ considerably between the two subclasses, we analyzed six species of Plectidae with particular reference to *C. elegans*. Our embryological studies reveal a number of basic similarities of Plectidae with Secernentea but not with Adenophorea, and thus support the conclusions drawn from molecular data. On the other hand, Plectidae express certain developmental features not found in *C. elegans.* Prominent characteristics are (a) the strict left-right divisions of somatic founder cells generating an obvious early bilateral symmetry and (b) an early gastrulation with a single gut precursor cell. These peculiarities are shared with some but not all representatives of the taxon Chromadoria.
To determine how crucial the standard type of gastrulation with two gut precursors is for those species that follow this pattern, we manipulated *C.elegans* embryos to gastrulate with a single blastomere like the Plectidae. It turned out that this is compatible with an essentially normal further embryogenesis, and thus cleavage of the gut precursor is obviously not required for proper ingression. As prominent differences exist among Secernentea concerning the potential to compensate for eliminated blastomeres, we tested this feature in one *Plectus* species. In contrast to our findings in *Acrobeloides nanus* (Cephalobidae), *Plectus* does not replace a lost cell but behaves like *C. elegans* in this respect.

235. Functional analysis of the *C. elegans* T-box gene family

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T-box genes are a group of developmentally important transcription factors united by a common DNA binding domain. T-box genes are present in all metazoan species so far analysed but are absent from yeast. There are 20 T-box genes in *C. elegans*, more than twice the number found in Drosophila. Many of the *C. elegans* T-box genes are highly diverged from those found in other species while others have clear orthologues present throughout the metazoan kingdom. One highly conserved T-box gene is *mab-9*, a member of the *tbx20* sub-family[1]. This was the first *C. elegans* T-box gene to be identified by mutation and is required for cell fate specification during hindgut and male tail development, and aspects of nervous system function. One other conserved T-box gene has recently been reported to be important for a particular muscle cell fate specification[2]. We have inactivated the remaining *C. elegans* T-box genes by RNAi and have found obvious phenotypes only in very few cases. These phenotypes include embryonic lethality, L1 lethality, and a Dpy phenotype with weakly penetrant male tail defects, and will be described in detail. The remaining T-box genes give no obvious phenotype by RNAi. Phylogenetic analysis reveals that several pairs of T-box genes are very similar to eachother and are therefore likely to be the result of recent duplications. This might suggest functional redundancy. Double RNAi experiments have revealed this to be the case with at least two of the T-box gene pairs (see also poster by Pocock *et al*). Study of the expression patterns of the whole T-box family may suggest other potential redundancy relationships which can be explored by RNAi. Comparison of the *C. elegans* T-box genes with the set of T-box genes now defined for *C. briggsae* is being used as a tool for defining potentially important regulatory regions present in orthologous genes.

[1] Woollard and Hodgkin, *Genes Dev* 14, 596-603, 2000. [2] Kostas and Fire, *Genes Dev* 16, 257-269, 2002

236. Taste perception by the nematode *Caenorhabditis elegans*

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We use *Caenorhabditis elegans* to study heterotrimeric G-protein mediated signal transduction. Only seven pairs of chemosensory cells are involved in the detection of tastants. Five pairs of cells were found to be important for attraction, ADF, ASE, ASG, ASI and ASK and two pairs of cells for aversion, ADL and ASH. Despite this limited number of chemosensory cells, the nematode is able to detect several different compounds and distinguish between them. This is most likely accomplished by the expression of multiple chemosensory receptors per cell. Furthermore, the observation that these cells express multiple different G protein alpha subunits implies that multiple signal transduction pathways might exist for perception per cell.
In this project we will determine which G alpha subunits are involved in taste, and whether these signals are modulated. We use a water soluble compound chemotaxis assay (Wicks et al 2000, Dev Biol 221, 295-307) to determine the effect of loss- or gain-of-function of the various alpha subunits. We searched for suitable compounds of the taste modalities salt, sweet and bitter. NaCl (salt) was clearly recognized by *C. elegans*. It is thought that NaCl perception relies on ion channels and that G proteins are not involved. However the loss-of-function mutant of *gpa-3* (a G alpha subunit) gives a weaker response to salt compared to the wild type, suggesting that G-protein signaling is involved.
Trehalose was a suitable sweet compound. In *Drosophila* trehalose acts via G-protein coupled receptors (GPCR). *C. elegans* avoids trehalose, however, mutations in the G-alpha subunit ODR-3 and GPA-10 affect trehalose aversion. This clearly shows that G proteins are involved.
An appropriate bitter compound is denatonium. In mammals the denatonium signaling pathway acts via GPCR. For *C. elegans* denatonium is a strong atractant, yet in time it becomes very repulsive to *C. elegans*. Mutations in the G-alpha subunits GPA-1, 2, 3, 10, 11, 14 and 15 affect chemotaxis to denatonium. We use genetic cell specific rescue (Massimo et al 2001, Int Worm Meeting, abstract 206) to determine which cells are involved in the perception of denatonium in our assay. Subsequently, we will determine which G alpha subunits are involved, and whether these signals are modulated.

237. Cloning of the *mab-2* gene of *C.elegans* using RNAi

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mab-2 (male abnormal) was originally identified in an EMS screen for males defective in mating ability. The phenotype is characterized by a deformed male tail which has one or more missing rays and defective V and T cell lineages. The mutation has been mapped using visible markers to the *dpy-5 unc-13* region of LGI but subsequent cosmid rescue attempts were unsuccessful. We therefore decided to attempt positional cloning by a reverse genetic approach using RNAi. We used a feeding RNAi library (from J.Ahringer) in order to quickly screen genes from the region for phenocopy of the *mab-2* mutant in a *him-8* background. A putative gene, F55F8.3 was found to give a *mab-2*-like phenotype in >90% of male F1 progeny and is therefore a good candidate for the gene mutated in *mab-2* worms. This gene had been previously screened for function in hermaphrodites using the same RNAi library and been found to produce a larval arrest (Lva) phenotype and indeed we observed an approximately 2-fold slower rate of growth compared to wildtype. The feeding result has been confirmed for F55F8.3 using injection of dsRNA. However, the later progeny of these injected hermaphrodites showed an embryonic lethal (Emb) phenotype in addition to the Lva and Mab phenotypes of the earlier progeny indicating a dosage effect: it may be that a high level of RNAi causes arrest at the embryonic stage whereas lower levels cause a delay in larval development and male tail abnormalities. This data is consistent with the fact that sequencing of F55F8.3 from *mab-2* worms showed no mutation in the coding sequence implicating either the promoter region, or other sites involved in regulation of expression, as likely sites of the *mab-2* mutation and that these may be hypomorphic alleles of an essential gene. Complementation tests are therefore being conducted to see if any of the many lethal (Let) genes mapped to this region are indeed allelic with *mab-2*. The other interpretation of these observations is that while giving a male tail phenotype when subjected to RNAi and mapping to exactly the same region as *mab-2*, F55F8.3 is not the gene defined by the *mab-2* (*e1241*) mutation.

Analysis of the genomic context of F55F8.3 showed that it is clustered with five other genes each with short intergenic regions and a putative 5' trans splice site just upstream of the presumed translational start site indicating that this region may be operonic. This raises the possibility that the RNAi is targeting the polycistronic pre-mRNA for degradation. Consistent with this, two other genes in this cluster also gave an Lva phenotype and one an Emb phenotype. However, if there was global degradation of pre-mRNA then all five genes would be expected to give these phenotypes; only one of the genes gives the *mab-2* phenotype when subjected to RNAi. It may however, indicate that these genes are functionally related. In order to see if F55F8.3 is down-regulated in the *mab-2* mutants we are doing semi-quantitative RT-PCR on the putative operon genes.

F55F8.3 contains WD-40 repeats, a protein-protein interaction motif, and is highly similar to the *S.cerevisiae pwp2* gene involved in growth, bud site selection and cell separation. Yeast two-hybrid screens are therefore an attractive means of revealing possible interacting proteins. It may be significant that another Lva gene in the operon also contains WD-40 repeats. Further work will also be done on characterizing the *mab-2* phenotype and the lineage defect implicated previously, using V and T cell markers.

238. *Caenorhabditis elegans* as a model organism in the study of nucleoside transporters.

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Nucleoside transporters (NTs) are essential in cells that lack *de novo* biosynthetic pathways. In addition, they have important roles in many adenosine-mediated physiological processes in mammals, including platelet aggregation and coronary vasodilation. Most eukaryotes possess multiple NTs, which can be grouped into two unrelated transporter families; the equilibrative nucleoside transporters (ENTs) and the concentrative transporters (CNTs). In order to understand the physiological functions of nucleoside transporters and the reasons underlying their biological diversity, we are using *C. elegans* as a model system. *C. elegans* possesses two genes encoding CNTs and six encoding putative ENTs.

As the first step towards understanding the rationale underlying this diversity of transporters, we expressed one of these, ZK809.4, in *Xenopus* oocytes and showed it to be a genuine ENT which we have designated CeENT1. It exhibits a broad substrate specificity for natural purine and pyrimidine nucleosides and also transports antiviral nucleoside analogues such as 3'-azido-3'-deoxythymidine (AZT). cDNAs for two other ENT genes, K09A9.3 and K02E11.1 (which we have designated CeENT2 and CeENT3 respectively) have been obtained from Yuji Kohara and have also been expressed in *Xenopus* oocytes. We have demonstrated that these are able to transport nucleosides, with adenosine being a good substrate for CeENT2 and adenosine and uridine being substrates for CeENT3. We have generated a further two cDNAs for the ENT genes F16H11.3 and C47A4.2 (designated CeENT4 and CeENT5 respectively). CeENT5 has been expressed in *Xenopus* oocytes and is able to transport a variety of substrates. We have also demonstrated that transport of uridine is affected by the inhibitor dipyridamole, but not by draflazine or dilazep.

We have used Green Fluorescent Protein reporter constructs to investigate the temporal-spatial expression patterns of NT genes. GFP expression of these reporters is observed principally in gut cells, although expression of some constructs was seen in other cells, such as the pharynx and vulval muscle cells. Additional clues to the biological roles of NTs have been obtained by double-stranded RNA interference (dsRNAi) and by obtaining deletion mutants from the *C. elegans* knockout consortium. RNAi experiments using dsRNA corresponding to CeENT1 and CeENT2 give rise to worms with an everted vulva and our experiments suggest that these two genes are co-redundant. Deletion mutants for CeENT2 and CeENT4 have been obtained from the knockout consortium and although the CeENT2 mutant appears to have no vulval phenotype, brood size is affected. Injection of CeENT2 deletion mutants with CeENT1 dsRNA also phenocopies the affect seen using CeENT1 and CeENT2 dsRNAs together. CeENT4 deletion worms show a severe phenotype, with everted vulva and vastly reduced brood sizes. These results suggest that at least 3 of the 6 ENTs are required in the worm for proper growth and development.

239. Genomic RNAi screens for genes influencing *C. elegans* diapause and longevity

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Pioneering work in *C. elegans* has shown that single-gene mutations can regulate the rate of aging. Notably, a subset of the genes that control lifespan are also involved in formation of a third stage diapause, the dauer larva. These genes define at least two endocrine pathways, insulin-like and nuclear hormone receptor signalling. Mutations that attenuate insulin-like signaling, (e.g. *daf-2*/insulin receptor mutants) promote longevity by activating the DAF-16/forkhead transcription factor. Longevity is modulated by a putative steroid hormone signalling pathway involving the cytochrome P450 gene DAF-9 and the nuclear hormone receptor DAF-12. Specifically, *daf-9* and *daf-12* mutants enhance the longevity of some *daf-2* alleles (class 2) but weakly suppress that of others (class 1).

To identify new genes that are involved in the regulation of aging and dauer formation, we initiated genomic RNAi screens using the existing chromosome I RNAi library (Fraser et al. (2000), Nature 408:325-330). Up to now, we have preliminarily found two genes whose RNAi knockdown extends lifespan strongly and several genes that affect lifespan weakly. In the screen for dauer phenotypes, we have identified several genes whose RNAi increases dauer formation in Daf-c mutant backgrounds.

To specifically address the role of known endocrine components, we also started a reverse genetic RNAi screen involving homologs of genes that act in mammalian steroid and peptide hormone pathways. None of the tested genes gave a dauer or aging phenotype so far. However, we found that the cytochrome P450 *cyp23a1* is essential for development and maintenance of gonadal structure and function. It is also indispensable for proper embryonic and larval development.

240. Environmental and genetic inputs regulate DAF-9 cytochrome P450 expression

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C. elegans larval development is dramatically controlled by the environment. In particular sensory cues, like food, temperature, and pheromone regulate the choice between reproductive growth and third stage dauer diapause. Sensory information is integrated by the nervous system and relayed to the soma by TGF-beta and insulin-like endocrine factors. A nuclear hormone receptor signaling pathway acts downstream or parallel to these endocrine inputs. Genetic and molecular data suggest that DAF-9/cytochrome P450 likely metabolizes a lipophilic hormone for the DAF-12 nuclear hormone receptor. *daf-9* mutants constitutively form dauer larvae, are sterile if they reach adult, and are modestly long-lived, suggesting that DAF-9(+) promotes reproductive development, fertility and short life. An important question is whether these deduced functions reflect endogenous regulation of the *daf-9* gene itself. To this end, we generated a rescuing *daf-9::GFP* fusion construct to examine its expression pattern and regulation.

Under conditions that promote reproductive growth, *daf-9::gfp* is expressed in IL1/URA neurons, the hypodermis, and the spermatheca, identifying potential endocrine tissues. Hypodermal expression first appears by mid -L2, a time point critical for the dauer decision. Ablation of the IL1/URA neurons causes upregulation of hypodermal *daf-9* and a bypass of diapause, revealing hormonal communication between these two tissues. Interestingly, we found that hypodermal expression is also contingent upon environmental cues. Under reproductive growth conditions, hypodermal expression is low. Weak dauer inducing conditions result in an upregulation, whereas strong dauer inducing conditions shut off hypodermal expression altogether. Similar trends are seen with conditional Daf-c mutants of *daf-7*/TGF-beta or *daf-2*/insulin-like receptor. Surprisingly, hypodermal *daf-9* regulation is strictly *daf-12(+)* dependent, suggesting that *daf-9* is feedback regulated by *daf-12.* By generating various promoter constructs we narrowed the regulatory region for hypodermal *daf-9* expression to 500 bp. We suggest that the sensitivity of hypodermal *daf-9* expression to environmental cues and genetic inputs reflects a homeostatic mechanism to mediate the all-or-none choice between reproductive growth and diapause.

241. A positive role for *lin-1* in *C. elegans* vulval development?

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Cells in the vulval equivalence group in the *C. elegans* hermaphrodite can adopt one of three different fates, 1°, 2° or 3°. The fates of these 6 cells, termed collectively the VPCs, are thought to be influenced by at least three different signalling events. A cell in the somatic gonad, the anchor cell induces P6.p to adopt the 1° cell fate. Lateral signalling between adjacent VPCs results in P5.p and P7.p adopting the 2° fate. The remaining cells, P3.p, P4.p and P8.p, adopt the 3° fate, it is thought because they do not receive sufficiently high levels of either the inductive or lateral signals to overcome an inhibitory signal from the neighbouring syncytial hypodermal cell hyp7.

Signalling between the anchor cell and P6.p is mediated by a signal transduction pathway involving *let-60* Ras and *mpk-1* MAP kinase. Previous work has shown that one likely target of this pathway is the ETS domain transcription factor LIN-1. Loss-of-function mutations in *lin-1* cause the Multivulva phenotype: P3.p, P4.p and P8.p are induced to adopt a vulval (1° or 2°) fate. Since the Multivulva phenotype caused by *lin-1*(lf) is epistatic to the Vulvaless phenotype caused by loss-of-function mutations in *mpk-1* MAP kinase, and since MPK-1 can directly phosphorylate LIN-1 *in vitro*, the current model is that the Ras/MAP kinase pathway mediates the induction of the 1° fate in part by phosphorylating and inactivating LIN-1, a negative regulator of this fate.

One molecular marker for the 1° fate is an FGF-family ligand EGL-17. GFP under the control of *egl-17* promoter sequences is expressed in P6.p and this expression is dependent upon *let-60* Ras. Activation of *let-60* in other VPCs causes ectopic expression of the reporter. Since *let-60* Ras appears to function in part by inactivating LIN-1, if *lin-1* regulated *egl-17*, then a reasonable expectation is that inactivation of *lin-1* might, like activation of *let-60*, cause increased expression of the EGL-17::GFP reporter. We have found, however, that *lin-1* null mutations have the converse effect: they abolish *egl-17::gfp* expression in P6.p. Thus *lin-1* is required positively for the expression of at least one marker for the 1° fate.

We reasoned that one possible explanation for this observation might be that loss of *lin-1* function might cause constitutive activation of the lateral signalling pathway which promotes the 2° fate. Consistent with a role for lateral signalling in negatively regulating *egl-17::gfp* expression in P6.p, expression of the marker was consistently higher than in wild type in worms homozygous for *n676n930*, a hypomorphic mutation in *lin-12*, which encodes the receptor for this pathway. However, the marker was not expressed in *lin-12(n676n930); lin-1(0)* double mutant hermaphrodites. *lin-1(0)* also suppressed the ectopic expression of *egl-17::gfp* caused by constitutive activation of *let-60* Ras. Together, these observations suggest that the role for LIN-1 in the induction of vulval fates might be more complicated than previously thought.

By comparing the sequence of the *C. elegans egl-17* promoter fragment with the analogous sequence from *C. briggsae*, we have identified 3 elements that show strong sequence conservation between the two species. We are presently assaying these and other fragments for their ability to direct expression of *gfp* in P6.p, and for their ability to be bound and regulated by LIN-1. We are also examining the effect of *lin-1* mutations on other markers for the 1° fate such as downregulation of LIN-12::GFP, and downregulation of *lip-1::gfp*.

242. The *Caenorhabditis elegans* homologue of human angiotensin converting enzyme: evidence for a non-peptidase role in larval development

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Angiotensin I-converting enzyme (ACE, EC 3.4.15.1, dipeptidyl dipeptidase A) is a zinc metallopeptidase that belongs to the M2 family of peptidases and cleaves dipeptides from the C-terminus of oligopeptides. It is best known for its pivotal role in blood homeostasis, converting angiotensin I to the vasoconstrictor, angiotensin II, and inactivating the vasodilator, bradykinin[1]. Targeted disruption of the ACE gene in mice revealed the involvement of ACE in several physiological and developmental processes in addition to blood homeostasis[2]. ACE has also been found in several invertebrates, including *Drosophila melanogaster*, where the enzyme is essential for hatching of the first instar larva[3,4]. Since invertebrates do not possess peptides structurally related to angiotensin I and bradykinin, understanding the function of invertebrate ACE is likely to reveal novel roles for this peptidase in biological processes.

A BLAST search of ACeDB revealed only one gene (*C42D8.5, ACE-related*) encoding a protein that was structurally related to human ACE and could be assigned on the basis of primary protein structure to the M2 family of peptidases. The conceptual ACE-related protein is 907 amino acids long and has a Mr of 101,087. The residues conserved between ACE-related and human ACE extend along a central 600 amino acid region and include all the cysteine residues that are presumably required for maintaining protein structure. Surprisingly, the crucial active site residues required for zinc co-ordination and catalysis are substituted with unrelated residues. It is therefore predicted that *C. elegans* ACE-related is not a functional peptidase.

Using the promoter region of *C42D8.5* translationally fused to GFP, we obtained a spatio/temporal expression pattern for *C. elegans ACE-related* . GFP fluoresence was restricted to the seam cells of the epidermis of larval stages of nematodes. Injection of *ACE-related* dsRNA into adult hermaphrodites resulted in 86% of progeny arrested as larvae. Arrested larvae appeared to be trapped within the L1 cuticle, having failed to ecdyse in the normal fashion. These data, taken together, suggest that the *C. elegans* ACE-related protein has an important role in larval moulting, probably through protein interactions at the cell surface. This is the first evidence for a physiological role for a non-peptidase member of the M2 family of proteins.

1. Soubrier, F., Hubert, C., Testut, P., Nadaud, S., Alhencgelas, F. and Corvol, P. (1993) J. Hypertension. **11**, 471-476
2. Hagaman, J. R., Moyer, J. S., Bachman, E. S., Sibony, M., Magyar, P. L., Welch, J. E., Smithies, O., Krege, J. H. and Obrien, D. A. (1998) Proceedings Of the National Academy Of Sciences Of the United States Of America. **95**, 2552-2557
3. Cornell, M. J., Williams, T. A., Lamango, N. S., Coates, D., Corvol, P., Soubrier, F., Hoheisel, J., Lehrach, H. and Isaac, R. E. (1995) J. Biol. Chem. **270**, 13613-13619
4. Siviter, R.J., Isaac, R.E. and Shirras, A.D. *in preparation.*

243. The *C. elegans* cyclic AMP-dependent protein kinase (PK-A) catalytic subunit gene (*kin-1*)

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PK-A is one of the major multifunctional ser/thr protein kinases found in eukaryotic organisms. The holoenzyme is comprised of two catalytic (C) and two regulatory (R) subunits. Both R and C subunits occur in multiple isoforms encoded by distinct genes. In many organisms, the genes that encode the C subunits also show alternative splicing behaviour. This generates an even broader array of C subunit heterogeneity. The diversity of C subunit polypeptides may enable this protein kinase to selectively target substrate polypeptides for phosphorylation.

Within the *C. elegans* genome, two genes (*kin-1* and *F47F2.1*) have been identified that encode PK-A C-subunit-like polypeptides. *Kin-1* is located on chromosome 1 and undergoes alternative splicing leading to isoforms of the C-subunit polypeptide with different C-terminal sequences. More recently, we have also demonstrated [Tabish, M., Clegg, R. A., Rees, H. H. & Fisher, M. J. (1999) 'Organization and alternative splicing of the *Caenorhabditis elegans* cyclic AMP-dependent protein kinase (PK-A) catalytic subunit gene (*kin-1*)' Biochem. J. **339,** 209-216] the occurrence of N-terminal alternative splicing events that may lead to the generation of as many as six distinct N-terminal domains in combination with either of the two different C-terminal sequences.

One of the novel N-terminal sequences contains a consensus sequence for myristoylation. Myristoylation is believed to encourage either protein: protein and/or protein: membrane interactions of the C-subunit, therefore, targeting its location and possibly, along with other factors, its substrate interaction. Here we describe the occurrence of myristoylated variants of the C-subunit in *C. elegans* and present evidence that they arise as a consequence of the predicted alternative splicing activity of the *kin-1* gene.

244. Computer prediction of cis-acting elements from co-regulated genes.

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The identification and analysis of cis-acting regulatory regions is an important approach with which to understand genome regulation. We are currently focusing upon the identification of potential transcription factor binding sites by seeking motifs which are common to the promoter sequences of putatively, co-regulated genes.

Analysis of gene expression using reporter gene fusions [http://129.11.204.86:591] has identified groups of genes which are expressed in the excretory cell. The sequences of the DNA fragments able to drive this expression have been analyzed using MEME [http://meme.sdsc.edu/meme/website/] and SPEXS software [http://ep.ebi.ac.uk/EP/SPEXS]. Output from the latter program was simpler but as vast number of motifs were identified, it was difficult to determine the significance of each motif in terms of regulating gene expression. In addition, SPEXS does not allow for degeneracy and the forward and reverse complements of a motif are considered to be two separate motifs rather than one. Furthermore, if SPEXS identifies two motifs, where the larger is identical to the smaller apart from the addition of an extra base pair, the score for the smaller motif does not take into account its appearance within the larger.

To overcome some of these deficiencies we have combined the reverse complements of each motif reducing the number of motifs requiring further analysis by a factor of two. In addition, we have devised a scoring strategy that incorporates different weightings for various factors. This has allowed motifs that occur frequently within 1 kb upstream from the translational start, and with high DNA sequence complexity, to be given the greater score as these are the most likely candidates for cis-acting regulatory elements.

For motifs that are identical other than in length, we add an appropriate proportion of the score for the larger motif to the score of the shorter motif. This therefore allows for degeneracy at the end position of the motif. Furthermore, scores for motifs that differ by a single base pair at an internal position but are otherwise identical are mutually increased by an appropriate factor. This strategy has allowed motifs, which fulfil the desired characteristics and are the most likely candidates for cis-acting elements to have the greater scores. Further work is ongoing to allow for degeneracy at 2 or more positions within the motif.

245. Cathepsin L cysteine protease is essential for embryogenesis in *C. elegans* and parasitic nematodes.

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C. elegans cathepsin L cysteine protease CPL-1 (encoded by cosmid T03E6.7) is essential for embryogenesis. Adult worms which are injected with *cpl-1* dsRNA or are homozygous mutant produce 100% dead embryos. The embryos arrest as a mass of cells and fail to undergo morphogenesis. This phenotype is strictly maternal. We are currently examing the precise role of cathepsin L during embryogenesis. Homozygous mutant larvae show no obvious developmental defects, although we have demonstrated that the *cpl-1* gene is expressed throughout development, with increased levels approx. four hours prior to each moult, suggesting a role in moulting (1). The absence of any larval phenotype suggests that this role is non-essential or redundant.

We have also identified highly similar cathepsin L genes in several species of animal and plant parasitic nematodes. The high level of sequence identity (80-90%) suggests that these are homologous enzymes. Importantly, we were able to rescue the *C. elegans cpl-1* RNAi and genetic mutant phenotype by injection of one of these parasite *cpl-1* genes. This demomstrates that the parasite enzymes are orthologues of the *C. elegans* cathepsin L. The essential role that this enzyme plays in nematode development identifies it as a potentially important target for controlling development and transmission of parasitic nematodes of animals and plants.

(1) Hashmi S, Britton C, Liu J, Guilliano DB, Oksov Y & Lustigman S. J. Biol. Chem. (2002) 277:3477-86

246. The role of the DPY-7 cuticular collagen in the exoskeleton of *Caenorhabditis elegans*.

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The *C.elegans* cuticle (exoskeleton) is a multi-layered extracellular matrix consisting predominantly of small collagen-like proteins that are extensively cross-linked. The cuticle is synthesised by the underlying hypodermis 5 times throughout the lifecycle of the animal. The collagen genes that contribute to the cuticle are under strict spatial and temporal regulation. Previous investigations utilising a monoclonal antibody specific for the cuticular collagen DPY-7 have indicated that this protein has an important structural role within the complex architecture of the exoskeleton. Using immunofluorescence we have shown that this collagen locates to circumferential stripes within the cuticle coincident with the annular furrow. An analysis of the *dpy-7 null* mutant by immunofluorescent and scanning electron microscopy has shown that there is no DPY-7 incorporated into the cuticle and that there is a concomitant lack of annulae on the surface of these animals.

We have investigated the localisation of the DPY-7 collagen in a variety of other cuticle collagen gene mutant backgrounds. We have identified a set of collagen genes that, when mutant, affect the localisation of the DPY-7 collagen. We have shown that a group of collagen genes expressed at the same time as *dpy-7* are required for the assembly of the DPY-7 stripes, and formation of annulae. We have also shown that collagen genes expressed later than *dpy-7* in cuticle synthesis are not required for DPY-7 assembly. Thus we conclude that a set of early expressed cuticle collagen genes are specifically involved in the synthesis of a cuticular substructure required for the presence of annulae on the cuticle surface, and that the collagens encoded by this subset of cuticular collagen genes are obligate partners.

247. Genome organization of *Microbacterium nematophilum* and possible determinants and mechanisms involved in pathogenicity for *C. elegans*

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M. nematophilum is a novel pathogen of *C. elegans* recently described by J. Hodgkin et al. (1). The bacterium is able to attach to the post-anal region of *C. elegans* and to induce massive swelling of the underlying tissues by an unknown mechanism. The disease causes constipation and slows growth of affected worms. *M. nematophilum* belongs to the Gram-positive coryneform group of bacteria and is poorly characterised.

We have determined the structure and size of the *M. nematopilum* genome. PFGE revealed that *M. nematophilum* possesses a circular chromosome of 2.85 Mb and a plasmid named pMN1 (60 kb). Restriction digests indicate that GC content is very high.

In order to search for possible virulence genes located on the plasmid we are sequencing a pMN1 library constructed in pUC18. Sequencing of more than 30 kb of pNM1 has identified a few candidates such as genes encoding superoxide dismutase, secretory thermolysin-like protease and ATP-dependent clp-protease. These enzymes are known to be important for virulence in certain other pathogenic bacteria. Investigation of activity of these genes and their possible roles in *M. nematophilum* pathogenesis is in progress.

Non-virulent strains of *M. nematophilum* have been obtained. Loss of pathogenicity is strongly correlated with decrease or loss of the yellow pigmentation of *M. nematophilum*. Restriction patterns of plasmids isolated from non-virulent strains are significantly different from those obtained for the original plasmid pMN1. These data suggest that pMN1 plasmid can undergo rearrangement or/and modification which may possibly be involved in changing the pathogenicity of *M. nematophilum*. These changes appear to be different from the known changes in methylation state of DNA between virulent and non- virulent variants shown for *Listeria monocytogenes* (2).

A phage infecting *M. nematophilum*, which is able to lyse its cells, has been discovered. Characterisation of this phage may provide a useful tool for developing other genetic approaches for this bacterium.

1. Hodgkin, J., Kuwabara, P. E., Corneliussen, B. *Current Biology* (2000) 10, 1615-1618. A novel bacterial pathogen, *Microbacterium nematophilum*, induces morphological change in the nematode *C. elegans.*
2. Wei, Z., Kathariou , S. *Applied and Enviromental Microbiology* (1997) 63 (8), 3085-3089. Host-Mediated Modification of Sau3AI Restriction in *Listeria monocytogenes*: Prevalence in Epidemic-Associated Strains.

248. A GFP-fusion to the cuticular collagen, COL-19, its expression pattern, and its efficacy in the visualisation of ECM disruption.

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The impermeable *C. elegans* cuticle, an extracellular matrix (ECM) that surrounds the whole worm, is composed of an extensively cross-linked network of trimeric small collagen-like proteins whose great tensile strength and elasticity facilitate the ECM's role in morphogenesis, motility and providing a protective barrier between the animal and the outside environment. From the complete *C. elegans* sequence, more than 150 of these collagen genes have been identified each of which possess specific temporal and spatial patterns of expression.

The integral role of collagens in the ECM make them a useful marker for the study of ECM defects. Consequently, a GFP-collagen fusion has been constructed using the adult-specific, hypodermally-synthesised collagen, COL-19. This COL-19-GFP translational fusion was integrated to form the strain TP12 in which the COL-19 fluoresces green under UV light enabling the pattern of its expression to be visualised. A number of crosses have been carried out between this strain and many mutant strains in order to visualise the effect of the mutations on the pattern of COL-19 expression. These studies have shown significant COL-19 disruption in some dumpy, blistered and squat mutants (mutants that are normally associated with ECM defects). A common characteristic of many of these mutants was that most severe COL-19-GFP disruption was observed in the area overlying the seam cells. Such disruption in the collagen mutant, *dpy-5*, exemplifies the hypothesis that suggests the presence of a single aberrant collagen, in this case DPY-5, is sufficient to lead to global collagen disruption in the cuticle. Seam cell-localised disruption was also evident in the strain *dpy-11*, a mutant for the enzyme thioredoxin which is believed to play a role in collagen biosynthesis. The observed COL-19-GFP disruption confirms the importance of this enzyme in the correct expression of the collagen as well as the efficacy of this construct as a marker for ECM defects.

This strain has also been used with RNA-interference to transiently suppress the expression of genes with potential roles in collagen synthesis. In this way, through identifying genes which give a disrupted COL-19 phenotype, we hope to be able to implicate them in playing a role in ECM synthesis.

Elucidation of the mechanism of cuticle formation in *C. elegans* is a major objective of our laboratory since individual components may provide targets for chemotherapy against the ECM-synthesising machinery of parasitic nematodes.

249. Characterisation of mutants with an altered E Lineage in *Caenorhabditis elegans*

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The *Caenorhabditis elegans* intestine consists of twenty cells and is exclusively derived from the E-cell lineage. These twenty cells are generated during embryogenesis. During larval development, no more cell divisions occur in E. However a nuclear division occurs in most cells during L1 making these cells binucleate. Endoreduplication of gut nuclei once during each larval stage resulting in a ploidy of 32 in adult nuclei. During a forward genetic screen to identify mutants with an altered number of gut nuclei, we identified a gain-of-function mutation in the cell cycle phosphatase, Cdc25.1, which causes hyperplasia of the intestine[i]. This deregulated proliferation is exclusive to the intestine and can be controlled by administered RNAi. In the same screen, we identified a second independent mutation, *lin-62*, which although has a similar mutant phenotype to *cdc25(gf)*, displays distinct genetic behaviour. Data will be presented in our efforts to clone this gene.

1 Clucas C., Cabello, J., Bussing, I., Schnabel, R., and Johnstone, I.L. Oncogenic potential of a *C.elegans* cdc25 gene is demonstrated by gain of function allele. *EmboJ* **21**., 665-674.

250. Genes regulating social feeding behaviour in *C. elegans*.

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Natural isolates of *C. elegans* exhibit either social or solitary feeding behaviour[1,2,3,4]. Social strains aggregate and feed together on a bacterial lawn; solitary strains show no aggregation and feed in isolation. This variation is associated with a single amino acid change in NPR-1, a seven transmembrane receptor related to mammalian neuropeptide Y receptors. Null mutations in *npr-1* transform solitary wild strains into strongly social animals.

To identify molecules required to transduce sensory signals leading to social feeding, we mutagenised social *npr-1(null)* animals and selected mutants that feed alone. From three mutagenesis screens (19200 haploid genomes screened), in both N2 and CB4856 (a snip-SNP-bearing strain) backgrounds, we isolated 150 mutants that show varying degrees of suppression of social feeding. Ninety of these mutants exhibit strong suppression of social feeding, and are being mapped and analysed further (see also abstract by Tremain and de Bono). Among these mutants are alleles representing 7 known suppressors of social behaviour (*osm-9*, *ocr-2*, *tax-4*, *tax-6*, *eat-4*, *odr-8* and *lin-32*) that function in other sensory pathways.

For two of the ‘novel’ complementation groups, one represented by 5 alleles, and a second represented by 2 alleles, the physical location of the affected genes has been refined to less than 50 kb by snip-SNP mapping. Mutants in both complementation groups show strong suppression of both aggregation and bordering behaviours, but do not show uncoordination in movement and they have no defects in olfaction, male mating, avoidance of high osmolarity, or dye-filling. Transgenic rescue experiments are in progress to define the molecular identity of these genes.

[1]Cassada R.. Burrowing, Spontaneous Mutants, etc. with Another Wildtype Strain. Worm Breeder's Gazette 9(3): 29;
[2]Davis M.W. and Avery L.. Social Behavior in *bor-1* Mutants Depends on Bacterial Smell. Worm Breeder's Gazette 11(5): 69;
[3]Hodgkin J. and Doniach T. (1997). Natural variation and copulatory plug formation in *Caenorhabditis elegans*. Genetics 146: 149-164;
[4]de Bono M. and Bargmann C.I. (1998). Natural variation in a neuropeptide Y receptor homolog modifies social behavior and food response in *C. elegans*. Cell 94: 679-689.

251. The mechanism of action of the neuropeptide AF1 in *C.elegans*

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AF1 (KNEFIRFamide) was the first neuropeptide to be isolated from a nematode [1]. It has a potent stimulatory action on the body wall muscle of *Ascaris*. Furthermore, it has a potent stimulatory action on the pharyngeal muscle of *C.elegans* [2]. A *C.elegans* gene, *flp-8*, encoding multiple copies of AF1 has been identified [3], however deletion of this gene does not result in any obvious phenotype (Kim & Li, Soc. Neurosci. Abs. 1999). To delineate the putative physiological role of this peptide in the control of locomotion and feeding, and to define the signalling pathway, we are comparing the response to AF1 in wild type and mutant strains.

We have previously shown that the pharyngeal response to AF1 is diminished in *snb-1* (*md248*), which is defective in neurotransmission, and is consistent with the report that *flp-8* is only expressed in extrapharyngeal neurones. Mutants with deficits in catecholamine transmission did not show an altered response to AF1 compared to wild-type. However, in EPG recordings, the response to 100 nM AF1 was abolished in *eat-2* mutants which are defective in cholinergic signalling from the putative pacemaker neurone MC [4]. Thus the peptide AF1 is likely to increase the activity of MC and thereby increase the frequency of pumping. It is interesting that the effect of AF1 is apparently independent of the action of 5-HT, which is considered the main determinant of increased pumping in response to food. We are now considering the question, how does AF1 stimulate MC?

This project is supported by a BBSRC GAIN award to LHD and JFB.

1. Cowden, C., Stretton, A.W. & Davis, R.E. *Neuron* **2**, 1465-1473 (1989).
2. Rogers, C.M., Franks, C.J., Walker, R.J., Burke, J.F. & Holden-Dye, L. *J.Neurobiol.* **49**, 235-244 (2001).
3. Nelson, L.S., Kim, K.Y., Memmott, R.E. & Li, C. *Mol.Brain Res.* **58**, 103-111 (1998).
4. Avery, L. *Genetics* **133**, 897-917 (1993).

252. Functional role for glutamate-gated chloride channel subunits in the pharynx of *C. elegans.*

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The molecular target of the widely used anthelmintic, ivermectin, is a glutamate-gated chloride channel. In *C. elegans*, six genes encode subunits of this ligand-gated ion channel. As ivermectin affects nematode feeding it is evident that this family of genes play a role in regulating the pharynx. In order to define the role of the individual genes we are carrying out a systematic investigation employing immunocytochemical techniques with subunit specific antibodies and combining this with functional analysis of mutant strains or worms treated with RNAi for specific subunits. Furthermore, we plan to express putative functional orthologues of the *C. elegans* genes from the parasitic nematode *Haemonchus contortus* in mutant strains of *C. elegans* to further define this gene family in nematodes.

The pharyngeal muscle of wild-type worms (N2 strain) exhibit a desensitising depolarisation to bath applied glutamate (EC_{50}= 166µM). This response is mediated by at least two of the GluCl subunits. The *avr-15* gene product GluClα2 has been shown to play a pivotal role in the wild-type pharyngeal response to glutamate, conferring both a high affinity for glutamate and rapid desensitisation (1). The GluClβ subunit has also been demonstrated to be present in pharyngeal muscle using reporter gene constructs (2). RNAi directed against GluClβ subunit mRNA resulted in a reduced affinity to glutamate in *avr-15* mutant worms (EC_{50}=696µM) and also reduced the maximal response to glutamate. RNAi for GluClβ exhibited no significant difference in the glutamate response with regards to maximal depolarisation or the percentage desensitisation of the response, but display significantly faster desensitisation kinetics, measured by the $t_{1/2}$, across a range of glutamate concentrations.

Using subunit specific antibodies, we have localised the GBR2B subunit to a number of neurones in the enteric nervous system but not the pharyngeal muscle itself. A similar staining pattern was observed using an antibody recognising both A and B splice variants of the GBR2 subunit. Consistent with this presynaptic location of the GBR2 subunit, we have observed a small but significant difference in the response to glutamate in *avr-14* compared to wild type.

Currently we are assessing the effect of over-expressing the putative *H. contortus avr-15* orthologue HG5 in the pharynx of *avr-15* mutant worms, using intracellular recording techniques. The pharyngeal muscle of *avr-15* continues to pump in the presence of up to 10mM ivermectin, and it is presumed that expression of a functional GluClα2 orthologue in these worms should rescue ivermectin sensitivity.

1) Pemberton *et al* (2001) Mol. Pharmacol. 59: 1-7
2) Laughton *et al* (1997) J. Exp. Biol. 200 (10): 1509-1514