

## ECWM Submissions

- Sperm cues and polarization of the anterior-posterior axis in *C. elegans*  
Rebecca Lyczak, Sarah Kirkegaard, Bruce Bowerman  
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- Dopamine signals through the G protein Galphao in *C. elegans*  
Daniel L. Chase, Michael R. Koelle  
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- Identifying the downstream effector(s) of GOA-1, the *C. elegans* ortholog of the major human brain G alpha protein.  
Antony M Jose, Michael R Koelle  
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- Characterization of the 12 *C. elegans* Regulator of G Protein Signaling (RGS) Proteins  
Heather A. Hess, Michael R. Koelle  
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- An N-terminal region of *C. elegans* RGS proteins EGL-10 and EAT-16 directs inhibition of Go alpha versus Gq alpha signaling  
Georgia A. Patikoglou, Michael R. Koelle  
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- sma-9 functions in TGF-beta /Sma/Mab pathway and encodes a zinc finger protein homologous to *Drosophila* Schnurri  
Jun Liang, Ling Yu, Cathy Savage-Dunn  
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- Molecular cloning of an embryonic lethal gene (jh1) on chromosome I in *C. elegans*  
Wonhae Lee, Joohong Ahnn  
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- Heparan sulfate side chain modifying enzymes have important roles in neuronal development  
Hannes Bülow, Oliver Hobert  
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- Genome-wide RNAi screen for genes that control germ cell apoptosis in the nematode *C. elegans*  
Guillaume Lettre, Ekaterini Kritikou, Martin Jaeggi, Andrea Calixto, Andrew Fraser, Ravi Kamath, Julie Ahringer, Michael O. Hengartner  
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- Calcineurin, a component of G-protein coupled phosphorylation pathways, is involved in movement, fertility, egg laying, and growth in *C. elegans*  
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  - Sexual Transformation of *Caenorhabditis* hybrids.  
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  - Functional analysis of the *him-3* gene family  
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Frédérique Gay, Dominica Calvo, Miao-Chia Lo, Julian Ceron, Rueyling Lin, Yang Shi  
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**467205. Sperm cues and polarization of the anterior-posterior axis in *C. elegans***

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The anterior-posterior (A-P) axis is established shortly after fertilization. Sperm entry into the oocyte triggers the completion of oocyte meiosis, and the position of the sperm pronuclear/centrosome complex provides a cue that polarizes the embryo. Little is known about the nature of the sperm cues used during these early events, although the sperm donated centrosome and/or its associated microtubules play a critical role in axis specification. In order to identify components of these cues, we screened for mutants with defects in sperm triggered events. In this screen we identified six alleles of a gene we call *scu-1* (sperm cue abnormal) which may encode a component of the sperm cue.

*scu-1* mutants produce embryos with defects in A-P axis specification. Wild-type embryos exhibit numerous asymmetries during the first cleavage including polarized cortical contractions, cytoplasmic flows, posterior meeting of the pronuclei, and an asymmetric division. *scu-1* embryos show defects in all these processes; localized contractions and directed flows are absent, the pronuclei meet centrally, and the first cleavage is often symmetric. *scu-1* embryos also lack the close contact between the sperm pronuclear/centrosome complex with the posterior cortex, an association implicated in the early polarization event. *scu-1* acts upstream of the PAR proteins for their proper localization to the cortex. Surprisingly, these polarity defects can be rescued by mating with wild-type males, indicating that SCU-1 may enter the embryo in sperm.

In addition to a lack of polarity, *scu-1* embryos have defects in meiotic exit and centrosome behavior. The oocyte DNA completes meiosis and extrudes two polar-bodies similar to wild-type; however, the DNA remains condensed and the pronuclear envelopes form only after a long delay. During this time, the sperm and oocyte DNA fail to retain contact with the cortex, resulting in aberrant pronuclear positioning. Similarly, the centrosomes do not stay in the posterior end of the embryo, moving quickly away from the posterior cortex. In some embryos, the centrosome nucleation of microtubules is uncoupled from the cell cycle stage of the DNA. Analysis of the *scu-1* mutant may provide insights into how the cell cycle is coupled with the establishment of polarity.

**469558. Dopamine signals through the G protein G<sub>α</sub>o in *C. elegans***

**Daniel L. Chase**, Michael R. Koelle

Yale University

Abnormal signaling by the neurotransmitter dopamine has been implicated in a variety of human disorders including schizophrenia and Parkinson's disease. In mammals dopamine binds to and activates two classes of G protein-coupled receptors (D1-like and D2-like) which couple to the G<sub>α</sub> proteins G<sub>s</sub> and G<sub>i</sub>. These G<sub>α</sub> proteins effect cellular changes at least in part through the modulation of intracellular cAMP levels. Recent evidence however, suggests that dopamine might act through additional non-cAMP-dependent signaling pathways.

Dopamine also functions as a neurotransmitter in *C. elegans*. Mutants lacking dopamine have a defect in slowing locomotion upon exposure to food<sup>1</sup>. The locomotion of *C. elegans* exposed to exogenous dopamine is inhibited in a dose-dependent manner, with higher concentrations of dopamine causing paralysis of the animal<sup>2</sup>. We have carried out genetic screens for mutants that are resistant to the paralytic effects of exogenous dopamine to identify molecules and signaling pathways required for dopamine signaling in *C. elegans*. Our screens show that mutants lacking the G<sub>α</sub>o protein GOA-1 or its putative downstream signaling component DGK-1 fail to respond to exogenous dopamine treatment. G<sub>α</sub>o signaling in *C. elegans* is antagonized by G<sub>α</sub>q (EGL-30) signaling. We found that mutants defective in EAT-16 or GPB-2, members of the RGS protein complex that inhibits G<sub>α</sub>q, also fail to respond to dopamine. Thus hyper-activating G<sub>α</sub>q signaling or blocking G<sub>α</sub>o signaling can produce dopamine-resistant animals. In addition to identifying conserved G<sub>α</sub>o and G<sub>α</sub>q signaling components we have isolated mutations in at least two new genes required for dopamine signaling in *C. elegans*.

These results suggest that in *C. elegans* G<sub>α</sub>o signaling pathways are required for dopamine signaling *in vivo*. Whether dopamine signals through G<sub>α</sub>o-mediated signaling pathways in mammals awaits further study.

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**636789. Identifying the downstream effector(s) of GOA-1, the *C. elegans* ortholog of the major human brain G alpha protein.**

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The most abundant G protein of the human brain,  $G_{\alpha o}$ , signals through an unknown mechanism. GOA-1, the *C. elegans* ortholog of the human  $G_{\alpha o}$ , is highly similar (~80% identical) to its mammalian counterpart, and is expressed throughout the *C. elegans* nervous system. GOA-1 signaling modulates a variety of behaviors in *C. elegans*, including locomotion and egg laying. It is expected that mutations in the downstream components of the *goa-1* signaling pathway will result in defects in these behaviors similar to those seen in *goa-1* mutants. Genetic screens designed to identify such mutations have been carried out in our laboratory. These have identified alleles of some known genes (e.g. *vs24*, an allele of *dgk-1*, encoding a diacylglycerol kinase) as well as alleles of novel genes (e.g. *vs39*) (Amy Bany, pers. comm.). *dgk-1* is the predominant gene identified in such screens. Further, genetic screens for mutations that suppress the defects of constitutively active GOA-1 result in many alleles of *dgk-1* (1). Epistasis analysis suggests that DGK-1 acts downstream of or parallel to GOA-1 (2). DGK-1 is 38% identical to DGK $\theta$ , an enzyme expressed in the human brain (2). DGK-1, like GOA-1, is expressed throughout the *C. elegans* nervous system (2). Guided by these observations, we decided to test the possibility that DGK-1 is the direct downstream effector of GOA-1.

To this end, the GOA-1 and DGK-1 proteins were expressed recombinantly and purified. An *in vitro* assay for the activity of DGK-1 was developed. Purified DGK-1 shows diacylglycerol kinase activity, but biochemical analysis of this protein is complicated by its tendency to aggregate. We are refining purification strategies to obtain aggregation-free protein.

As a complementary approach, we plan to use immunoprecipitation to test for direct interaction between GOA-1 and DGK-1 in worm extracts. We have generated antibodies against DGK-1. Preliminary western blotting experiments indicate that a significant amount of DGK-1 is soluble. While this is in agreement with the fact that the mammalian homologue, DGK $\theta$ , was purified from rat brains as a soluble protein (3), it is surprising since the enzyme acts on a substrate, diacylglycerol, that is present in the cell membrane. It is possible that GOA-1 recruits the soluble DGK-1 to the membrane. We plan to use immunofluorescence to look for changes in the localization of DGK-1 in *goa-1* mutants. We would also like to detect any changes in DGK-1 activity in the *goa-1* mutant. Hence, we are currently fine-tuning an assay that detects DGK activity in worm extracts.

The defects observed in *goa-1* mutants appear to be more severe than those seen in the *dgk-1* mutants (2), raising the possibility that GOA-1 has more than one effector in *C. elegans*. Hence, we are also mapping and molecularly cloning the gene corresponding to the lesion *vs39*, identified in our lab in a screen for mutants that phenocopy *goa-1* mutants.

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**850787. Characterization of the 12 *C. elegans* Regulator of G Protein Signaling (RGS) Proteins**

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Heterotrimeric G proteins mediate signaling through G protein-coupled receptors and are composed of three subunits (alpha, beta, gamma). To elicit the appropriate cellular response, the duration of signaling by the G protein must be tightly regulated. The Regulator of G protein Signaling (RGS) proteins stimulate the slow intrinsic GTPase activity of the G alpha, thus rapidly attenuating signaling.

Exactly 12 RGS proteins are found in *C. elegans* and 24 in humans. Approximately 20 G alpha proteins are found in both worms and mammals. One might expect each RGS protein to regulate a specific G alpha protein. However, many mammalian RGS proteins act on the same G proteins in vitro. This apparent lack of specificity in vitro may be addressed by analyzing the function of these proteins in vivo. For example, spatial and temporal expression patterns may determine the function of the RGS proteins.

We are using reverse genetics to identify the functions and specificities of the RGS proteins in vivo. We have generated RGS knockout mutants by screening a ~1 million genome knockout library made in our lab and we now have mutants for ten RGS genes. Our previous analysis showed that the EAT-16 RGS protein controls behavior by inhibiting G alpha q, while the EGL-10, RGS-1, and RGS-2 proteins all regulate behavior by inhibiting G alpha o. Our new knockout strains for five RGS genes are viable and fertile and show no gross defects. This suggests that these RGS mutants may either have subtle defects or the RGS proteins function redundantly. Analysis of expression patterns for all the RGS genes shows extensive overlap, supporting the idea of redundancy. We are currently constructing double mutants between RGS genes that are the most similar in sequence and that overlap in expression in an attempt to uncover redundant functions.

One RGS gene, *rgs-7*, is expressed in the germline and in early embryos, and the *rgs-7* knockout strain shows maternal effect embryonic lethality. The G alpha o and GPA-16 G proteins are required for embryonic viability since they control centrosome function during early mitotic cell divisions. We are testing the idea that RGS-7 controls centrosome function by regulating these G proteins in early embryos.

**726368. An N-terminal region of *C. elegans* RGS proteins EGL-10 and EAT-16 directs inhibition of Go alpha versus Gq alpha signaling**

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

Molecular analysis of the EGL-10 N-terminal and GGL-RGS fragments showed that the *in vivo* protein levels of these fragments double when they are coexpressed, suggesting these proteins mutually stabilize each other, perhaps by forming a complex. The GGL-RGS fragment was found to be 50% insoluble whether in the presence or absence of the N-terminal fragment. While the N-terminal fragment was almost completely insoluble and could not be solubilized by many nonionic detergents, it did exhibit differential detergent solubility depending on the presence or absence of the GGL-RGS fragment. Using sucrose density centrifugation, we found a significant portion of the EGL-10 N-terminal fragment may be localized to lipid rafts, which are detergent insoluble membrane domains in which G proteins are known to cluster. This result raises the exciting possibility that EGL-10 may specifically inhibit G protein signaling through its selective localization to these specialized membrane domains.

Finally, using EGL-10/EAT-16 chimeras we found that the N-terminal or GGL-RGS domain of either protein is capable of inhibiting either GOA-1 or EGL-30, and that G protein specificity was highly dependant on the linker between the two domains. These data suggest that key factors contributing to EGL-10/EAT-16 function may be the localization to lipid rafts via the N-terminal domain, and the manner in which the GGL-RGS region is linked to the N-terminal region.

**188566. *sma-9* functions in TGF- $\beta$  /Sma/Mab pathway and encodes a zinc finger protein homologous to *Drosophila* Schnurri**

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*sma-9* functions in TGF- $\beta$  / Sma/Mab pathway and encodes a zinc finger protein homologous to *Drosophila* Schnurri

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The Sma/Mab pathway, a TGF- $\beta$  related pathway, regulates body length and male tail pattern in *C. elegans*. Mutants of *dbl-1* ligand, *daf-4* type II receptor, *sma-6* type I receptor, and *sma-2*, *sma-3*, *sma-4* Smads display similar small body length and abnormal male tail sensory ray fusion. Although the major components were identified, we expected the existence of additional cofactors that are critical for the signaling. In a genetic screen in R.W Padgett's lab, one of the new genes identified was *sma-9*. *sma-9* mutants show similar body length and abnormal male tail as the other identified *sma* mutants.

We cloned *sma-9* by transformation rescue and sequencing of mutant alleles. *sma-9* encodes a zinc finger protein homologous to *Drosophila* Schnurri. Schnurri is required for DPP pathway and acts as a transcriptional cofactor of *Drosophila* Smads protein MAD. The SMA-9 N-terminal region has a glutamine rich sequence that may have transcriptional activity; the C-terminal region includes seven zinc finger motif organized into three pairs. Four nonsense alleles have been identified. cDNA clones and clones from RT-PCR display an excellent alternative splicing of *sma-9*. Some forms contain an SL1 spliced leader, but others do not. Both the N-terminal and the C-terminal regions have cDNA variant forms. It is possible that different SMA-9 isoforms have different functions. Finally, We determined the *sma-9* expression pattern using transcriptional reporters. The *sma-9* expression pattern shows an overlap with *sma-2*, *sma-3*, *sma-6* and *daf-4*: the pharynx, intestine, seam cell, and vulva, from L1 larvae to adults, supporting its role as a downstream component of the Sma/Mab pathway.

**809710. Molecular cloning of an embryonic lethal gene (jh1) on chromosome I in *C. elegans***

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Six lethal mutations near the *unc-29* locus on chromosome I of *Caenorhabditis elegans* have been isolated previously. Among these mutants, one particular mutant (*jh1*) showed embryonic lethal phenotype that shows specific defects in posterior body morphology during early development. Following the outcrosses, three-factor crosses were conducted to place the *jh1* mutation on a precise genetic map near the *unc-29* locus on chromosome I using *dpy-5(e61)*, *dpy-5(e61) unc-13(e-1091)* as genetic markers. The results of three factor crosses have placed the *jh1* mutation in the region between 0.42 and 0.64 map unit right from the center of chromosome I, which corresponds to approximately a 300 Kb region in the physical map. Along with the results of three-factor crosses, cosmid rescue experiments were performed by microinjection of 8 cosmids, which are located within the genetically mapped position. Among 8 cosmids, only the cosmid B0414 decreased the embryonic lethality in the subsequent generations, suggesting that genes in the cosmid B0414 could rescue the mutation. We are currently injecting subclones of this cosmid the gene that rescues the lethal mutations.

**908260. Heparan sulfate side chain modifying enzymes have important roles in neuronal development**

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Development and correct patterning of the nervous system requires complex interactions between neurons and epidermal cells. This is achieved by the concerted action and integration of many different signaling pathways. Recently, heparan sulfate proteoglycans (HSPG) have been shown to be important modulators in several of these pathways. While HSPG's are divergent in their proteinaceous backbone, all forms contain heparan sulfate side chains (hence the name) that show complex patterns of secondary modification of the saccharide entities, mostly including sulfations and epimerizations. The general importance of sulfations for nervous system development has been suggested by in vitro experiments, however in vivo data is completely lacking. In a suppressor screen of a gain of function phenotype that is induced by overexpression of a heparan sulfate-binding axon guidance/branching cue, we have isolated mutants in two enzymes that catalyze secondary modifications in heparan sulfate side chains, namely the heparan 6O-sulfotransferase and the glucuronic acid C5 epimerase. We show that both mutants have overlapping yet distinct defects in various aspects of their neuroanatomy. We describe the expression patterns of these enzymes and provide genetic evidence that documents how specific modifications can affect specific signaling pathways. Our analysis represents the first in vivo analysis of this class of enzymes.

**41516. Genome-wide RNAi screen for genes that control germ cell apoptosis in the nematode *C. elegans***

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Apoptosis can be defined as a physiological mechanism that removes unneeded, harmful, or damaged cells in a regulated manner. It has important developmental functions, and mis-regulation of apoptosis has been associated with various pathologies (e.g. cancer, strokes, neurodegenerative disorders, etc.). Apoptosis is part of the normal developmental program of the nematode *C. elegans*: out of the 1090 somatic cells that are born in a hermaphrodite, exactly 131 will die by apoptosis. Several genetic screens have identified genes required for somatic cell death in the worm. Apoptosis also occurs stochastically in the *C. elegans* germ line. Although the core apoptotic machinery is the same for physiological germ cell death than for somatic cell death, genetic studies have shown that the regulators are different. Indeed, the BH3 domain protein EGL-1 is essential for cell death in the soma, but is not required for germ line apoptosis.

In order to identify genes that regulate physiological germ cell death in *C. elegans*, we undertook a genome-wide RNAi screen. We have completed our primary screen of the *C. elegans* genome (~16,700 genes), and have found several dozen genes that cause more germ cell deaths when inactivated by RNAi. We expect that sequence analysis of these candidates and epistatic analyses will allow us to categorize them into functional groups, with the ultimate goal of better understanding the different pathways that might regulate physiological germ cell death.

### **308859. IDENTIFICATION OF LOCALIZATION DOMAINS OF UNC-43**

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Glutamate is the most abundant excitatory neurotransmitter in the brain, and glutamatergic synapses play a critical role in learning, memory, and developmental plasticity of the central nervous system. It is critical to understand how glutamatergic synapses are formed and regulated in the CNS in order to develop novel applications for the diagnosis, treatment, and prevention of diseases of the CNS.

Rongo and Kaplan established a potent model system in the nematode *C. elegans* for studying synapse formation by focusing on the glutamatergic synapses between a sensory neuron and its interneuron target. An important regulator of GLR-1 synapses is the UNC-43 Ca<sup>2+</sup> and calmodulin dependent kinase (CaMKII). UNC-43 regulates the transport of GLR-1 from neuron cell bodies to synapses: nematode neurons that lack UNC-43 fail to transport GLR-1 to synapses and accumulate GLR-1 in their cell bodies. UNC-43 is localized to synapses, and this localization is required for its function. We previously demonstrated that UNC-43 is localized to synapses. To characterize UNC-43 localization further, we have taken advantage of known mutations from several biochemical studies of CaMKII- these mutations have not been previously used to study CaMKII synaptic localization. Using the mammalian studies as a guide, we introduced the corresponding amino acid substitutions into a GFP-tagged UNC-43 transgene. We have introduced the transgenes into *unc-43* null mutants and examined the effect of the mutations on UNC-43::GFP localization. Our goal is to narrow down a region of the UNC-43 protein that targets it to synapses. The CaMKII enzyme contains two domains capable of binding to substrate: site A, which contains the catalytic domains, and site B, which is noncatalytic. There is an autoinhibitory domain that keeps the enzyme quiescent in the absence of Ca<sup>2+</sup>. This domain is made up of a pseudosubstrate loop, which binds to the catalytic domain, and helix alpha-R1, which binds to site B and contains the T286 regulatory residue. Upon Ca<sup>2+</sup> influx, CaM binds and displaces the pseudosubstrate domain, allowing the catalytic domain to phosphorylate T286. Phosphorylation of T286 displaces alpha-R1 from both sites A and B, freeing the kinase to now phosphorylate substrates. Any of these domains could bind to trans-acting factors that localize the kinase, and several amino acid substitutions have been shown to impair the ability of these domains to bind their substrates. We have identified several intramolecular interactions that facilitate UNC-43/CaMKII translocation to and from the synapse. Our results suggest that it is not the relative level of Ca<sup>2+</sup> independence of UNC-43 per se that results in synaptic delocalization. Rather, we believe that we have disrupted key residues that interact with the proteins that regulate the synaptic localization of UNC-43.

**672144. Functional analysis of AMPA-type glutamate receptor cytosolic tail sequences and their contribution to receptor localization at synapses**

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The modulation of AMPA-type glutamate receptor localization to central nervous system synapses is an important component of synaptic plasticity, and can be triggered by LTP (Long Term Potentiation) and CaMKII (Type II calcium-calmodulin-dependent protein kinase) activity. (1, 2). In mammalian hippocampal neurons, the different AMPA receptor subunits GluR1, GluR2, GluR3, and GluR4 are proposed to form heteromultimers, and individual subunits can confer localization specificity to the multimeric channels that they comprise (2,3). One likely explanation for such subunit specificity is that the targeting of AMPA receptors to the synapses is probably due to the interaction of PDZ domain-containing proteins and the receptor subunit cytosolic tail sequences (4). In *C. elegans*, there are four AMPA receptor subunits that are expressed in the command interneurons, including, *glr-1*, *glr-2*, *glr-4* and *glr-5* (5). We are using the molecular, genetic, and cell biological approaches to examine the delivering of AMPA receptors. We hope to identify the PDZ domain-containing proteins that interact with the tail sequences of AMPA receptors in *C. elegans* in order to understand the localization and regulation of these receptors in response to plasticity at synapses.

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(5) Brockie et al. J. Neuroscience (2001), vol. 21(5), p. 1510



**881426. Genetic mapping and Characterization of Bisphenol-A Resistant Mutants in *Caenorhabditis elegans*.**

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Endocrine disrupters are exogenous substances, which can influence endocrine function in humans and other animals. Environmental exposure to these chemicals has been reported to modify sexual development and reproductive function in amphibians, crustacea, and fish. Thus, studies on the effect of endocrine disrupters are important to understand its mechanism.

Bisphenol-A(BPA) is one of the endocrine disrupters which can bind to estrogen receptor. First, we studied effect of BPA on the survival of *C. elegans*. We found that about 90% of the worms died when worms were treated with 90mM BPA plate for 3 hours. In contrast, only 1-2 % of worms died on the control plates. Then we performed EMS mutagenesis to isolate mutants which become resistant to BPA condition. We have isolated mutants showing 70% ~90% survival on BPA treated condition. Currently these candidate mutants are being mapped by using genetic markers. One of these candidate, *bpr-1(jh11)*, has been mapped to LG IV near the *dpy-20* locus. This mutant shows a weak Unc phenotype and reproduce normal number of progeny. Another candidate, *bpr-2(jh12)* shows an egg retention phenotype and shows much decreased brood size. Interestingly, we found that both mutants show moderate resistance to nonylphenol, which is another type of alkylphenol. However, these resistance to nonylphenol was not as specific as to BPA, which suggest that these two mutants may have mutations for BPA specific resistance.

**622898. GENES REQUIRED FOR AMPA-TYPE GLUTAMATE RECEPTOR LOCALIZATION IN *C. ELEGANS***

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Synaptic plasticity at excitatory synapses in the central nervous system couples the cellular events of protein phosphorylation, localization, and turnover of neurotransmitter receptors with the organismal events of learning and memory (1). For example, the mammalian AMPA-type glutamate receptor GluR1 has been shown to be targeted to post-synaptic membranes during Long Term Potentiation (LTP) by the calmodulin-dependent kinase II (CaMKII), a process that is dependent on PDZ scaffolding proteins (2). To get a better understanding of AMPA-receptor localization and synaptic plasticity, we are studying glutamate receptor localization in *C. elegans*. A functional GLR-1::GFP fusion protein has allowed visualization of GLR-1, an AMPA-type glutamate receptor subunit expressed in the *C. elegans* CNS. Many aspects of receptor targeting have been shown to be conserved, with localization dependent on LIN-10, a PDZ protein (3) and CaMKII (4). Combining this integrated construct with a forward genetic approach, we have screened for and identified mutants that mislocalize GLR-1. We are currently mapping the more interesting mutations using a combination of phenotypic and single nucleotide polymorphism markers.

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3. C. Rongo et al., *Cell* 94, 751 (1998).
4. C. Rongo and J.M. Kaplan, *Nature* 402, 195 (1999).

**842249. Vacuolar-type H<sup>+</sup>-ATPase E subunit is required for embryogenesis and yolk transfer in *C. elegans*.**

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Vacuolar H<sup>+</sup>ATPase (V-ATPase) is a multi-subunit proton pump composed of peripheral V<sub>1</sub> sector and membrane-bound V<sub>o</sub> sector. It is localized in the membrane of intracellular acidic organelles including endosomes, lysosome, golgi apparatus and clathrin coated vesicle as well as in plasma membrane. Because of its regulation in acidification, V-ATPase is required for many intracellular processes such as, receptor mediated endocytosis, zymogen activation, accumulation of neurotransmitters and protein sorting. We have characterized the E subunit of V-ATPase in *C. elegans*. This subunit is one of the well conserved subunit showing 57 % identity in amino acid sequences to human homologue, ATP6E. GFP expression and antibody staining showed that *vha-8* is abundantly expressed in large H-shaped excretory cell, which is consistent with other subunit expression patterns. RNA mediated interference (both dsRNA injection and bacterial feeding) targeted to *vha-8* resulted in embryonic lethal or larval arrest phenotypes. In addition, we also observed endomitotic oocytes and defect in receptor-mediated yolk transfer in Po animals. Interestingly, RNAi affected animals showed resistance to high osmotic environment compared to wild type. We are in the process of characterizing these phenotypes. The observed phenotypes are possibly caused by disrupted pH homeostasis due to the defective V-ATPase. Our results suggest that E subunit of V-ATPase is involved in embryogenesis and receptor mediated endocytosis.

**522872. The Golgi P-type Ca<sup>2+</sup> ATPase pump PMR1 regulates calcium homeostasis in *C.elegans***

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PMR1 is a Golgi P-type Ca<sup>2+</sup> ATPase pump located in the Golgi membrane. In human, mutations in human PMR1 homologue (ATP2C1) cause Hailey-Hailey disease, which is an autosomal dominant skin disease characterized by persistent blisters and erosions of the skin. Yeast *pmr1* supplies the secretory pathway with Ca<sup>2+</sup> and Mn<sup>2+</sup> required for glycosylation, sorting, and ER associated protein degradation. The *C.elegans* genome database revealed the presence of a predicted single *CePMR1* orthologue gene on the cosmid CC4(LGI) and two predicted isoforms by alternative splicing. These putative proteins show about 55% identity in amino acid sequence with that of human and high conservation with other species.

GFP fused to the promoter region of *CePMR1* was expressed in intestine, hypodermal cells and spermatheca (gonad in male) from early embryonic stages to the adult stage. 3' RACE experiments isolated three unique isoforms of *CePMR1*. RNAi targeted to a common region of three isoforms resulted in a lower survival rate than wild-type in calcium-depleted conditions while RNAi targeted to a unique region of the largest isoform results in a higher survival rate than the common isoform RNAi. Several transgenic truncated forms of *CePMR1* which mimic the human dominant mutant form of Hailey-Hailey disease in *C. elegans* results in low survival rates in calcium-depletion conditions. These results confirm the role of PMR1 in calcium homeostasis. We are currently performing stage-specific northern analysis and isoform-specific in situ hybridization to elucidate function of each isoforms.

**533515. Calcineurin, a component of G-protein coupled phosphorylation pathways, is involved in movement, fertility, egg laying, and growth in *C. elegans***

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Calcineurin is a  $\text{Ca}^{2+}$ -calmodulin-dependent serine/threonine protein phosphatase that has been implicated in various signaling pathways. Here we report the identification and characterization of calcineurin genes in *C. elegans* (*cna-1/tax-6* and *cnb-1*) which share high homology with *Drosophila* and mammalian calcineurin genes. *C. elegans* calcineurin binds calcium and functions as a heterodimeric protein phosphatase establishing its biochemical conservation in the nematode. GFP promoter analysis, whole-mount immunostaining and immuno-EM analysis was used to show calcineurin expression in hypodermal seam cells, body-wall muscle, vulva muscle, neuronal cells, and in sperm and the spermatheca. *cnb-1* null mutants isolated by target-selected mutagenesis showed pleiotropic defects including low fertility, lethargic movement, and delayed egg-laying. Interestingly, these characteristic defects resembled phenotypes observed in gain-of-function mutants of *unc-43*/ $\text{Ca}^{2+}$ -calmodulin-dependent protein kinase II (CaMKII) and *goa-1*/ $\text{G}_o$ -protein alpha-subunit. Mutants of these three genes display serotonin-mediated and levamisole-mediated egg laying defects suggesting a role for all three genes in vulva muscle function. Double mutants of *cnb-1* and *unc-43(gf)* displayed a synergistic severity of movement and egg-laying defects. These data suggest that calcineurin has an antagonistic role in CaMKII-regulated G-protein-coupled phosphorylation signaling pathways in *C. elegans*.

**575902. Potent and Specific Inhibition of Calcineurin by the Down Syndrome Critical Region 1 Homologue *rcn-1* in *C. elegans***

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Down Syndrome Critical Region (DSCR1) is a gene located on human chromosome 21 and has been implicated in such disease models as Alzheimer's and Down Syndrome. The protein encoded by DSCR1 (MCIP1) and its homologue in yeast were shown to bind and inhibit the protein phosphatase calcineurin. In addition, members of this protein family, termed calcipressins, were transcriptionally regulated by calcineurin, forming a conserved negative feedback inhibition loop. Recently, a study in which MCIP1 was overexpressed in cardiac hypertrophic hearts of mice showed specific inhibition of hypertrophic conditions, suggesting alternative treatment methods of cardiac hypertrophy using this endogenous inhibitor of calcineurin. In light of these observations, we sought to characterize *rcn-1*, the DSCR1 homologue in *C. elegans*. *rcn-1* is expressed throughout embryonic stages to adulthood in hypodermal cells, nerve cords and various neurons throughout the body, vulva epithelial cells, marginal cells of the pharynx, and diagonal muscle, sensory rays, and spicules of the male tail. Observations of *rcn-1* expression by GFP and RT-PCR analysis in calcineurin mutant backgrounds suggest that *rcn-1* may be upregulated by calcineurin activity. RCN-1 specifically binds to calcineurin A from *C. elegans* lysate in a calcium-dependent manner, and potently inhibits bovine calcineurin phosphatase activity dose-dependently confirming its conserved role as a calcipressin. Calcineurin promoter-driven RCN-1 overexpression in wild-type animals results in calcineurin-deficient phenotypes such as small body size, cuticle defects, fertility defects, slow growth, and serotonin-resistant egg-laying defects. In addition, a constitutively active calcineurin A mutant, *cna-1(gf)*, was isolated by target-selected mutagenesis, and showed hyperactive serotonin-mediated egg laying. Calcineurin promoter-driven RCN-1 overexpression in *cna-1(gf)* animals restored the abnormal egg-laying response to normal serotonin-mediated egg laying levels. These results confirm a potent and specific inhibition of calcineurin *in vitro* and *in vivo*.

**229184. A Genetic Screen for G Protein Signaling Components, Neurotransmitters, and Neurons that Inhibit Egg-Laying Behavior in *C. elegans*.**

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Yale University

Neurotransmitters can signal by activating heterotrimeric G proteins that act through poorly understood pathways. In *C. elegans*, homologs of the Go (GOA-1) and Gq (EGL-30) G protein alpha subunits are expressed throughout the nervous system and act in opposition to each other to regulate egg-laying behavior. We screened 39,000 mutagenized haploid genomes for mutations that confer a hyperactive egg-laying phenotype similar to that caused by loss-of-function mutations in GOA-1. Studying the genes identified by these mutations should help us gain a more complete cellular and molecular understanding of how neurotransmitters, acting through G proteins, regulate behavior.

We isolated mutants with the desired hyperactive egg-laying phenotype, including mutations in the known G protein signaling genes *eat-16* (one allele) and *dgk-1* (four alleles). Twelve additional isolates, representing at least five genes. Nine of these isolates fall into a new class of mutants that are hyperactive for egg laying but not locomotion. Two mutations have been finely mapped, and rescue experiments are in progress.

As a supplement to our screen, we surveyed 20 *Unc* mutants reported to have the hyperactive egg-laying phenotype. Only seven of these had a phenotype as strong as the mutants recovered in our screen. These seven all have defects in the VC neurons, which synapse onto the egg-laying muscles and whose role in egg-laying behavior has remained obscure. *unc-17* and *unc-4*, as described below, have signaling defects in the VCs. The five others (*unc-5*, *unc-42*, *unc-75*, *unc-76*, *unc-115*) were known to have defects in neuron guidance and fasciculation. Using a VC-specific GFP reporter in these mutant backgrounds showed that all have defects in VC neuron morphology, ranging from mild to severe. These results are consistent with the model in which the VC neurons can function to inhibit egg-laying behavior.

The transcription factor UNC-4 was identified both in our survey of the *Uncs* and in our screen. UNC-4 was previously studied for its role in determining the synaptic input of certain motor neurons involved in locomotion. However, UNC-4 is also expressed in the VC neurons. Expressing the *unc-4* cDNA in the VC neurons rescues the hyperactive egg-laying phenotype of *unc-4* mutants, suggesting that UNC-4 is necessary in the VC neurons for their proper function. It was recently reported that the VC neurons of *unc-4* mutants no longer express the proteins that synthesize (CHA-1) or package (UNC-17) the neurotransmitter acetylcholine (1). We determined that both *unc-17* and *cha-1* mutants are hyperactive for their egg-laying behavior, and expression of CHA-1 in the VC neurons can partially rescue the egg-laying defect of *cha-1* mutants. Our results suggest that acetylcholine released from the VC neurons can inhibit egg-laying behavior.

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**948044. Ray pattern and P3.p cell lineage evolution in *Caenorhabditis*.**

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Ray pattern is one of the most variable morphological characters in rhabditid nematodes. *C. elegans* and *C. remanei* exhibit a 2(1)3+3 pattern in which ray 3 is separate from other rays and located at the anus. This pattern is ancestral in the 'elegans group' of *Caenorhabditis* (Sudhaus and Kiontke, 1996). A derived 2/4+3 ray pattern in which ray 3 is posterior of the anus and frequently fused with ray 4 is diagnostic for *C. briggsae*. However, this pattern is not fixed and three *C. briggsae* strains have been identified that exhibit both the derived and ancestral patterns (Baird, 2001). Using this variation, it should be possible to identify the genes responsible for ray pattern evolution in *Caenorhabditis*. Phenotypic segregation has demonstrated that ray pattern variation in *C. briggsae* results from allelic variation at two or three major-effect genes. Analyses of mutant phenotypes in *C. elegans* suggest that ray pattern evolution may result from alterations of HOM-C/Hox gene expression patterns (Chow and Emmons, 1994). Alterations in anteroposterior positional information also may account for evolutionary changes in the P3.p cell lineage. In *C. briggsae*, P3.p cell division frequency exhibits strain-specific variation (Dellatre and Félix, 2001). This variation correlates with ray pattern variation in *C. briggsae* males. To confirm this correlation, ray pattern and P3.p cell division frequency are being tested for cosegregation in crosses between variant strains. Single nucleotide polymorphisms (SNPs) from several *C. briggsae* strains are being identified at the Washington University Genome Sequencing Center. These SNPs will be used to map the genes responsible for ray pattern and P3.p cell division frequency variation. Initial experiments will focus on candidate genes known to regulate HOM-C/Hox gene expression patterns. If no linkage to candidates is detected, recombination mapping will proceed with SNPs distributed throughout the genome.

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**697861. Identification and characterisation of novel genes involved in the engulfment of apoptotic cell corpses in *C. elegans***

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Apoptosis or programmed cell death plays an important role in the development and homeostasis of multicellular organisms. The final phase of the death programme - namely the engulfment of apoptotic cells - is an evolutionarily conserved process that prevents release of potentially toxic intracellular contents into the surrounding tissue. So far, seven genes have been identified that are required for efficient engulfment of apoptotic cells in the nematode *C. elegans*. The function of *ced-7* appears to be specific for embryonic cell death, since mutations in *ced-7* significantly perturb the engulfment of cell corpses in embryos, but not in larvae. Thus, it is likely that there are additional genes involved in the engulfment of apoptotic cell corpses, which specifically promote engulfment during larval development, and which have not been identified so far, because they might not cause an engulfment defect by their own.

To identify new genes that are involved in the removal of apoptotic cells and to further investigate the role of *ced-7*, we are performing a *ced-7* enhancer screen. So far, we have screened 7'000 haploid genomes and we have found six mutants that have an enhanced engulfment defect. These mutations are presently being mapped to chromosomes and tested for complementation with the known engulfment genes.

Since our screen is performed in a sensitised background - an approach that has not been used so far - we hope that we will identify not only alleles of known engulfment genes, but also isolate mutations in previously unidentified genes.

**101227. Effects of mutations in *C. elegans* beta integrin cytoplasmic tyrosine residues**

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Heterodimeric integrin receptors play vital roles in bi-directional signaling during tissue development, organization, remodeling, and repair. The  $\beta$  integrin subunit cytoplasmic domain is essential for transmission of many of these signals. Unlike vertebrates, which have multiple  $\beta$  subunit genes, the nematode *Caenorhabditis elegans* expresses only one  $\beta$  subunit ( $\beta$ pat-3) and a null mutation in this gene causes embryonic lethality. To determine the functions of  $\beta$ pat-3 during larval development and in adult tissues, we have established several *C. elegans* rescued lines expressing  $\beta$ PAT-3 integrin with specific cytoplasmic tail mutations. A wild type  $\beta$ pat-3 allele (*pat-3(+)*) and alleles with Tyr to Phe mutations (*pat-3(Y792F)*, *pat-3(Y804F)* and *pat-3(YYFF)*) in the cytoplasmic domain were able to rescue *pat-3* null animals. Animals rescued with mutant alleles of  $\beta$ pat-3 integrin display cell migration defects. During normal *C. elegans* development, the two arms of the hermaphrodite gonad extend to form a U-shaped structure, but in the *pat-3(Y792F)*, *pat-3(Y804F)* and *pat-3(YYFF)* mutant rescued lines, gonad morphology is abnormal. In the mutant animals, gonadal distal tip cells do not follow the normal path along the dorsal body wall resulting in mislocalized and misshapen gonad arms. Integrins are known to be involved in the assembly and stabilization of muscle cytoskeleton. Immunofluorescence staining of rescued lines with anti-PAT-3 antibodies showed that wild type and mutant  $\beta$  integrins were each distributed in a dotted pattern along the length of muscle cells, indicative of proper localization to dense bodies/focal adhesions. Rescued animals show normal movement and egg laying, indicating that muscle functions are not affected by tyrosine mutations of the  $\beta$ PAT-3 tail. Detailed phenotypic analysis of rescued animals will be used to more fully define the role of the  $\beta$ PAT-3 tail tyrosine residues in tissue-specific integrin functions.

**327465. Role of the RBCC-NHL genes in *C. elegans* temporal development**

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Temporal development of the hypodermal cells in *C. elegans* is regulated by a group of genes that comprise the heterochronic pathway. *lin-29* is the most downstream regulatory gene known in the pathway and it encodes a transcription factor that accumulates in the last larval stage to initiate the transition to adulthood. Because *lin-29* mRNA is present as early as L2, it seems that translation to protein is inhibited until the appropriate developmental time. Negative regulation of LIN-29 is achieved through *lin-41*, a gene acting upstream of *lin-29* in the heterochronic pathway. LIN-41 belongs to the RBCC (Ring finger, B-box, and coiled coil domains) family, many members of which have been implicated in human diseases. LIN-41 also contains a region of six 44 amino acid repeats referred to as the NHL domain that is believed to mediate protein-protein interactions. The heterochronic phenotype associated with *lin-41* is neither 100% expressive nor 100% penetrant, indicating that there may be other genes working in parallel to *lin-41* in the late stages of development. We have identified *nhl-2*, an RBCC-NHL family member that displays a heterochronic phenotype when knocked down by RNA interference. Its placement in the heterochronic pathway and its function are currently under study.

**388220. The *C. elegans* maternal-effect gene *clk-2* is essential for embryonic development, encodes a protein homologous to yeast Tel2p and affects telomere length.**

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The *C. elegans* maternal-effect *clk* genes are involved in the temporal control of development and behavior. We will present our detailed genetic and molecular characterization of *clk-2*. A recessive temperature-sensitive mutation in *clk-2* affects embryonic and post-embryonic development, reproduction, adult behaviors, and life span, yet virtually all phenotypes can be fully maternally rescued. While some aspects of *clk-2* function are required throughout the life cycle, including for fertility, embryonic development strictly requires the activity of maternal *clk-2* only during a narrow time window between oocyte maturation and the 2 to 4-cell stage.

Positional cloning of *clk-2* reveals that it encodes a protein homologous to *S. cerevisiae* Tel2p. In yeast, the gene TEL2 regulates telomere length and participates in gene silencing at subtelomeric regions. *clk-2* mutants have elongated telomeres, and *clk-2* overexpression leads to telomere shortening. A functional CLK-2::GFP fusion protein is cytoplasmic in worms, suggesting that CLK-2 has an indirect effect on telomeres. The phenotype of the *clk-2* mutants could be the result of altered patterns of gene expression. We are currently exploring the relationships between the various *clk-2* phenotypes and investigating whether the maternal effect is the result of epigenetic mechanisms comparable to subtelomeric silencing.

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**819134. MAU-2: a novel eukaryotic protein required for the guidance of cellular and axonal migrations along both body axes in *Caenorhabditis elegans*.**

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The gene *mau-2* (maternal effect uncoordinated) is defined by four recessive viable-maternal effect mutations (1), which lead to severe uncoordination and defective egg-laying. The locomotory defects are fully maternally rescued by the presence of a wild-type copy of the gene in the mother, while the egg-laying defect is not. Thus, it appears that *mau-2* functions early in development for the wiring of the locomotory system, as well as later during the morphogenesis of the egg-laying system. Mutations in *mau-2* result in the mispositioning of numerous migrating neurons and axons, as well as of mesodermal cells, including pioneer axons, along both body axes. Thus, *mau-2* is required for the guided migration of cells and axons during the development of the nervous system (2). We have molecularly identified the gene *mau-2* and it encodes a novel protein that displays significant similarity to predicted proteins in *Drosophila*, zebrafish, mammals, and *Arabidopsis*, but whose functions have not been characterized. From late gastrulating embryos to mid-embryogenesis, a functional MAU-2::GFP fusion is expressed ubiquitously. It then becomes predominantly expressed in the nervous system in late embryos, larvae and adults, but it is also detected in muscles and hypodermis. In all cases, the protein is found in the cytoplasm. Given that *mau-2* is expressed in cells which are affected in *mau-2* mutants, it is possible that *mau-2* acts cell-autonomously, participating in the intracellular interpretation of guidance cues.

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**954661. Nematode titins: giant polypeptides composed of multiple repeating motifs expressed in muscle**

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Vertebrate titin (also known as connectin) is a 3 X 10<sup>6</sup> Da polypeptide, single molecules of which span half a sarcomere, from Z-disc to M-line. Titin is likely to have two functions: (1) Its elasticity provides nearly all of the resting tension of muscle and restores the sarcomere to its relaxed length. (2) It acts as a molecular scaffold for assembly, especially of the thick filaments and A-bands. Until now, the closest titin homologs in *C. elegans* muscle were known to be twitchin (754,000 Da) and UNC-89 (732,000 Da), each located in the A-bands. Here, we report that there are previously unknown proteins that are even more similar to vertebrate titins, and these are encoded by a gene on chromosome V. AceDB and later, WormBase, have annotated 4-5 separate genes, namely, W06H8.3, H05O09.1, Y38B5A.A, F12F3.3 and F12F3.2. Our analysis, however, indicates that these "genes" comprise one transcriptional unit spanning 81,354 bp, encoding 3 overlapping mRNAs. The gene structure was predicted by GeneMark.hmm and then we confirmed all 67 exons by sequencing RT-PCR products, partial cDNA clones and 5' and 3' RACE products. The Ce titin gene encodes polypeptides of 2.2 MDa, 1.2 MDa and 301 kDa. The 2.2 MDa isoform resembles twitchin and UNC-89 in that it contains multiple Ig (56) and Fn3 (11) domains, and a single MLCK-like protein kinase domain. In addition, however, the 2.2 MDa isoform contains 4 classes of short, 14-51 residue, repeat motifs arranged mostly in many tandem copies. An interesting aspect of the two largest isoforms, is that in contrast to other members of the twitchin / titin family, there are multiple regions which are likely to form coiled-coil structure. One of these tandem repeat regions is similar to the PEVK regions of vertebrate and fly titins. As the PEVK region of vertebrate titins is considered to be the main elastic element of the titins, this indicates that the repeat motifs in the Ce titins may have a similar elastic function. In transgenic animals, the first ~100 residues of the largest isoforms targets to dense bodies, the worm analogs of Z-discs. Antibodies localize the two largest isoforms to the I-band, between the dense bodies. Thus, we propose that the 2.2 MDa and 1.2 MDa Ce titins are anchored at one dense body and extend to the next dense body, possibly by forming multimers. This model is consistent with previous EM observation of filaments connecting adjacent dense bodies in the muscle of the related nematode *Ascaris*. Finally, we demonstrate that the protein kinase domain of two Ce titin isoforms has protein kinase activity *in vitro*, and this activity is regulated by a novel mechanism. We have begun to characterize a 1460 bp deletion (*gk135*), which begins in intron 14 and ends in intron 16, recently isolated by the Vancouver *C. elegans* Gene Knockout Lab.

### **391192. TWO MODIFIER SCREENS FOR NEW GENES INVOLVED IN PROGRAMMED CELL DEATH**

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Most genes involved in the execution of programmed cell death (PCD) in *C. elegans* have been identified by screening for mutations that cause the survival of cells that normally die. The cloning of these genes has resulted in the identification of many of the key components that control PCD. To identify new genes that play a role in PCD, we are performing two modifier screens using a *lin-11::gfp* reporter that is expressed in Pn.aap cells. In the ventral cord of wild-type animals, six of the Pn.aap cells survive and express *lin-11*, while the six others undergo PCD. By contrast, in mutants defective for killing, such as strong loss-of-function mutations in *ced-3*, all 12 Pn.aap cells survive and express *lin-11*. The survival of Pn.aap cells can be easily monitored in strains carrying the *lin-11::gfp* reporter using a fluorescence-equipped dissecting microscope.

The first modifier screen, a *ced-4* suppression screen, is designed to identify genes that protect cells from undergoing PCD. We are screening for a reduction in the number of GFP-positive cells (*i.e.*, an increase in PCD) in the ventral cords of animals bearing a partial loss-of-function mutation in *ced-4*. To date, approximately 40,500 mutagenized genomes have been screened for zygotic defects in cell survival: 5,000 were screened clonally and 35,500 non-clonally. Two strong suppressors have been obtained. The first suppressor, *n3418*, identified in the clonal screen, confers recessive suppression and sterility. We have mapped the mutation to a small region on chromosome III. The second suppressor, *n3696*, identified in the non-clonal screen, confers dominant suppression and is an intragenic mutation in *ced-4*.

The second modifier screen, a *ced-3* enhancer screen, is designed to identify genes that play a subtle role in the execution of PCD. Again using the *lin-11::gfp* reporter, we are screening for an increase in the number of GFP-positive cells (*i.e.*, a decrease in PCD) in the ventral cords of animals bearing a partial loss-of-function mutation in *ced-3*. We have screened approximately 35,000 genomes non-clonally and isolated 212 enhancers. Of these, at least 13 are alleles of *ced-7*, 12 are alleles of *ced-3*, six are alleles of *ced-4*, and four are alleles of *ced-9*. Twenty-five of the remaining 170 isolates have obvious engulfment defects. On the basis of complementation tests and map position, we have identified at least four new cell-killing genes.

**808531. UNC-98 is a Zn finger protein, which localizes to muscle cell nuclei and focal adhesion structures, and is required for myofibril organization**

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The muscle phenotype of *Unc-98* mutant animals, when viewed by polarized light, was first described by Zengel and Epstein (1980) as having poorly organized myofibrils and birefringent "needle-like" structures at the ends of muscle cells. Consistent with these findings, examination of these animals by EM revealed shortened M-lines and broken dense bodies (Z-line analogs). The needle-like structures, originally thought to be accumulations of thin filaments by EM, stained with neither phalloidin (actin staining) nor antibodies to myosin. In addition, immunofluorescence with antibodies to alpha-actinin and vinculin revealed a less regular staining of dense bodies as compared to wild type. The original mutant allele of *unc-98*, (*su130*), was recovered as motility defective. Though not obvious by casual observation, this mutant does have slower motility when compared to w.t. ( $p < .0001$ ) in a sensitive, liquid motility assay. We have since recovered two additional alleles of *unc-98* (*sf19*, *sfDf1*), both with motility defects. We began genetic mapping using deficiencies, duplications, and 3-factor mapping. After placing *unc-98* to a narrow interval between *dpy-7* and *unc-18*, we tested for transgenic rescue of *unc-98* using cosmids in the region. We obtained rescue of the mutant muscle phenotype with cosmid F08C6 and then with an 8kb PCR fragment predicted to contain just a single gene, F08C6.7. The cDNA sequence of F08C6.7 revealed a gene of 7 exons, which translates into a protein of 310 amino acids. Double stranded RNA from the full-length cDNA was injected to produce RNAi resulting in a phenotype identical to that of the existing mutants. Sequencing of two alleles revealed that both are 3' intron splice acceptor site mutations (replacing AG with AA) that may result in aberrant splicing products but may also produce simply lower levels of the full-length protein. By western analysis we see antibody binding to an UNC-98 protein present in w.t. but clearly absent in the mutants. We can conclude that these mutants are loss of function, producing significantly lower levels of the full-length UNC-98 protein, if any at all. By genetic criteria, our worst allele *sf19* is not null; the motility phenotype worsens when placed over a deficiency.

The UNC-98 protein contains four C2H2 zinc finger domains and predicted nuclear localization and export signals (primarily at the N-terminus). Transgenic lines containing an UNC-98::GFP fusion protein (full length UNC-98 plus promoter) were created to determine UNC-98 localization. UNC-98 localizes to M-line and dense bodies, but also localizes to the nucleus, excluding the nucleolus. Immunofluorescence with antibodies to UNC-98 resulted in prominent M-line staining but undetectable levels of staining in either the dense body or nucleus. The absence of antibody staining, likely due to antigen masking or lower protein levels in these areas of the cell, was recovered by utilizing a transgenically rescued line with higher expression levels of the UNC-98 protein. To date, there has only been one other muscle specific protein found in both the myofibrils and the nucleus - UNC-97, a LIM domain protein (Hobert et al, 1999). Remarkably, UNC-98 showed strong interaction with UNC-97 when used as bait to screen a yeast 2-hybrid "bookshelf" of known dense body and M-line components. Upon further examination, we found this interaction to require the first two LIM domains of UNC-97 and all four zinc fingers from UNC-98. Curious as to how important it was to have all zinc fingers for proper localization of UNC-98, we developed four GFP constructs each missing one, two, three, or four zinc fingers. Individual constructs were injected and resulting transgenic lines examined for localization. Interestingly, in all cases, nuclear localization was maintained, dense body localization was less prominent, and previously prominent M-line localization was now diffusely localized throughout the A-band. Our working hypothesis is that UNC-98 functions both as a focal adhesion protein and a transcription factor, or acts together with transcription factors, to influence gene expression. We plan to 1) conduct microarray experiments to determine which genes might be regulated by UNC-98, 2) examine the role of the NLS and NES's, 3) further examine the UNC-98-UNC-97 interactions, and 4) obtain additional alleles of *unc-98*.



**349307. EGL-47 is a putative G protein-coupled receptor found in the synaptic termini of HSN neurons that stimulate egg-laying behavior in *C. elegans***

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Egg laying is a simple behavior in *C. elegans* that extensive genetic studies have shown to be under the control of two heterotrimeric G proteins. The neurotransmitter receptors that act through these G proteins are not known. *egl-47* was identified by two dominant mutations that block egg laying, phenocopying ablation of the HSN neurons that stimulate egg laying (1). The HSN neurons are morphologically normal in *egl-47* mutants, suggesting the function, not the development of these neurons has been blocked. While the G proteins that control egg laying also affect many other behaviors, *egl-47* appears to only affect egg laying.

We mapped *egl-47* to a 97 kb interval and found that a PCR product from within this interval generated from *egl-47 (gf)* was able to block egg laying when transformed into wild-type worms. One gene in this PCR product contained mutations in the *egl-47(gf)* mutants, as well as second-site mutations in two intragenic *egl-47* revertents, identifying the gene C50H2.2 as the *egl-47* gene. Analysis of *egl-47* mRNA revealed that *egl-47* uses two promoters and generates transcripts encoding proteins with different N termini and a common C terminus. The common C terminus appears to contain seven transmembrane spanning domains, suggesting *egl-47* encodes two G protein-coupled receptors with different extracellular N-terminal domains that may bind different ligands. Only a small percentage of G protein coupled receptors have known ligands, and EGL-47 is not similar enough to any of those to predict its ligand. The fact that EGL-47 appears to be a receptor that blocks egg laying suggests it may couple to GOA-1, the G protein that blocks egg laying. *goa-1(null); egl-47(gf)* double mutants show hyperactive egg laying, exactly like the *goa-1(null)* single mutant, consistent with this model.

Both *egl-47 (gf)* mutants have an A to V change in a putative transmembrane domain of the EGL-47 protein. Mutations in analogous positions in several characterized receptors result in constitutive receptor activation. Deletion of the *egl-47* gene causes no obvious phenotypic defects, suggesting that the normal function of EGL-47 may be redundant or subtle, although no close *egl-47* homologs are evident in the *C. elegans* genome sequence. The *egl-47* promoter was used to express full-length EGL-47::GFP fusion protein in *C. elegans*. Punctate fluorescence was seen in the HSNs, apparently at synapses these neurons make in the egg-laying system. A few other puncta were visible in the head. These observations support the model that EGL-47 couples to the G protein GOA-1 in the HSN to inhibit egg laying. They also suggest why EGL-47, being expressed in only a few cells, affects fewer behaviors than GOA-1, which is widely expressed.

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**622880. A new isoform of UNC-89 with two MLCK-like protein kinase domains**

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Mutants in the *C. elegans* gene *unc-89* have disorganized myofibrils in which thick filaments are not organized into A-bands, and for most alleles, there are no M-lines. *unc-89* encodes a giant 732,000 Da polypeptide that is a member of the intracellular branch of the Ig superfamily (Benian et al., 1996). UNC-89 is composed of 53 Ig domains and several signal transduction domains, including a dbl-homology (DH) domain near its N-terminus. Recently, a possible human analog of UNC-89 called "obscurin" has been identified (Young et al., 2001). Teichmann and Chothia (2000) and WormBase predicted that gene C24G7.5 encodes an Ig superfamily member that is expressed in muscle. The predicted initiator methionine codon for C24G7.5 is only 2.7 kb downstream of the 3' end of *unc-89*. With a combination of GeneMark.hmm exon predictions and RT-PCR, we have been able to demonstrate that C24G7.5 is not a separate gene, but rather a set of alternatively spliced exons for *unc-89*. Splicing treats the 680 bp 3'UTR and stop codon of the previous *unc-89* as an intron, and continues into the C24G7.5 exons. Whereas the previously characterized UNC-89 (UNC-89-S) is 6,632 residues, the new isoform (UNC-89-L) is potentially 8,082 residues. The C-terminal extension of UNC-89-L contains a protein kinase, 640 residues of low sequence complexity, an Ig, a Fn3, and a second protein kinase domain. The two kinase domains of UNC-89-L have highest homology to twitchin, MLCKs, and titin, especially in the large subunit. However, they show significant dissimilarity in subdomains I and II that suggest that both kinase domains might lack phosphotransferase activity. Using RT-PCR we can show that both kinase domains are included in an UNC-89-L polypeptide.

RNAi, by injection, using sequence unique to either *unc-89-L* or *unc-89-S*, gives polarized light phenotypes very similar to typical *unc-89* mutants. Previously, we have noted that *unc-89*; *unc-22* double mutants, in certain allele combinations, are completely paralyzed as adults. We obtained the same adult paralysis in the progeny of *unc-22* null animals that had been injected with dsRNA from either *unc-89-L* or *unc-89-S*. Antibodies (EU133) specific for UNC-89-L were generated to the region lying between the 2 protein kinase domains. These antibodies demonstrate expression of UNC-89-L in both body wall and pharyngeal muscles, much like the previously generated UNC-89 antibodies, EU30. Antibodies (EU73) were generated to the DH-PH domains near the N-terminus shared by both isoforms. By use of deconvolution microscopy, we have noted in certain segments of the mid-A-band region, that the EU73 and EU133 epitopes are distinct and non-overlapping, separated by ~0.4µm. We are trying to interpret this result in terms of how UNC-89 polypeptides are organized in the A-band. Interestingly, although our previous western blots seemed to show a single polypeptide of the size of twitchin, consistent with our previous calculation that UNC-89-S is 732 kDa, we have been running lower percentage polyacrylamide gels, and can now detect 2 polypeptides. Although we now know that UNC-89-L is expressed in both muscle types, we do not yet know whether UNC-89-S has the same pattern of expression. This is because the only sequence unique for the *unc-89-S* transcript is the 3'-UTR. Thus, we are attempting to localize each transcript by RNA in situ hybridization.

**261484. Cathepsin Z is expressed in major hypodermal cells of *C. elegans* and has a role in worm's molting/development**

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We identified two *C. elegans* homologues *Ce-cpz-1* and *Ce-cpz-2* of *Onchocerca volvulus* cathepsin Z-like enzyme. *O. volvulus* is the causal agent of river blindness in human. In *O. volvulus* it was postulated that Ov-CPZ (LOVCP) has a role in molting of L3 to L4 larvae. We used *C. elegans* as a model to study the functional roles of CPZ-1 (82% identity to Ov-CPZ) in *C. elegans* and thus to elaborate on its potential function of this gene family in nematodes. We generated several lines of transgenic worms by injecting either *gfp* or *LacZ* reporter constructs containing a 2.1 kb encoding sequences plus 1.1 or 2.3 kb upstream sequences of the *Ce-cpz-1* gene (*Ce-cpz-1:gfp* or *lacZ*) into wild type *C. elegans* and studied the expression pattern of the gene during worm's development. The expression of *cpz-1:gfp* or *lacZ* fusion gene was in the hypodermal and pharyngeal muscle cells the same regions where the native protein was identified; few cells in early embryo. At late-stage embryos and other larval stages CPZ-1 localized in the hypodermal region as well as in the pharyngeal lining of the worms. In thin sections of *C. elegans*, the native CPZ-1 was present in the cuticular region of the worm and in molting larvae the protein was localized between the old and the new cuticle similar to what was observed in *O. volvulus*. To test whether *Ce-cpz-1* is essential for worm's development, we injected dsRNA prepared from relevant cDNA of *Ce-cpz-1* gene into the gonad of young adult hermaphrodite. We found that 100% of injected hermaphrodites gradually became paralyzed over three day period after injection, produced several embryos and later died. Embryonic defects and lethality was seen also in laid embryos. 60% of L4 soaked animals had phenotypic defects very similar to injected animals. Although embryonic defects and lethality were observed in some laid embryos, majority of the embryos escaped lethality, developed to L2, but had severe defect in the L2/L3 molt. To determine more precisely whether *Ce-cpz-1* has a role in larval molts, L1 larvae were soaked in dsRNA. A number of phenotypes were observed 8-24 hours after transfer of dsRNA treated larvae onto agar plates. Majority of the soaked L1 had significant but transient reduced growth, and some of the affected larvae did not go through L1/L2 molt. We suggest that CPZ-1 functions in molting either by taking part in the process of ecdysis or in the synthesis of new cuticle, similar to what was observed in *O. volvulus*. Its role however, during embryonic development is still under investigation.

**599471. Hypodermal expression of *sma-3* is essential for body length regulation in *C. elegans***

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The Sma/Mab pathway is one of the TGF-beta related pathways in *C. elegans*. The loss of function with any known factor (*dbl-1*, *daf-4*, *sma-6*, *sma-2*, *sma-3*, *sma-4*) in this pathway results in small body size and male tail ray fusions. Our model for this pathway is based on biochemical studies in related signal transductions. When the ligand DBL-1 binds on type II receptor DAF-4 and type I receptor SMA-6, the always active DAF-4 phosphorylates SMA-6. SMA-2 and SMA-3 belong to R-smads which are phosphorylated by activated SMA-6 during the signaling. After the activation, they form a heteromeric complex with Co-smad, SMA-4. The complex goes into nucleus and regulates the transcription of target genes.

We made two kinds of *sma-3::GFP* translational fusion constructs. GFP was inserted at the N-terminus or C-terminus of SMA-3 protein. The N-terminal construct showed the activity of rescuing body length and male tail ray fusions in the mutant *sma-3(wk30)*. By observing the GFP fluorescence distribution, we found that both of the *sma-3::GFP* constructs were expressed in pharynx, intestine and hypodermis. They localized in the nucleus. To determine if *sma-3* expression in all of the tissues is critical for body length regulation or not, we did two kinds of analyses. First, with co-injection of *sur-5::GFP* and *sma-3* genomic fragment into *sma-3(wk30)*, we got various kinds mosaic worms. We found that with the expression of *sma-3* in hypodermis, the worms have wild type body size. But, without *sma-3* hypodermal expression, all of the worms are small whether *sma-3* is expressed in intestine, pharynx or not. Second, we did the directed expression of *sma-3*. In the *sma-3::GFP* N-terminal construct, the *sma-3* promoter was removed and tissue specific expression promoters were put in. The promoters were *elt-3*, *dpy-7*, *vha-7* (hypodermal), *elt-2*, *vha-6* (intestinal) and *myo-2* (pharyngeal). We got the co-expression in pharynx and intestine by using *tmy-1* isoform III promoter or the co-injection of *myo-2* and *vha-6* constructs. From the fluorescence images, we found that the constructs were expressed in the regions we expected. The measurement of the worm length was done. The data shows the hypodermal expression of *sma-3* could mostly restore the body length, similar to the *sma-3* promoter. But, the intestinal expression of *sma-3* has no effect. And the *sma-3* expression in pharynx or both in intestine and pharynx can only rescue the body length a little, not significantly. So, the hypodermal expression of *sma-3* is necessary and sufficient to restore the body length of *sma-3* mutant.

During the process, we found that the C-terminal *sma-3::GFP*, although it has little activity, has much brighter fluorescence by comparing with N-terminal *sma-3::GFP* at the same concentration. The co-injection of the two *sma-3::GFP* fusion genes gives a result similar to N-terminal construct alone, i.e., it can rescue *sma-3(wk30)* mutant but has weak fluorescence. It indicates that the intensity of GFP fluorescence is negatively regulated by *sma-3* activity. The idea is confirmed by the co-injection of *sma-3* genomic fragment and C-terminal *sma-3::GFP* fusion gene. It also gives a weak fluorescence and totally rescues the *sma-3(wk30)* mutant. Currently, we are investigating whether the *sma-3* feed back loop depends on other components in the pathway.

### **347980. A GENETIC PATHWAY FOR THE CONTROL OF THE SEXUALLY DIMORPHIC DEATHS OF THE CEM NEURONS**

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During wild-type hermaphrodite development, 131 somatic cells undergo programmed cell death. While many genes involved in the execution of cell death have been identified, the mechanisms that control the commitment of specific cells to undergo programmed cell death are poorly understood. To date, mutations in four genes, *ces-1*, *-2*, and *-3* (cell death specification), and *egl-1*, have been found to affect specifically the deaths of particular cells. *ces-1* and *ces-2* encode transcription factors. Mutations in a transcriptional regulatory element of *egl-1*, which encodes a protein required for all somatic cell deaths, cause inappropriate expression of *egl-1* in the HSNs in hermaphrodites, resulting in their deaths.

To identify additional genes that act in the specification of cell death, we have performed a genetic screen for hermaphrodites in which the male-specific CEM neurons fail to undergo programmed cell death. The CEM neurons die during normal hermaphrodite development but survive and differentiate in males. The reporter *pkd-2::gfp* (kindly provided by Maureen Barr and Paul Sternberg) is expressed in the CEMs of males and in the CEMs of *ced-3(n717)* hermaphrodites, which are defective in essentially all programmed cell death. By using the *pkd-2::gfp* reporter as a marker for CEM survival, we were able to screen efficiently for survival of a single cell using a dissecting microscope equipped with fluorescence optics. A screen of 60,000 mutagenized haploid genomes yielded at least 154 independent mutations that cause survival of the CEMs, including 42 alleles of known cell-death genes and at least 64 mutations that cause sexual transformation. Among the isolates from our screen are nine mutations in four genes either new or not previously known to control CEM survival. These four genes act in three distinct stages of the CEM death decision. A new locus, defined by two isolates, acts in the determination of the sexual identity of the CEMs. This gene appears to be required to repress masculinization and acts upstream in the sex determination pathway to control both CEM death and HSN survival. Two genes seem to act to determine faithful expression of the CEM identity. Two isolates are alleles of the *Pax6* homolog *vab-3*, which is apparently required for efficient CEM cell death. Two isolates define a gene required for proper neuronal differentiation; these mutants are kinker Unc and have ventral cord defects, in addition to their defect in CEM death. Interestingly, mutants in this gene are very weakly Vab, and these mutations cause a strong Vab phenotype in combination with weak alleles of *vab-3*. On the basis of their broader defects in neuronal differentiation, we propose that these two genes are required for proper CEM differentiation rather than for specifically controlling CEM death. Finally, a new locus, defined by three isolates, causes strong CEM survival but not other obvious defects, and is not suppressed by mutations in sex determination. This gene may act downstream of sex determination and the determination of CEM identity to control CEM programmed cell death.

**582224. Sexual Transformation of *Caenorhabditis* hybrids.**

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As a recurrent pattern of speciation, Haldane's Rule asserts "When in the F1 offspring of two different animal races one sex is absent, rare, or sterile, that sex is the heterozygous sex" (Haldane, 1922). The broad adherence to Haldane's Rule implies that speciation in diverse taxa occurs through similar mechanisms. In *Caenorhabditis*, Haldane's Rule results from sexual transformation of F1 hybrids. From crosses of *C. briggsae* AF16 males to *C. remanei* EM464 females, all adult F1 hybrids were female. This gender bias resulted from sexual transformation, not male-specific lethality. This was ascertained using a single-worm PCR assay to detect the *C. briggsae* homolog of the X-linked *unc-18* gene, *Cb\_unc-18*. *Cb\_unc-18* was detected in only half of the adult hybrids consistent with a karyotypic ratio of 1:1. This hybrid sexual transformation phenotype is partially suppressed by the *C. briggsae* HK104 and *C. remanei* PB228 variant strains. When either of these strains was used, diplo-X females and haplo-X intersexes were obtained. The effects of HK104 and PB228 were cumulative. From crosses of *C. briggsae* HK104 males to *C. remanei* PB228 females, haplo-X hybrids were male rather than intersexual. These results implicate defects in sex determination as a reproductive isolation mechanism in *Caenorhabditis*. Genetic studies of the HK104 and PB228 variants will be used to identify the dysgenic interactions that cause this reproductive barrier. Those genes involved will be mapped using single-nucleotide polymorphism (SNP) markers. *C. remanei* SNPs will be obtained from comparisons of PB228 and EM464 sequence data. To facilitate this, a PB228 genomic library has been constructed. Random clones from this library will be sequenced. The corresponding regions of the EM464 genome will be amplified and sequenced. *C. briggsae* SNPs will soon be available from the Genome Sequencing Center at Washington University. Ultimately, the objective of this research is to characterize the molecular mechanisms that implement this mode of reproductive isolation.

Haldane, J. B. S. (1922) Sex ratio and unisexual sterility in hybrid animals. J. Genetics 12: 101-109.

**926964. Investigating the role of *cnk-1* in Ras signaling**

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How does Ras activate Raf? Despite extensive investigations the exact mechanism by which Ras activates Raf has not been identified, but what is clear is that there are many proteins implicated in functioning at this step in the pathway that may facilitate the activation of Raf. Mutations in *Drosophila* CNK (Connector eNhancer of KSR) were identified as enhancers of a dominant negative KSR rough eye phenotype (1). Genetic epistasis suggests that CNK functions a step close to Ras and Raf, and binding studies indicate that CNK can bind Raf. Interestingly a rat CNK homolog, Maguin-1, was simultaneously identified in a two-hybrid screen as an interactor with a neuronal membrane associated Guanylate kinase, S-SCAM, suggesting CNK proteins may have functions outside of Ras signaling (2).

*C. elegans* has one CNK homolog R01H10.8, which we have named *cnk-1*. We have found that *cnk-1(RNAi)* has no discernible phenotypes in a wild-type background, though has a strong synthetic rod-like larval lethal phenotypes in *ksr-1(n2526)* animals, and weaker Ras-like phenotypes in the *sur-8* and *lin-45* raf hypomorphic backgrounds (3, 4). Thus *cnk-1* functions as a positive regulator of Ras signaling in *C. elegans*. We are currently seeking to obtain mutations in *cnk-1* to determine if the RNAi phenotypes represent its true requirements for Ras-dependent developmental events.

*cnk-1* predicted to encode a 796 amino acid, multi-domain protein with N-terminal SAM (Sterile Alpha Motif), CRIC (CNK specific domain), and PDZ (PSD-90, Disc large, ZO-1) domains, and a C-terminal PH (Pleckstrin Homology) domain. All these domains, except for the CRIC whose function is unknown, are protein interaction domains, suggesting that CNK-1 may function as a scaffold. To identify CNK-1 interacting proteins we have cloned both full length and truncated forms of *cnk-1* into yeast two-hybrid vectors and are in the process of testing CNK-1 for interactions with components of the Ras pathway, as well as initiating a two-hybrid screen. The progress of these and other experiments will be reported at the meeting.

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3. Sundaram, M., Goldman, A. and Howard, R. (1999) International Worm Meeting abstract 817.
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**531622. Functional analysis of the him-3 gene family**

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During meiosis, the two specialized cell divisions that allow eukaryotes to reproduce sexually, chromosomes must undergo a complex structural changes. Following DNA replication, the sister chromatids must be organized about a proteinaceous axis called a chromosome core or axial element. Once each chromosome finds its homologous partner, the cores become aligned and anchored by the polymerization of a complex protein network called the synaptonemal complex (SC) between them. Although the formation of the axial element is absolutely required for the correct segregation of chromosomes at the end of meiosis, little is known about its molecular components, especially in higher eukaryotes. Among them, the *C. elegans* him-3 gene has been shown to encode a component of the chromosome core that is required for the formation of the SC, for reciprocal recombination and for correct chromosome segregation at meiosis (Zetka et al, 1999). Three other him-3-like genes are encoded in the nematode genome and suggest the presence of a gene family involved in chromosome structure. Our RNA interference experiments indicate that these proteins are also involved in chromosome segregation at meiosis. Two-hybrid experiments suggest that one of these interacts with HIM-3, raising the possibility that some members of this family may physically interact in concert to mediate chromosome morphogenesis. To investigate the function of these genes during meiosis we are currently using both a reverse genetic strategy and genetic non-complementation screens with deficiencies in the regions of each homolog to isolate new mutations mimicking the RNAi phenotype. Several candidates have already been isolated and are currently under analysis. To characterize the localization and expression of each protein during meiosis, we are raising antibodies against each homolog to be used in cytogenetic analysis. The characterization of this gene family will better our understanding of the relationships between vital meiotic processes such as chromosome pairing, chromosome morphogenesis, recombination, and chromosome segregation.

This work is supported by a grant by the Natural Sciences and Engineering Council (NSERC) of Canada to M.C.Z.



**984034. A NOVEL PHENOTYPE OF TRANSGENE MISEXPRESSION YIELDS NEW INSIGHT INTO THE SYNMOV GENES**

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In the course of a screen to identify mutants defective in the control of the sex-specific deaths of the CEM neurons using the reporter *pkd-2::gfp* (see abstract by Schwartz and Horvitz), we found 29 independent isolates that had strong GFP expression in the pharynx, a tissue that does not normally express the reporter. Including further clonal and nonclonal screens, we have now identified 68 mutants with this phenotype. This transgene misexpression is not dependent on chromosomal integration, high transgene copy number, or choice of co-injection marker, and the phenotype can be seen with at least one other GFP reporter that contains a different promoter.

We found that mutations in certain synMuv (synthetic Multivulva) genes (see abstracts by Ceol and Horvitz) could produce this phenotype. The synMuv genes act to inhibit vulval development. Animals mutant in both a class A gene and a class B synMuv gene, but not animals mutant in one or more class A genes or in one or more class B genes, display a Multivulva phenotype. Several class B synMuv genes have been cloned and shown to encode genes with homologs implicated in transcriptional silencing and chromatin modification. The synMuv genes able to cause *pkd-2::gfp* expression in the pharynx include one member of the synMuv A pathway and two members of the synMuv B pathway, a result that does not conform to the finding that the two pathways act separately and in parallel to prevent vulval cell fates. None of the 20 other synMuv genes tested caused this phenotype. Of the 68 mutants with this phenotype, 67 appear to be alleles of these three genes. A fourth gene, defined by a single allele, *n3599*, caused an identical phenotype of transgene misexpression. *n3599* mutants are not synMuv A or synMuv B. Interestingly, *n3599* is synthetically nonviable with a subset of synMuv B mutations, including *lin-35 Rb*. This subset does not correspond to other subsets previously shown to be associated with other phenotypes, including the synMuv B mutants shown to be synthetically lethal with the cell cycle regulator *fzr-1* (Fay, Keenan, and Han, Genes and Development 16: 503-517, 2002). It is possible that the synMuv genes synthetically lethal with *n3599* share a normal function distinct from both vulval development and cell cycle regulation, but likely involving transcriptional repression. Further investigation into these transgene-misexpression and synthetic-lethal phenotypes may define new transcriptional silencing complexes that include novel proteins and proteins previously implicated in silencing but acting in novel combinations.

### **34764. Reversal Behavior in *C.elegans***

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In the laboratory environment, the *C.elegans* exhibits a characteristic locomotory pattern consisting of long forward movement interrupted by two types of turns: omega turns and reversals. In a reversal event, the worm moves backward up to several body lengths for up to a few seconds, and then moves forward again in a new direction.

We have shown that worms' reversal behavior was influenced by several environmental and intrinsic variables. Worms reversed slower on older (drier) plates, or on plates with food, or in lower temperature, or in lower humidity, or with eggs in the body. Adults reversed slower than larvae worms. Hermaphrodites reversed slower than males. The effect of texture of the surface worms moving was also examined but further work is still in progress before we can give a clear conclusion.

A striking result of our experiments is that when worms were first placed on a plate with a pick, their reversal frequency was suppressed for up to one minute compared to the subsequent two reversals recorded. However, the latter two reversals were similar. Also, the suppression always existed no matter what the environmental and intrinsic variables looked like or how they changed. Preliminary results indicated that harsh touch was sufficient but not necessary to induce this reversal suppression. We are investigating this phenomenon further.

We also examined the effect of another reversal-inducing stimuli, heat, on the worms' reversal behavior. We found that moving a hot pick near the worm's head could induce reversal but did not suppress the reversal frequency, indicating that merely inducing reversal can not by itself suppress the subsequent reversal frequency; rather, it depends on the type of sensory input that induced the reversal.

**230243. Molecular Studies and Electrophysiological Characterization of Cyclic Nucleotide Gated Channels in *Caenorhabditis elegans***

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Cyclic nucleotide-gated (CNG) Channels are nonselective cation channels and activated by the direct binding of cyclic nucleotides such as cAMP and cGMP. These channels are composed of tetramer of alpha-subunits or alpha- and beta-subunits and belong to the superfamily of voltage-gated ion channels. In the vertebrate, high levels of these channel messages were detected in photoreceptor, olfactory sensory neurons. CNG channels play important roles in sensory transduction.

In *C. elegans*, there are six candidate genes for the CNG channel. Two CNG channels (TAX-4, TAX-2) were well characterized previously. These channels are required for thermosensation, chemosensation, and normal outgrowth and guidance of some sensory neuron axons. Others (F14H8.6, F38E11.7, Y76B12C.1, C23H5.7) have not been studied yet. We began to investigate functional properties of these CNG channels including their electrophysiological characteristics. We have isolated two deletion mutants, *jh111* and *jh113* (mutations in F14H8.6 and F38E11.7 respectively). The *jh111* mutants display axon outgrowth defects in phasmid sensory neurons. We are currently examining these mutants for any defects in chemotaxis. We are also conducting electrophysiological recording after expressing these genes in heterologous system.

**727096. A large scale screen for *dpy* mutants in *Pristionchus pacificus***

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*Pristionchus pacificus* has been established as a satellite organism for studying developmental processes and comparing them to *C. elegans*. Cell fate specification and gene functioning during vulva formation have evolved substantially between these two nematodes. Most vulva defective mutants in *Pristionchus pacificus* cannot easily be assigned for gene functions from the knowledge of *C. elegans*.

To facilitate the cloning of these mutants we are generating genetic and physical maps of *Pristionchus pacificus*. Our work presented here is focussing on the construction of a genetic map using *Dpy* mutants. We screened 50,000 gametes and identified 50 *dpy* mutants. In order to map these mutants to the six chromosomes in *Pristionchus pacificus*, we are using the SNP markers of the genetic linkage map (Srinivasan et al, submitted). Of the 50 mutants, 34 have already been assigned to different chromosomes. Complementation analysis of 29 of these 34 mutants showed that they fall into 8 complementation groups. We are currently fine-mapping those *dpy* mutants with regard to the SNP markers. The remaining 21 *dpy* mutants are currently assigned to individual chromosomes. We estimate a total number between 12 and 15 complementation groups with a *dpy* phenotype.

In *C. elegans*, saturated screens identified 26 complementation groups with a *dpy* phenotype when mutated, 6 of which are located on the X chromosome. In contrast, in *Pristionchus pacificus* we find only one complementation group with one allele which assigned to the X chromosome.

The data emanating from the genetic *dpy* map from the genetic and physical map projects will facilitate further genetic and molecular studies in *Pristionchus pacificus*. Specifically, the genetic *dpy* map will allow in-depth genetic characterization of mutants in *Pristionchus pacificus* isolated in various screens, thereby establishing it as a second general nematode model system.

**307592. Programmed cell death in *Pristionchus pacificus***

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To study the evolution of developmental processes, we analyse vulva development in *P. pacificus* and compare it to *C. elegans*. In *P. pacificus*, 7 of the 12 ventral epidermal cells undergo apoptosis, whereas the corresponding cells in *C. elegans* fuse with the surrounding hypodermis. Phylogenetic analyses suggest that the cell death of these epidermal cells represents a derived character. To understand the evolutionary changes responsible for these evolutionary alterations in the apoptotic mechanism, we are characterising cell death mutants in *P. pacificus*. Genetic screens lead to the isolation of mutations in the cell death pathway in *P. pacificus* that result in the survival of P(1-4,9-11).p. Complementation tests have shown, that these mutants fall into two complementation groups, one of which has been proven to encode the *P. pacificus* ortholog of *ced-3* (Sommer et al., 1998). The second complementation group had been originally characterized as *ipa-2* (inhibitor of P-ectoblast apoptosis) and is a good candidate for the *Ppa-ced-4* homolog.

We have mapped *ipa-2* to chromosome III in the neighborhood of *pal-1* and have isolated the BAC clone 7-E-22 by hybridization with a *Ppa-pal-1* probe. Sequencing this BAC clone indicated small-scale synteny to *C. elegans* in that region. However, we could not identify a *ced-4* like molecule on that BAC. Further mapping placed *ipa-2* between the two SNP markers S55 and *tra-1*, which are adjacent to the *pal-1* locus. Further fine mapping is in progress to clone *ipa-2* by BAC walking.

**653150. Requirement for a p38 MAP Kinase Signaling Pathway in *C. elegans* Immunity**

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We used a *Caenorhabditis elegans*-*Pseudomonas aeruginosa* pathogenesis system to perform a genetic screen for *C. elegans* mutants with enhanced susceptibility to pathogens (*esp*). The screen identified two genes required for host defense: *esp-2*, encoding SEK-1, a *C. elegans* MAP kinase kinase homologous to the mammalian MKK3/6 and MKK4 classes of MAP kinase kinases, and *esp-8*, encoding NSY-1, an ortholog of the mammalian MAP kinase kinase kinase, ASK-1. *esp-2/sek-1* and *esp-8/nsy-1* mutants showed diminished levels of p38 MAP kinase activation, and RNAi of a *C. elegans* p38 MAP kinase ortholog, *pmk-1*, gave an *Esp* phenotype. These findings provide direct genetic evidence for the involvement of the evolutionarily conserved p38 MAP kinase pathway in *C. elegans* immunity. Combined with data from other organisms, our results suggest that a stress-activated MAP kinase signaling cassette is an ancient feature of innate immune responses in diverse species.

**520778. Acetylation regulates the subcellular localization of POP-1, a *C. elegans* cell fate-determining transcription factor**

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LEF/TCF family of HMG-box transcription factors are conserved from *C. elegans* to human, and are nuclear effectors of the Wnt signaling pathway. This pathway not only controls embryonic patterning and cell fate decisions in various species, but is also involved in carcinogenesis. During early *C. elegans* embryogenesis, the Wnt signaling controls anterior/posterior polarity of the EMS blastomere, the common precursor of MS (mesodermal) and E (endodermal) cells. POP-1, the *C. elegans* ortholog of LEF/TCF factors, plays a crucial role in the specification of the anterior MS blastomere, at least in part through suppressing the expression an early E cell fate determining gene, end-1. In the posterior E cell, POP-1 nuclear level is downregulated by the Wnt signaling, leading to the activation of end-1 and the initiation of the endoderm differentiation programs. Although phosphorylation appears to be involved in the Wnt signaling-mediated downregulation of POP-1, the molecular mechanisms regulating POP-1 biological activity remain largely unknown.

We investigated acetylation in the regulation of POP-1 functions, and showed that the human histone acetyltransferase CBP, as well as its *C. elegans* ortholog CBP-1, interact and acetylate POP-1 in vitro and in vivo. Mutational analysis identified three acetylation sites (lysines K185, K187 and K188) adjacent to the HMG-box DNA binding domain of POP-1. Although CBP also acetylates the neighboring K187 and K188 residues, K185 appears to be the major site targeted by CBP in vitro. We provide evidence that K185 is also acetylated by CBP-1 in worm embryos. To study the functional consequences of POP-1 acetylation, we analyzed the subcellular distribution of wild-type (wt) POP-1 and acetylation-defective mutants (triple, double, or individual mutations of the 3 K to A or R) in mammalian cells. We found that wt POP-1 is subjected to an active nuclear export mechanism, and shuttles between nuclear and cytoplasmic compartments although it predominantly localizes in the nucleus of most of the cells. In contrast, acetylation-defective mutants are exclusively cytoplasmically localized. Consistent with a role for acetylation in regulating the subcellular distribution of POP-1, inhibition of histone deacetylase activities promotes nuclear accumulation of wt POP-1. Distinct sets of experiments allowed us to demonstrate that POP-1 mutants are not only more efficiently exported than wt POP-1 but also display a defect in nuclear entry, and that all 3 K are required for proper nuclear localization of POP-1 in mammalian cells. Consistently, we found that the acetylation-defective POP-1 mutants (3K to A or R) are also mislocalized in *C. elegans* embryos, and fail to rescue the pharyngeal defects and embryonic lethal phenotype of the pop-1 (zu189) genetic mutant. Taken together, these results identify a novel physical and functional interaction between an histone acetyltransferase and the cell fate-determining transcription factor POP-1, and highlight a crucial role for acetylation in the regulation of POP-1 biological activity during *C. elegans* embryogenesis.

**965658. CED-13, a novel BH3 domain protein, regulates radiation-induced and developmental programmed cell death in *C. elegans*.**

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Programmed cell death (PCD) is a common cell fate in the development of multicellular organisms. In *C. elegans*, for example, 131 of the 1090 somatic cells that are born undergo PCD, and nearly half of the cells born in the *C. elegans* germline have a similar fate. Although much has been learned about the machinery that executes PCD in *C. elegans* and in mammals, only in a few examples have the signal transduction pathways leading to activation of this machinery been elucidated. Current models suggest that most, if not all PCDs are controlled by three main gene families. In *C. elegans* these are represented by *ced-9* (*bcl-2* family member), *ced-4* (similar to mammalian *Apaf-1*), and *ced-3* (a caspase family member). These genes act in a pathway such that *ced-9* inhibits *ced-4*, which in turn activates *ced-3*, promoting PCD. We are interested in defining molecular signaling events that trigger activation of this pathway.

We have identified a novel gene, *ced-13* (formerly *cip-1*), whose protein product can interact with the CED-9 protein. We have shown that CED-13 protein binds to CED-9 in a two-hybrid assay. Co-precipitation experiments using a GST-CED-9 fusion protein and <sup>35</sup>S-labelled CED-13 also indicate that CED-9 and CED-13 may normally interact. Sequence inspection suggests that CED-13 contains a BH3 (*bcl-2* homology region 3) domain that may mediate interaction with CED-9, a hypothesis supported by our binding studies. The *C. elegans* BH3 domain protein EGL-1 interacts with CED-9 in a similar manner.

To define the function of *ced-13* we overexpressed the gene using *C. elegans* heat shock promoters. We found that embryos overexpressing *ced-13* are dead. This inviability can be rescued by loss-of-function mutations in the *ced-3*, or *ced-4* genes, or by a gain-of-function mutation in *ced-9*. These observations suggest that CED-13 acts upstream of the core PCD machinery to promote PCD.

We isolated a deletion allele of *ced-13* that lacks a 1.3 kb DNA segment containing the gene. *ced-13* mutants are weakly defective in developmental PCD. Specifically, double mutants of *ced-13* and a weak *ced-3* mutation are more defective for PCD than mutants carrying either single mutation. More strikingly, however, whereas radiation normally induces many cell deaths in the germlines of wild-type adults, *ced-13* mutants exhibit no radiation-induced PCD. These results support the notion that *ced-13* is an important activator of PCD in *C. elegans*.

Our preliminary results suggest that *ced-13* may be transcriptionally induced following irradiation, and that this induction may be dependent on the *C. elegans* p53-related gene *cep-1*. Thus, our studies suggest that a radiation-response signal transduction pathway impinges on *ced-13* to regulate PCD in the germline. Interestingly, the *egl-1* gene shows a partial defect in PCD following irradiation and a severe defect in developmental PCD. We propose that *egl-1* and *ced-13* together regulate both types of death.



**803363. *daf-6*, a gene regulating morphology of the glial-like amphid sheath cells, encodes a Patched-related protein.**

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Glia account for over 90% of the cells populating the human brain, and glia-derived tumors are the most common and most lethal human brain tumors. Surprisingly, compared to their neuronal counterparts, little is known about glial cell development, function, or morphogenesis. Growing evidence suggests that glia play active roles in regulating synaptic transmission and neuronal current propagation, and are likely to play key roles in many, if not all, aspects of nervous system function.

*C. elegans* hermaphrodites contain 23 neuron-associated sheath cells that bear striking similarities to vertebrate glia. Reminiscent of astrocytes, they possess large cell bodies with multiple small projections and a long main projection that enwraps the dendritic processes of sensory neurons. Recent availability of *C. elegans* glial reporter genes (1), the sequencing of the entire *C. elegans* genome, and the facility with which genetic studies can be undertaken in this organism make it an excellent system to study basic aspects of glial cell biology. *C. elegans* anterior and posterior sensory structures are composed of three cell types: sensory neurons responsive to odor, taste, osmolarity, mechanical stimuli and heat; sheath cells that associate with the neurons and envelope the distal ends of their dendritic processes; and socket cells involved, in part, in generating a pore through which chemical signals can be sensed by the ensheathed neurons. Two bilaterally symmetric sensory structures, the amphids and phasmids, seem to be important for most chemosensation in *C. elegans*. Because the neurons of the amphids and phasmids have been well characterized, we have chosen to study their accompanying glia as models for understanding glial cell development and function.

Using a GFP reporter gene expressed in amphid sheath cells (1) we examined the association of amphid neurons and sheath cells by confocal microscopy. Our results show that three domains of interactions can be defined. A domain near the cell bodies where the sheath cell and neurons do not interact, a domain in which the sheath cell process interacts with some neurons, but does not fully ensheath all neurons, and a domain in which all neurons are fully ensheathed by the sheath cell. These observations are consistent with previous electron microscope studies (2).

To understand how these domains are formed and what their functional significance is we are cloning the *daf-6* gene. Previous work showed that *daf-6* mutants exhibit a progressive proliferation of the ensheathing portion of the amphid sheath cells concomitant with gradual retraction of neuronal dendrites away from the tip of the nose (3). These results suggest that *daf-6* may normally inhibit amphid sheath cell growth. Bob Herman showed by mosaic analysis that *daf-6* function is likely to be required within the amphid sheath cells (4). To clone *daf-6* we examined open reading frames in the *daf-6* region of *daf-6(e1377)* animals for mutations. We found that the *patched*-related gene *ptr-7* contained a mutation converting a Trp codon to a stop codon at codon 149. We are currently testing rescue of *daf-6* using the *ptr-7* cosmid.

Hedgehog signaling through the Patched receptor can promote cell growth and proliferation and may have a role in the interactions of neurons with Schwann cell myelin sheaths. Furthermore, alterations in human Patched1 and the downstream transcriptional regulator Gli1 are associated with glial tumors. That Patched normally inhibits the hedgehog signaling pathway is consistent with the *daf-6* phenotype of extra cell growth.

In addition to characterizing *daf-6* we have begun to explore glial cell function and morphogenesis using a combination of laser ablation, time-lapse microscopy, genomic and genetic approaches.

(1) R. Roubin, personal communication.

(2) Ward, S., *et al.*, J. Comp. Neurol. 160: 313-337.

(3) Albert, P., *et al.*, J. Comp. Neurol. 198: 435-451.

(4) Herman, R. K., Genetics 116: 377-388.

**989413. An RNAi-based screen identifies putative mediators and targets of the *let-7* small temporal RNA**

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Small, temporally regulated RNAs (stRNAs) of 21-22 nucleotides control the timing of developmental events in *C. elegans* and, presumably, other metazoans through posttranscriptional regulation of target gene expression. Recently, almost 200 related RNAs, termed micro-RNAs (miRNAs), have been identified from various organisms including worms, flies and humans. While this discovery suggests the wide-spread use of miRNAs in the regulation of gene expression, little is known about their mode of action. To improve our understanding of miRNAs we devised a functional genomics approach based on RNA-interference (RNAi) by feeding to identify interaction partners of the *let-7* stRNA. Screening a previously described bacterial library, which covers 2,400 genes on *C. elegans* chromosome I (A.G. Fraser *et al.* (2000), *Nature* **408**: 325-330), we identified ca. 50 suppressors of the lethality of a temperature-sensitive *let-7* allele. Intriguingly, the largest class of suppressors code for components of the translation machinery, supporting the notion that *let-7* regulate its target genes by inhibiting target-mRNA translation. In addition, we have identified a number of potential targets of *let-7*. Importantly, since most of the suppressors conferred sterility or embryonic lethality they would likely have eluded traditional genetic approaches of identification. Finally, the observation that about one fifth of the identified suppressors had no previously detected post-embryonic phenotype, suggests that RNAi-based modifier screens are powerful tools for detection of novel gene functions.

**874571. *eor-1* and *eor-2* act downstream of the Ras and Wnt pathways during vulval and P12 development**

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In *C. elegans*, a Ras/ERK signaling pathway controls multiple cell fate decisions and cooperates with a Wnt/B-catenin signaling pathway to specify vulval and P12 cell fates in a Hox-dependent manner. *eor-1* and *eor-2* are two new positively-acting nuclear components of the Ras and Wnt pathways. Mutations in *eor-1* and *eor-2* suppress gain-of-function mutations in *mpk-1* ERK and loss-of-function mutations in *pry-1* Axin, suggesting that *eor-1* and *eor-2* act at the convergence of the Ras and Wnt pathways. *eor-1* and *eor-2* function redundantly with the Mediator complex gene *sur-2* and the functionally-related gene *lin-25*, such that removal of both *eor-1/eor-2* and *sur-2/lin-25* mimics the removal of a main Ras pathway component. Furthermore, the *eor-1* and *eor-2* mutant backgrounds reveal a positive role for the Elk1-related gene *lin-1*, and suggest that this positive role of *lin-1* involves *sur-2* and *lin-25*. *eor-1* encodes the ortholog of human PLZF, a BTB/zinc finger transcription factor that is fused to RARalpha in acute promyelocytic leukemia. *eor-2* encodes a novel protein. *eor-1* and *eor-2* appear to function closely together and cooperate with Hox genes to promote the expression of Ras- and Wnt-responsive genes.

**172437. Functional Study of *Caenorhabditis elegans* Cadherins**

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Cell-cell interaction is one of the fundamental processes required for multicellular organism development. Cadherins represent a major superfamily of transmembrane glycoproteins that mediate calcium-dependent homophilic cell-cell adhesion. Studies from other organisms suggest that cadherins are candidates for synapse formation during nervous system development. We and other labs have studied 12 predicted cadherin-related genes in *C. elegans*. We call ten of them typical cadherins, while flamingo belongs to serpentine family B, and calsyntenin is a member of the calsyntenin subfamily. Their expression patterns have been surveyed by using promoter::GFP reporters. Most of the ten typical cadherins are expressed in the epidermis, six are expressed in the nervous system with highly restricted patterns - none of them is expressed in more than six groups of neurons. While flamingo is expressed exclusively in the nervous system with expression in more than 20 groups of neurons and calsyntenin is

expressed in all neurons as well as ovarian sheath. Localization studies using GFP-tagged calsyntenin proteins indicate that calsyntenin are localized at synapses. Our results also suggest that calsyntenin is proteolytically processed. Its N-terminal fragment is released and accumulates in coelomocytes, while the C-terminal stump is internalized where it persists for a long time in cell bodies and neurite. The physiological and developmental roles of calsyntenin in synaptic formation and gonadal function will be pursued.

**936016. Functions of the septins in *C. elegans* development**

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Septins are a family of GTPases involved in cytokinesis in diverse organisms. They are also expressed in post-mitotic cells, suggestive of other cellular functions. *C. elegans* have two septins, encoded by the *unc-59* and *unc-61* genes. Loss of septin function results in approximately 20% embryonic lethality. Up to 50% of those larvae that hatch do not survive the first larval (L1) stage due to defective formation of the pharynx. Some pharynges appear to not have properly elongated and are not attached to the buccal cavity, while others appear morphologically normal, but are also unattached. Preliminary studies reveal no obvious early failures in cell division, indicating a potential role for septins in pharyngeal organogenesis. UNC-59 appears to be associated with AJM-1 containing junctional complexes in the embryonic pharynx, and is able to localize correctly in *unc-61* mutants. In contrast, UNC-61 is enriched in the embryonic nervous system, particularly in the nerve ring and ventral cord. UNC-61 localization to the nervous system requires *unc-59* function. Assays of locomotory behavior on newly hatched L1s reveal that mutations in either septin frequently cause uncoordination. As all of the ventral cord neurons are present, these defects cannot be attributed to cytokinesis failures in the ventral nerve cord. This is the first *in vivo* evidence of a role for septins in neuronal development or function. We are using neuronal drugs and GFP reporters to determine if septins are required for neuronal development, function, or both. Newly hatched septin mutant L1s expressing *unc-47::GFP* to reveal GABAergic neuronal morphology display abnormalities including absent, misdirected or abnormally positioned processes, defasciculation of the ventral cord, discontinuities in the ventral cord, punctate ventral and dorsal cords, and abnormal numbers of GABAergic neurons. The incidence of these defects in process guidance is proportional to the percentage of larvae that are defective in locomotory behavior, and are likely to be responsible for the uncoordinated phenotype.

## 201718. Effects of Hyperactivation of EGL-15 on Sex Muscle Development

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*C. elegans* has long been recognized as an excellent model organism in which to study the genetics of muscle formation. The hermaphrodite sex muscles offer additional advantages for elucidating the various processes involved in myogenesis. First, they are derived post-embryonically from a single mesoblast, M. Second, their functionality is not essential for viability. Third, defects in the function of sex muscles result in an obvious egg laying-defective (Egl) phenotype. These animals fill with unlaidd eggs that ultimately hatch within their mothers, forming "bags-of-worms".

Normal muscle development involves a number of cellular processes, including cell fate specification, myoblast migration and proliferation, myocyte differentiation and proper muscle attachment. Cell culture experiments and studies of vertebrate embryogenesis have shown that FGFs help regulate many of these processes. Specifically, FGFs have been shown to induce muscle cell migration and proliferation and to repress myocyte differentiation.

Similarly, the *C. elegans* FGFR, EGL-15, is essential for normal sex muscle development. EGL-15 has a well-characterized role in the mechanisms used to guide the migrations of the sex myoblasts (SMs) the progenitors of the sex muscles. However, *egl-15* expression is not limited to the period of SM migration; rather, it is expressed at several stages in the M and SM lineages. These data suggest that EGL-15 might function at multiple steps in sex muscle development.

We have used a constitutively hyperactivated form of EGL-15 [*egl-15(neu\*)*] to explore the role of EGL-15 in sex muscle development. Transgenic animals bearing *egl-15(neu\*)* generally display a Clear (Clr) phenotype. However, when this Clr phenotype is suppressed by a mutation in *soc-2*, the animals display a completely penetrant Egl phenotype. Analysis of the sex muscles in these animals indicates that the cells are present, but they fail to express markers characteristic of mature muscle cells. Thus, it appears that hyperactivation of EGL-15 inhibits sex muscle differentiation. Interestingly, the remaining muscles in the worm appear normal.

*egl-15(neu\*)* causes additional defects within the M lineage, including SM position defects and early cell fate determination defects. These effects are consistent with the expression of EGL-15 at various points in the M lineage and an instructive role for EGL-15 in SM migration.

The parallels between the ability of FGF to antagonize vertebrate myogenesis and the inhibition of sex muscle differentiation by *egl-15(neu\*)* allows us to use the fully penetrant Egl phenotype to characterize the role of FGF signaling in muscle development. A mutagenesis screen to isolate suppressors of the Egl phenotype would be expected to identify mutations that compromise the signaling pathway acting downstream of EGL-15 to inhibit sex muscle differentiation. Interestingly, mutations in some of the components of the EGL-15 signaling pathway involved in its essential function do not restore normal egg-laying function of *egl-15(neu\*)* animals. Thus, this screen may isolate mutations in EGL-15 signaling components that are specific to the process of muscle development.

In a screen of animals representing 50,000 EMS-mutagenized haploid genomes, we isolated 15 suppressors of the Egl defect of *egl-15(neu\*)*. These mutations are all partial suppressors. Thus, it appears that egg-laying functionality has only been partially restored. Direct examination of the sex muscles in the suppressed lines reveals the nature of the partially penetrant effects of the mutants. These animals display various degrees of sex muscle differentiation. While some worms display WT muscle structure, many worms can only generate disorganized and morphologically abnormal sex muscles. Interestingly, the mutants demonstrate mature sex muscles both in the correct as well as incorrect positions. Thus, these mutants can correct the muscle differentiation defect but not the cell specification or sex myoblast migration defects found in the non-mutagenized parent. Therefore, it is likely that the genes responsible for mediating cell migration and fate specification are different from those required for mediating differentiation.

**965182. *clk-1* and ubiquinone biosynthesis**

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Mutations in the *clk-1* gene of *C. elegans* affects lifespan and numerous physiological rates, including behavioral rates (defecation, pharyngeal pumping, and swimming), developmental rates (embryonic and post-embryonic), and reproduction. *clk-1* encodes a 187 amino acid protein localized in mitochondria. Interestingly, *clk-1* is implicated in the synthesis of ubiquinone (Q), an important electron transporter, especially in mitochondria. The involvement of CLK-1 in Q biosynthesis in *C. elegans* has been demonstrated by the finding that *clk-1* mutants do not synthesize Q (Miyadera *et al.*, 2001; Jonassen *et al.*, 2001). Rather, they accumulate demethoxyubiquinone (DMQ), a Q-synthesis intermediate that is able to sustain mitochondrial respiration in worms (Miyadera *et al.*, 2001), as well as in mammals (Levavasseur *et al.*, 2001). Remarkably, although only DMQ is present in all three *clk-1* alleles, *clk-1(e2519)*, which is a point mutant, produces a much weaker phenotype. This suggests that the lack of Q cannot solely account for the *Clk-1* phenotype. Recently, it has also been found that *clk-1* mutants are unable to grow on a Q-deficient bacterial strain (Jonassen *et al.*, 2001). In order to understand the impact of Q metabolism on *clk-1* mutants, we systematically examined the phenotype of *clk-1* worms, when fed with bacteria deficient in each of the genes implicated in Q biosynthesis in *E. coli* (*ubi* genes). Our results confirm that exogenous Q is necessary for *clk-1* mutants fertility and development. This suggests that dietary Q from wild-type bacteria is capable of being used in a key cellular process that requires Q. To study this process further, we have generated a *C. elegans* knock-out (KO) in the *coq-3* gene, which encodes another enzyme that participates in ubiquinone biosynthesis. However, contrary to *clk-1* mutants, *coq-3* mutants do not appear to accumulate a Q-synthesis intermediate that is competent for respiration. In fact, *coq-3* KO mutants display a lethal phenotype, which suggests that exogenous Q cannot replace all of the Q functions. Taken together, our results suggest a model in which Q plays distinct roles at mitochondrial and non-mitochondrial sites (Hihi *et al.*, 2002). In particular, Q is necessary at non-mitochondrial sites for development. Consistent with our model, we have found that *clk-1* is necessary for development in mammals, since a *clk-1* mouse KO is lethal early during development, even though respiration is almost normal (Levavasseur *et al.*, 2001). This indicates that DMQ cannot functionally replace Q at some non-mitochondrial sites, and that these sites are probably conserved between species. Our model suggests how the Q biosynthesis defect relates to the phenotype of *clk-1* mutants. We are currently using genetic methods to determine the nature of the non-mitochondrial sites of Q action.

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**705538. THE C. ELEGANS ORFEOME CLONING PROJECT : VERSION 1.0**

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

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



**669380. Two homologues of *rde-1*, *alg-1* and *alg-2*, are implicated in RNA interference and microRNA pathways.**

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In a variety of organisms, double stranded RNA (dsRNA) has been shown to induce a gene silencing phenomenon called RNA interference (RNAi). During RNAi long trigger dsRNA molecules are processed into small 22-24 nucleotide (nt) RNAs (siRNAs) that are thought to guide the destruction of complementary cellular RNAs. In previous genetic screens, we have identified several *rde* (RNA interference-deficient) 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, plants and *Drosophila*. To date, we have identified 27 homologues of *rde-1* in *C. elegans* genome. Among these, *alg-1* and *alg-2* were previously shown to be required for the processing and the activity of two natural small RNAs (miRNAs), *lin-4* and *let-7*, that function in development [Grishok *et al*, Cell 106: 23-34 (2001)]. Here we show that ALG-1 and ALG-2 interact with miRNAs (i.e. LIN-4 and LET-7) in vivo. Surprisingly, although as reported previously *alg-1* and *alg-2* are not required for somatic RNAi we find that these genes are required for RNAi in the germline. These findings suggest that RDE-1 homologs function in RNAi at more than one step and reveal yet another connection between the RNAi and miRNA pathways.

**278351. FGF Regulation of Fluid Balance in *C. elegans***

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*clr-1* encodes a receptor tyrosine phosphatase (RTP) that negatively regulates an EGL-15 FGFR signaling pathway in *C. elegans*. The pseudocoelomic cavity of *clr-1* mutants fills with clear fluid, leading to the characteristic Clear (Clr) phenotype. The Clr phenotype suggests that the balance of EGL-15 signaling is essential for fluid homeostasis in worms. Animals that completely lose EGL-15 function arrest early in larval development, and less severe reduction of EGL-15 function results in a Scrawny (Scr) phenotype. To understand how FGFR signaling controls fluid homeostasis in *C. elegans*, we are investigating the cellular basis of these phenotypic defects.

A number of approaches have been taken to investigate the cellular site of EGL-15 and CLR-1 function. Although these approaches have not given completely consistent results, several lines of experimentation suggest that CLR-1 acts in the excretory cell to regulate EGL-15 signaling. First, a *clr-1::gfp* reporter is expressed robustly in the excretory cell as well as a number of other cells. Second, mosaic analysis of *clr-1* indicates that *clr-1* activity is required in the AB.plp sublineage (C.Z. Borland, unpublished data); this lineage gives rise to a group of cells that include the excretory cell. Consistent with these results, laser ablation of the excretory cell has been shown to result in a similar Clr phenotype (F.K. Nelson and D.L. Riddle, 1984). These data suggest that CLR-1 is normally expressed and functions in the excretory cell.

To explore this further, we have used tissue-specific promoters to identify cells in which CLR-1 or EGL-15 can be expressed to rescue their respective mutant phenotypes. Promoters (*vha-1p* and *itr-1p*) that drive expression in the excretory cell can drive full *clr-1* rescue activity, while a body wall muscle promoter (*unc-54p*) cannot. Surprisingly, expression of CLR-1 by neuronal promoters (*snb-1p* and *unc-14p*) can also rescue the Clr phenotype, suggesting a potential role of CLR-1 in neurons. It is unlikely that CLR-1 acts in neurons within the AB.plp lineage; most neurons derived from the AB.plp lineage have bilaterally symmetrical cells derived from the AB.prp lineage, which presumably carry out the exact same biological function.

As a complementary approach, we have dissected the endogenous *egl-15* promoter by deletion analysis to look for important regulatory elements. A 200bp sequence (E15) was found to be essential for full-scale *egl-15* promoter activity. Moreover, the E15 enhancer fragment is able to drive *egl-15* rescue activity when fused to a minimal *pes-10* promoter. Therefore, this E15 element is both necessary and sufficient for complete *egl-15* promoter activity. Intriguingly, a GFP reporter under the control of two copies of this enhancer element (*2E15::pes-10::GFP*) is expressed mainly in hypodermal cells. Surprisingly, neither the *lin-26* nor the *jam-1* hypodermal promoters can drive *clr-1* or *egl-15* rescue activity, but these negative results may have failed for other reasons.

Although our data do not pinpoint a single site of action of EGL-15 function, it is striking that the three potential sites of rescue, neurons, hypodermal cells, and the excretory cell, all contribute to the tissue domain surrounding the excretory canal outside of the basal lamina. Additional studies are in progress to understand FGF-regulated fluid flux in worms.

**399916. Anaphase Promoting Complex function during meiosis in *Caenorhabditis elegans*.**

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The Anaphase Promoting Complex or Cyclosome (APC/C) is a multisubunit E3 ubiquitin ligase, which causes the destruction of cell-cycle regulators leading to the segregation of chromosomes at anaphase and exit from mitosis. Previously, we and others identified nine genes which encode subunits of the *C. elegans* APC/C, based on sequence homology, RNA-mediated interference (RNAi), and/or temperature-sensitive (ts) mutational analysis (1-3). The analysis of these genes was the first demonstration of a requirement for the APC/C for progression through meiosis I in eukaryotes.

This study focuses on the function of APC/C during meiosis I. Peptide-specific antisera raised against *C. elegans* CDC-23 (APC-8) suggest that during meiotic prophase, the APC/C is diffusely localized to the nucleus, with some concentration on chromosomes in diakinesis. At metaphase I, APC/C localization shifts to the meiotic spindle. A similar pattern is observed in mitotically dividing embryos, in which APC/C is nuclear-associated during interphase but centrosome-associated at metaphase and anaphase. These localization patterns are consistent with a role for APC/C in chromosome segregation during mitosis and meiosis.

Some temperature-sensitive alleles of *cdc-23* arrest only at the metaphase-to-anaphase transition of meiosis I in oocytes, whereas others have mitotic and/or spermatogenesis defects as well. This suggests that the APC/C might have oocyte meiotic-specific functions in addition to its well-characterized mitotic functions. To address this issue, we conducted a screen for suppressors of *mat-3 (or180)*, an oocyte meiosis-specific ts allele of *cdc-23*. At the non-permissive temperature of 25<sup>0</sup>, *mat-3 (or180)* hermaphrodites arrest as one-cell meiotic embryos. Among 250,000 mutagenized haploid genomes, we identified 11 alleles that partially suppress the oocyte meiotic-specific defect of *mat-3 (or180)*. One allele, *som-1 (av4)*, is a dominant extragenic suppressor of *mat-3 (or180)* on LG I. At 25<sup>0</sup>, *mat-3 (or180); som-1 (av4)* animals gives rise to ca. 10% viable embryos within a given brood, whereas nearly 100% of the embryos are multicellular. Many of these multicellular embryos are clearly dividing abnormally. In addition, *mat-3 (or180); som-1 (av4)* animals display a high-incidence-of-males (him) phenotype at 25<sup>0</sup>. Taken together, these phenotypes suggest that *som-1 (av4)* allows aberrant chromosome segregation to occur in order for *mat-3 (or180)* animals to reproduce. We postulate that the gene represented by *som-1 (av4)* encodes a novel, meiotic-specific substrate or regulator of the APC/C.

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**456343. Germinal Center Kinase (GCK-1) affects meiotic prophase progression in the *Caenorhabditis elegans* germline**

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The *Caenorhabditis elegans* Aurora A kinase, AIR-1, is associated with mitotic centrosomes and is required for chromosome segregation. Using AIR-1 as the bait in a yeast two-hybrid screen, we cloned a novel member of the Ste20/PAK group of kinases (T19A5.2) which we have designated as germinal center kinase (*gck-1*). The Ste20p group of kinases has been divided into two families (PAKs and GCKs) based on the location of the kinase domain. The absence of a PAK homology domain as well as the presence of an N-terminal kinase domain and a unique C-terminus classifies GCK-1 as a member of the germinal center kinase (GCK) family. This family is comprised of eight subfamilies, several of which have been shown to activate the MAP kinase pathway. *gck-1* falls into the third GCK (GCK-III) subfamily; however, the GCK-III subfamily proteins have not been previously shown to activate any of the MAP kinase pathways.

To determine GCK-1 localization, a rabbit polyclonal antibody was raised against recombinant GCK-1 fused with maltose binding protein (MBP). GCK-1 is associated with P-granules which localize to the germ line precursor cells of the developing *C. elegans* embryo. Additionally, GCK-1 localizes to the mitotic cleavage furrow in embryonic somatic cells. To determine the role of GCK-1 in *C. elegans* development, we performed RNA-mediated interference (RNAi). *gck-1(RNAi)* results in sterility due to improper meiotic prophase progression. A wild-type *C. elegans* gonad consists of two U-shaped tubes with mitotic nuclei at the distal end. As the nuclei move proximally within a common cytoplasm they enter pachytene of meiotic prophase I where they remain for a period of time before entering into diakinesis. Linearly arranged diakinetically become large cellularized oocytes as they progress proximally towards the spermatheca. The bivalents in the most proximal oocytes can be visualized with a phosphohistone H3 antibody. The oocytes are fertilized in the spermatheca, and then pass into the uterus where the maternal nucleus meiotic divisions I and II are followed by several mitotic cell cycles before the embryos are extruded into the environment. Alpha-tubulin and DAPI staining of *C. elegans gck-1(RNAi)* gonads reveals what appear to be multiple rows of small cellularized diakinetically in the proximal end of the gonad. However, only some of the small cellularized bodies have phosphohistone H3 positive bivalents and these cells are randomly distributed in the proximal gonad rather restricted to the most proximal end, as in wild type. The cell cycle stage of the non-staining small cellularized bodies is presently undefined.

The *gck-1(RNAi)* germ line progression defects are similar to the phenotype resulting from over-activation of *let-60(Ras)* in the MAP kinase pathway. The MAP kinase pathway is required for pachytene exit in the *C. elegans* gonad. Since GCK-1 shares some homology with other protein kinases that are known to be involved in the MAP kinase pathway, genetic epistatic studies are underway to determine the relationship between GCK-1 and the MAP kinase pathway. Additional studies are being conducted to further define the relationship of *gck-1* with other mutants that have germ line progression defects. Future experiments will determine the interaction between GCK-1, the MAP kinase pathway, and the AIR-1 kinase.

**502570. Evidence for imprinted X chromosome silencing in early *C. elegans* embryos.**

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Dosage compensation is the essential process that equalizes X-linked gene expression in organisms whose sexes differ numerically in their chromosomal constitutions. This process has been studied extensively in very diverse groups of animals, including mammals, nematodes and insects, and in each case, the chromosome-wide regulation is closely associated with sex-specific modification of chromatin structure. In *Caenorhabditis elegans*, transcription from both X-chromosomes is down regulated by half in XX hermaphrodites to equal expression in XO males. Dosage compensation in XX embryos does not become established until, approximately, the 30 cell stage. Although much is known about the initiation and maintenance of dosage compensation, it currently remains unclear whether any mechanism exists to overcome the X chromosome dosage imbalance in the early embryo, before the establishment of these processes.

An important level of control determining the transcriptional status of an individual gene or large chromosomal region can be imposed through the regulation of chromatin structure. Previous work in our lab examining the chromatin organization in germ cells of *C. elegans* demonstrated that the histones on the X chromosome show a dramatic reduction in specific posttranslational modifications that correlate with transcriptional activation. In order to determine whether the X-chromosome specific silencing established in the germ cells is heritable, we examined the chromatin organization of the X chromosome and the autosomes during the earliest cleavage divisions. Unexpectedly, we found that in early XX embryos, a single chromosome lacks specific histone modifications that indicate a transcriptionally active state. Combined histone antibody / DNA FISH analysis of early embryos demonstrates that the exceptional chromosome is always the sperm-derived X chromosome, and that this epigenetic mark persists through the earliest cleavage divisions until approximately the 20-30 cell stage.

Our results provide evidence for a previously undescribed mechanism of X-chromosome regulation in XX embryos which functions before the establishment of somatic dosage compensation in *C. elegans*. Specifically, our data suggests a model in which a transient X chromosome imprint may provide sex chromosome dosage compensation in the early embryo by maintaining a silenced chromatin status established during gametogenesis. That is, passage through the male germline establishes a silencing epigenetic imprint on the X chromosome that persists through fertilization and early cleavage divisions until dosage compensation can be initiated in the XX embryo. Indeed, decay of the imprint may provide the dosage signal to activate somatic compensation. Meta-stable continuation of germ line silencing of the paternal X chromosome may be a conserved mechanism of X chromosome regulation in the early XX embryo.

**602680. AN UNUSUAL MUTATION IN CDK-1 REVEALS THAT THE MAJOR EMBRYONIC CYCLIN DEPENDENT KINASE HAS A ROLE IN THE REGULATION OF CELL POLARITY**

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CDK-1, previously called NCC-1, has been shown to be required for both meiosis and mitosis in the early embryo. Hypomorphic mutations show zygotic defects including sterility, uncoordinated movements and small size due to loss of post-embryonic cell divisions. RNAi revealed that removal of maternal and zygotic cdk-1 leads to a one cell arrest (Boxem and van den Heuvel, 1999). In contrast, a mutation we discovered in screens for maternal effect mutations affecting cell fate, cdk-1(ne236), has no discernible cell cycle defect and instead alters the polarity of the EMS cell division. The EMS blastomere of *C. elegans* divides asymmetrically after orientating its spindle along the plane of its previous division and produces two daughters with distinct cell fates. Both the EMS cell fate and its division orientation are specified via signaling from the neighboring blastomere P2. The cdk-1(ne236) mutation completely converts the normally anterior/posterior orientation of the EMS division to left/right, even in intact embryos. Besides the change of EMS spindle orientation, this mutation also causes cell fate changes. ne236 interacts with some components of wg/WNT signaling, enhancing partially penetrant gutless phenotypes. In addition, this mutation causes the C blastomere to produce cell types normally made by EMS. Despite the cell fate and EMS spindle defects, cdk-1(ne236) mutants maintain all measurable cell cycle functions and make well differentiated embryos with the normal number of cells. The mutation has no obvious zygotic defects, is 100% maternal effect lethal, and is fully recessive to wild type. We therefore propose that rather than completely eliminating CDK-1 function, the mutation is affecting one specific aspect of CDK-1 function. We are using further forward and reverse genetics to identify downstream components of CDK-1 in spindle orientation and cell fate in the early *C. elegans* embryo. Our genetic experiments have revealed that the C fate defect is suppressible by lowering the dose of some of the *C. elegans* cyclin B genes. This is intriguing since the cdk-1(ne236) mutation is in a residue predicted to make contact with cyclins. In addition, we have found that destabilizing microtubules can partially restore the EMS spindle orientation. This suggests that in the mutant embryos the CDK-1-Cyclin B interaction is subtly altered, perhaps leading to a stabilization of the microtubules. We are carrying out biochemical assays to test this model\*.

\* We thank Dayalan Srinivasan, Mike Boxem and Sander van den Heuvel for sharing unpublished reagents.

**14979. Evolution and function of the secretin family of serpentine receptors**

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The secretin family of serpentine receptors is an ancient family that has members in *C. elegans*, *Drosophila*, and vertebrates. Our alignment data suggests at least five member genes in the common ancestor of these lineages. Further duplications of each of the genes within the individual lineages lead to further expansion of the family. The five subfamily branches with their *C. elegans* members are flamingo-cefla(f15b9.7), latrophilin(lat-1(b0457.1) and lat-2(b0286.2)), calcitonin receptor(c13b9.4), corticotrophin releasing factor receptor(zk643.3), and secretin receptor(c18b12.2). Most of the studied members of this family are involved in regulated secretion in response to a peptide ligand. We are currently attempting to understand the organismal biology of this family in *C. elegans* by expression and phenotypic analysis and by linking these receptors to the peptide ligands that activate them.

One of the aspects that we are focusing on is the latrophilin subfamily. Lat-1 expression starts after gastrulation and continues into the adult. It is expressed in the hypodermal, pharyngeal and some neuronal cells in the embryo. During larval development it is expressed in mechanosensory and interneurons in the head and tail, gland cell in pharynx, gonadal sheath cells, spermatheca, uterine epithelial cells and intestinal cells. We are focusing on the following aspects of the expression pattern: expression in alternating dorsal hypodermal cells during intercalation, expression and localization to the apical surface in the pharyngeal primordium cells, expression in ventral cord neurons and gland cell in the dauer, and expression in the reproductive organs. Phenotypes from RNAi have correlated with some of these expression patterns. These include elongation defects, abnormal pharyngeal development and attachment, small eggs, and ovulation defects in the injected animal. Lat-2 is expressed in the g1 gland cell and arcade cells in the head in which it localizes to the syncytial end of the process, and the expression cycles with the molts.

**766822. Exclusion of germ plasm components from somatic lineages by localized protein degradation.**

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In *Drosophila*, *C. elegans*, *Xenopus* and Zebrafish embryos, germ plasm components segregate with the germ lineage and are not maintained in somatic lineages. Here we show that, in *C. elegans*, this process involves active degradation of germ plasm components in somatic lineages. PIE-1, POS-1 and MEX-1 are germ plasm components that share a pair of CCCH zinc fingers (ZF1 and ZF2). When fused to GFP, ZF1s from each protein cause GFP to be degraded specifically in somatic blastomeres. Consistent with a role in protein degradation, mutations in ZF1 cause abnormal stabilization of PIE-1 in somatic blastomeres. We have identified a novel protein ZIF-1 that binds to the ZF1 of PIE-1. Depletion of ZIF-1 by RNAi causes PIE-1, POS-1 and MEX-1 to be maintained in somatic lineages and results in embryonic lethality. A yeast two-hybrid screen revealed that ZIF-1 can bind to Elongin C. Elongin C in mammals functions with Cul2 as an E3 ubiquitin ligase that targets specific proteins for ubiquitination and degradation. Consistent with this role, RNAi depletion of Elongin C, Cul2 or the E2 ubiquitin conjugation enzyme Ubc5 blocks ZF1-dependent degradation in somatic blastomeres. These results suggest that a ZIF-1/Elongin C/Cul2/Ubc5 complex targets ZF1 containing proteins for degradation in somatic cells. We propose that localization of germ plasm components involves both targeting to the germ lineage and rapid degradation in somatic lineages.

Many thanks to Edward Kipreos for the cul-2 clone and helpful discussions.



**551260. Molecular localization of *nT1* breakpoints and the role of EGL-18 and ELT-6 GATA factors in vulval development**

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*egl-18* and *elt-6*, adjacent genes encoding GATA factors, are redundantly required for seam cell development and vulval development.<sup>1,2</sup> *egl-18* chromosomal mutants, in which *elt-6* is presumably active, show partially penetrant lethality and relatively mild defects in vulval development. In contrast, RNA-mediated interference (RNAi) of both *egl-18* and *elt-6* activity results in fully penetrant lethality, and animals rescued for lethality by a transgene with a partial promoter show a strong vulvaless phenotype: vulval precursor cells (VPCs) often fuse with the surrounding epidermis or undergo fewer than normal number of cell divisions.

*nT1* was isolated in a screen for vulvaless mutants, and is a reciprocal translocation between chromosomes IV and V.<sup>3</sup> Examination of *nT1* homozygous animals carrying a transgenic *ajm-1::GFP* adherens junction marker indicates that most VPCs fuse with the surrounding epidermis in the late L2 and early L3 stages. *nT1* mutants are thin and grow slowly, but exhibit only a small percentage of lethality. Because the *nT1* vulval phenotype resembled that observed in *egl-18/elt-6* RNAi animals, and because an *nT1* breakpoint was mapped near *egl-18/elt-6* on LGIV,<sup>4</sup> we hypothesized that the *nT1* phenotypes might be due to a disruption of *egl-18* and *elt-6* function in the vulva. Two lines of evidence support the hypothesis. First, *nT1* does not complement *egl-18*: *nT1/egl-18* animals exhibit an intermediate level of vulval defects. Second, through a series of PCR analyses and a subsequent inverse-PCR analysis, we have located the breakpoints of *nT1* near *egl-18* on LGIV, approximately 12Kb upstream of the *egl-18* ATG, and in the *act-3* gene on LGV. Our hypothesis is that there is an important vulval enhancer for *egl-18/elt-6* further upstream of the *nT1* breakpoint. We are currently attempting to find the possible vulval enhancer by examining expression patterns of a series of enhancer constructs and testing *egl-18* transgenes for rescue of the *nT1* vulvaless phenotype. If *nT1* indeed represents a vulva-specific *egl-18 elt-6* double mutant, it will confirm previous RNAi results suggesting that EGL-18 and ELT-6 are important regulators of cell fusion and fates in the vulva,<sup>2</sup> and will be very useful in helping us understand the role these GATA factors play during vulval development.

<sup>1</sup> Koh and Rothman, Development 128: 2867-2880, 2001

<sup>2</sup> Koh et. al., IWM, 2002

<sup>3</sup> Ferguson and Horvitz, Genetics 110:17-72, 1985

<sup>4</sup> Clark and Baillie, WBG 10(1): 81

**361270. A screen for suppressors of rod-like lethality of *lin-1 eor-1* double mutants**

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Ras/MAPK signaling pathway regulates several aspects of *C. elegans* development, including development of the excretory system and the vulva. Loss or reduction of ras-mediated signaling input causes rod-like larval lethality or a vulvaless phenotype. In an attempt to identify new components of Ras signaling pathway, we have initiated a genetic screen for suppressors of rod-like lethality of *lin-1 eor-1* double mutants. *lin-1* encodes an ETS transcription factor and is best known for its negative role in Ras signaling pathway as evidenced by a multi-vulva phenotype in loss-of-function mutants. *eor-1* encodes a BTB/Zinc finger protein, similar to human promyelocytic leukemia zinc finger protein (hPLZF), and has a positive role in Ras signaling.<sup>1</sup> *eor-1* was identified in a genetic screen for enhancers of a weak *lin-45 raf* mutation, and loss-of-function mutations result in a low percentage of egg-laying defects and rod-like lethality.<sup>2</sup> Surprisingly, *lin-1 eor-1* double mutants show fully-penetrant rod-like larval lethality, suggesting *lin-1* may have a positive role in Ras-mediated signal transduction.<sup>1</sup>

We mutagenized a strain of *lin-1(e1275) eor-1(cs28)* double mutants rescued for lethality by a wild-type *eor-1* transgene. In our initial screens of approximately 1500 EMS mutagenized genomes, we have isolated three candidate suppressors of *lin-1 eor-1* lethality. *cs56 V* is a dominant suppressor: heterozygous *cs56* worms survive in *lin-1(e1275) eor-1(cs28)* background, and homozygotes in *lin-1(+)* *eor-1(+)* background either arrest as late larvae or become sterile adults. *cs57 IV* is a recessive suppressor, and is linked to *lin-1* and *eor-1*: its phenotype in *lin-1(+)* *eor-1(+)* background has not yet been determined. *cs58 IV* is a dominant suppressor: heterozygous *cs58* worms survive in *lin-1(e1275) eor-1(cs28)* background, and *cs58 lin-1(e1275) eor-1(cs28)* triple mutants arrest as lumpy, non-rod-like L2-stage larvae. Further characterization and cloning of these mutations are underway.

<sup>1</sup>Howard and Sundaram, IWM, 2001.

<sup>2</sup>Rocheleau et. al., Genetics, in press.

**150690. ASYMMETRIC PROTEIN LOCALIZATION IN ZYGOTES: A ROLE FOR PROTEIN DEGRADATION?**

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PIE-1 is a CCCH finger protein, which, like P granules, becomes enriched in the posterior half of the 1-cell embryo before the first cleavage. We are interested in the mechanisms that mediate this asymmetric localization. Using a PIE-1:GFP fusion, Kim Reese has shown that PIE-1 localization in the zygote depends on a 160 amino-acid domain at the carboxy-terminal end of the protein (Reese et al, 2000). She also found that PIE-1 can bind to MEX-5 and MEX-6 in a yeast two hybrid assay (K. Reese, unpublished). The Priess lab has shown that MEX-5 and MEX-6 are two redundant CCCH finger proteins, which become localized to the anterior end of the zygote, in a domain opposite that of PIE-1 (Schubert et al., 2001; K. Reese, unpublished results). MEX-5 and MEX-6 are required for PIE-1 localization: in mex-5;mex-6 double mutants, PIE-1 remains uniformly distributed in the zygote (Schubert et al., 2001). We wondered whether the physical interaction between MEX-5/6 and PIE-1 is required for PIE-1 localization.

To address this question, we analyzed PIE-1 deletion derivatives for their ability to 1) localize in vivo and 2) bind MEX-5 and MEX-6 in the yeast 2-hybrid system. We found that a deletion into the 160aa carboxy-terminal domain completely eliminates PIE-1's ability to localize in zygotes and also eliminates the PIE-1/MEX-5/6 interaction. This result supports the idea that this interaction is required for localization in vivo. However, we also observed pie-1 mutants that could bind MEX-5/6 in yeast but were severely impaired in their ability to localize in vivo. These results suggest that binding to MEX-5/6, although necessary, may not be sufficient for localization.

Given that MEX-5/6 localize in a pattern opposite that of PIE-1, how could an interaction between the two be required for PIE-1 localization? MEX-5/6 and PIE-1 start out uniformly distributed throughout the cytoplasm. We propose that as MEX-5/6 becomes enriched in the anterior, it binds to PIE-1 and targets it for degradation locally, thereby restricting PIE-1 to the posterior.

**961957. Does the heterochronic gene *lin-41* interact with the *C. elegans* homologues of the spatial patterning genes, *nanos* and *pumilio*?**

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Developmental timing is controlled by heterochronic genes, mutations in which cause changes in the relative timing of developmental events. Genetic studies in *C. elegans* have ordered the known heterochronic genes in a regulatory pathway. Late acting genes in this pathway include the small untranslated RNA, *let-7*; two of its target genes, *lin-41* and *hbl-1* (the *C. elegans* *hunchback* gene); and the transcription factor *lin-29*. LIN-29 is thought to be responsible for coordinating specific cellular fates at the larval to adult transition. Its expression appears to be post-transcriptionally repressed by *lin-41* and *hbl-1* until the L4 stage when LIN-29 protein is first observed. *let-7* is thought to relieve *lin-29* repression by binding to complementary regions in the 3 UTRs of *lin-41* and *hbl-1*, and thereby down regulating expression of *lin-41* and *hbl-1*. We are testing the hypothesis that *lin-41* acts through *hbl-1* to regulate expression of *lin-29*.

LIN-41 belongs to the same RBCC-NHL family of proteins as the *Drosophila* Brain Tumor (Brat) protein. Intriguingly, Brat is involved in the translational repression of *Drosophila hunchback*, supporting the role of LIN-41 as a translational repressor. To facilitate this repression, Brat interacts with Nanos and Pumilio proteins at specific regulatory elements in the *hunchback* 3 UTR called Nanos Response Elements (NRE). We are therefore taking an RNAi approach to look for genetic interactions between *lin-41* and the *C.elegans* homologues corresponding to *Drosophila nanos* and *pumilio*.

**518836. Using RNAi to identify regulators of neural differentiation gene *pag-3***

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Mutations in the *pag-3* gene result in an uncoordinated phenotype and misexpression of touch neuron-specific markers in the BDU cells. PAG-3 is a zinc finger protein expressed in several different neuron types, including the touch cell receptors, the BDU interneurons and the motor neuron lineages. Mutations of *pag-3* cause cell lineage defects in the ventral nerve cord. Reiterations of the division of the Pn.aa cells give rise to extra cell deaths and extra VC motor neurons and the loss of VA and VB cells. *pag-3* is expressed in the Pn.aa cells, the Pn.aaa/p cells and their final descendants, the VA, VB and VC motor neurons.

We are testing candidate upstream regulators of *pag-3* using RNAi on animals carrying integrated *pag-3::gfp* array *IsIs5*. To precisely stage the animals during the P cell divisions, we have crossed in integrated *hlh-8::gfp* array *ayIs6*, which stains the M myoblast cells during their concurrent divisions. The candidate regulators we are testing include three basic helix-loop-helix genes: *lin-32*, *hlh-2* and *hlh-3*. *pag-3* is the *C. elegans* homolog of *Drosophila senseless*, which is downstream of *atonal*, the homolog of *lin-32*. Based on their patterns of expression, *hlh-2* and *hlh-3* may regulate *pag-3*. Since *pag-3* is expressed in cells which undergo anterior/posterior divisions, we are also testing the effect of *pop-1* RNAi on *pag-3::gfp* in hatched larvae.

An alternative to this directed approach is to screen for suppressors of the *pag-3* uncoordinated phenotype. Large plates will be prepared with a food target at one end. Mutagenized *pag-3* animals carrying an integrated touch neuron-specific marker will be placed on the opposite side of the plates, and the first worms to reach the target will be selected. Revertants of the motility phenotype will be examined for BDU misexpression of the touch neuron marker. The suppressors we isolate will be separated into those that do or do not suppress the misexpression phenotype.

**923282. Regulation of Programmed Cell Death in *C. elegans* Tail Spike Cells**

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While a core pathway governing apoptosis in *C. elegans* has been identified, the regulation of this process in specific cells remains largely unexplored. The majority of the 131 somatic cells in *C. elegans* that undergo programmed cell death (PCD) die several minutes after being generated. The tail spike cells, a pair of cells implicated in formation of the tail spike, differ in that they persist for several hours before undergoing apoptosis. During this time, the pair of cells fuses and extends a cytoplasmic process that projects towards the rear of the animal. The elaborate sequence of events that occurs prior to the tail spike cell's demise suggests that tail spike PCD may not be cell-intrinsic, but that it may instead rely upon upstream cellular events to signal the initiation of PCD. We wish to determine whether completion of the tail spike cell's complex cellular program is a necessary precursor to PCD, and to further understand the molecular mechanisms underlying the regulation of apoptosis in this cell.

Post-translational control of CED-3 and homologous mammalian caspases via activation by upstream factors is well-established; however, the role of transcriptional control of these proteases remains unaddressed. Using a GFP reporter construct driven by a minimal *ced-3* promoter, we demonstrated that *ced-3* is transcriptionally upregulated in the tail spike cells shortly before they die. Transcriptional activation of *ced-3* may thus be a key step in the initiation of tail spike PCD. We wish to understand the relationship between *ced-3* upregulation and tail spike fusion and process extension, and to identify factors responsible for increased *ced-3* expression. To this end, we plan to isolate a tail spike-specific promoter region. We showed that the *C. briggsae ced-3* promoter shares several regions of homology with its *C. elegans* counterpart. These regions will be used to guide our promoter analysis studies. Using time-lapse movies of *ced-3::gfp* expression, we hope to follow *ced-3* transcriptional upregulation more closely. The temporal relationship between *ced-3* transcriptional upregulation and tail spike fusion will be assessed in embryos expressing both *ced-3::rfp* and *ajm-1::gfp* reporter constructs; AJM-1 localizes to adherens junctions and thus facilitates identification of fusing cells. The role of fusion in mediating *ced-3* upregulation and tail spike cell death will be assessed in *eff-1* animals, which are deficient for cell fusion, and in worms in which one of the tail spike cells has been ablated. We hope to learn whether tail spike cell death is dependent upon cues originating from the completion of specific cellular events, and to identify these cues by performing a screen for genes that when mutant lead to aberrant *ced-3* expression in the tail spike cells. We expect to identify transcription factors as well as any cell-cell signaling components that may be involved in mediating tail spike cell death.

**916547. Genetic and molecular analysis of polyglutamine neurotoxicity in *C. elegans***

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At least eight hereditary neurodegenerative disorders, including Huntington's Disease, have been identified in which the disease locus expresses a protein that contains an expanded glutamine tract. The mechanism by which these polyglutamine (polyQ) repeats cause neurodegeneration and cell death is unknown. We have established a *Caenorhabditis elegans* model system to identify proteins involved in polyglutamine (polyQ) neurotoxicity. N-terminal fragments of the Huntington's Disease protein huntingtin (Htn) containing polyQ tracts of 2, 23, 95 or 150 residues are expressed in the ASH sensory neurons (PNAS 96, 179-184, 1999). Expression of Htn-Q150, but not Htn-Q2, Htn-Q23 or Htn-Q95, causes age dependent ASH degeneration without ASH cell death in aged animals. ASH neurodegeneration was determined by the ability of the exposed sensory endings of ASH neurons to uptake lipophilic dyes. ASH cell death was assessed using immunohistochemical techniques or GFP expression. PolyQ-mediated ASH neurodegeneration was partially dependent on *ced-3* caspase function and modestly dependent on *crt-1* function suggesting the involvement of both the apoptotic and necrotic-like cell death pathways. We are currently investigating the role of the cell survival pathways in our *C. elegans* model of polyQ-mediated neurodegeneration. To identify genes that normally protect neurons from polyQ-mediated neurodegeneration, we performed an F2 EMS screen for mutations in genes that exacerbated polyQ-mediated ASH neurodegeneration. After screening 30,000 mutagenized animals, 5 mutant strains were identified that carried mutations in the *polyQ enhancer-1* (*pqe-1*) gene. In *pqe-1* mutant animals, ASH neuronal cell death is dependent upon the presence of expanded polyQ tracts (Htn-Q95, Htn-Q150), as ASH neurons expressing N-terminal fragments of huntingtin with shorter polyQ tracts (Htn-Q2, Htn-Q23) are unaffected. We found that PQE-1 proteins containing a glutamine/proline-rich and a charged domain are critical for *pqe-1* function. Overexpression of PQE-1 protects *C. elegans* neurons from the toxic effects of an expanded huntingtin fragment. Overt phenotypes have not been observed in *pqe-1* mutant animals other than enhancement of polyQ neurotoxicity. Mutations in *pqe-1* do not impair ASH survival and function, or accelerate apoptotic or necrotic-like cell death pathways. Molecular and biochemical experiments are underway to address the connection between PQE-1 function and polyQ neurotoxicity. To identify genes that are required for poly-Q mediated neurodegeneration, we have initiated a genetic screen to isolate mutations that suppress polyQ-mediated ASH neurodegeneration. Identification and characterization of genes isolated from these genetic screens will provide insight into pathogenic mechanisms underlying polyQ-induced neurodegeneration and cell death. Finally, we are using the well characterized *C. elegans* nervous system to gain insight into the cellular mechanism of neurodegeneration caused by mutations in *Nna-1* (Science 295, 1904-1906, 2002). We are currently investigating the role of the two *C. elegans* *Nna-1*-related genes in the process of neurodegeneration.

**951350. Potential Roles of CBP, CREB, and HDAC in Polyglutamine Induced Neurodegeneration in *C. elegans***

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Huntington's disease and at least eight other neurodegenerative diseases result from expansions of polyglutamine tracts. Though it is clear that these expansions are toxic in many cell types, the molecular basis of polyglutamine induced cell death in the nervous system is unknown. Inhibitors of histone deacetylases (HDAC) reduce polyglutamine induced cell death in cell culture and *Drosophila*. (McCampbell, 2001; Steffan, 2001). A transcription activator, cAMP response element binding protein (CREB), and a histone acetyltransferase, CREB binding protein (CBP) have been found in mutant polyglutamine aggregates. (McCampbell, 2000) These data suggest that expanded polyglutamine may alter transcription by sequestering glutamine rich proteins such as CBP and CREB perturbing their function. This hypothesis predicts that 1) overexpression of CBP and CREB protects cells from polyglutamine induced degeneration and 2) CBP or CREB loss of function mutations enhance polyglutamine induced neurodegeneration in *C. elegans*. We have established a *C. elegans* model to address the molecular mechanism of polyglutamine toxicity. N-terminal fragments of human huntingtin with varying lengths of glutamine stretches were expressed in *C. elegans* sensory neurons. (Faber, 1999) Up to 30% of the neurons expressing mutant huntingtin fragment degenerate in 8 day old animals. A putative role of CREB, CBP, and HDAC in polyglutamine induced neurodegeneration is being examined using loss of function alleles. Additional experiments are underway to look at overexpression of these genes. These experiments will further define the role of the CREB/CBP pathway in polyglutamine expansion diseases.



**890931. Identification of Effectors of *zyg-1*-Dependent Centrosome Duplication**

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The centrosome is the main microtubule organizing center of the cell and a major determinant of mitotic spindle form. The replication of this organelle must be tightly controlled so that it duplicates precisely once per cell cycle. Despite the importance of this event, the molecular mechanisms that regulate it remain largely unknown. In *C. elegans*, the ZYG-1 protein kinase localizes to centrosomes and is required for centrosome duplication. To identify other factors that act with ZYG-1 to control centrosome duplication, we have taken two approaches. First, using a conditional allele we have screened for suppressor mutations that allow growth of homozygotes at the restrictive temperature. In a screen of 314,000 EMS-mutagenized haploid genomes, we identified 51 candidate suppressors. Our initial analysis indicates that this set contains both dominant and recessive suppressors. Second, we have performed a yeast two-hybrid screen to identify open reading frames that exhibit a *zyg-1*-dependent interaction. Nine such candidates have been confirmed and are currently being analyzed. Further analysis of these genes should allow us to identify factors that play critical roles in centrosome replication.

**616994. Two vacuolar ATPase B subunits exist in the nematode genome, and *spe-5* encodes one that is required for spermatogenesis**

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Mature spermatozoa in all animals are streamlined, specialized cells that contain only the components required to find, approach, and fertilize oocytes. In *C. elegans*, asymmetric segregation of cytoplasm occurs at the end of spermatogenesis when all the essential components are placed into haploid spermatids budding from a residual body. The residual body contains unnecessary components and will eventually be reabsorbed. A specialized, Golgi-derived organelle called the fibrous body-membranous organelle (FB-MO) contains proteins necessary for functional spermatozoa. All FB-MOs segregate into spermatids that are budding from a residual body, and mutants affecting the morphogenesis or proper segregation of FB-MOs have been identified.

*spe-5* mutants rarely form spermatids and instead form terminal spermatocytes that have completed meiosis. These spermatocytes have an abnormal cratered appearance caused by the partial distension of the MO double membrane from the FB. The *spe-5* gene encodes a B subunit of the vacuolar (H<sup>+</sup>)-ATPase, which is a multisubunit machine that hydrolyzes ATP to pump protons across a membrane and establish a proton gradient. Experiments using pH-sensitive indicators have shown that the MOs in spermatids have a low luminal pH (K. HILL and S. W. L'HERNAULT, unpubl.), so V-ATPases and therefore SPE-5 may be located on the MOs. Antibodies and GFP fusion constructs will be used to confirm the localization of SPE-5. A gene located on the X chromosome encodes another B subunit with 85% amino acid identity to SPE-5, and it is not yet known if it can functionally replace *spe-5*. The existence of two B subunits in the genome suggests that while both likely contribute to the activity of the V-ATPase, they may differ from each other by specialized functions. This hypothesis initially will be addressed by a two-hybrid assay that may reveal different protein binding specificities.

**560375. LOCALIZATION DYNAMICS OF THE A/P POLARITY MACHINERY IN THE 1-CELL EMBRYO.**

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Polarization of the *C. elegans* zygote depends on 6 cortically enriched proteins (PAR-1/6) and two cytoplasmic proteins (MEX-5/6) that function together to localize determinants (e.g. PIE-1) in response to a polarity cue associated with the sperm asters. Using time-lapse fluorescence microscopy, we have analyzed the localization dynamics of PAR-2, PAR-6, MEX-5, MEX-6 and PIE-1 in wild-type and mutant embryos. These studies reveal that polarization of the *C. elegans* zygote involves two genetically and temporally distinct phases. In the first phase, the sperm asters exclude PAR-6/PAR-3/PKC3 complex from the nearby cortex, allowing PAR-2 to accumulate in what will become the posterior end of the embryo. This first phase requires the activity of the non-muscle myosin NMY-2, and the 14-3-3 protein PAR-5. In the second phase, the ring finger protein PAR-2 maintains anterior-posterior polarity by excluding the PAR-6/PAR-3/PKC3 complex from the posterior. Our results also suggest that PIE-1 localization depends on local inhibition of MEX-5/6 by the posteriorly-enriched PAR-1 kinase.

**311993. The *C. elegans* Schnurri homolog SMA-9 in postembryonic mesodermal cell fate specification**

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We are interested in understanding how mesodermal cell fates are specified. The postembryonic mesodermal lineage, the M lineage, provides a valuable system for these studies. During postembryonic development, the single blast cell M divides characteristically and reproducibly to generate 14 striated bodywall muscles, 2 un-differentiated sex myoblasts that give rise to 16 non-striated sex muscles (vulval and uterine muscles), and two non-muscle cells (coelomocytes). Previous work from us and others have shown that correct patterning of the M lineage involves both lineage specific intrinsic factors and cell-cell signaling. We are interested in identifying additional factors in M lineage fate specification and characterizing functional interactions among these different factors.

Using GFP-based mutagenesis screens, we have isolated a number of mutants that have defects in M lineage patterning. Three of these mutants were allelic to *sma-9*, a mutation affecting the body size of the animal. *sma-9* mutations caused a coelomocyte to sex myoblast fate transformation in the M lineage. We have cloned *sma-9* and shown that its product contains multiple C2H2 type zinc fingers and is the *C. elegans* homolog of the *Drosophila* Schnurri protein (see abstract by J. Liang and C. Savage-Dunn). Schnurri (Shn) in flies has been shown to play critical roles in the TGF-beta signaling pathway. The TGF-beta signaling pathway, however, does not appear to play an important role in M lineage cell fate specification. Instead, we observed intriguing genetic interactions between *sma-9* and *lin-12*. Our current efforts are directed towards a better understanding of how SMA-9 functions in M lineage fate specification.

## 206820. Characterization of the Molecular Determinants of EGL-15 FGFR Signaling Specificity

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The *C. elegans* fibroblast growth factor receptor (FGFR) EGL-15 is involved in two major functions: an early essential function and a cell migration guidance event. A host of hypomorphic *egl-15* mutations have been identified. Most can be placed into an allelic series based on the degree of essential function activity. However, four mutations that specifically affect SM chemoattraction cannot be fit into this allelic series. These mutations have identified molecular determinants of the EGL-15 FGFR that specify its role in cell migration guidance.

The *C. elegans* FGFR contains an extra domain in its extracellular portion when compared to other FGFRs. This domain, termed the EGL-15-specific insert, is encoded entirely by exon 5 of *egl-15*. We have discovered an alternative exon 5, exon 5A, that lies between the original known exon 5 and exon 6. Alternative splicing at exon 5 creates two different isoforms of EGL-15: EGL-15(5A) containing exon 5A; and EGL-15(5B), containing the original exon 5, now termed exon 5B. Three of the four migration-specific alleles of *egl-15* have mutations in exon 5A that would eliminate the EGL-15(5A) isoform in these mutants. Based on the specific phenotypes of these mutants and additional transgenic experiments, EGL-15(5A) is required for SM chemoattraction, whereas EGL-15(5B) is both necessary and sufficient for the essential function mediated by this receptor.

*C. elegans* has two FGF ligands for EGL-15: EGL-17 and LET-756. The phenotypic consequences due to loss of these ligands mimics loss of the individual receptor isoforms. EGL-17, like EGL-15(5A), is required specifically for SM migration, while LET-756, like EGL-15(5B), plays an essential role. These similarities suggest a model in which isoform function is specified by the activating ligand. To test this model, we determined whether the ligands are functionally interchangeable with respect to the two processes mediated by EGL-15.

Through a series of promoter swapping experiments we have demonstrated that the ligands are at least partially interchangeable but that signaling specificity is also conferred by the receptor isoform independent of the ligand used. When expressed from the *egl-17* promoter, LET-756 can act as a chemoattractant to rescue the SM migration defect of *egl-17(n1377)* mutants, although not as robustly as EGL-17. Conversely, EGL-17 can act as the ligand for the essential function in place of LET-756, although less robustly than LET-756. Importantly, the ability of either ligand to mediate chemoattraction is completely dependent on EGL-15(5A) activity. While both ligands mediate the essential function independent of EGL-15(5A). A significant part of functional specificity is therefore encoded within the receptor isoform.

We have begun to address how the 5A and 5B domains alter EGL-15 function. By removing both alternative exon 5 sequences [EGL-15( $\Delta$ 5)], we have recreated the structure of other FGFRs. This construct retains all of the functions of EGL-15(5B) but not the SM chemoattraction function of EGL-15(5A). This result suggests that the 5A domain adds the SM chemoattraction function to the basal EGL-15( $\Delta$ 5) FGFR, and that the 5B domain is not necessary for effective mediation of the essential function. Consistent with this conclusion, a comparison between the *C. briggsae* and *C. elegans* 5A and 5B domains has revealed that 5A is more conserved (89% identical) than 5B (64% identical). We are trying to identify residues critical for 5A function by genetic screens and site-directed mutagenesis.

The fourth migration-specific allele, *egl-15(n1457)*, identifies the carboxy-terminal domain as critical specifically for SM chemoattraction. This allele contains a nonsense mutation that truncates both isoforms by removing the C-terminal region, yet the only apparent defect is in SM migration. This observation suggests that the C-terminal region provides a binding site for proteins that mediate SM chemoattraction. We are refining the region by structure-function analysis and identifying interacting partners through a two-hybrid screen.

By analyzing the functions of these two specificity determinants on EGL-15, we hope to understand the mechanisms by which EGL-15 can mediate its different functions.

### 770147. MicroRNAs in *C. elegans*

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Two small temporal RNAs, *lin-4* and *let-7*, control developmental timing in *Caenorhabditis elegans*. These two regulatory RNAs are members of a large class of 21-24-nucleotide non-coding RNAs, called microRNAs (miRNAs). We have cloned more than 70 miRNAs in *C. elegans* and confirmed their expression by Northern. We have also developed the program MicroScan, which computationally identifies most of the cloned miRNAs as well as dozens of additional candidates. This computational analysis indicates that there are no more than 140 miRNAs in *C. elegans*, which represents 0.7 percent of the predicted *C. elegans* genes. We are investigating the expression of the additional predicted miRNAs.

Nearly all the *C. elegans* miRNA genes are conserved in *C. briggsae*, and some are conserved in *Drosophila* and human. Quantitation of the number of miRNA molecules per animal indicates that their cloning frequencies (ranging from 1 to >300) roughly correlate with their molecular abundance. MicroRNAs have diverse expression patterns during development: miRNAs encoded in a single genomic cluster are coexpressed during embryogenesis; three *let-7* paralogs are temporally coexpressed with *let-7* in L3; other miRNAs begin to be expressed during each of the other stages of larval development; and still other miRNAs are expressed constitutively throughout development. The abundance of miRNA genes, their evolutionary conservation, and their expression patterns imply that, as a class, miRNAs have broad regulatory functions in animals.

**815339. Maternal-effect suppressors of the *cdc-25.1(gf)*-dependent cell cycle defects in the intestinal lineage of *C. elegans*.**

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In order to understand the nature of developmental pathways that regulate the cell cycle in specific cell types, our lab has been studying mutants that affect cell division in the intestinal lineage. From a screen to isolate such mutants, we have characterized a dominant, maternal-effect, gain-of-function mutation in the cell cycle phosphatase, *cdc-25.1*, which specifically affects intestinal cells. *cdc-25.1(gf)* mutants undergo an extra round of mitosis between the 8E and 16E (E descendants/intestinal cells) stages of embryonic development, resulting in a doubling of the total number of intestinal cells in adult worms. This mutant phenotype is caused by a G47D substitution in the N-terminal region, which has been shown to be the regulatory region of the phosphatase from studies performed in other systems. Anti-CDC-25.1 staining is detected in all nuclei of early embryos before gastrulation (28-cell stage), in both wild-type and *cdc-25.1(gf)* animals, however, the gain-of-function protein is expressed up to and beyond the 100-cell stage, unlike its wild-type counterpart.

As little is known regarding the developmental regulation of *cdc-25.1* N-terminal domain, we set out to identify regulatory mechanisms that would address why intestinal cells are uniquely competent to respond to this gain-of-function phosphatase as well as and why the CDC-25.1(gf) protein is not downregulated at the appropriate time. In order to understand the mechanism of regulation of *cdc-25.1*, we performed a semi-clonal screen for viable maternal-effect mutants that suppress or enhance the extra intestinal cell phenotype of *cdc-25.1(gf)* worms carrying a GFP reporter specific to the intestinal lineage, *elt-2::GFP*.

Following the analysis of 5,456 haploid genomes, we report the isolation of five maternal-effect, recessive mutations that suppress the *cdc-25.1(gf)* mutant phenotype. All five of the suppressors were shown to complement in the F2 generation, four of which are extragenic to the *cdc-25.1* locus. One intragenic suppressor was isolated and was found to carry a L273F substitution in a highly conserved catalytic domain of the CDC-25.1 protein in addition to the original G47D lesion. Only one of the suppressors displays an additional impenetrant sterile phenotype, and the other suppressors have no apparent phenotype when outcrossed from the *cdc-25.1(gf)* background. With the exception of the intragenic suppressor, all suppressors were shown to segregate independently from chromosome I markers. A snip-SNP approach was used to map the suppressor *rr40* to the central region of chromosome III. Three-point mapping is presently underway.

**867051. Developmental control of nuclear divisions in the *C. elegans* intestine.**

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The intestinal lineage of *C. elegans* undergoes three different developmentally regulated cell cycles throughout the life cycle. First, the founder E cell undergoes several mitotic divisions to give rise to 20 intestinal cells at the end of embryogenesis. Then, during the first larval stage, intestinal cells of the hindgut perform a round of nuclear division without cytokinesis to become binucleate. Following this division, all intestinal cells undergo endoreplication at each molt to become polyploid at the adult stage. In a screen designed to isolate developmental regulators of the cell cycle, we isolated an allele (*rr33*) based on its ability to phenocopy the *cki-1* (RNAi) phenotype. This mutant undergoes an extra round of nuclear division early in the L2 stage and gives rise to tetranucleate intestinal cells. Intestinal cell numbers are unchanged at the hatch in *rr33*, but when they reach adulthood they have  $50.33 \pm 4.64$  ( $n=39$ ) intestinal nuclei with a maximum of 63 nuclei compared to the 32-34 nuclei observed in N2. Through lineage analysis we found that all the posterior intestinal cells are capable of executing an extra round of nuclear division early in the L2 stage with no obvious bias among the intestinal rings. Genetic analysis revealed that this allele is recessive and demonstrates a maternal effect, but can be zygotically rescued. Mapping of *rr33* using SNIP-SNP mapping and three-factor approaches predict that this mutation is on the right arm of chromosome I between the markers *dpy-5* and *unc-13*. Transformation rescue is underway to determine the molecular identity of the *rr33* mutation.

In order to better understand the genetic basis of how this switch from mitosis, to karyokinesis, to endocycles occurs in this lineage, we have performed a maternal effect screen using an *elt-2::GFP* marker for animals that either phenocopy *rr33* mutant or mutants with less intestinal nuclei. After screening 1500 haploid genomes we isolated three mutants with less intestinal nuclei that fall into three different complementation groups. All these mutants hatch with the wild-type complement of intestinal cells but are unable to perform the first nuclear division at the L1 stage. Further characterization and mapping of these mutants is underway in the laboratory.



**222957. IDENTIFICATION AND CHARACTERIZATION OF SYNMOV SUPPRESSORS, INCLUDING A HOMOLOG OF THE CHROMATIN-REMODELING ATPASE ISWI**

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The receptor tyrosine kinase (RTK)/Ras, Notch, and Wnt signal transduction pathways play important roles in the formation of the *C. elegans* vulva by influencing developmental fates. Another group of genes that function in the specification of vulval cell fates by antagonizing the RTK/Ras pathway is the synthetic Multivulva (synMuv) genes. The synMuv genes act in two functionally redundant classes, A and B, to negatively regulate vulval development. Animals with a mutation in one or more genes within the same class are non-Muv. By contrast, animals with mutations in both class A and class B genes are Muv. Among some of the identified class B gene products are counterparts of Rb (*lin-35*), the Rb-associated protein RbAp48 (*lin-53*), the heterodimeric transcription factors DP (*dpl-1*) and E2F (*efl-1*), and the chromatin-associated proteins HDAC (*hda-1*), Mi-2 (*let-418*), and HP1 (*hpl-2*). The three molecularly characterized synMuv A genes encode novel proteins of uncharacterized function.

To understand more about the interactions between the synMuv A and B pathways and their link(s) to the RTK/Ras pathway, two screens for synMuv suppressors were performed. In these screens, 80 suppressors of the synMuv phenotype of *lin-15AB(n765ts)* and 41 suppressors of the *lin-53(n833); lin-15A(n767)* synMuv phenotype were isolated. We will present the mapping, complementation, and genetic characterization of some of these suppressors.

One suppressor of *lin-53(n833); lin-15A(n767)* was cloned and found to be a homolog of the chromatin-remodeling ATPase ISWI. RNAi of the ISWI homolog suppresses the Muv phenotype of most, if not all, synMuv combinations. Therefore, the ISWI homolog may be required for the ectopic induction of vulval fates observed in synMuv mutants. Genetic characterization is underway to confirm these observations. Additionally, we are seeking a deletion allele and producing an antibody.

**749370. FUNCTIONAL STUDIES OF THE CLASS B SYNMUVS, GENES REQUIRED FOR NEGATIVE REGULATION OF VULVAL INDUCTION, AND CHARACTERIZATION OF THE CLASS B SYNMUV GENE *lin-61***

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The receptor tyrosine kinase/Ras pathway necessary for vulval induction in *C. elegans* is negatively regulated by two redundant pathways defined by the synthetic Multivulva (synMuv) class A and class B genes. Mutations in members of either class alone do not result in a Multivulva phenotype, but animals containing loss-of-function mutations in both a class A and a class B gene are Muv. The identified synMuv class A genes encode novel proteins. Many of the class B synMuv genes, including *lin-35* Rb, *dpl-1* DP, *efl-1* E2F, *lin-53* RbAp48, *hda-1* HDAC, and *let-418* Mi-2, have homologs that are known to be involved in chromatin-modification.

We have molecularly characterized a new class B gene, *lin-61*. LIN-61 is predicted to contain three MBT repeats, which are poorly defined sequences of approximately 100 base pairs found in a number of nuclear proteins, including the *Drosophila* Polycomb group protein Sex Comb on Midleg. We have identified the molecular lesions in all ten *lin-61* alleles. *n3809*, a nonsense mutation at amino acid 159, is predicted to delete two-thirds of the protein and is a presumptive null. Animals homozygous for *lin-61(n3809)* are viable. We are using antibodies and translational GFP constructs to characterize the expression pattern of LIN-61. Additionally, we are trying to identify proteins that interact with LIN-61 by performing a yeast two-hybrid screen.

Some of the proteins acting in the synMuv B pathway have been shown to physically interact *in vitro* (1,2) or in yeast two-hybrid assays (3), indicating that many synMuv proteins may function together in transcriptional regulatory complexes. We are attempting to use co-immunoprecipitation experiments to further explore *in vivo* physical interactions among the synMuv proteins. These studies may allow us to assign functionality to some of the novel class B genes, to analyze the effects of various synMuv mutations on physical interactions, to identify potential sub-complexes among the synMuv proteins, and possibly to determine whether class A and class B proteins play redundant roles in the formation of chromatin-modifying complexes.

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3. Walhout et al. (2000) Science **287**:116.

**46712. The interactors of CKI-2 suggest a novel CDK inhibitor regulatory mechanism in *C. elegans***

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In *C. elegans*, two CIP/KIP family CDK inhibitors (CKIs) are encoded by the *cki-1* and *cki-2* genes found in tandem on chromosome II. Inactivation of *cki-2* by *RNAi* (RNA mediated interference) or cosuppression produces an impenetrant embryonic arrest phenotype. It is unclear whether *cki-2* functions as a negative cell cycle regulator, nor is it clear whether the embryonic lethality seen in *cki-2 (RNAi)* or cosuppressed worms is due to cell cycle defects. To gain further insight into *cki-2* function, we performed a yeast two-hybrid screen in order to identify interacting partners of CKI-2. We have identified three CKI-2 interactors: W03D2.4 (CePCNA), C06A5.9 (CeRING), and K12C11.2 (CeSUMO-1).

To identify how these proteins interact, we performed directional yeast two-hybrid assays that showed CeSUMO-1 also interacted with CeRING. However, CeSUMO-1 did not interact with CKI-1, which implies that CeSUMO-1 regulate CKI-2 specifically. Because CeSUMO-1 interacted with both CeRING and CKI-2, and that the CePCNA binding domain is located near CeRING binding domain on CKI-2, we postulate that CeRING might antagonize the CKI-2/CePCNA interaction through some dynamic regulation potentially mediated by CeSUMO-1. This possibility was supported by competitive inhibition analysis using inducible expression of CeSUMO-1 in yeast. We showed that CeSUMO-1 antagonized the CKI-2/CeRING interaction, but not the CKI-2/CePCNA interaction.

Since other known SUMO-1 binding proteins obtained from yeast two-hybrid screens have been reported to be *bona fide* substrates of SUMO-1 conjugation *in vivo*, we are investigating whether CKI-2 is also modified by CeSUMO-1 *in vivo*. To better understand the role of these regulatory interactions on CKI-2 function in early embryonic development, we are using a *pie-1* promoter-based germline expression system to visualize maternal CKI-2::GFP and how this may be affected in *CeSUMO-1 (RNAi)* worms.

The nature of the interacting partners of CKI-2 suggests that these proteins regulate CKI-2 during *C. elegans* development through SUMOylation, which highlights a novel regulatory mechanism for CIP/KIP family proteins.

**907027. Roles of PP2A and PAR-1 in vulval development.**

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*sur-6* encodes a PR55 regulatory subunit of the heterotrimeric PP2A phosphatase that positively regulates Ras signaling during vulval precursor cell fate specification and acts between Ras and Raf. The two missense mutants upon which this analysis was based cause a low percent Vul phenotype and no other phenotypes. However RNAi analysis indicates that *sur-6* has a role in embryonic development. We have obtained a deletion allele *sur-6(sv30)*, from Simon Tuck's Lab (Umea Univ), which deletes 5 of its 6 WD40 repeats. *sv30* mutants are maternal effect embryonic lethal and are more Vul than the other alleles, but not as severely Vul as mutants in the main Ras/Raf/MAPK pathway. The genetic interactions of *sur-6(sv30)* with other regulators of the Ras pathway are similar to those seen with the missense alleles. Hence there appears to be no absolute requirement for SUR-6 in modulation of the Ras pathway and the missense alleles affect the Ras signaling functions of *sur-6* more severely than its other functions.

In other systems PR55/SUR-6 can either inhibit or promote the catalytic activity of PP2A. The Kohara and Baillie labs have shown that *let-92* encodes the catalytic subunit of PP2A. We have shown that *let-92* also acts positively in the context of vulval development. This implies that SUR-6 promotes the catalytic activity of PP2A which positively regulates the Ras/MAPK cascade, perhaps by removing inhibitory phosphates from a substrate such as LIN-45 Raf or KSR-1.

The ser/thr kinases AKT, SGK and C-TAK1/PAR-1 have been identified biochemically as candidate inhibitory kinases that act on RAF or KSR to down-regulate the Ras/Raf/ MAPK cascade in mammalian tissue culture cells. Using RNAi in sensitized genetic backgrounds we have found no evidence so far that AKT and SGK have a role in vulval development. However we find that *par-1(b274lf)/par-1(zu310ts)* trans-heterozygotes are weakly Muv. Additionally we find that *par-1(lf)* reverts the suppressed (non-Muv) phenotype of *sur-6(ku123)*; *let-60(n1046gf)* and *let-60(n1046gf)*; *ksr-1(n2526)* double mutants. By contrast, *par-1(lf)* does not reduce the ability of *lin-45(ku112)* to suppress the *let-60gf* Muv phenotype. Therefore *par-1* has an inhibitory role in vulval development. We are currently investigating the relationship between PP2A and PAR-1 in vulval development.

**628630. F54C4.3 is an overexpression suppressor of *mat-3*/APC8**

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"*vex-2(ku233)*" mutants are fully viable but semi-sterile and have a variety of vulval defects, including variably sized Pn.pxx nuclei. *ku233* appears to be a very weak hypomorphic allele of the Anaphase Promoting Complex (APC/C) gene *mat-3* since: 1) *ku233* fails to complement *mat-3(ax68ts)*; 2) a non-complementation screen with *ku233* identified two new *mat-3* alleles, *cs53* and *cs54*; 3) *ku233* mutant defects are rescued by a PCR product containing the *mat-3* operon; and 4) *ku233* mutants contain two base pair substitutions ~400 bp upstream of the *mat-3* operon. Thus, it appears that the developmental defects of *ku233* mutants are caused by a slight perturbation in the expression of a general cell cycle regulator, *mat-3*.

Interestingly, we previously showed that *ku233* mutant defects could be rescued by transgenes containing a different gene, F54C4.3 - in other words, F54C4.3 is an overexpression suppressor of *mat-3(ku233)*. Furthermore, RNAi against F54C4.3 causes many post-embryonic cell division defects. F54C4.3 encodes a protein with an A/T hook and five C2H2 zinc fingers, and is not closely related to known genes from any other organism. We are investigating a possible role for F54C4.3 in cell cycle regulation.

**367894. *hbl-1*, a homologue of *Drosophila hunchback*, regulates temporal patterning during *C. elegans* post-embryonic development**

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While the genes involved in spatial patterning have been extensively studied, we are just beginning to understand the genes that control development along the axis of time. Thus far, the best characterized genetic pathway that regulates temporal identity is the heterochronic pathway of *C. elegans*. The developmental transition between the fourth larval stage and the adult stage in *C. elegans* is under the control of the heterochronic genes *let-7*, *lin-41*, and *lin-29*. *let-7* encodes an untranslated micro-RNA (miRNA); it is thought to post-transcriptionally regulate the expression of its target genes (e.g. *lin-41*) by binding to complementary sites in the 3'UTRs of their mRNAs.

We isolated a precocious allele of *hbl-1* in a genetic screen for heterochronic mutants. Our data suggests that *hbl-1*, a homologue of *Drosophila hunchback*, is another late-acting member of the heterochronic pathway. Like *lin-41*, *hbl-1*'s expression is likely to be regulated by *let-7* through the 3'UTR of its mRNA. Furthermore, *hbl-1* may act in parallel or in conjunction with *lin-41* to repress adult fates during larval stages. Studies in *Drosophila* have shown that *Brain Tumor* regulates *hunchback* expression post-transcriptionally via interactions with sites in the *hunchback* mRNA; significantly, *Brain Tumor* is a member of the same RBCC protein family as *lin-41*. Taken together, these observations suggest an enticing explanation for the genetic interaction observed between *lin-41* and *hbl-1*: it is possible that *lin-41* post-transcriptionally regulates *hbl-1* expression via a similar mechanism.

Since *hunchback* is best known for its embryonic role in spatial patterning in the fly, it is intriguing that we have characterized a post-embryonic role for *hbl-1* in temporal patterning in the worm. Our data supports the hypothesis that spatial and temporal patterning programs may utilize common genetic players, and may thus share regulatory mechanisms. Studies of how *hbl-1* functions in the heterochronic pathway in *C. elegans* will lend insight into the potential for as yet unexplored roles for homologues of *hbl-1* and of the other heterochronic genes in developmental patterning across phylogeny.

**334181. Molecular connections between developmental timing and circadian timing: The *C. elegans* homologues of the circadian genes *doubletime* and *timeless* regulate post-embryonic developmental timing**

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Spatial patterning during animal development is genetically controlled by genes such as the *Hox* cluster. Similarly, the temporal aspect of developmental patterning is under the genetic control of heterochronic genes. The most extensively investigated heterochronic pathway defines a somatic clock that controls the timing of cell proliferation and differentiation during *C. elegans* post-embryonic development.

*lin-42*, a component of the *C. elegans* heterochronic pathway, is the homolog of *Drosophila* and mammalian *period*, a key regulator of circadian rhythms <sup>(1)</sup>. Circadian timing genes regulate the timing of gene expression over a 24-hour period. *lin-42* thus defines a molecular link between two different types of biological clocks: developmental timing and circadian timing. This connection raises the possibility that other *C. elegans* homologues of circadian genes may be involved in the control of developmental timing. Indeed, the *C. elegans* genome contains a number of homologues of circadian timing genes in addition to *lin-42*. These homologues are not known to play a role in the recently defined circadian rhythms of *C. elegans* <sup>(2)(3)</sup>. We have investigated the possible function of these homologues in control of developmental timing by using RNA interference (RNAi) to knock-down of gene expression. We present evidence that *kin-20* and *tim-1*, the *C. elegans* homologues of the *Drosophila* circadian timing genes *doubletime* and *timeless*, regulate the developmental clock that specifies correct temporal cell fates. We present a model, based on genetic epistasis, of the possible ordering of these circadian timing homologues in the *C. elegans* heterochronic pathway.

The connections that we are uncovering between circadian and developmental timing pathways suggest that they may utilize conserved molecules and mechanisms of temporal regulation. An intriguing possibility raised by our findings is that developmental timing genes played an ancestral role to circadian timing, or vice versa.

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**494922. NHR-6: a nuclear receptor transcription factor required for ovulation.**

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The *C. elegans* genome encodes greater than 270 nuclear receptor (NR) genes (Sluder et al, 1999). Among these are fifteen genes that, based on comparative sequence analysis, encode nuclear receptor proteins conserved across metazoan phyla (Sluder et al, 1999). One of these conserved NR genes is *nhr-6*, which encodes the only *C. elegans* member of the NGFI-B NR sub-family (Wilson et al, 1992; Kostrouch et al, 1995; Sluder et al, 1999).

We have initiated a functional analysis of *nhr-6*. Data from RNAi experiments and analysis of the deletion mutant *lg6001* have established a role for *nhr-6* in gonad development and/or function. Specifically, *nhr-6(RNAi)* and *nhr-6(lg6001)* animals have extremely low brood sizes and lay abnormally shaped eggs. Further analysis demonstrated that these phenotypes were due to an ovulation defect. Oocytes undergoing ovulation in *lg6001* hermaphrodites frequently fragment entering the spermatheca. Only a fraction of the oocyte fragments become fertilized and many of these fail to develop properly. Oocyte fragments that remain in the gonad can become endomitotic (Emo phenotype). An *nhr-6::GFP* transgene is expressed in the spermatheca, suggesting a role for *nhr-6* in spermathecal development and/or function.

Currently, our analysis of *nhr-6* is focused on the following: 1) a detailed characterization of *nhr-6* expression and the ovulation defect observed in *lg6001* mutants; 2) the *in vivo* significance of a conserved Akt/PKB phosphorylation site within the DNA binding domain of NHR-6; and 3) a comparative biochemical analysis of NHR-6 and an NGFI-B homolog (DiNHR-2) from the filarial nematode *Dirofilaria immitis*. The results of these investigations will be presented.

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**889677. *cgh-1*, a conserved germline predicted RNA helicase required for gametogenesis and oocyte survival in *C. elegans***

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In *C. elegans* approximately half of all developing oocytes die by apoptosis during the late pachytene stage of meiosis. This physiological germline cell death is triggered by an unknown regulatory pathway that is distinct from mechanisms that induce cell death in somatic cells, or in response to genotoxic stress. Evidence suggests that these apoptotic oocytes normally donate their cytoplasm to the survivors, and thereby may function as nurse cells. We have determined that in *C. elegans*, *cgh-1* is required to prevent essentially all developing oocytes from undergoing apoptosis through the physiological mechanism (Navarro, et al., Development 128, 3221-32, 2001). CGH-1 is a predicted DEAD box RNA helicase that is associated with germline and early embryonic P granules, and is also present in additional granules in the gonad core that persist in the early embryo in a pattern that parallels maternal mRNA. Consistent with our findings, evidence indicates that in *Drosophila* and *Xenopus*, CGH-1 orthologs associate with maternal mRNA. When germline apoptosis is prevented, *cgh-1(RNAi)* oocytes develop normally in many respects but cannot be fertilized, indicating that *cgh-1* is required for oocyte function.

We have hypothesized that in *cgh-1(RNAi)* oocytes, the physiological germline apoptosis mechanism is triggered by a specific abnormality in oocyte or maternal gene regulation, and not by a generalized abnormality in P granule function or a non-specific impairment of oogenesis. To test this model further, we have investigated whether germline cell death is also increased by defects in the constitutive P granule components PGL-1, and the GLH DEAD box helicases. We have determined that sterility associated with the *pgl-1(bn102)* mutation is not accompanied by elevated levels of germ cell death. In addition, germline cell death is almost undetectable in sterile *glh-1; glh-4(RNAi)* hermaphrodites. The latter finding is intriguing because CGH-1 protein levels are very low or undetectable in a significant proportion of these animals. It is possible that the low levels of CGH-1 protein remaining in *glh-1; glh-4(RNAi)* hermaphrodites is sufficient to prevent excessive germ cell death, but the sharply reduced level of overall germ cell death observed suggests that it is more likely that most *glh-1; glh-4(RNAi)* germ cells do not mature to the stage at which apoptosis normally occurs. Further supporting the model that physiological germ cell death is not triggered non-specifically by defective oogenesis, germ cell apoptosis is not elevated in *gld-2(q497)* animals, in which gametogenesis is highly abnormal.

CGH-1 protein levels accumulate dramatically as developing oocytes enter meiosis and the central gonad core is formed. We have tested whether this increase in CGH-1 expression is specifically dependent upon mechanisms that direct entry into meiosis. In *gld-1(q485)* mutants, developing meiotic germ cells reenter mitosis and form a tumorous gonad. We have observed that while CGH-1 protein initially accumulates appropriately in *gld-1(q485)* mutants, in the tumorous portion of their gonads CGH-1 levels are barely detectable. In *gld-1(q485); gld-2(q497)* double mutants, in which germline cells fail to enter meiosis, CGH-1 expression is consistently low throughout the gonad, as is characteristic of normal mitotic germ cells. These findings are consistent with the model that the increase in CGH-1 levels that normally occurs in developing oocytes is specifically associated with the process of meiosis. Together, our findings support the view that *cgh-1* is required during meiosis for normal function of a specific regulatory mechanism that determines whether a developing oocyte will survive or undergo apoptosis.

### 136435. Phosphorylation of the carboxy-terminus of ICP-1 by AIR-2 kinase stimulates

#### AIR-2 kinase activity

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Despite the central importance of the mitotic spindle in cell division, the regulation of mitotic spindle formation and function is not well understood. Our lab has previously identified a *C. elegans* orthologue of the Aurora kinase family, AIR-2, that is required for proper chromosome segregation and cytokinesis. AIR-2 localizes to chromosomes at metaphase, the central spindle at anaphase, and to the cytokinesis remnant at telophase.<sup>(1)</sup> This dynamic localization pattern is shared by a group of proteins known as chromosomal passengers, which includes the *C. elegans* inner centromeric protein ICP-1. ICP-1 co-localizes with AIR-2, and may act to target AIR-2 as it is required to localize AIR-2 *in vivo*, and directly binds AIR-2 *in vitro*.<sup>(2)</sup> Furthermore, *icp-1(RNAi)* results in a phenotype that is indistinguishable from *air-2(RNAi)*.<sup>(2)</sup> Given these results, we hypothesized that ICP-1 is a direct substrate of AIR-2.

Here we show that recombinant AIR-2 specifically phosphorylates recombinant ICP-1 *in vitro*. To localize the site of AIR-2 phosphorylation in ICP-1, constructs encoding the amino-terminus (IN), middle (IM), and carboxy-terminus (IC) of ICP-1 were created. *In vitro* kinase assays revealed that only the IC fragment could be phosphorylated by AIR-2. Site-direct mutagenesis of two adjacent serines in the IC fragment that are highly conserved across eukaryotes strongly reduced the level of phosphorylation by AIR-2. Phosphorylation of these serines was additive as mutating each residue separately caused only partial reduction in phosphorylation. Interestingly, both full length ICP-1 and the IC fragment, but not the IN or IM fragments, induce an increase in the kinase activity of AIR-2 towards the generic substrate MBP. This induction requires that ICP-1 be phosphorylated by AIR-2 as mutation of both of the serines at the AIR-2 phosphorylation site in either full-length ICP-1 or the IC fragment abolished the ability to increase AIR-2 activity. In conclusion, ICP-1 is a direct substrate of AIR-2 kinase, and phosphorylation of ICP-1 feeds back to potentiate the kinase activity of AIR-2. We are currently creating transgenic lines that express GFP fusions of either wildtype or phosphorylation-site mutant ICP-1 to determine the role of AIR-2 mediated phosphorylation in ICP-1 localization and function *in vivo*.

<sup>(1)</sup> Schumacher et al., (1998) **Journal of Cell Biology**143:1635-1646.

<sup>(2)</sup> Kaitna et al.,(2000) **Current Biology**10:1172-1181.

**210807. A new genetic screen to identify genes that regulate life span**

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To identify new genes, that affect life span, we did a genetic screen looking for genes that extend life span when increased in gene dosage. We reasoned that increasing the dosage of any gene whose activity is rate limiting for the aging process should extend the life span of the worm. We increased gene dosage by analyzing duplication strains where the dosage of genes covered by the duplication is increased by 50% compared to wild type. Therefore, our genetic screen was a life span survey of duplication strains.

The initial survey of duplications covered approximately 50% of the *C. elegans* genome from a total of 35 strains tested for life span (Tissenbaum and Guarente 2001). From this survey, we identified a new gene in the insulin-like signaling pathway, *sir-2.1* (Tissenbaum and Guarente 2001). This gene is the *C. elegans* homolog to yeast SIR2. In yeast, SIR2 also regulates life span by gene dosage (Kaeberlein, McVey et al. 1999).

We have obtained duplication strains covering the rest of the genome. Currently, we have tested an additional 40 duplication strains for life span and we have three new regions that extend the mean life span. We are now narrowing down the region responsible for the life span extension as well as continuing the screen to cover the rest of the genome.

\* these two authors contributed equally to this project

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**946689. Nematodes harboring a longevity gene mutation display a time-limited (mid-life) protection of the age-related deterioration of muscle cell nuclei**

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As nematodes age, a variety of tissue-specific changes occur that coincide with the deterioration of the animal. The most prominent tissue-specific changes occur in the muscle tissue. As observed at both the light microscope and electron microscope levels, aging muscle cells lose cytoplasmic volume, sarcomeres become disorganized in their packing morphology, lipid inclusions become apparent, and muscle cell nuclei become misshapen and mottled, with an increase in the size of the nucleolus.

A large number of genes have been identified that influence nematode lifespan. However, very little is known about how these genes affect the actual aging of the worm. Understanding the relationships between aging and lifespan remains important to understanding the biology of aging. In a first attempt to look at the effect of a longevity mutation on aging, we monitored the age-related changes in muscle cell nuclei morphologies from wild-type (N2) and *age-1* mutant nematodes. Our findings indicate that the age-related deterioration of muscle cell nuclei is less severe on an *age-1* mutant background. This protective effect, however, is apparent only during a window of time representing the middle age of the nematode. We envision a model of aging whereby lifespan is determined by the deterioration of key tissues, such as muscle, with age.

## **255432. STRUCTURE FUNCTION ANALYSIS OF GLUTAMATE-GATED CHLORIDE CHANNELS**

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Glutamate-gated chloride channels (GluCl) belong to the family of ligand-gated ion channels that includes nicotinic acetylcholine, serotonin, GABA and glycine receptors. The subunit composition of GluCl in vivo is not clear. By analogy with other ligand-gated channels of the same family we assume that they possess a pentameric structure probably consisting of at least a and b subunits. *Caenorhabditis elegans* has different GluCl channels formed by different a subunits. Five GluCl subunits genes have been characterized so far. They encode the subunit proteins a1, a2, a3, a4 and b. The subunits share the same structure; a large extracellular amino-terminal domain, four membrane-spanning domains (TM1-TM4), a cytoplasmic loop between TM3 and TM4, and an extracellular carboxy terminal. The best understood GluCl channel, which consists of a2 and b subunit, is expressed in the muscle cells of the pharynx. Here the channels mediate the response to the motor neuron M3.

To better understand why worms have different a-type channel subunits, we used the myo-2 promoter to express various a-type subunits in the pharynx. Our results show that a1 and a3 subunits are unable to substitute for a2. To determine the difference between a subunits and to identify the minimal rescuing domain of a2 which confers the ability to restore M3 neurotransmission, chimeras between a2 and a1 or a3 subunits were generated. Despite the high sequence homology in the a1-M3 domains, our results suggest that the transmembrane domain of a2 together with the intracellular loop and carboxy terminal is necessary to rescue the activity of the channel. The conformation of the amino terminal extracellular domain is also essential for the activity. In fact a chimeric extracellular domain construct is not able to restore the activity. Work is in progress to understand if the intracellular loop between TM3 and TM4 is important for the proper localization and thus for the activity of the channel.

**751231. Microarray Analysis of MAP Kinase Signaling in the Germline of *C. elegans***

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Our lab uses microarray analysis to study germline development in *C. elegans*. Through microarrays, we can examine the global transcriptional response of all genes to a specific condition or mutation. Previous microarray experiments have compared global gene expression patterns of wild type animals to mutant animals lacking a germline. Analysis of these data has identified a set of genes that are germline enriched.

The Ras/MAP kinase pathway is involved in many developmental processes in *C. elegans*, including meiotic progression. We are examining the effects of MAP kinase signaling using a temperature sensitive MAP kinase mutant allele, *mpk-1(ga111)*, which produces a phenotype only in the germline. When these mutant animals are raised at the restrictive temperature, germ cells arrest at the pachytene stage of meiosis. By comparing gene expression of these MAP kinase mutants to control animals through microarray analysis, we can examine the global genome response to loss of MAP kinase signaling in the germline. To do this, we have compared mRNA extracts from control and *mpk-1* mutant adults raised at the restrictive temperature and obtained a set of candidate genes that are enriched in the control as compared to the *mpk-1* animals. We have also shown that *mpk-1(ga111)* adults can resume MAP kinase signaling, as evidenced by resumption of meiotic progression, when shifted to the permissive temperature. Oocytes are detected in these animals 12 hours after shifting and oocyte production is similar to wild type by 20 hours. Thus, we have shifted the control and mutant animals to the permissive temperature and compared their gene expression after 9 hours by microarray. The sets of genes that show changes in the expression ratio, control/*mpk-1*, after 9 hours at the permissive temperature provide information about the temporal nature of the gene expression changes that are transcriptionally regulated by MAP kinase in the germline. These two conditions define two groups of MAP kinase responsive genes. The early-induced category includes genes that are enriched in the control/*mpk-1* comparison before shifting, but are no longer enriched after 9 hours at the permissive temperature and are candidates for direct targets of MAP kinase signaling. The late-induced category includes genes that are enriched at both timepoints. Using the results of these experiments, we were able to define 50 early-induced and 162 late-induced genes. Of these, 35 and 52 genes are germline-enriched, respectively. We are currently examining the regulation and function of the 35 germline-enriched, early-induced genes. This experiment will be extended with a complete timecourse microarray analysis including timepoints from 4 to 20 hours after shifting to the permissive temperature.

**350474. Establishment and maintenance of directional left/right asymmetry in the bilateral taste receptor neurons ASEL and ASER**

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Most animals display some level of left/right asymmetry in otherwise bilaterally symmetric nervous systems. Where do these asymmetries exist, how are they established, and how are they maintained? In *C. elegans*, some bilateral analogs such as the taste receptor neurons, ASEL/R, and the odorsensory neurons, AWCL/R, exhibit left/right asymmetry in their gene expression patterns. Other bilateral analogs such as the QL/R cells and P11/P12 neuroblasts, have distinct migration patterns. Furthermore, numerous bilateral analogs break symmetry when both neurons of a pair send their axonal processes into the right fascicle of the ventral nerve cord. The focus of our studies is the directional(biased) asymmetry observed in the ASE neurons.

Unlike the antisymmetry(random pattern) of *str-2* expression observed in the AWC neurons(Troemel et al., 1999), specific transcription factors and receptors are expressed in a determinate manner in the ASE neurons. In wild type adult animals, the guanylyl cyclase receptors, *gcy-6* and *gcy-7*, and the LIM homeobox gene, *lim-6*, are expressed in ASEL(Yu et al., 1997; Hobert et al., 1999). Another guanylyl cyclase, receptor, *gcy-5*, is expressed exclusively in ASER(Yu et al., 1997). Using genetic and laser ablation techniques, we have begun efforts to characterize this interesting example of directional asymmetry.

Initial genetic screens and candidate gene testing focused on identification of genes affecting asymmetric expression in a *lim-6* GFP reporter strain. Using these approaches, we discovered that mutations in the cyclic nucleotide-gated channel subunits, *tax-2* and *tax-4*, and mutations in the small GTPase *ras*, *let-60*, affect *lim-6* expression. We also found a set of transcriptions factors that appear to play a role in *lim-6* expression. We are currently mapping an additional mutant which we have named *lsy-1* (*lim-6* expression symmetrized).

Since some of these mutants did not affect *gcy-7* or *gcy-5* expression, we next initiated a genetic screen for mutants affecting expression in a *gcy-7* GFP reporter strain. We have found mutants which comprise six novel complementation groups. Three of these mutants(*lsy-2*, -3, -4) decrease *lim-6* and *gcy-7* expression in ASEL and turn on expression of *gcy-5* in ASEL. Since these mutants affect the expression of the putative ion receptors *gcy-5* and *gcy-7*, we have tested these mutants in chemotaxis assays and have discovered defects. We are currently mapping these mutations using SNP mapping and three factor analysis.

Establishment of ASE asymmetry may occur via a cell autonomous mechanism in which the specific pattern of cell divisions determines the cell fate, a cell non-autonomous mechanism in which other cells signal to ASEL or ASER to express specific genes asymmetrically, or a combination of these mechanisms. Using laser ablations, we are attempting to identify the cellular focus of the machinery involved in ASE asymmetry establishment. Using a top down approach, we have ablated cells in early embryos and later observed the worms for changes in *gcy-5* expression using a GFP transcriptional reporter strain. Our data suggests that cells of the AB lineage send an inhibitory signal to either ASEL or its precursors, preventing *gcy-5* expression. We plan to continue down these lineages to ascertain the identity of the putative signaling cell(s) and the temporal nature of the signal. We also plan to use various other lineage specific GFP and RFP reporter strains to verify the lineage identity in ablated embryos.

**559812. Temporal and spatial control of initial meiotic entry in the *C. elegans* germ line**

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In many organisms, germline polarity is established when a subset of previously mitotic germ cells differentiate and enter meiosis at a distinct developmental time and position. Initial meiotic entry has been the focus of our investigations in the *C. elegans* hermaphrodite, where it normally occurs in the L3 at the proximal-most part of the germ line. Using a forward genetic approach we have isolated mutants that cause a proximal proliferation (Pro) phenotype, in which the proximal-most cells of the adult germ line - proximal to mature gametes - are mitotic. Characterization of our Pro mutants indicates that they are defective in initial meiotic entry. These defects lead to a change in the normal pattern of germline development and ultimately cause ectopic proliferation in the proximal region of the adult germ line. This analysis provides us with new insight into mechanisms that govern timing and position of initial meiosis.

Specifically, we have identified and analyzed four mutations in two loci that cause a Pro phenotype with spatially displaced and/or temporally delayed initial meiotic entry. Three of these are *glp-1(Pro)* mutants and a fourth defines a novel *pro* locus. The *glp-1(Pro)* phenotype is temperature sensitive and the alleles differ genetically from any previously described alleles of *glp-1*: they are phenotypically gain-of-function, but are effectively competed by *glp-1(+)*. In addition, the *glp-1(Pro)* phenotype is independent of the ligand LAG-2, and dependent on the downstream effector, LAG-1. This phenotype led us to consider and investigate an early role for LAG-2 in the proximal gonad in the timing of initial meiotic entry. The other *pro* locus is defined by one allele that confers a temperature sensitive Pro phenotype. This locus has been mapped to a small interval on LGII. Together, these and other Pro mutants will further our understanding of mechanisms that govern the precise timing and position of initial meiotic entry.



### 174783. Tubulin and centrosome rotation in the early *C. elegans* embryo

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The early *C. elegans* embryo is well suited for the study of microtubule function as it undergoes a variety of microtubule dependent processes during its first cell division. These processes include meiosis, centrosome duplication, establishment of embryonic polarity, maternal pronuclear migration, centrosome/pronuclear complex centration and rotation, mitosis, cytokinesis, and daughter nuclei positioning. We have used RNAi and genetic alleles of a beta tubulin gene to investigate the requirements for specific beta and alpha tubulin isoforms in the early *C. elegans* embryo.

We initially isolated a temperature sensitive mutant, *qt1*, which fails to undergo centrosome rotations in the P0, P1, and EMS blastomeres of the early embryo and determined that the *qt1* mutation affects *tbb-2*, a beta tubulin gene. Interestingly, *tbb-2(qt1)* complements another allele of *tbb-2*, *t1623* (Gonczy et al., 1999). *tbb-2(t1623)* embryos also fail to undergo centrosome rotation in P0 but have a higher rate of defects in pronuclear migration and meiosis than *tbb-2(qt1)*. To define the null phenotype, we used RNAi to deplete *tbb-2* gene function from wild-type embryos. *tbb-2(RNAi)* embryos divide normally except for an exaggerated spindle rocking in the first cell division. Two *tbb-2* deletion alleles obtained from the *C. elegans* Knockout Consortium show a similar early embryonic phenotype. Using RNAi to deplete *tbb-2* in *qt1* and *t1623* mutant embryos rescues the centrosome rotation defects indicating that *qt1* and *t1623* are gain-of-function alleles. Staining *qt1* and *t1623* embryos with an anti-tubulin antibody reveals additional differences in their phenotypes. In both mutants, spindle orientation in P0 is disrupted due to centrosome rotation failure, but some *t1623* embryos have stunted microtubules and microtubule kinetochore attachment often fails. In contrast, *qt1* microtubules look wild type. Thus, these two beta tubulin mutations appear to be affecting microtubule structure in different ways, perhaps by altering the dynamic instability of the microtubules or interfering with microtubule binding partners.

To further investigate tubulin function, we examined the role of other tubulins present in the early embryo. Microarray analysis indicates that two beta tubulin genes and two alpha tubulin genes are expressed abundantly in the early *C. elegans* embryo. To determine if any of these tubulin subunits have specific functions, we used RNAi to deplete these gene products individually and in various combinations in wild-type embryos. *tbb-1(RNAi)*, *tba-1(RNAi)*, and *tba-2(RNAi)* embryos have no early embryonic phenotypes, while *tbb-2(RNAi)* embryos show the vigorous spindle rocking described above. As expected, *tbb-1(RNAi) tbb-2(RNA-1)* and *tba-1(RNAi) tba-2(RNAi)* embryos make no spindle structures, verifying the lack of cross-reactivity of our RNAi. We also used RNAi to remove all four pair-wise combinations of the alpha and beta tubulins to investigate whether different alpha and beta tubulin heterodimers might have specific functions. This experiment was suggested by the observation that, like *tbb-2(RNAi)*, *tba-2 (RNAi)* rescues the *tbb-2(qt1)* phenotype indicating that alpha and beta tubulins might have specific partners. We found embryos missing the four combinations of alpha and beta microtubules divide like wild-type embryos with a low level of meiotic and centrosome rotation defects. These results indicate that the alpha and beta tubulins are functionally redundant in the early embryo.

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**994715. Identification of Loci Required for the Establishment of Anterior/Posterior Polarity in *C. elegans***

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The CCCH zinc finger protein PIE-1 is an essential regulator of germ cell fate that segregates with the germ lineage in early *C. elegans* embryos. PIE-1 protein is initially found throughout the cytoplasm of newly fertilized embryos but becomes enriched in the posterior cytoplasm before the onset of the first mitosis. As a result PIE-1 is inherited predominantly by P1 in the 2-cell stage. To identify trans-acting factors needed to carry out this process as well as genes with a more general role in the establishment of A/P polarity, we decided to screen a collection of 964 temperature sensitive maternal-effect lethal mutants for mutants that mislocalize PIE-1.

From the screen, 18 mutants were identified with consistent PIE-1 localization defects and many others with mild PIE-1 defects. Currently, we are 1) outcrossing those 18 mutants to a PIE-1: GFP line to make time-lapse normarski/GFP movies; 2) conducting complementation tests and 3) doing linkage analysis and 3-factor mapping. Preliminary time lapse analysis reveals two interesting phenotypic classes: mutants where PIE-1 fails to localize early but eventually does so during mitosis, and mutants where PIE-1 localizes early but becomes delocalized during mitosis. These two classes suggests the existence of distinct mechanisms for establishment and maintenance of PIE-1 asymmetry.

**288470. A novel gene *sym-1* regulates synaptic differentiation in *C.elegans***

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Neurons form interfaces at the synapse, an asymmetric structure consisting of pre-synaptic and post-synaptic cell terminals in direct apposition to each other. We are interested in identifying genes that regulate the development of presynaptic structures using the *C.elegans* GABAergic neuromuscular junction (NMJ) as a model system. To visualize presynaptic terminals, we use a vesicle associated synaptobrevin::GFP (SNB-1::GFP) marker expressed by a GABAergic neuron specific promoter [1,2]. Wild type animals that express SNB-1::GFP have uniform spaced and sized fluorescent puncta.

*rpm-1* (regulator of presynaptic morphology) was identified in a genetic screen for mutations which affect the morphology of SNB-1::GFP puncta[3]. Some GABAergic NMJs in *rpm-1* mutants contain excessive number of active zones, suggesting that *rpm-1* is a negative regulator of presynaptic differentiation. RPM-1 is a large presynaptic protein with a putative guanine nucleotide exchange factor domain and a RING zinc finger domain. RING zinc finger domains have been identified in E3 ubiquitin ligase complexes [4]. This suggests that *rpm-1* may have E3 ubiquitin ligase activity for selective protein degradation.

To understand how *rpm-1* regulates presynaptic differentiation, I used a genetic approach to identify the genetic pathways mediated by *rpm-1*. I have identified a new gene *sym-1* (synapse morphology) that functions in the *rpm-1* pathway. *sym-1* encodes an F-box and SPRY domain protein. Similar to *rpm-1* mutants, *sym-1* null animals have irregular clustering of SNB-1::GFP and no obvious behavioral abnormalities. The majority of F-box proteins recruit substrates to ubiquitin ligase complexes for ubiquitination [5, 6, 7]. We propose a model where SYM-1 recruits specific protein targets to an RPM-1 containing E3 ubiquitin ligase complex. SYM-1/RPM-1 complex could mediate localized ubiquitin proteolysis at presynaptic termini and the tightly regulated turnover of unknown protein factors essential for the establishment of normal synaptic structures. My future goals focus on the functional analysis of the *sym-1* gene and the identification of its substrate.

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**719938. In vivo imaging of neuronal responses to touch and taste.**

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Genetic analysis of behavior has provided important insights into the mechanisms underlying behaviors such as touch avoidance and chemotaxis. However, to fully understand these processes 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 in *C. elegans*; however, because of their relatively slow kinetics and small signal size, it has been difficult to use indicators 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 found that application of a mechanical stimulus near the sensory dendrite of ALM or PLM leads to a rapid increase in intracellular calcium in the process and cell body of that neuron. Recordings of calcium transients from mutants defective in the putative mechanotransduction channel MEC-4 failed to show calcium influx in response to a "light touch" stimulus (a rapid poking with a flexible probe) but often showed calcium transients in response to a "harsh touch" stimulus (a slower poking with a solid probe). These results suggest that MEC-4 plays a specific role in mechanotransduction, and that the touch neurons may contain a *mec-4*-independent sensory modality involved in harsh touch detection.

We have also used *cameleon*-based calcium imaging to detect responses to soluble repellant stimuli by the polymodal ASH sensory neurons. Large calcium transients were detected in response to several aversive soluble tastants (quinine, SDS, and copper ions) and to high osmotic strength (glycerol); as in the touch neurons, the calcium increase was rapid and the return to baseline more gradual. Altering the duration of the repellant stimulus strongly affected the duration but not the amplitude of the ASH calcium transient. The ASH neurons showed significant sensory adaptation in their calcium responses to both copper and glycerol; the extent of this adaptation was highly dependent on both the duration of the stimulus and the interstimulus interval. We have begun to investigate the effects of molecules thought to participate in ASH sensory transduction (*OSM-9*) and adaptation (*TAX-6*) on chemosensory calcium transients; results of these experiments will be presented.

**204308. A genetic screen to isolate suppressors of a temperature-sensitive mutation of the separase gene in *C. elegans*.**

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The anaphase promoting complex or cyclosome (APC/C) drives chromosome segregation during the metaphase-to-anaphase transition in meiosis and mitosis. The APC/C is an E3 ubiquitin ligase that targets proteins for destruction by the 26S proteasome. One key substrate in other organisms is securin, an inhibitor of separase. Separase is the proteolytic enzyme that cleaves the cohesion proteins that hold chromosomes together during metaphase. Degradation of securin by APC/C frees separase so that it has access to the cohesion proteins. The destruction of these cohesion proteins by separase allows anaphase to occur.

Siomos et al. (2001) have shown that the *C. elegans* separase gene, *sep-1*, is required for chromosome separation during meiosis I. *sep-1* RNAi causes embryonic lethality where chromosomes are not properly separated at meiosis I. DNA replication continues to occur leading to multinucleate 1-cell embryos. Here we report a suppressor screen using their temperature-sensitive allele of the *sep-1* gene. EMS mutagenesis was used to isolate suppressors in the F2 generation of *C. elegans*. Presently, three candidate suppressors have been isolated that allow growth and survival at the non-permissive temperature. We anticipate that these suppressor mutations may define proteins that either interact with separase or are meiotic substrates of separase.

Siomos et al., 2001. Current Biology 11: 1825-1835.

**573532. *egl-41*, which may act in sex determination, is identical to *sel-10*, which encodes an F-box protein and negative regulator of *lin-12*/Notch**Sibylle Jäger<sup>1</sup>, Hillel Schwartz<sup>2</sup>, Bob Horvitz<sup>2</sup>, Barbara Conrad<sup>1</sup><sup>1</sup>Max-Planck-Institute of Neurobiology, Martinsried, Germany<sup>2</sup>HHMI, Dept. Biology, MIT, Cambridge, U.S.A.

Hermaphrodites carrying dominant mutations in the gene *egl-41* (*egl*, egg-laying defective) lack the hermaphrodite-specific HSN neurons but have the male-specific CEM neurons and therefore are weakly masculinized (1, 2). Our analyses with a deficiency (*nDf42*) and a duplication (*ctDp8*) that span the *egl-41* locus indicate that *egl-41* is not haplo-insufficient and that the dominant mutations cause an altered function that is antagonized by wild-type activity. The existing *egl-41* alleles (*n1069*, *n1074*, *n1077*, *e2055*, *n3717*) therefore most likely represent gain-of-function (gf) mutations causing altered function. It has previously been shown that *egl-41*(gf) mutations enhance the masculinizing effect of weak loss-of-function (lf) mutations in *tra-2* (*tra*, transformer) and suppress the feminizing effect of a *tra-2*(mx) (mx, mixed character) allele (1, 3). In addition, a null mutation of *fem-1* (*fem*, feminization) was found to be epistatic to *egl-41*(gf) mutations (3). Our analyses furthermore indicate that lf mutations of *fem-2* and *fem-3* are epistatic to *egl-41*(gf) mutations as well and that *egl-41*(gf) mutations are epistatic to a *her-1* (*her*, hermaphroditization) mutation. We suggest the gene defined by *egl-41*(gf) mutations is part of the genetic pathway that specifies sexual fate and acts between *her-1* and the *fem* genes. To determine the lf phenotype of *egl-41* and to facilitate the cloning of the gene by transformation rescue, we performed an F1 screen for dominant revertants of the phenotype caused by the *egl-41*(gf) mutation *n1077*. We screened 20,000 haploid genomes and identified one mutation, *bc189*, that is closely linked to the *egl-41* locus. *bc189* completely suppresses the *egl-41*(*n1077*gf) phenotype but causes no obvious additional abnormalities. We therefore cloned the gene by combining fine mapping, using SNPs, and our observation that the dominant phenotype of *egl-41*(*n1077*gf) is antagonized by wild-type activity. *egl-41* proved to be identical to a gene previously characterized, *sel-10* (*sel*, suppressor/enhancer of *lin-12*). *sel-10* was identified as a negative regulator of *lin-12*/Notch (*lin*, lineage abnormal) signaling and encodes an F-box protein (4, 5). The *egl-41*(gf) mutations as well as *bc189* are missense mutations in the *sel-10* ORF (G566R and D482N, respectively). We will therefore now refer to *egl-41* as *sel-10*. *sel-10*(*bc189 n1077*) suppresses a partial lf mutation of *lin-12*, enhances a gf mutation of *lin-12*, and suppresses a lf mutation of *sel-12* and therefore genetically behaves like the canonical lf allele of *sel-10*, *ar41*. Surprisingly, using the same criteria, *sel-10*(*n1077*gf) also behaves like a *sel-10*(lf) mutation. Furthermore, preliminary data indicate that, just like *sel-10*(gf) mutations, *bc189 n1077* as well as *ar41* enhance the masculinizing effects of weak *tra-2*(lf) mutations. These results suggest that *sel-10* is normally involved in the determination of sexual fate and that *sel-10*(gf) mutations share certain characteristics with *sel-10*(lf) mutations. Using biochemical and molecular approaches, we are currently seeking potential targets of the F-box protein SEL-10 in the sex determination pathway.

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**777946. *C. elegans* DAZ-1 is necessary in early prophase of female meiosis**

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The *DAZ* family genes, which encode an RNA-binding protein with a single RRM (RNA recognition motif), are conserved among multicellular eukaryotes. They are involved in germ cell development, either spermatogenesis or oogenesis, or both, depending on the host species. *C. elegans* has a single member of this family, named *daz-1*. We have previously shown that *daz-1* null hermaphrodites are sterile, due to the arrest of oogenesis at the pachytene stage of meiosis I. In contrast, spermatogenesis is not affected in either hermaphrodites or males that are defective in *daz-1*.

Analysis using antibodies against the DAZ-1 protein revealed that DAZ-1 is expressed only in the gonad, most abundantly at the mitotic and the transition zones in hermaphrodites. Female germ nuclei assume characteristic crescent shape at the transition zone in wild-type worms, but those in *daz-1* hermaphrodites appeared to be morphologically irregular. To facilitate analysis of the function of DAZ-1 in mitotic and early meiotic germ cells, we examined ultrastructure of these cells in wild-type and *daz-1* hermaphrodites by transmission electron-microscopy. We found that loss of *daz-1* function resulted in a reduction of the size of nucleoli in meiotic germ cells. This observation was confirmed by immunostaining of a nucleolar marker protein. In addition, the spatial organization of the meiotic nuclei in the *daz-1* gonad was abnormal. In wild-type hermaphrodites, partially cellularized germ nuclei were located at the periphery of the gonad, leaving an anucleate cytoplasmic core (rachis) at the center of the early meiotic region. In contrast, such a core was absent in the *daz-1* gonad. All germ nuclei in the mutant gonad were cellularized, and were located uniformly.

The smaller size of nucleoli in the *daz-1* gonad was suspected to correlate with reduced synthesis of ribosomes in this mutant. However, our trials to reduce the general translation activity in the gonad by RNAi failed to reproduce the same phenotype as *daz-1*, suggesting that DAZ-1 is unlikely to affect the general translation activity. We speculate that DAZ-1 may regulate expression of specific genes post-transcriptionally in mitotic and early meiotic germ cells, the products of which are required for the progression of meiotic prophase.

**727169. *pom-1* is a Conserved Regulator of Cell Polarity and Cell Division in the *C. elegans* Embryo and Fission Yeast**

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The 1-cell *C. elegans* embryo undergoes an asymmetric cell division that establishes the fundamental distinction between the germline and the soma. This process is initiated by specification of the anterior/posterior (A/P) axis at fertilization, in which the site of sperm entry defines the future posterior pole of the embryo. A group of cortical proteins encoded by the *par* genes respond to this cue by adopting an asymmetric pattern of localization, leading, in turn, to the polarized distribution of cytoplasmic determinants. Specifically, two cytoplasmic CCCH zinc finger proteins, MEX-5 and MEX-6, localize to the anterior of the embryo and trigger the posterior enrichment of the germline determinants PIE-1 and P granules. Despite the identification of several genes that regulate this process, we lack an understanding of their underlying molecular mechanisms.

To investigate these mechanisms, we have conducted an RNAi screen for new regulators of A/P polarity. Unlike previous screens, which relied on mutants with symmetric cleavages to identify polarity genes, our screen uses a PIE-1:GFP fusion to monitor A/P polarity. Because PIE-1 asymmetry is visible in the zygote before the first cleavage, this approach avoids biases against genes that function in both cell polarity and cell division. Indeed, our screen resulted in the identification of just such a gene - F49E11.1, which we have named *pom-1* (see below).

We find that *pom-1(RNAi)* zygotes exhibit pronounced defects in PIE-1 and P granule localization, while other aspects of A/P polarity remain intact. Specifically, time-lapse movies of *pom-1(RNAi)* embryos expressing the PIE-1:GFP fusion protein revealed that PIE-1 fails to localize to the posterior during pronuclear migration, but does exhibit transient localization upon assembly of the mitotic spindle. PGL-1:GFP movies revealed that P granules do not exhibit this transient 'mitotic rescue' and instead, remain uniformly distributed throughout the embryo in the absence of *pom-1*. Surprisingly, however, asymmetric localization of the PAR proteins and MEX-5/6 remains intact in *pom-1(RNAi)* embryos. Moreover, cytoplasmic flows and pseudocleavage appear unaffected. During mitosis, *pom-1(RNAi)* embryos exhibit pronounced defects in cell division, including mispositioning of the mitotic spindle and abnormal cytokinesis. Histone:GFP movies have revealed that there are also defects in chromosome segregation in the absence of *pom-1* function. We find that a *pom-1* deletion (kindly provided by Bill Raich) results in phenotypes that are identical to those observed by RNAi. Thus, *pom-1* regulates both cell polarity and cell division in the 1-cell embryo.

The distribution of the POM-1 protein is consistent with these two functions. It is uniformly cortical in oocytes and newly fertilized embryos, but coalesces into discrete cortical foci just prior to the onset of PIE-1 and P granule localization. During mitosis, POM-1 localizes to the centrosomes and the midzone of the mitotic spindle.

Intriguingly, POM-1 is a member of the highly conserved dual-specificity tyrosine kinase, or DYRK, family. One family member, Pom1p, was found to regulate cell polarity and cell division in *S. pombe* (Bahler and Pringle, 1997). Thus, our findings reveal an unexpected link between A/P polarity in *C. elegans* and cell polarity in *S. pombe* that may extend to other organisms as well.



**514638. One Goal - three Approaches: Exploring Genetics of Necrotic-like Cell Death in *C. elegans***

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Necrotic cell death is a main contributor to neurodegenerative conditions in humans, yet the mechanisms underlying necrosis are poorly understood. In *C. elegans*, a number of genetic defects can cause necrotic-like cell degeneration, including dominant alleles specifying hyperactive variants of the MEC-4 and DEG-1 degenerin ion-channel subunits, a dominant allele of the *deg-3* gene encoding a non-desensitizing acetylcholine receptor subunit, and a transgenically-expressed constitutively-activated mutant of the G-protein subunit  $G\alpha_S$ . The morphological changes induced by these cytotoxic insults include cell swelling and vacuolization, and are microscopically distinct from apoptotic cell death. In addition, key executors of apoptosis are not required for the initiation or progression of necrotic cell death. Interestingly, the membranous inclusions occurring at the early stage of necrosis resemble ultrastructural features seen during some excitotoxic cell death in mammalian models and aberrant aggregates are common features in several human neurodegenerative disorders.

We are focusing on a gain-of-function mutation in the touch mediating degenerin ion-channel subunit *mec-4(d)* to study molecular requirements for necrotic-like cell death. When mutated, the MEC-4(D) ion channel is locked open, leading to increased cation influx, which triggers the degeneration of the touch cells.

In a screen for suppressors of ectopic *mec-4(d)* expression, we isolated 13 suppressor alleles in previously unknown loci influencing cell. One of the loci was identified as the  $Ca^{2+}$ -binding protein calreticulin [Xu K. et al., 2001], suggesting elevated intracellular  $Ca^{2+}$  plays a critical (and conserved) role in necrosis. Here we report on the progress of the mapping and characterization of the remaining suppressor alleles. Our long term goal is to clone these loci to learn about molecular details of necrotic death mechanisms.

In a systematic attempt to identify additional key executors of necrotic cell death, we are using a commercially available RNAi feeding library, which targets the expression of 2,800 transcripts on chromosome I. We feed *mec-4(d)* worms with each individual RNAi clone and subsequently score for suppression of touch neuron degeneration. Halfway through this screen, we identified 10 clones with significant suppressor effects. Several of the genes we identified are involved in  $Ca^{2+}$ -regulation. This includes calpains.

In ischemic brain injury (e.g., in stroke) in mammals, abnormally high concentrations of intracellular  $Ca^{2+}$  trigger a cascade of proteolytic events, that lead eventually to the dismantling of the affected cells. Calpains are a group of  $Ca^{2+}$  regulated cysteine proteases, that participate in mammalian necrosis. The completed sequence of the worm genome predicts 16 calpain-like genes with homology to mammalian calpains. We are in the course of examining the involvement of each of these genes in the progression of necrotic-like cell death induced by *mec-4(d)* using RNAi. Our goal is to order calpains into a "pathway" with other genes that influence the death process.

**41526. Serotonin modulation of a chemosensory circuit in *C. elegans***

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

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

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#### 437099. Phenotypic characterization of *dsc* mutants

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The *clk-1* gene is required for the regulation of biological timing. Mutations in *clk-1* result in slowed behavioral rhythms, slowed developmental rates and a lengthened life span (1, 2). In addition, *clk-1* mutants show a defect in responding to changes in temperature. Wild type worms which have been raised to adulthood at 20°C can adjust their defecation cycle length after a shift to a new temperature: the cycle gets shorter when worms are shifted to a higher temperature and longer when worms are shifted to a lower temperature. In contrast, the defecation cycle of *clk-1* mutants is not affected by changes in temperature. The length of the defecation cycle of *clk-1(qm30)* mutants raised at 20°C is 90 sec when worms are shifted and scored at 15, 20 and 25°C. This indicates that *clk-1* is necessary for adjusting the defecation cycle length in response to changes in temperature (3).

Recently, a number of suppressor mutants, which are called *dsc* for **d**efecation **s**uppressor of *clk-1*, were identified. There are two classes of *dsc* mutants. Mutations in class 1 genes (*dsc-3* and *dsc-4*) suppress the slow defecation cycle of *clk-1* mutants and restore the ability of react to changes in temperature. However, mutations in the class 2 genes (*dsc-1*, *dsc-2* and *dsc-7*) only suppress the slow defecation at 20°C, but do not suppress after a shift to a new temperature (3).

We examined the interactions between *dsc* mutations for their effect on defecation. Mutations in the two class 1 genes, *dsc-3* and *dsc-4*, do not have an additive effect. The defecation cycle length of the *clk-1; dsc-4 dsc-3* triple mutant is almost the same as that of the *clk-1; dsc-3* and *clk-1; dsc-4* double mutants. This result suggests that both class 1 genes may regulate the same process. In contrast, mutations in the class 2 genes have additive effects with the mutations in the class 1 genes. For example, the defecation of the *clk-1; dsc-4; dsc-1* triple mutant is faster than that of *clk-1; dsc-1* and *clk-1; dsc-4* double mutants. This result suggests that the class 1 and class 2 genes may regulate different processes. In addition, class 1 genes are epistatic to class 2 genes for their ability to restore temperature sensitivity to the *clk-1* mutants. The defecation cycle of *clk-1; dsc-4; dsc-1* is faster at 25°C than at 20°C. The existence of the 2 classes of suppressors suggests that the defecation cycle may be regulated by two different mechanisms.

The *dsc* mutants were isolated as suppressors of the slow defecation phenotype of *clk-1* mutants. However, *clk-1* mutants show several additional phenotypes, such as other slow behavioral rhythms, a slow developmental rate and a long life span. We studied whether other *clk-1* phenotypes are also suppressed by *dsc* mutations. Overall the *dsc* mutations cannot suppress the slow development of *clk-1* mutants or some other slow behavior. But, one of the *dsc* mutants, *dsc-4* was analyzed in greater detail. *dsc-4* cannot suppress the slow pumping and slow post embryonic development of *clk-1* mutants. Surprisingly however, the slow egg-laying rate and slow embryonic development of *clk-1* were suppressed by *dsc-4(qm182)*. Thus, *dsc-4* can suppress a variety of *clk-1* phenotypes, but not all. This observation suggests that the *clk-1* mutations produce a pleiotropic phenotype by at least two different mechanisms: a mechanism that can be suppressed by *dsc-4* and a mechanism that cannot.

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**553566. Post-PIE-1 Chromatin Reorganization in the Embryonic Germline of *C. elegans***

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The ability of a multicellular species to faithfully propagate relies upon the ability of a germ lineage to both refrain from differentiation during embryogenesis, and simultaneously remain capable of renewing an entire organism in subsequent generations. The germ cell must therefore retain its "integrity" through the continuous cycle from fertilization through gametogenesis. To begin to understand the mechanisms governing this totipotency, it is necessary to determine how germ cells fundamentally differ from somatic cells.

In early *C. elegans* embryos, new mRNA synthesis occurs only in somatic blastomeres and is repressed in the germ lineage. This transcriptional repression is dependent on the protein PIE-1 until it disappears at approximately the 100-cell stage. Premature removal of PIE-1 results in transcriptional activation of the germ cells and subsequent transformation of the germ lineage into a somatic lineage. This demonstrates the need for transcriptional repression in maintaining the embryonic germline in an undifferentiated state. PIE-1 is thought to act by blocking mRNA elongation, as the elongating form of RNA polymerase II is absent from the germ lineage until PIE-1 disappears. We wanted to further investigate this early embryonic repression by studying the chromatin organization in the early germline.

Chromatin organization is one level of regulating gene expression. DNA is packaged into nucleosomes by the four core histone proteins H2A, H2B, H3 and H4. These core histone proteins have N-terminal tails, which can be modified in various ways including phosphorylation, acetylation, methylation, ubiquitination, and ADP ribosylation. It is believed these modifications may act in different combinations to alter the access of different regulatory proteins to the DNA. These different combinations can be read as a "histone code" and specify transcriptional activity or repression.

As the germ lineage is transcriptionally repressed in early embryos, we expected that these cells would be lacking histone modifications that correlate with transcriptional activation. Surprisingly, this was not the case, as the histones in both the somatic and early germline blastomeres exhibited similar modifications. A striking difference, however, was observed after the division of P<sub>4</sub> to Z2 and Z3, coincident with the known time of disappearance of PIE-1. After their birth, and continuing until after hatching, Z2 and Z3 are the only two cells in the embryo which exhibit gross differences in the "histone code" of their genomes.

We hypothesize that in early embryos there are two distinct and sequential repressive mechanisms that guard germ cell identity. The first requires PIE-1, which may prevent productive transcription at either the elongation or re-initiation steps. We suggest that a second mechanism, which begins after the disappearance of PIE-1, involves chromatin reorganization. Once PIE-1 is removed, Z2 and Z3 become transcriptionally competent; perhaps one product of the resulting initial transcription serves to signal chromatin remodeling to continue germline repression. We are testing a variety of candidate genes for roles in this process, as well as designing screens for a less biased approach to identify components.

**524077. *dre-1*, a heterochronic gene affecting gonadal and extragonadal developmental age**

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The heterochronic loci specify developmental age of various tissues and ensure the proper succession of temporal fates. We have been focusing on regulatory circuits that regulate third and later larval stage fates. The nuclear hormone receptor *daf-12* specifies L3 options of reproductive growth and dauer formation. Null mutants of *daf-12* are fully dauer defective, but have impenetrant delayed heterochronic phenotypes in gonadal and extragonadal tissues, suggesting that overlapping functions must work together with *daf-12* to specify reproductive growth. In screens for mutants that enhance the gonadal heterochronic phenotypes of *daf-12* null alleles we picked up *dre-1*.

In *dre-1daf-12* double mutants, the delayed heterochronic gonadal phenotype is dramatically enhanced. Distal tip cells fail their L3 dorsal movements and instead migrate into head and tail on the ventral body wall.

*dre-1* on its own displays variable and impenetrant gonadal cell migration phenotypes, including extra turns or ventrally located gonadal arms. Some alleles also have impenetrant heterochronic phenotypes in the seam cells. Notably, some cells express adult alae precociously at the L3 molt. In addition, many adult animals display gapped adult alae and undivided seam cells at the L4 molt, revealing an underlying problem in seam cell differentiation. We performed epistasis experiments with various heterochronic mutants and found that precocious mutants *lin-14*, *lin-28*, and *lin-42* abolished the alae gap phenotype. Epistasis experiments with delayed mutants are in progress. *dre-1* maps to the center of chromosome V. We have obtained YAC rescue, and are now injecting cosmids that cover this region.

Another locus, *lin-46*, also enhances *daf-12* heterochronic phenotypes. *lin-46* was first identified by Moss and Ambros, and displays impenetrant delayed L3 and Adult seam phenotypes. *daf-12* null mutants also show impenetrant L3 seam phenotypes. Double mutants display synergistic and penetrant L3 and Adult seam defects.

In summary, *lin-46* and *dre-1* act in parallel to *daf-12* to regulate third and later stage programs in gonadal and extragonadal tissues. The DAF-12 nuclear receptor may hormonally coordinate development in these various tissues by working together with distinct heterochronic gene products.

**194953. Mechanism of *cog-2* function**

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*cog-2* (connection-of-gonad defective) mutants were isolated from a screen for egg-laying-defective (Egl) mutants with vulval morphology defects. *cog-2* encodes a Sox family transcription factor that is required for the establishment of a functional vulval-uterine connection. In *cog-2* mutants, the uterine seam cell (utse) fails to execute a developmentally programmed cell fusion to the anchor cell (AC). This fusion is required for opening a channel between the vulva and the uterus. Although initial specification of utse fate occurs in *cog-2* mutants, nuclei of the utse syncytium sometimes execute an extra division and fail to migrate to terminal positions within the utse. These abnormalities may result from a failure to maintain utse fate, which in turn prevents fusion to the AC. Investigation of the mechanism of *cog-2* function can provide insight into the role of Sox protein target selection and regulation and the role of cell fusions in organogenesis.

We aim to identify regulatory targets, binding partners, and/or genetic interactions of COG-2 using genetic and molecular approaches. At the genetic level, we have identified multiple suppressors of *cog-2* mutants. We have found that two such suppressors can restore egg-laying activity to approximately 25% of the suppressed animals, whereas only 3% of non-suppressed *cog-2* mutants have egg-laying activity. We plan to present mapping data and phenotypic analysis of these mutants. In addition, we are characterizing other mutants with AC-utse fusion defects. At the molecular level, we are developing reagents to be used for a yeast two-hybrid screen to identify potential COG-2 binding partners.

**979004. COORDINATE REGULATION OF GLUTAMATE RECEPTORS AND *ncs-1* IN INTERNEURONS BY *fax-1* AND *unc-42***

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The *C. elegans fax-1* gene encodes a nuclear receptor homolog of the human PNR gene. PNR has been implicated in the specification of photoreceptor identities in the human retina. In *C. elegans*, *fax-1* functions in regulating aspects of neuron identity, including expression of *flp-1* and axon pathfinding decisions in the AVK interneurons. The paired-like homeobox gene *unc-42* is similarly required for specification of neuron identity, including expression of *flp-1* and axon pathfinding in the AVK neurons, and axon pathfinding and expression of glutamate receptors *glr-1*, *glr-4* and *glr-5* in the AVA, AVD and AVE interneurons.

We determined the expression pattern of *fax-1* through immunofluorescence studies using anti-FAX-1 antisera. *fax-1* is expressed in 18 neurons, the distal tip cells, and 4 "secondary" vulval cells. Expression in non-neuronal cells is limited to those stages during which these cells are undergoing migration or morphogenetic movements; the dtc's from L2 through L4, and the vulval cells only during L4. However, *fax-1* deletion mutants do not have obvious gonadal or vulval defects. Expression in the nervous system appears to be very consistent from mid-embryogenesis through adulthood. FAX-1 protein is detected in the AVA, AVB, AVE, AIY, AVK, DVA, MI, and RIC neurons, plus two pairs of anterior neurons that have not yet been positively identified.

We are interested in identifying genes that may be regulated by *fax-1* and, given the overlapping expression pattern, the regulatory relationship between *fax-1* and *unc-42*. The *ncs-1* gene encodes a neuron-specific calcium sensor and is expressed in the AVK interneurons. We observed no expression of *ncs-1::gfp* in the AVK neurons of *fax-1* and *unc-42* mutants, suggesting that both transcriptional regulators control the expression of *ncs-1*. FAX-1 immunoreactivity was reduced, but not absent, in the AVK's of *unc-42* mutants, suggesting that expression of *fax-1* is partially dependent on *unc-42* in this cell type. Therefore, *unc-42* may regulate *ncs-1* via *fax-1*.

We examined expression of glutamate receptor genes in several neurons in *fax-1* mutants. *fax-1* is required for expression of the two NMDA receptor subunits, *nmr-1* and *nmr-2*, in the AVA and AVE interneurons, but not the AVD and RIM neurons (in which *fax-1* is not expressed). However, *fax-1* is not required for expression of the non-NMDA receptor subunits, *glr-1* and *glr-5* in AVA and AVE interneurons. Unlike the AVK neuron, expression of FAX-1 protein appeared normal in the AVA and AVE interneurons of *unc-42* mutants. *unc-42* is required for normal pathfinding by the AVA, AVD and/or AVE interneurons, but *fax-1* is not. Therefore, in contrast to AVK, where *fax-1* and *unc-42* appear to function in linear or overlapping pathways to regulate neuron identity, in the AVA and AVE interneurons *fax-1* and *unc-42* appear to play parallel regulatory roles to regulate different aspects of neuron identity.

*fax-1* may regulate these genes either directly or indirectly. The *nmr-1*, *nmr-2* and *ncs-1* genes contain four, six, and five (respectively) potential monomeric *fax-1* binding sites (AAGTCA core common to this class of nhr). None of these genes contain potential dimeric binding sites. We are taking two major approaches to identify other genes that are regulated by *fax-1*. First, we are preparing to perform a yeast one-hybrid screen. Preliminary experiments are underway to test the system using potential binding sites in *fax-1*'s promoter and sites known to be bound by PNR. Second, we are performing microarray analysis in collaboration with the Kim Lab to identify candidate genes that are differentially-regulated in *fax-1* mutants. We are currently evaluating approximately 100 candidates that are two-fold or more under-represented in *fax-1* L1's versus wild-type L1's.

**253709. Identification of functions for downstream targets of the dauer-regulating TGF-beta pathway in *Caenorhabditis elegans***

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*Caenorhabditis elegans* development is environmentally regulated. Under favorable environmental conditions, the *C. elegans* life cycle consists of four larval stages (L1- L4) in the progression to an adult. However, if environmental conditions are unfavorable, a worm may arrest development following the L2 stage and become a dauer larva. Dauers have several morphological and physiological alterations that make them well adapted for long-term survival and resistant to harsh environmental conditions.

Genetic studies of dauer formation have focused on the identification of genes that affect the dauer formation. Genes that affect dauer formation fall into two phenotypic classes: one is dauer-constitutive (Daf-c), which mutants enter dauer inappropriately, and the other is dauer-defective (Daf-d) which mutants fail to form dauer under inducing conditions. Dauer formation is regulated by three parallel pathways including a transforming-growth-factor- $\beta$ -like pathway. Much of the TGF- $\beta$  intracellular signaling mechanism has been demonstrated, but the target genes responding to the signaling pathway have not yet been elucidated.

Our lab has identified over a thousand genes that show significant difference in expression level in wild type vs. Daf-c TGF- $\beta$  pathway mutants (see abstract by Liu et al.). We would like to understand the function of these regulated genes in the dauer formation process. We are using the RNAi feeding method to knockdown expression of these genes. A ts *daf-4* allele is used in our assay, allowing us to perform the assay at temperatures that give a weak Daf-c phenotype, so that we can see an enhancement or suppression of dauer formation.

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**855576. *mig-14* functions in multiple developmental processes regulated by Wnt signaling**

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P3.p P8.p express the Hox gene *lin-39* and become the vulval precursor cells (VPCs). Activation of the Ras and Notch pathways in the VPCs causes them to adopt induced cell fates and divide to form the vulva. A Wnt signaling pathway also functions in VPC fate specification, via regulation of the Hox gene *lin-39*. Wnt signaling was first implicated in VPC fate specification with the identification *bar-1*. *bar-1* encodes a beta-catenin/armadillo-related protein that is required for expression of the Hox gene *lin-39* in the VPCs. In *bar-1* mutants, loss of LIN-39 can cause too few VPCs to adopt induced fates. In addition to *bar-1* (beta-catenin), other Wnt pathway components, including *apc-1* (APC), *pry-1* (Axin), and *pop-1* (TCF), also function in VPC fate specification. Besides the defects in vulval development, *bar-1* mutants also have defects in the migration of the progeny of the neuroblast Q<sub>L</sub> and in cell fate specification by P12. Both of these processes are known to be regulated by Wnt signaling.

Like *bar-1*, a single mutation in a locus previously called *pvl-2(ga62)* was identified in the screen for mutants with a Pvl phenotype. *pvl-2(ga62)* mutants display defects in VPC fate specification, migration of the progeny of Q<sub>L</sub> and in fate specification by P12 like those of *bar-1* mutants, suggesting that *pvl-2* may function as a component of a Wnt signaling pathway. This is further supported by the fact that *ga62* fails to complement *mom-3*, which was identified by the Bowerman lab in a screen for endoderm/mesoderm specification mutants. *mom-3* mutants display maternal effect lethality resulting from an E to MS cell fate transformation, and *pvl-2/mom-3* animals die as embryos showing that the two loci are allelic. *ga62* also fails to complement *mig-14(mu71)* for both the Egl and P12 phenotypes. *mig-14(mu71)* was identified by the Kenyon lab in a screen for Q<sub>L</sub> neuroblast migration mutants. This locus, previously known as *mig-14* (Kenyon, Nishiwaki), *mom-3* (Bowerman), *let-553* (Hodgkin), and *pvl-2* (Eisenmann) will now be called *mig-14*. As *mig-14* functions in multiple processes regulated by Wnt signaling, we are attempting to map and clone this locus. In collaboration with the Bowerman lab, we have mapped *mig-14* to the interval on LGIIR between the *jsp304* and *jc101* polymorphisms. We have obtained rescue with a YAC which covers this interval. Currently, SNP mapping is being employed to further localize the *mig-14* locus. Details of progress will be reported. As there are no known Wnt pathway components or regulators in the genetic interval where this locus lies, *mig-14* may encode a novel Wnt pathway component.

**390572. Gene Regulation by TGF-beta Signaling In *C. elegans*.**

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In *C. elegans*, there are two well characterized TGF- $\beta$  signaling pathways, the Sma/Mab (small/male abnormal) and Daf (dauer formation) pathways. These pathways share a common type II receptor (*daf-4*) but utilize different ligands, type I receptors and smads. We have performed multiple experiments using cDNA microarrays to examine the changes in gene expression caused by TGF- $\beta$  signaling mutants. We have compared expression between *daf-4* and wild type animals as they enter dauer, as well as *sma-6* and *dbl-1* overexpressing animals. This gives us the opportunity to compare and contrast global expression changes that are related to both TGF- $\beta$  signaling pathways in *C. elegans* and specifically to the Sma/Mab pathway that we study. Our preliminary analyses of these results show roughly 800 genes to be regulated in our experiments. Significantly, only 50 genes show regulation common to both experiments. These expression changes are likely to represent genuine functional regulation by TGF- $\beta$  signaling and may indicate immediate downstream targets. We are currently in the process of confirming these results by Northern blot analysis. In addition to our continuing analysis of these experiments, we plan to do targeted studies of these 50 genes including RNA interference knockouts to try and determine their function. We are also proposing additional microarray experiments, including a developmental timecourse and experiments involving downstream components of TGF- $\beta$  signaling.

### 337246. EXPRESSION OF *nhr-111*, AN APPARENT *C. elegans*-SPECIFIC NUCLEAR HORMONE RECEPTOR

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*nhr-111* is a predicted gene (F44G3.9) that is a member of the superfamily of nuclear hormone receptors. Nuclear hormone receptors function by regulating the transcription of other downstream genes. *nhr-111* bears significant sequence homology to nuclear receptors that are involved in nervous system development in several species, such as *fax-1* of *C. elegans*, PNR and TLX of vertebrates, and *tailless* of *Drosophila*. *nhr-111* has a high degree of sequence homology to *fax-1* in the ligand-binding domain, but is more diverged from *fax-1* in the DNA-binding domain. Therefore, *nhr-111* may have been created by duplication of the *fax-1* locus, followed by divergence of DNA-binding domain sequences to create a new DNA-binding activity. Alternatively, *nhr-111* may have been created by a cassette-type mechanism in which the *fax-1* ligand-binding domain exons were duplicated and fused to DNA-binding domain exons descended from another nuclear receptor. The nearly-complete *C. briggsae* genomic sequence does not contain a gene that is closely related to *nhr-111*, raising the interesting possibility that *nhr-111* is a nuclear receptor member that evolved after the relatively recent *elegans-briggsae* divergence. Another predicted gene, Y60A3A.15, bears close relationship to the *nhr-111* ligand-binding domain, but lacks a DNA-binding domain of any kind. This predicted gene also does not appear in the *C. briggsae* sequence.

We constructed *nhr-111::gfp* transgenes to determine the time and place of *nhr-111* expression. *nhr-111* is expressed in embryos and early larvae in four cells: a pair of neurons in the ventral ganglion of the head (that have not yet been positively identified) and a pair of cells in the midbody that appear to be somatic gonad precursors. Thus, like *odr-7*, *nhr-111* appears to have a very restricted pattern of expression, suggesting that it may be involved in regulating highly-specific developmental or physiological processes. While we do not yet know the function of *nhr-111*, it may participate in a process that differentiates *C. elegans* from *C. briggsae* and other Rhabditidae.

**508347. Identifying genes regulated by the TGF-beta mediated dauer pathway in *Caenorhabditis elegans* using cDNA microarrays**

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Dauer formation in *Caenorhabditis elegans* is regulated by several environmental stimuli, including food, pheromone and temperature. Three parallel pathways, including a transforming-growth-factor- $\beta$ -like pathway, regulate dauer formation. Members of the TGF- $\beta$  superfamily play critical roles in cell growth and differentiation, and in embryogenesis. In *C. elegans* these pathways are essential regulators of dauer formation, body-size determination, male copulatory structures, and axonal guidance, but the precise pathways remain unclear. Repeat experiments suggest that the dauer TGF- $\beta$  pathway functions in neurons to control the dauer decision (Inoue & Thomas (2000) Dev Biol 217: 192). We would like to understand the output of this pathway in neurons, and how the neurons signal other cells in the body to control dauer formation. We used microarray analysis of 17,871 genes to determine changes in gene expression caused by TGF- $\beta$  pathway mutations. In these experiments, 734 genes were repressed, and 499 genes were induced at least 2.145-fold at 99% confidence level. Two hundred eighty-seven genes were regulated more than 4-fold and 54 genes were regulated more than ten-fold. More than 90% of the genes identified in this analysis have not been reported previously as dauer-regulated genes. The identified genes reveal that the TGF- $\beta$  pathway affects many aspects of cell physiology including cell growth, protection, cytoskeletal organization and signaling. Regulated genes include several types of transcription factors, as well as cell surface and cytoplasmic signal transducers. Identified targets possibly linked to TGF- $\beta$ 's effects on cell growth include the large group of ribosomal protein and aromatic amino acid permeases, as well as the translation elongation factor EF-2 protein. The TGF- $\beta$ -mediated genes with protective function include genes such as superoxide dismutase and many dauer-induced cytochrome P450's, which may serve to protect the worms from toxin, or to control signaling by modifying steroid hormones. Among the signaling molecules identified as targets, *daf-12*, one of the key genes in dauer formation signaling pathways, was induced; whereas *daf-2*, the gene that regulates dauer formation with *daf-12*, was repressed. Dauer-induced genes and dauer-repressed genes have different expression patterns in a gene expression map having 48 mountains which contain sets of highly correlated genes (See Kim et al., (2001) Science (2001) 2087-2092). We are using RNAi to suppress some of these regulated genes to test their function in dauer formation (see abstract by Pan & Patterson). We are also using computational analysis to identify putative transcriptional regulatory elements in these regulated genes (see abstract by Jani & Patterson). We will discuss what we have learned about dauer formation from our microarray analysis, and our plans to use further experiments to sort direct targets of TGF- $\beta$  signaling from indirect targets.

**604719. STEROL REQUIREMENTS AND DISTRIBUTION IN npc-1 NULL C. ELEGANS**

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The human NPC1 gene encodes an active membrane transporter of fatty acids and other hydrophobic molecules. Mutation in this gene causes Niemann-Pick disease, characterized by inappropriate lipid (especially cholesterol) accumulation. Deletion of the homologous *C. elegans* gene, *npc-1*, caused hypersensitivity to cholesterol deprivation<sup>1</sup>. Cholesterol (delta 5 sterol) is metabolized by *C. elegans* to 7-dehydrocholesterol (7DHC; delta 5, delta 7 sterol), lathosterol (delta 7 sterol) and two 4alpha-methylsterols (4MS), through a pathway that may include, or lead to, one or more functionally active, required sterols. Either 7DHC or lathosterol can completely replace cholesterol to support *C. elegans* growth and reproduction, but 4MS's can do so only partially<sup>2,3</sup>. We have determined the responses of *npc-1* null animals to minimum levels of cholesterol, 7DHC, lathosterol and 4MS's. Compared with wild-type, *npc-1* null animals required ca. 3-fold higher levels of all sterols to achieve comparable growth and reproductive effects. Thus, the *npc-1* null phenotype could not be suppressed by replacement of cholesterol with any of its most abundant metabolites. We have previously shown by filipin staining that sterol accumulates in five distinct cells of cholesterol-grown *C. elegans* - two amphid socket cells, two phasmid socket cells and the excretory gland cell - and is also found in the intestine<sup>3</sup>. We now report that filipin stains the same five cells in chitinized embryos, which have never had an opportunity to ingest sterol. Intestinal staining is also seen, but is much weaker than in foraging animals. Animals raised on 7DHC or lathosterol stain similarly to those grown on cholesterol. The same five cells are stained to comparable intensity in cholesterol-grown *npc-1* null animals. Sterol accumulation patterns in *npc-1* null animals and embryos grown on 7DHC and lathosterol will also be presented.

<sup>1</sup>Sym et al., Curr. Biol. 10, 527, 2000; <sup>2</sup>Lozano et al., Lipids, 22, 84, 1987; <sup>3</sup>Merris et al., in revision.

**937213. Expression and localization of UNC-13 proteins in *C. elegans***

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We are analyzing the expression patterns of different forms of UNC-13 proteins. *unc-13* mutants are severely uncoordinated and are resistant to acetylcholinesterase inhibitors. The most abundant protein product expressed from this gene, UNC-13 LR, localizes to synapses (Kohn, 2000). UNC-13 interacts with other presynaptic proteins and affects vesicle priming (Richmond, 2001). While some mammalian homologues are found in the nervous system (Brose, 1995), the human homologue is expressed in kidneys (Song, 1999). The *C. elegans unc-13* gene is predicted to express several different protein products including UNC-13 LR, UNC-13 LMR, and UNC-13 MR. L represents the left region of the protein, M represents the middle, and R represents the right. Mutations in the *unc-13 L* region of the gene alter expression of some protein products and result in uncoordinated movement. A large deletion in the *unc-13 R* region, which would eliminate all protein products, results in lethality (Kohn, 2000). We are developing antibodies to determine the localization of the different forms of UNC-13 proteins to explore the possibility that these proteins have different functions. Specifically, we are making antibodies that recognize the UNC-13 M and UNC-13 R regions. We are interested in determining whether all forms of the protein are found in neurons, and where proteins are localized within neurons. Knowledge of the localization patterns of the proteins will enable us to explore possible functions of these proteins.

**14564. Cameleon-reported Insights into Touch Receptor Channel Function in Vivo**

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Mechanical signaling, such as underlies the sense of touch, is the least understood type of signal transduction. Elegant genetic analyses of gentle body touch sensation conducted by Chalfie and colleagues have provided data supporting a molecular model of touch transduction. In this model, a candidate mechanically-gated touch-transducing channel (made up of MEC-4 and MEC-10 subunits of the DEG/ENaC channel superfamily) associates with additional MEC proteins inside and outside the cell. These contacts tether the channel to extracellular and intracellular proteins in associations that exert gating tension. A real challenge in testing the working model of touch transduction is in finding an appropriate system for electrophysiological testing of mechanosensitive gating. Expression in heterologous systems such as oocytes is problematic because several proteins, both inside and outside the cell, must contact the channel to deliver gating precise forces. Direct patching of touch neurons is also technically difficult given that tiny neurons are tightly tied to the cuticle within a specialized extracellular matrix. For this reason, we have been interested in in vivo assays of channel function, such as made possible by the work with gene-based sensors of neuronal activity such as calcium sensing cameleons.

In previous work, Kerr et al. (Neuron 26:583-594) reported effective use of a cameleon to report transient calcium changes in muscle and neurons. The cameleon houses a YFP and a CFP connected by a calcium binding domain. When calcium is bound to the cameleon, a conformational change allows for fluorescence resonance energy transfer so that ratiometric measurements of YFP and CFP signals can be used to indicate changes in calcium concentrations.

Since our preliminary studies (IWM 2001, abstract 659), we have made several improvements to our assay system. First, we have reconstructed a mec-4 promoter/cameleon reporter that has increased fluorescence signal, integrated the transgene, and repeatedly outcrossed strains so that now we get consistent and substantial measurements from touch neurons. Second, we have modified our mechanical "worm poker" to deliver precise gentle touch stimuli.

For gentle touch stimuli, we find that mec-4 null mutations completely abolish responses. However, a response to harsh touch can still be elicited, indicating that ion channels can still be activated in tested mechanosensory neurons and suggesting that the six body touch receptor neurons also house channels that respond to harsh touch. We are testing effects of mec-4, mec-2 (a stomatin-related protein that has been shown to potentiate MEC-4 channel activity in oocytes (Goodman et al., Nature 415:1039)) and mec-6 (needed for stable MEC-4 channel expression) mutations on the response. Since the touch receptor channel is highly likely to be a sodium channel, the calcium influx in response to touch may seem initially paradoxical. We expect that in response to touch receptor MEC-4/MEC-10 channel activation, sodium influx depolarizes the cell thereby activating other channels.

In summary, cameleon-based reporting is a novel method for monitoring neuronal responses to gentle touch in vivo and preliminary data indicates mec-4 mutants are defective in this response.

**913050. *C. elegans* G protein-coupled receptor kinase, *grk-2*, and chemosensation**

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We are interested in how sensory neurons detect different stimuli, and we use the ASH sensory circuit in *C. elegans* to study this question. The ASH neurons detect high osmolarity, touch to the nose, and noxious odorants such as 1-octanol and benzaldehyde. To address the molecular pathways involved in octanol detection, we conducted an EMS screen to obtain octanol avoidance mutants. Six mutant strains were identified that are defective in octanol avoidance.

One recessive mutant strain, *rt97*, is severely defective in octanol response, and mildly defective for osmotic avoidance, but normal for nose touch response. *rt97* animals are also severely defective in response to odorants sensed by the AWA and AWC sensory neurons suggesting that the *rt97* mutation perturbs chemical sensation mediated by many neurons. *rt97* corresponds to a T354I mutation in the conserved kinase domain of a putative *C. elegans* homolog of G protein-coupled receptor kinase (GRK), *grk-2*. A promoter::GFP reporter construct for *grk-2* is expressed in many neurons in the head and tail including the ASH neurons, as well as in vulval muscles and the ventral nerve cord.

G protein-coupled receptor kinases (GRKs) are a family of serine/threonine protein kinases that phosphorylate activated G protein-coupled receptors (GPCRs). In mammals, GRKs are known to play a major role in receptor desensitization. Two GRKs are found in the *C. elegans* genome; *grk-2* is most similar to mammalian GRK2/3 (65% identity), and *grk-1* is similar to mammalian GRK5 and GRK6. We are characterizing *grk-2* further, and are addressing why a mutation in *grk-2* causes defective olfactory response in *C. elegans*. Characterization of *grk-2* in *C. elegans* should help us to understand how the GRKs regulate olfactory response.



**411734. Regulation of Early Germline Proliferation in *C. elegans***

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Primordial germ cell proliferation is essential to amplify the germ line and establish a stem cell population from which gametes differentiate. We are using *C. elegans* as a model system to study the developmental molecular mechanisms that control early germ cell divisions. In *C. elegans* a pair of primordial germ cells (PGCs), Z2 and Z3, are born at ~100-cell stage of embryogenesis, arrive in the somatic gonad primordium, and begin to proliferate in the last half of the first larval stage. These cells then give rise to the thousands of nuclei of the adult germ line. What developmental cues trigger the proliferation of the PGCs and govern meiotic competence in the germ line?

Soma-to-germ line interactions have been implicated in germline development in many organisms, including *C. elegans*. The receptor GLP-1, a LIN-12/Notch family member, promotes germline proliferation in response to a ligand produced by a somatic cell, the distal tip cell. Cell ablation studies (Kimble and White 1981) and genetic studies (Austin and Kimble, 1987), however, have demonstrated that GLP-1 independent cell-cell communication exists between germ line and somatic gonad precursors. The PGCs (Z2 and Z3) neither divide nor enter meiosis after an early L1 ablation of the flanking somatic gonad precursors, Z1 and Z4 (Kimble and White 1981). Thus cell-cell interaction is necessary for (1) the first several rounds of germ cell divisions and (2) competence to enter meiosis. In attempts to identify the molecular pathway responsible for this interaction, we isolated several Nog (no apparent germ line) mutants in at least 2 loci that superficially phenocopy the effects of the Z1 and Z4 ablation on the germ line, while leaving the somatic gonad intact. One Nog mutant, *ar228*, has a recessive and highly penetrant Nog phenotype. In these mutants, Z2 and Z3 are present in the L1 but there are no apparent germ cells in later larval stages or in the adult. This mutation has been mapped to a region containing less than 100 ORFs. Through molecular characterization and identification of the locus defined by *ar228*, we hope to gain a molecular understanding of early germline proliferation, meiotic competence, and/or cell-cell communication within the gonad primordium.

**271278. STRUCTURAL DIFFERENCES IN THE GOLGI APPARATUSES OF WILD-TYPE AND *unc-20* MUTANT ADULTS**

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Mutations in the *C. elegans* gene *unc-20* result in axon outgrowth and pathfinding defects in several neurons, hypersensitivity to serotonin, and uncoordinated movement. At the current time the molecular identity of *unc-20* is unknown, however genetic mapping data suggest that *unc-20* is either C15C7.1 or C15C7.2, both of which are predicted Golgi complex proteins. We used transmission electron microscopy to learn more about the function of the *unc-20* in relation to Golgi complex structure and axon bundling. There were clear differences between wild-type and *unc-20* mutant Golgi complexes in two cell types, the coelomocytes and pharyngeal marginal cells. In both tissues, the lengths of individual Golgi cisternae of *unc-20* mutants were significantly shorter than wild-type, and prominent vesicles were present adjacent to cisternae, which was not observed in wild-type. This phenotype is suggestive of a defect in vesicle trafficking between adjacent cisternae. However, we have not ruled out the possibility that these defects are due to the *smg-1* mutant background in the strain examined. Despite defects in outgrowth and pathfinding by some neurons in *unc-20* mutants, overall nerve bundling does not appear to be disrupted in *unc-20* mutants.

In addition, the ultrastructure of Golgi apparatuses in wild-type *C. elegans* were found to vary among different tissues. Golgi apparatuses were relatively scarce in the intestine, spermatheca and hypodermal seam cells and had two to four loosely-stacked cisternae. Golgi apparatuses were plentiful in coelomocytes and pharyngeal marginal cells and had two to eleven tightly-stacked well-organized cisternae. Golgi apparatuses of neurons were also scarce, had three to four tightly-stacked, but disorganized, cisternae. Abundant clear vesicles were found adjacent to the cisternae in neuron Golgi apparatuses. Intestinal, seam cell, coelomocyte, and marginal cell Golgi apparatuses often had a single large vesicle associated with their trans face, while spermatheca and neuron apparatuses did not. This study reveals considerable structural heterogeneity of Golgi apparatuses among different tissues. These tissue-specific differences may reflect underlying differences in protein trafficking or processing, and/or differences in mechanisms of cisternal maturation.

We have amplified cDNA and genomic DNA from *unc-20(e112)* mutants without detecting a mutation in either C15C7.1 or C15C7.2. Thus the hypothesis that *unc-20* encodes a protein involved in Golgi structure or function has not yet been confirmed. We are continuing with a molecular analysis of *unc-20(r977)*, a *smg*-dependent allele that is likely to have a mutation in the open reading-frame.

#### **494851. Analysis of linker cell death**

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Apoptosis can sculpt tissues during development, may prevent cancer and could potentially play a role in neurodegeneration. Genetic approaches using *C. elegans* have identified a conserved genetic pathway required for cellular suicide, which includes molecules like caspases. The apoptotic cell undergoes an engulfment process, which enables a neighboring cell to swallow the dying cell. An unresolved question is the nature of the signaling between the dying and engulfing cell.

Male worms have a linker cell at the tip of the developing gonad. This cell leads migration of the gonad to the end of the intestine. When it reaches its final destination, the linker cell dies and is engulfed by either U.lp or U.rp. It appears that the engulfing cell may play an active role in the demise of the linker cell, since the linker cell will survive if either the engulfing cell is killed by laser ablation or the linker cell is prevented from reaching the engulfing cell in a migratory mutant background (1). Strikingly, the linker cell is one of only a couple of cells that can die even in the presence of a strong loss-of-function allele of the *ced-3* caspase (2). This observation suggests that a novel mechanism remains to be uncovered, which facilitates caspase-independent cell death in *C. elegans*.

A GFP:*lag-2* fusion protein provides a marker for the linker cell (courtesy of J. Kimble). We are using these transgenic worms for 4-D microscopy to study linker cell death in a living animal. This approach enables fine temporal and spatial resolution of the death and engulfment of the linker cell. We are studying the migration and death of the linker cell in wild-type animals and in apoptotic mutants. The strains examined; *ced-2*, *ced-6*, *ced-7*, *ced-9* and *ced-13*, encompass both the core apoptotic pathway and also engulfment mutations. Preliminary observations suggest that in certain mutant backgrounds, linker cell death is delayed but not abrogated. An intriguing observation was what appeared to be cytoplasmic processes from dying linker cells contacting an adjacent cell, perhaps the engulfing cell. We plan to use the GFP marker strain to screen for mutants to identify novel components in this unusual cell death process and in particular to try to unravel the relationship between death and engulfment.

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**773851. *pvl-5* maintains hypodermal cell identity by preventing apoptosis during *C. elegans* vulval development.**

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Programmed Cell Death (PCD) or Apoptosis is a tightly regulated, evolutionarily conserved process. The PCD regulatory network has increased in complexity, from a relatively simplistic linear pathway involving few protein domains in *C. elegans* to a complex network of protein-protein interactions between vastly expanded families of proteins with diverse domains in vertebrates. The nematode, *C. elegans*, has been a powerful model organism for dissecting and elucidating the core intrinsic PCD pathway. We describe here a gene involved in preventing apoptosis of Pn.p cells via a novel pathway.

During the L1, 12 neuroectoblast cells, the P cells migrate into the ventral midline where they divide to generate a Pn.a neuroblast and a Pn.p hypodermal cell. Six of the twelve Pn.p cells (P3.p-P8.p) become the vulval precursor cells (VPCs) while the remaining six fuse with the surrounding hypodermis. A single recessive allele, *pvl-5(ga87)*, was identified in a screen for mutants affecting vulval development. In *pvl-5* mutants there are an average of 7.0 large Pn.p nuclei [n=172, range=4-10] in the ventral midline compared to 11 in wild-type animals. Consequently, there are fewer than six VPCs and usually fewer than 22 cells available to form the vulva. Analyses using *ajm-1::GFP*, a marker that outlines the boundaries of hypodermal cells, and lineage analyses in *pvl-5* animals show that the 12 Pn.p cells are born correctly in *pvl-5* mutants, but later undergo abnormal cell death around the L1 molt. The corpses of the dying cells are refractile and ovoid with a concavity in the center, which is distinct from the more typical button-shaped morphology of dying neurons. This cell death defect is suppressed by loss of function mutations in *ced-3* caspase and a gain of function mutation in *ced-9*, a Bcl-2 homology anti-apoptotic protein, suggesting that the Pn.p cells are undergoing PCD. Interestingly, this cell death defect is not suppressed by *ced-4(lf)*, an APAF-1 homolog. This is surprising given that loss of CED-4, which is required for the activation of CED-3, suppresses all known developmental programmed cell death in the worm. This suggests that in the *C. elegans* vulva, the loss of *pvl-5* function leads to the activation of PCD by a mechanism distinct from that seen in other somatic cells. Mutations in two genes, *lin-24* and *lin-33* lead to abnormal death of the Pn.p cells. The cell deaths in *pvl-5* are distinct from those caused by the semi-dominant mutations in *lin-24* and *lin-33* in that they are suppressible by *ced-3(lf)* and *ced-9(gf)* but not by mutations in genes involved in corpse clearance. Interestingly in *Pristionchus pacificus*, the cells outside the equivalence group undergo PCD while in *C. elegans* they fuse with the surrounding hypodermis. It is possible that *C. elegans* had acquired the *pvl-5* function that keeps the Pn.p cell from undergoing apoptosis or that *P. pacificus* had lost this function leading to the cells undergoing PCD in the absence of *lin-39*. To understand the molecular nature of the *pvl-5* gene product we are trying to clone the *pvl-5* gene by cosmid rescue. We have identified two genomic regions that rescue *pvl-5* and results will be presented.

**450533. The role of LIN-5 and GoLoco domain proteins in spindle position determination**

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During cell division, the mitotic spindle is responsible for chromosome segregation and determination of the cleavage furrow plane. In response to developmental cues, the spindle position can be altered to generate cells with different sizes and developmental fates. To better understand spindle positioning during development, 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. 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 several 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 G $\alpha$  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* G $\alpha$  homologs, *goa-1* and *gpa-16* (Gotta and Ahringer, 2001), all show a high degree of similarity. In each case, RNAi does not prevent 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, and the proper localization of LAP-1,2 depends upon the presence of LIN-5. 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.

**705163. *daf-5*, a TGF-beta signaling output controls stem cell division in dauer larvae**

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The reproductive/dauer developmental decision is controlled by environmental cues, such as food availability, pheromone and temperature. A TGF-beta-related pathway promotes reproductive over dauer development. Mutations in *daf-7* (TGF-beta-like ligand), *daf-1* and *daf-4* (type I and type II receptor kinases), and *daf-8* and *daf-14* (Smad transcription factors) induce dauer at restrictive temperature regardless of environmental cues. The Daf-c phenotype of these mutants is suppressed by mutations in *daf-3* (Smad) or *daf-5*.

Epistasis analysis suggests that *daf-5* and *daf-3* are either antagonized by this TGF-beta-related pathway or act in a parallel pathway. Our initial mapping data implied DAF-5 was a new component of TGF-beta pathway rather than another Smad. Therefore, we expected the unveiling of the molecular identity and function of DAF-5 would shed new light on the TGF-beta signaling mechanisms.

Our cloning of *daf-5* showed that *daf-5* encodes a novel protein with weak similarity to chromatin remodeling proteins such as Sno/Ski and ACF-1. Sequencing of twenty *daf-5* alleles revealed a mutation hotspot. Both translational and transcriptional *daf-5::GFP* fusions were most prominently expressed in the nuclei of the neurons including ganglia in the head and tail. Also, transgenic animals show GFP expression in the hypodermis, pharynx, muscles, intestine, and distal tip cells. This observation is consistent with expression and functional data from the Riddle and Thomas labs that indicate that the TGF-beta receptors function in the nervous system. Using nervous-system-specific and other promoters, we are testing in which tissues *daf-5* must be expressed to rescue a *daf-5* phenotype.

DAF-5 and DAF-3 have been shown to interact with each other in the yeast-two-hybrid system (Hu, Tewari, Ruvkun and Vidal, 2001 IWM abstract 346). We are collaborating to identify the domains of DAF-5 required for the DAF-5/DAF-3 interactions. We then plan to perform immuno-precipitation from extracts of *C. elegans* to see if DAF-5 and DAF-3 physically interact during different developmental stages and to determine if the interaction is controlled by the TGF-beta signaling.

Hong et al (1998) showed that dauer cell cycle arrest in the *daf-7* mutant is dependent on the cell cycle regulator *cki-1*. We used *rnr-1::GFP* (C. Zimmerman & R. Padgett, personal communication) and *cki-1::GFP* (Hong, 1998) to examine the seam cell division in dauer larvae or early L3. Results suggest that DAF-5 is required for developmental cell cycle arrest in seam cells of dauer larvae and that the TGF-beta pathway controls *cki-1* via *daf-5*.

**494121. Mechanism of *lin-39* regulation by the Wnt and Ras pathways during vulval development**

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We are studying how extracellular signaling regulates cell fate specification during vulval development. The vulva arises from a group of six Vulval Precursor Cells (VPCs) located in the ventral midline of the body. The Hox gene *lin-39* is expressed in the VPCs and is required for their cell fate specification. At the L3 stage, activation of a conserved RTK/Ras signaling pathway induces vulval formation and upregulates LIN-39 levels. A Wnt signaling pathway also acts during VPC fate determination and is known to regulate *lin-39* levels. Therefore, *lin-39* is regulated by Wnt and Ras pathways. Our goal is to understand how these signaling pathways converge in the regulation of the Hox gene *lin-39* to specify VPC fates. We are taking three approaches to discern whether regulation of *lin-39* by the Wnt and the Ras pathways is transcriptional or post-transcriptional.

First, we built a transcriptional and a translational *lin-39::GFP* fusions containing the entire *lin-39* genomic sequence by recombination of GFP into a YAC containing *lin-39*. GFP expression from these constructs recapitulates the expression pattern of *lin-39* in ventral cord neurons, sex myoblast descendants, and VPCs. At the time of vulval induction GFP level is upregulated in P6.p in both *lin-39TL::GFP* and *lin-39TN::GFP*. This result suggests that the Wnt and Ras pathways regulate *lin-39* at the transcriptional level. We are currently crossing *lin-39TL::GFP* and *lin-39TN::GFP* into Wnt and Ras mutants to understand this regulation in more detail.

Second, we are dissecting the *lin-39* promoter to look for enhancer elements required for *lin-39* regulation. We cloned *lin-39* genomic regions into a *pes10::GFP* reporter. Our result shows that TCF3, a 1.3 kb fragment located approximately 6 kb upstream of the transcription starting site is sufficient for the expression of GFP in the VPCs, mainly in P6.p at the time of vulval induction. We are currently dissecting TCF3 in order to identify specific elements required for Wnt or Ras regulation.

Third, the LIN-39 protein sequence has two phosphorylation sites for MAP kinase, suggesting that phosphorylation might be required for *lin-39* regulation. Our result showed that 6HIS-LIN-39 purified from *E. coli* was phosphorylated by vertebrate MAP kinase *in vitro*. Mutations in both sites abolished phosphorylation mediated by MAP kinase. We are currently taking an *in vivo* approach to test if phosphorylation is involved in *lin-39* regulation at the time of vulval induction.

**371538. A DOPAMINE RECEPTOR MODULATING MECHANOSENSORY RESPONSES IN *C.elegans*.**

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Various dopamine-modulated behaviors, including motor response, reward and information processing, are subject to sensitization and habituation. Mal-adaptation of these processes are thought to play a role in learning and memory processes as related to addictions and psychosis. The underlying mechanisms of these processes are still poorly understood, and genetic approaches with *C. elegans* may allow the study of these mechanisms for dopamine mediated responses. A large body of evidence suggests that dopamine functions as a neurotransmitter in *C. elegans* modulating various behaviors, including egg laying, food sensing, and motility. Here we report the identification and functional characterization of a *C. elegans* dopamine receptor. The *dar-1* gene encodes two alternatively spliced forms of the receptor recognizing catecholamines, with dopamine displaying the highest potency. Other amines did not activate the receptor. The absence of (nor)epinephrine in *C. elegans* indicates that dopamine is the likely endogenous ligand for this receptor. Functional coupling of the receptor was dependent on Gas. The *dar-1* gene displays a restricted neural expression pattern including several neurons in the nerve ring and mechanosensory neurons like ALM and PLM. A deletion mutant for *dar-1* (*dar-1(ev748)*) displayed altered behavioral responses to mechanosensory stimuli, but not previously established dopamine-related behaviors. The *dar-1* mutant showed a greatly enhanced facilitation of accelerations in response to tap habituation training. This phenotype was rescued by a *dar-1* wild-type transgene indicating that the DAR-1 receptor is required for modulation of mechanosensory behavioral plasticity.



**492963. Identification of Genes Specifically Required for Ventralward Axon Guidance in *C. elegans***

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Several conserved classes of guidance molecules direct axon guidance in *C. elegans* and other organisms. The UNC-6/netrin diffusible guidance cue is required for axon guidance along the dorsal-ventral axis in *C. elegans*, *Drosophila* and vertebrates. The UNC-5 guidance receptor in combination with UNC-40/DCC mediates repulsive response to the UNC-6 which is expressed in ventral nerve cord and ventral epidermis, to orient growing axons in a dorsal direction.

Attractive responses to UNC-6 require UNC-40 but not UNC-5. We and the laboratory of C.

Bargmann have reported that SAX-3/Robo mediates repulsive response to SLT-1/Slit which is expressed in dorsal body wall muscles, to orient axons in a ventral direction. *eva-1*, enhancer of ventralward axon guidance-1, has AVM ventralward axon guidance defects. We have rescued *eva-1* mutations with overlapping cosmids. We have found that the *eva-1* mutation enhances the AVM and PVM guidance defects of *unc-6* null or *unc-40* null alleles, suggesting that EVA-1

functions in an axon guidance pathway that acts in parallel with UNC-6 and UNC-40. We have also found that *eva-1* mutation doesn't enhance the AVM nor PVM guidance defects of *slt-1* null allele or *sax-3* strongest allele (*ky123*), suggesting that EVA-1 functions in the same pathway as SLT-1 and SAX-3.

**839057. 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 aim at formalizing 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.

For additional information, demonstration and references see:

<http://www.wisdom.weizmann.ac.il/~kam/CelegansModel/CelegansModel.htm>

#### **463408. An Eph Receptor Sperm-Sensing Control Mechanism for Oocyte Meiotic Maturation**

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During sexual reproduction in most animals, oocytes arrest in meiotic prophase and resume meiosis (meiotic maturation or M-phase entry) in response to sperm or somatic cell signals (1). Despite progress in delineating mitogen-activated protein kinase (MAPK) and CDK/cyclin activation pathways involved in meiotic maturation, it is less clear how these pathways are regulated at the cell surface. In *Caenorhabditis elegans*, oocytes and somatic gonadal sheath cells sense the presence of sperm in the reproductive tract and prepare for fertilization (2). When sperm are absent, oocytes arrest in meiotic prophase for days (2). Sperm promote oocyte M-phase entry and MAPK activation using the major sperm protein (MSP) as a signaling molecule (3). MSP also functions in sperm locomotion, playing a role analogous to actin (4). Thus during evolution, MSP has acquired extracellular signaling and intracellular functions for reproduction.

We present multiple lines of evidence that the VAB-1 Eph receptor protein-tyrosine kinase and a somatic gonadal sheath cell-dependent pathway, defined by the POU-homeobox gene *ceh-18*, negatively regulate oocyte M-phase entry and MAPK activation. MSP antagonizes these inhibitory circuits, in part by binding VAB-1 on oocytes and sheath cells. Eliminating *vab-1* and *ceh-18* function removes the dependence of meiotic maturation and ovulation on the presence of sperm. Therefore, this meiotic control mechanism resembles a cell cycle checkpoint (5) and may confer a selective advantage to hermaphrodites and females by conserving metabolically costly oocytes when sperm are unavailable for fertilization. MSP-domain proteins are found throughout the metazoa, including six in the human genome, and may regulate contact-dependent ephrin/Eph receptor signaling pathways.

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**645297. Identifying new genes involved in TGFbeta signaling in *C. elegans***

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All animals, including humans are affected by various outside stimuli and respond accordingly to those stimuli. *Caenorhabditis elegans* senses environmental cues (pheromone, food and temperature) and develops accordingly into a reproductive adult or a dauer larva. The decision as to which developmental path is followed is controlled by a TGFbeta signaling pathway.

The components of the TGFbeta pathway in *C. elegans* include a ligand encoded by *daf-7* and two receptors encoded by *daf-1* and *daf-4*. *daf-8*, *daf-14* and *daf-3* code for transcription factors called Smads. (Daf stands for dauer formation.) Mutations in *daf-7*, *daf-1*, *daf-4*, *daf-8* and *daf-14* will result in a dauer-constitutive (Daf-c) animal (one which will go into dauer even under conditions which do not induce dauer development in wild type). Mutations in *daf-3* result in a dauer-defective (Daf-d) animal, which suppresses the Daf-c phenotype. Other mutations have also been identified that are Daf-d and/or suppress the Daf-c phenotype of TGFbeta pathway mutants. They include *daf-5*, *daf-12* and *scd-1*. *scd* stands for suppressor of constitutive dauer.

The above model of the dauer pathway describes the members of the pathway and the path of the TGFbeta signal. The mechanism by which the TGFbeta pathway regulates transcription of dauer specific or non-dauer genes is still not understood. Our genetic analysis suggests that there are other, still unidentified factors in the TGFbeta pathway (such as transcription factors or co-factors) that regulate transcription of these genes. Identification of these factors might help to explain in more detail the transcriptional events.

Our search for the new genes concentrated downstream of the TGFbeta pathway. Screens were performed yielding eighty suppressors of the *daf-7* (Daf-c) mutation. I tested thirteen suppressors for penetrance and complementation of four other known Daf-c suppressors - *daf-3*, *daf-5*, *daf-12* and *scd-1*. Our tests indicate that we have isolated alleles of these known suppressors, as well as four alleles that do not complement any of these mutants. We will test these alleles for complementation with the other two known suppressors: *scd-2* and *scd-3*.

I have also started to map selected suppressors using a snip-SNP method, which utilizes presence of SNPs (single nucleotide polymorphisms) in Bristol N2 and Hawaii CB4856 strains.

**572971. Regulation of body size by *lon-1* and *kin-29* downstream of the *dbl-1* signaling pathway**

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Sma/Mab pathway signaling in *C. elegans* regulates proper body size formation. The components known to be involved in this TGF-beta-like signaling pathway consist of the ligand (*dbl-1*), two receptor serine/threonine kinases (*sma-6* and *daf-4*) and transcriptional regulators known as Smads (*sma-2*, -3, and -4). Smads have previously been shown to translocate to the nucleus upon receptor stimulation, bind DNA and affect target gene transcription. Mutations in any of these components result in animals that are approximately 30% shorter in length than wild-type animals. Two genes isolated from EMS screens performed in our lab, *lon-1* and *kin-29*, oppositely affect proper body size formation. *kin-29* is small, similar to the mutations seen in the known Sma/Mab pathway signaling components (also identified as *sns-8* by A. Lanjuin and P. Sengupta), while *lon-1* is approximately 25% longer than wild-type. We observe that *lon-1* is epistatic to Sma/Mab pathway signaling components and find that it encodes a novel protein with homology to the CRISP (cysteine rich secretory protein) family of proteins. *lon-1* is expressed in the hypodermis and is capable of regulating proper body size formation when expressed specifically in hypodermal tissues. We see that loss of pathway signaling results in an up-regulation of *lon-1* mRNA expression, suggesting that the Smads are unable to bind the *lon-1* promoter and repress its transcription. Recently, the Smads have been shown to bind tubulin; microtubule disruption then results in Smad nuclear accumulation and transcriptional regulation of a TGF-beta -dependent reporter gene. This suggests that the Smads are released from the microtubules, become phosphorylated by the receptor serine/threonine kinase and therefore translocate to the nucleus and affect target gene transcription. We hypothesize that *kin-29* is the component necessary for the release of the Smads from the microtubules. We have cloned *kin-29* and find that it encodes a serine/threonine kinase showing homology to members of the EMK (ELKL motif kinase) family. These proteins have been shown to phosphorylate MAPs (microtubule associated proteins), thereby affecting microtubule stability. We are currently investigating the role *kin-29* plays in microtubule stability and regulation of Smad activity.

**43430. A predicted innexin, LEP-1, is required for male tail tip morphogenesis in *C. elegans***

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We are interested in the genetic and molecular mechanisms that control male tail tip morphogenesis. The male tail tip of *C. elegans* undergoes morphogenesis during the fourth larval stage (L4 stage) of postembryonic development. The four hypodermal cells that form the tail tip coordinately fuse and retract in the males, generating a blunt-ended (peloderan) tail tip in the adult. In some species related to *C. elegans*, the tail tip cells fail to retract, producing a conical, pointed (leptoderan) tail tip. Because this failure of tip retraction does not affect morphogenesis of other male tail structures, these two processes are regulated independently.

To further understand the genetic mechanisms that underlie male tail tip morphogenesis in *C. elegans*, we are characterizing fourteen new mutants in which the tail tip cells fail to retract. The tail tip of these mutants resembles that of leptoderan nematodes (Lep phenotype) in both morphology and development. The first gene cloned from this screen is *lep-1*. The *lep-1* gene controls male tail tip morphogenesis in *C. elegans* (Yang, 2000). The protein product of *lep-1* might be part of a signaling pathway involved in tail tip morphogenesis since it is a predicted innexin (gap junction component). Members of the innexin protein family are structural components of invertebrate gap junctions and are analogous to vertebrate connexins.

I am currently working on the functional characterization of the *lep-1* gene during *C. elegans* development. Up to now, most of our work has been done with a reduced function (*rf*) allele of *lep-1* (*bx37*). Although the phenotype of this allele is reduced male tail tip retraction, one important goal is to determine if *lep-1* is only required for postembryonic tail tip morphogenesis. To determine the developmental consequences of completely inactivating the *lep-1* gene, I am screening for a null allele of *lep-1*. To characterize its spatiotemporal expression pattern, I am also in the process of generating transgenic lines with transcriptional and translational fusions between *lep-1* and *gfp*. This will test the hypothesis that *lep-1* is expressed in the tail tip cells themselves and that LEP-1 innexin assembles at the boundaries between cells. To test that LEP-1 can self-assemble into gap junctions that function to "electrically" couple cells, I plan to inject expression constructs into *Xenopus* oocyte and conduct cell line expression experiments. Overall, these studies will help us to understand the role of this innexin in postembryonic morphogenesis.

**315337. Body size regulation in *C. elegans* -Progress in cloning *lon-4*, *lon-5*, *lon-6* and *lon-7*.**

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We are interested in understanding how body size is regulated in *C. elegans*. The final size of an organism or organ can in principle be influenced by the number of cells, their volume and the amount of extracellular matrix between them. In *C. elegans* growth occurs in several stages. During embryogenesis, cell division occurs without an increase in total volume of the embryo. During larval development on the other hand, growth is associated both with an increase in the size of certain cells and with an increase in cell number. Finally during the adult stage, body size increases without an increase in cell number. Measured in terms of volume, 43% of the total growth of *C. elegans* occurs during the larval stages and 57% occurs during the adult stage. It has been shown that even though cell division ceases during the adult stage, nuclei in the hypodermis undergo several rounds of endoreduplication during this stage. Since in other systems an increase in cell ploidy results in an increase in cell size, it has been suggested that the increase in body size during the adult stage is at least in part due to an increase in endoreduplication in hypodermal tissue. Consistent with this idea, mutations in *dbl-1*, a gene encoding a TGF-beta family ligand, reduce both hypodermal endoreduplication and body size, and mutations in *lon-1*, a gene that is negatively regulated by the TGF-beta pathway, causes an increase in endoreduplication. Mutations in *dbl-1* do not appear to affect the number of somatic cells.

In order to learn more about how body size is regulated we are characterizing several genes that can mutate to give rise to a Lon phenotype similar to that caused by overexpression of *dbl-1* or loss-of-function mutations in *lon-1*. We have previously shown that *lon-3* encodes a cuticle collagen that causes an increase or decrease in body length when under or overexpressed. *lon-3* mutations affect body length independently of their effects on endoreduplication. We are presently cloning *lon-4*, *lon-5*, *lon-6* and *lon-7*. We have mapped *lon-4* to a region close to *lin-29* on chromosome II, and *lon-5* and *lon-6* to small intervals on X. In a reverse genetic approach to understand how body size is regulated in *C. elegans* we have generated a deletion allele, *sv31*, of a homologue of S6 kinase. Wild-type worms starved, after the critical time point when they can enter dauer, grow to a final body size considerably less than wild type. In other organisms S6K is thought to act as a sensor of the nutritional status of the cell, altering cell growth in response to nutrients by regulating the efficiency of translation. In *Drosophila* for example, mutations in S6K cause a reduction in cell and body size, as does raising flies with reduced nutrients. Consistent with the results from these other systems, *sv31* homozygotes grow more slowly than wild type, have a reduced brood size, and grow to a final size that is slightly less than that to which wild-type worms grow. Interestingly *sv31* mutant worms also have a darker appearance, probably due to the increased size of fat droplets in the intestine. This suggests that they have a metabolic defect resulting in increased fat accumulation. We are presently conducting experiments to characterize the *sv31* phenotype in more detail.

**970434. The role of UNC-103, an ERG K channel, in electrical excitability in *C. elegans***

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Human ether-a-go-go related gene (HERG) encodes a K channel involved in repolarization of the cardiac action potential. Mutations in HERG result in long QT syndrome, a condition that can lead to life-threatening arrhythmias. *unc-103* is the *C. elegans* homologue of HERG, sharing 70% amino acid identity in the highly conserved transmembrane and pore domains. A gain-of-function mutation (gf), A334T, occurs in an amino acid in transmembrane domain S6 that is conserved in all species variants of ERG isolated to date. *unc-103* (gf) displays a profound mutant phenotype characterized by pharyngeal pumping arrhythmias, egg-laying and locomotion defects, consistent with a hyperpolarizing effect of an inappropriately activated K channel. Quantitation of pharyngeal pauses in (gf) mutant worms reveal a 10 fold increase in pause length, from  $0.3 \pm 0.1$  to  $13.0 \pm 2.7$  seconds in Wild-type and mutant worms respectively ( $p < 0.05$ ). This pumping defect is partially rescued by the HERG-specific blocker d-sotalol, with 100 mM d-sotalol treatment decreasing pause length by nearly 50% from  $14.9 \pm 5.1$  to  $7.6 \pm 1.7$  seconds in control and drug-treated worms respectively. This result suggests that the drug-binding pocket of ERG may be conserved between worms and humans. Locomotion assays reveal that both the (gf) mutation and a loss-of-function (lf) mutation (n1213) in *unc-103* result in impaired movement as determined in liquid thrashing assays. Wild-type worms thrashed at a rate of  $45.4 \pm 0.8$  thrashes/sec while the (gf) worm thrashing rate was slowed over ten-fold to  $3.1 \pm 0.4$  thrashes/sec and the (lf) rate was  $36.9 \pm 1.9$  thrashes/sec ( $p < 0.05$  for (gf) and (lf) when compared to Wild-type). Whereas (gf) worms are unable to lay eggs, *unc-103* (lf) worms are impaired in their ability to retain eggs, consistent with a depolarizing effect that may result from the absence of this K channel. These data suggest *unc-103* plays an essential role in feeding, locomotion and egg-laying. In an effort to understand the molecular nature of the mutant phenotype, we have engineered the analogous *unc-103* (gf) mutation into HERG (A653T) and expressed this clone in *Xenopus* oocytes to study under voltage-clamp conditions. HERG A653T displays a 30 mV hyperpolarizing shift in the I(V) when compared to Wild-type HERG. This profound change in gating kinetics would result in a channel passing considerable outward current at negative membrane potentials. This hyperpolarizing shift may be responsible for setting resting membrane potentials too hyperpolarized in the tissues where it is expressed. Indeed, we find that expression of this mutant channel significantly hyperpolarizes the *Xenopus* oocyte  $V_m$  by 17 mV when compared to Wild-type HERG-expressing oocytes ( $-80.6 \pm 0.8$  and  $-63.6 \pm 0.6$  for A653T and Wild-type HERG respectively,  $p < 0.05$ ). We propose that *unc-103* plays a critical role in establishing resting membrane potentials in various tissues and that mutations in this gene disrupt normal resting potentials resulting in the mutant phenotypes observed. We are currently looking at d-sotalol effects on the mutant channel expressed in *Xenopus* oocytes and plan to use the *unc-103* (gf) worms as a background for a repressor screen to identify interacting proteins.



**404981. A *Pc-G* like gene, *sop-2*, is required for maintaining the repressed state of Hox genes in *C. elegans***

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The role of *Polycomb*-group genes (*Pc-G*) in maintaining the repressed state of Hox genes is evolutionarily conserved from *Drosophila* to mammals at both structural and functional level. *Pc-G* proteins form at least two biochemically separable complexes, Esc-E(Z) and Pc-Ph, which appear to have distinct functions in the repression mediated by *Pc-G*. The Esc-E(Z) complex consists of Extra sex combs(Esc) and Enhancer of zeste [E(Z)]. The Pc-Ph complex consists the products of *Pc*, *Posterior sex combs(Psc)*, *polyhomeotic(ph)*, and *Sex combs on midleg(Scm)*. In *C. elegans* only the homologs of E(Z) and Esc have been identified, and are encoded by *mes-2* and *mes-6*, respectively. However, instead in regulating Hox gene expression, the function of *mes-2* and *mes-6* is mainly limited to germline development and silencing of tandem transgenic arrays. Homologs of other *Pc-G* proteins have not been identified in *C. elegans* genome. How the repressed state of Hox gene is maintained in *C. elegans* is unknown.

We have isolated one mutation, *sop-2(bx91)*, which causes ectopic expression of all four Hox cluster genes, *lin-39*, *ceh-13*, *mab-5* and *egl-5*, resulting in massive homeotic transformations. For example, the development of a pair of seam cells, V1 to V6, is affected in *sop-2(bx91)* mutants. During normal development, V1 to V4 produce alae, whereas V5 and V6 generate 6 pairs of male sensory organs in the tail, called rays. V5 generates ray 1. V6 gives rise to rays 2 to 6. In *sop-2* males, rays as well as little fan-like cuticular structures, derived from anterior seam cells V1-V4, are formed in anterior body region, and V5 generates one or more ectopic rays. The anterior descendants of V6 often adopt the fate of posterior descendants of V6 in *sop-2* mutants, resulting in duplication of rays. Generation of ectopic rays from V1-V5 and duplication of V6-rays are dependent on *mab-5* and *egl-5* activity. *sop-2* also regulates the expression of *lin-39* in specifying serotonergic neurons in the male ventral cord. Like *Pc-G* mutants, *sop-2(bx91)* also causes other defects that may not be due to the ectopic expression of Hox genes. For example, *bx91* causes larval lethality at 25 degree, Muv, and partial hermaphrodite to male sex transformation.

We found that *sop-2* encodes a novel protein with no clear motifs. However, the C-terminal of SOP-2 has a weak similarity to a SPM domain, which is also present in and required for the function of *Pc-G* proteins Ph and Scm. A highly conserved Proline in this domain has been mutated to Serine in *bx91*, suggesting that this domain is important for *sop-2* function. Like the expression of *Pc-G* genes in other systems, *sop-2* is widely expressed and its product forms speckled domains in the rim of the nucleus. All these studies suggest that a highly divergent Polycomb like complex may be existed in *C. elegans* to fulfill the Pc-Ph function. We are currently performing yeast two-hybrid to identify the SOP-2 interacting proteins.

#### 928544. Cellular physiology of cultured *C. elegans* mechanosensory neurons

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

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

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

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

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

**897007. *C. elegans* SKN-1 links developmental specification of the feeding/digestive system to mechanisms that orchestrate one of its most ancestral functions, resistance to oxidative stress**

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The *C. elegans* transcription factor SKN-1 initiates formation of the feeding and digestive system by specifying the fate of one of the first four embryonic cells, then it later has postembryonic functions that have not been elucidated. We have previously found that in parts of its DNA binding and transcription activation regions, SKN-1 is related to the dimeric vertebrate NRF bZIP proteins, which are not present in *C. elegans*. This similarity suggests that SKN-1 might represent an NRF protein ortholog, even though it binds DNA through a unique and highly divergent monomeric mechanism. Vertebrate NRF proteins are required to activate oxidative stress-induced enzyme genes directly and to resist oxidative stresses, indicating that *skn-1* might have similar functions.

Supporting this model, we have now determined that homozygous *skn-1* mutants are differentially sensitive to the oxidative stress inducer paraquat, and have a reduced lifespan. *skn-1* is expressed postembryonically in the intestine and the ASI neuron, which regulates entry into the dauer stage, a larval form with reduced metabolic needs. In the intestine, and similarly to NRF proteins, a SKN-1::GFP fusion translocates into the nucleus in response to oxidative stress. In *C. elegans*, multiple SKN-1 binding sites are present near the 5' start site of numerous NRF target gene orthologs. These *C. elegans* genes include gamma-glutamylcysteine synthetase (*gcs-1*), which is essential for glutathione synthesis. In wild type *C. elegans*, a *gcs-1::gfp* transgene is expressed at high levels in the pharynx and ASI neuron, and at low levels in the intestine, but its intestinal expression is induced robustly by either paraquat or heat. In both the intestine and ASI neuron, GCS-1::GFP expression is abolished in *skn-1* mutants, and is dependent upon a SKN-1 binding site within a composite element which is similar to that through which SKN-1 activates its embryonic target genes *med-1* and *med-2*. We conclude that SKN-1 and NRF proteins have preserved some parallel target genes and functions, and that SKN-1 acts through conserved transcription mechanisms that are required not only for the initial specification of the feeding and digestive system, but also for one of its most ancestral functions. The cell fate specification roles of SKN-1 and possibly NRF proteins thus may have an ancient functional origin in oxidative and other metabolic stress responses.

**165577. Cell fusion in *C. elegans*: Function of a novel protein EFF-1.**

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Cell fusion leads to the formation of multinucleate syncytia. This has been demonstrated in mammalian tissues (i.e. muscle, placental, and bone) as well as in the hypodermis of the nematode *C. elegans*\*. The mechanism of cell fusion has remained a mystery in every organism. We have recently identified a gene required for cell fusion (*eff-1*; epithelial fusion failure) in *C. elegans*. EFF-1 is a predicted type I integral membrane glycoprotein. EFF-1 activity appears to be solely restricted to the fusion of adjacent lipid bilayers. Mutations within the extracellular domain of EFF-1 do not seem to affect prefusion events (differentiation, adhesion, etc.). At the embryonic level *eff-1* mutants cannot fuse dorsal or ventral hypodermis. However, as the worms mature through the adult stage, these same cells fuse to form a large multinucleate syncytium (139 nuclei). *eff-1* mutant larvae can also be phenotyped by a small ball at the end of the tail (referred to as *eff*-tails), presumably resulting from a failed embryonic fusion of two epithelial cells normally destined for apoptotic death following fusion. As the worm matures and elongates, other morphological structures are subject to cell fusions (i.e. Vulva, lateral seam cells). Although *eff-1* mutant worms are delayed in fusing their cells within these tissues they are remarkably viable, allowing us to analyze this dynamic process at all stages of development.

I propose to study the structure and function of the EFF-1 protein. According to protein BLAST searches EFF-1 is not a member of any known protein family. We are employing three approaches to deduce the functional domains and motifs of EFF-1. (1) We are mutating putative structural and functional domains (Phospholipase A2 active site, Fusion peptide), via site directed mutagenesis, within a wild type copy of the *eff-1* gene known to rescue *eff-1* mutant worms, and microinjecting the products into mutant worms to test for rescue. I postulate that the mutation of essential residues within these sites will alter the function of EFF-1 to a less functional form, thus preventing rescue. (2) We have isolated several new alleles by mutating male worms with the mutagen ethylmethanesulfonate (EMS). I hypothesize that accumulation and analysis of new alleles will help in identifying functionally significant regions in EFF-1. In addition, a strain homozygous for a strict *eff-1* null genotype should arise following repeated random mutagenesis. (3) I also propose to identify EFF-1 isoforms and characterize their role in cell fusion. The hypothesis is that functionally significant alternate forms of EFF-1 could exist which were not pulled down in our initial cloning efforts. cDNAs from known spliced form products will be tested for their ability to rescue *eff-1* mutant strains. Data derived from these three sets of experiments will give us a clearer picture of the functional structure of EFF-1, which will lead us to predict a role for EFF-1 within a mechanism for lipid membrane fusion.

\* Mohler WA, Shemer G, del Campo JJ, Valansi C, Opoku-Serebuoh E, Scranton V, Assaf N, White JG, Podbilewicz B. (2002) The type I membrane protein EFF-1 is essential for developmental cell fusion. *Dev Cell* Mar;2(3):355-62.

**648199. Characterizing *bar-1* function and regulation during *C. elegans* vulval development**

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*bar-1* was identified in a screen for mutants affecting vulval development. In *bar-1* mutants, the Vulval Precursor Cells (VPCs) show defects in cell fate specification and adopt Fused fates more often than in wild-type animals. *bar-1* was cloned and found to encode a *C. elegans* homolog of the  $\beta$ -catenin/Armadillo family of proteins that have two functions: cell adhesion and Wnt signaling. *bar-1* is required to maintain levels of *lin-39Hox* in the VPCs and *mab-5Hox* in the Q neuroblasts to enable these cells to adopt correct cell fates, suggesting a role for *bar-1* in Wnt signaling. *pry-1*, an Axin homolog and a negative regulator of Wnt signaling, functions upstream of *bar-1* in the above processes. *pry-1* mutants display phenotypes associated with overactivation of the Wnt pathway and hence would be a good tool to isolate suppressors that could potentially identify other genes functioning in Wnt signaling in these processes. We have identified six suppressors from screening 5000 haploid genomes. We are in the process of characterizing the phenotypes of these suppressors and will determine if any of them are *bar-1* alleles. In addition to *bar-1(ga80)* isolated in our lab, there are seven more alleles of *bar-1* that were isolated from screens done in the Kenyon lab and have not been further characterized. Efforts are underway to determine the nature of mutations in these alleles that leads to differences in severity of phenotypes amongst them.

We know from work in our lab and others that *bar-1* functions through a Wnt signaling pathway to enable the VPCs to adopt correct cell fates. However, we know very little about how *bar-1* is regulated in the vulva to begin with. We used a series of *bar-1* promoter deletions made as transcriptional GFP fusions to determine the spatio-temporal expression patterns in the vulva and other places. Using the above analysis, we found a 1.1 kb piece of the *bar-1* promoter that was sufficient to drive *bar-1* expression in the VPCs and another 1.1 kb piece that is sufficient to drive expression in the ventral cord neurons. We are in the process of analyzing smaller deletions within these 1.1 kb regions to find minimal enhancer elements required for expression in the VPCs and ventral cord neurons in the hope of identifying transcription factors that bind to these sites and regulate *bar-1* activity *in vivo*.

### 17771. A SCREEN FOR GENES SYNTHETICALLY LETHAL WITH *lin-35* Rb

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The class B synMuv gene *lin-35* encodes a *C. elegans* protein similar to the product of the Retinoblastoma gene (Rb), a tumor suppressor. Many human solid tumors contain mutations in Rb or in genes encoding proteins that regulate Rb. *lin-35* mutant animals provide an in vivo model for mammalian cells harboring mutant Rb genes. Since *lin-35* mutations are not lethal, we are screening for genes with functions required for viability in *lin-35* mutants but not in wild-type animals to identify potential targets for cancer therapy. Such targets, if inactivated pharmacologically, could cause the specific death of Rb-deficient cells.

We are using the chromosome I RNAi library described by Fraser et al. (2000)<sup>1</sup> to screen for genes that are essential specifically in *lin-35(n745)* animals. *lin-35(n745)* contains an early stop codon and is considered a null allele<sup>2</sup>. We are comparing the phenotypes following RNAi of *lin-35(n745)* animals to the published results for N2<sup>1</sup>. At this point, we have screened 50% of chromosome I (1309 genes). We have seen severe phenotypes (embryonic lethality, sterility, larval arrest, larval lethality or severe growth delay) for 244 (18.6%) genes. Of those, 162 (12.4%) appeared to have the same or very similar phenotypes for *lin-35* and N2 animals. 82 (6.3%) of those tested apparently had severe phenotypes in *lin-35* but not in N2 animals, while 23 (1.8%) apparently had severe phenotypes in N2 but not in *lin-35* animals. Some of the RNAis of genes that caused severe phenotypes in *lin-35* animals but not N2 animals have been retested to confirm both the *lin-35* and N2 phenotypes. Of the 22 retested, 17 continued to display more severe phenotypes for *lin-35(n745)* animals. These genes do not fit into obvious classes based on homology. Extrapolating from the current data set, we expect to find 75-150 genes on chromosome I the RNAi of which are synthetically lethal with the *lin-35(n745)* mutation.

Because *lin-35(n745)* animals are less healthy than N2 animals (decreased brood size and rare sterile animals<sup>3,4</sup>), it is possible that some of the synthetic phenotypes seen in *lin-35* mutants but not in N2 animals are caused by the non-specific additive effects of two harmful mutations. Similarly, RNAi of some genes may affect one cell type and the *lin-35* mutation another so that together these two distinct defects result in severely affected animals. To identify genes the RNAi of which are cell autonomously synthetically lethal with *lin-35*, I am developing an assay to assess the effect of inactivation of *lin-35* in combination with inactivation of any of the genes that are identified in the primary screen within a single tissue.

<sup>1</sup>Fraser, A. G., Kamath, R. S., Zipperlen, P., Martinez-Campos, M., Sohrmann, M., and J. Ahringer. (2000). *Nature* 408: 325. <sup>2</sup>Lu, X., and H. R. Horvitz. (1998). *Cell* 95: 981. <sup>3</sup>Fay, D. S., Keenan, S., and M. Han. (2002). *Genes and Development* 16: 503. <sup>4</sup>M. Hurwitz and H. R. Horvitz, unpublished results.

**546450. Dual role of the E/Daughterless protein HLH-2 with respect to the AC/VU decision**

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The AC/VU decision is a simple example of lateral specification that occurs during the development of the somatic gonad. Two initially equivalent cells, Z1.ppp and Z4.aaa, communicate with each other via LIN-12, a receptor of the LIN-12/Notch family, and LAG-2, a ligand of the DSL family. As a result, one of the two cells becomes the anchor cell (AC) while the other becomes a ventral uterine precursor cell (VU). Although one of these cells always becomes the AC, which cell does so is random. Loss of activity of either *lag-2* or *lin-12* results in both cells taking on AC fate. Conversely, too much activity of *lag-2* or *lin-12* results in both cells taking on VU fate. We are interested in examining other genes that may play a role in this decision.

Our investigation has focused on *hlh-2*, which encodes a bHLH transcription factor that is homologous to *Drosophila* Daughterless and the mammalian E proteins. We have shown that a difference in HLH-2 expression is the first known indication that Z1.ppp and Z4.aaa are becoming different. RNAi studies indicate that *hlh-2* plays two different roles with respect to the AC/VU decision. First, we find that *hlh-2* activity is required in early L1 for the AC fate, as animals lacking *hlh-2* activity at this time do not form an AC. However, *hlh-2* is also required in early L2 in order to restrict the number of ACs formed from 2 to 1, as animals lacking *hlh-2* activity at this time exhibit a 2AC phenotype.

Several observations prompted us to ask whether the 2AC phenotype could indicate a role for HLH-2 in directly activating *lag-2* transcription. 1) HLH-2 is likely a transcriptional activator and is expressed in the same cell as *lag-2* during the AC/VU decision (the presumptive AC). 2) The 2AC phenotype is similar to the phenotype observed when *lag-2* activity is removed. 3) The *lag-2* promoter contains several potential binding sites for HLH-2. 4) In *Drosophila*, Daughterless and its dimerization partners are known to directly activate transcription of *Delta*, a homologue of *lag-2*.

RNAi experiments and mutation of potential HLH-2 binding sites in the *lag-2* promoter do suggest that HLH-2 is necessary for *lag-2* transcription in Z1.ppp and Z4.aaa during the AC/VU decision. Interestingly, *hlh-2* also appears necessary for *lag-2* expression in the mature AC. This is indicated by loss of *lag-2::lacZ* expression in the mature AC following *hlh-2(RNAi)*, as well as a phenotype indicative of loss of *lag-2* activity in the AC, failure of pi-cell induction.

In summary, *hlh-2* activity is necessary for two apparently separate roles in specification and function of the AC. The first is a requirement for AC fate, which our available evidence thus far suggests is a *lag-2*-independent role. The second is to activate *lag-2* transcription both during the AC/VU decision and in the mature AC.

## 220539. Developmental control of cell cycle in *C. elegans*

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

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

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

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



**309180. mig-15 acts with rac to control the orientation of vulval cell divisions and migrations in *C.elegans***

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mig-15 encodes a nematode ortholog of NIK, a murine serine/ threonine protein kinase of the Ste20 family<sup>1</sup>. Ste20 kinases encoded by misshapen (msn) mediate SAPK/JNK activation by functioning as MAP kinase kinase kinase kinases( MAP4ks) in *Drosophila*. MIG-15 may form a protein complex with other signaling proteins including receptor tyrosine kinases. It is reported that mutations in mig-15 disrupt embryonic and larval planar cell movements in the hypodermis, migration of the Q neuroblast descendants and outgrowth of the excretory canals<sup>1</sup>. MIG-15::GFP is localized to the adherens junctions during planar movement of hypodermal cells, and to the membrane sites where active signaling is likely occurring between the neighboring cells, suggesting that mig-15 may be a key regulator that integrate multiple signaling pathways and coordinate these with actin cytoskeleton rearrangement<sup>1</sup>.

We therefore investigated the role of mig-15 during vulval development. We found that mig-15 (*lf*) mutants have defects in vulval divisions and vulval cell migration. We have previously found that the small GTPase ced-10 rac and the rac-related gene mig-2 are redundantly required for the proper orientation of vulval cell divisions, and for vulval cell migrations<sup>2</sup>. To better understand the relationship between mig-15 and mig-2, we are testing if MIG-2:: GFP overexpression can suppress mig-15 mutant vulval defects.

1.Zhu, X., and E.M.Hedgecock (1998). MIG-15, a NIK ortholog of the STE20 family of serine / threonine protein kinases is involved in cell migration and cell signaling in *C.elegans*. Ph.D thesis. The Johns Hopkins University. 2.Kishore, R.S., and M.V.Sundaram (2002) ced-10 Rac and mig-2 function redundantly and act with unc-73 trio to control the orientation of vulval cell divisions and migrations in *Caenorhabditis elegans*. *Dev Biol* 241, 339-348.

#### 198246. Functional Studies of the *eff-1* Promoter

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Cell fusion is a common phenomenon in multicellular organisms, yet it is poorly understood. In the nematode *C. elegans*, the developing worm is very selective in choosing which cells and at what times cell fusion occurs. The recently discovered gene *eff-1*, which stands for epithelial fusion failure, is required for cell fusion in *C. elegans*.<sup>1</sup> Worms that have recessive mutations in *eff-1* exhibit a distinct larval phenotype - a constriction of the larval tail whip into a ball - that is a feature of cell fusion failures during morphogenesis. Though the requirement for this gene in cell fusion is known, nothing is known about how it is regulated in development. There are several genes, notably transcription factors, that play crucial roles in the fate decisions of fusing cells, and their expressions are restricted to specific cell types. Thus, it is likely that *eff-1* may be regulated by distinct transcription factors and upstream signaling pathways in each of the different cell types. The questions to ask are is *eff-1* indeed regulated differently in these cells, what upstream regulatory genes and which *cis*-regulatory elements in *eff-1* may account for specific regulation, and does the differential expression of these transcription factors have any role to play in *eff-1* function in these different cells?

These questions will be answered using molecular biology, microscopic and transgenic approaches. First, the temporal pattern of expression of *eff-1* will be determined. It is expected to be expressed in many tissues in the worm as it is required for cell fusion and roughly, a third of all cells in *C. elegans* fuse during development. We have begun to observe *eff-1* regulation via a transcriptional fusion of the *eff-1* promoter to a reporter fluorescent protein. Second, the determination of the length of the minimal promoter fragment that is required for the full expression of the gene will be necessary. This can be tested by observation of a Green Fluorescent Protein (GFP) reporter and by assaying for rescue of cell fusion defects of *eff-1* mutants during all stages of development. In addition, this helps in identifying the domains - putative transcription factor binding sites - within the promoter that are responsible for the function of the gene in a limited and well-defined region. The observation of the activity of wild type and mutant *eff-1* promoter expression in various transcription factor mutant worms will further aid in explaining how *eff-1* is regulated.

<sup>1</sup> Mohler WA, Shemer G, del Campo JJ, Valansi C, Opoku-Serebuoh E, Scranton V, Assaf N, White JG, Podbilewicz B. 2002 The type I membrane protein EFF-1 is essential for developmental cell fusion. *Dev Cell* 2:355-62.

**41697. A strategy for high-throughput gene mapping in *Caenorhabditis elegans*.**

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Forward genetic screens in model organisms remain a crucial tool for uncovering new information about biological systems. Unless a sequence tagged mutagen such as a transposon is used, these approaches require extensive mapping of a mutation in a gene of interest to discover the gene's identity. Traditional mapping methods in model systems often rely upon visible phenotypic markers for mapping mutations. However, SNP (single nucleotide polymorphism) markers are currently favored due to their relative abundance and usual lack of confounding interaction with the mutant phenotype. We present one tripartite strategy for rapid SNP-based cloning that takes full advantage of the availability of a sequenced *C. elegans* genome. We first confirm a set of 1,099 substitution SNP markers between the Bristol N2 and Hawaiian CB4856 strains, that span the genome with an average spacing of one marker every  $91 \pm 56$  kb. We then combine this resource with a tiered mapping strategy that progressively narrows the region containing the gene of interest. Finally, we utilize a high-throughput SNP assay that allows reliable and rapid genotyping with low marker development costs. This strategy affords rapid gene cloning in *C. elegans* and can be tailored for use in other model organisms with a sequenced genome.

### 749590. Finding Players in the CeTwist Pathway

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We are interested in understanding the function of the CeTwist protein in the mesoderm as a model for cell-fate specification during development. Previous characterization of a null allele in the CeTwist gene, *hlh-8* (*nr2061*), showed that this protein plays a role in non-striated muscle development (1). Animals with a null mutation in *hlh-8* do not properly form sex muscles, which leads to an egg-laying defective phenotype (1). A second semidominant allele *hlh-8* (*n2170*) has also been characterized (2). These animals are also egg-laying defective but the cellular defects suggest an earlier blockage in sex muscle development when compared with *hlh-8* (*nr2061*) animals. A suppressor screen is currently underway to identify mutations that can compensate for defects caused by the *n2170* allele. We expect this screen will identify proteins that physically interact with CeTwist or are downstream of CeTwist.

CeTwist is a member of the basic helix-loop-helix (bHLH) family of transcription factors. CeTwist can function as a heterodimer with CeE/DA, the product of the *hlh-2* gene, to turn on *egl-15* and *ceh-24* (1,3). We have recently identified *arg-1* (*apx-1* related gene) as another CeTwist target. In order to reveal a complete pathway of CeTwist function in *C. elegans* mesoderm development, it will be necessary to ultimately identify all downstream target genes of *hlh-8*. Using DNA microarrays, it is possible to observe global changes in gene expression of nearly every mRNA during development (4). Since *hlh-8* is expressed in at most 2% of cells at any given time during development, it may be difficult to obtain meaningful data using DNA microarrays by comparing mRNA from *hlh-8* null animals to mRNA from wild type animals. To circumvent this difficulty, we will overexpress both *hlh-8* and *hlh-2* from heat shock promoters and compare mRNA isolated from this strain to mRNA isolated from isogenic wild type animals. The three known targets genes of CeTwist: *egl-15*, *ceh-24*, and *arg-1* are overexpressed when both *hlh-8* and *hlh-2* are overexpressed from the heat shock promoter, and we predict unknown targets will also be overexpressed. To avoid measuring a secondary cascade of mRNA expression, the time of heat shock treatment will be optimized by studying the kinetics of mRNA induction of *egl-15*, *ceh-24*, and *arg-1* by RT-PCR after heat shock. We are currently building the appropriate strains for this study.

1. Corsi, A. K., Kostas, S. A., Fire, A. and Krause, M. (2000). *Caenorhabditis elegans* Twist plays an essential role in non-striated muscle development. *Development* 127, 2041-2051.
2. Corsi, A. K., Brodigan, T. M., Jorgensen, E. M. and Krause M. (2002). Characterization of a dominant negative *C. elegans* Twist mutant protein with implications for human Saethre-Chotzen syndrome. *Development* 129, 2761-2772.
3. Harfe, B. D., Gomes, A. V., Kenyon, C., Liu, J., Krause, M. and Fire, A. (1998). Analysis of a *Caenorhabditis elegans* Twist homolog identifies conserved and divergent aspects of mesodermal patterning. *Genes Dev* 12, 2623-2635.
4. Jiang, M., Ryu, J., Kiraly, M., Reinke, V. and Kim, S. K. (2001) Genome-wide analysis of developmental and sex-regulated gene expression profiles in *Caenorhabditis elegans*. *Proc Natl Acad Sci USA* 98, 218-223.

**176630. Identification of a new PHA-4 DNA binding sequence by site selection.**

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PHA-4 is a forkhead/winged helix transcription factor that is essential for pharyngeal organogenesis. PHA-4 is expressed in all five classes of pharyngeal cells as well as in the rectum, intestine and somatic gonad. The *pha-4* gene produces three PHA-4 protein isoforms called PHA-4A, B and C that differ only at their N-termini. To identify potential PHA-4 target genes expressed both inside and outside of the pharynx, the preferred PHA-4 binding sites of all three isoforms are being determined. After six rounds of DNA site selection from a pool of random oligonucleotides 20 bp long, baculovirus-produced PHA-4B selects the sequence TGTGG(C/T); this differs somewhat from the known TGTTTG PHA-4 binding site in the promoter of *myo-2*, pharyngeal myosin (1), and the high and low PHA-4 affinity sites in the promoters of pharyngeal expressed genes identified by Gaudet and Mango (2). This selected sequence is currently being inserted into the promoter of a GFP reporter gene to determine if it functions as an enhancer *in vivo*. Six rounds of selection of the PHA-4A and C isoforms have also been completed and the sequences are currently being cloned and analyzed.

(1) Kalb et al., Development 125, 2171-2180 (1998)

(2) Gaudet and Mango, Science 295, 821-825 (2002)

**477048. THE PUTATIVE REGULATORY SUBUNITS SUP-10 AND SUP-18 REGULATE THE SUP-9 TWO-PORE POTASSIUM CHANNEL THROUGH A MECHANISM THAT IS DISTINCT FROM THAT OF THE UNC-93 SUBUNIT**

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Rare altered-function mutations in the genes *unc-93*, *sup-9*, and *sup-10* result in the abnormal regulation of muscle contraction. These mutants move sluggishly and are unable to lay eggs. Loss-of-function mutations in any of these three genes completely suppress the phenotypes caused by any of the altered-function mutations, suggesting that all three genes act at the same step, possibly by encoding subunits of a protein complex. We have shown that *sup-9* encodes a two-pore K<sup>+</sup> channel subunit with similarity to the mammalian Two-pore Acid Sensitive K<sup>+</sup> channels TASK-1 and TASK-3. *unc-93* and *sup-10* encode novel putative transmembrane proteins that likely serve as regulatory subunits of SUP-9.

*sup-18* may encode a positive regulator of the *sup-9/sup-10/unc-93* channel complex. *sup-18(lf)* mutations fully suppress the Unc and Egl defects of *sup-10(gf)* mutants, while only partially suppressing those of *unc-93(gf)* or *sup-9(gf)* mutants, suggesting that *sup-18* may be preferentially required for the *sup-10(gf)* activity. In addition, we have found that *sup-18(lf)* mutations also only partially suppress the weaker defects of partial *sup-10(lf)* double mutants with *unc-93(gf)* mutations, indicating that the partial suppression of *unc-93(gf)* defects by *sup-18(lf)* is not caused by the greater severity of *unc-93(gf)* defects. We previously cloned *sup-18* and found that it encodes a type-one transmembrane protein, the cytoplasmic domain of which contains a nitroreductase domain. We found that the strong loss-of-function allele *sup-18(n1010)* mutates a highly conserved serine to an asparagine within the nitroreductase domain. The equivalent serine in a nitroreductase from *Thermus thermophilus* (*Ttnox*) contacts a tightly-bound FMN cofactor. We found that recombinant TtNOX carrying a serine-to-asparagine mutation at this site had severely reduced NADH oxidase activity *in vitro*, consistent with the hypothesis that SUP-18 has an enzymatic activity *in vivo*.

To explore the mechanisms by which *sup-10(gf)* and *unc-93(gf)* mutations activate *sup-9*, we have analyzed an unusual *sup-9* mutant. *sup-9(n1435)* suppresses fully the Unc and Egl defects of *sup-10(gf)* mutants but only weakly those of *unc-93(gf)* mutants, unlike null mutations in *sup-9* which fully suppress defects in both mutants. We hypothesized that *sup-9(n1435)* may be insensitive to *sup-18* and therefore displays the same differential suppression as *sup-18(lf)* mutations. Consistent with this model, the partial suppressive effects of *sup-9(n1435)* and *sup-18(lf)* towards *unc-93(gf)* defects were not additive in the triple mutant. The *sup-9(n1435)* mutation leads to a serine-to-phenylalanine substitution in the presumptive C-terminal cytoplasmic domain of *sup-9*. Using site-directed mutagenesis, we identified another residue in this domain that is required for *sup-18*- but not for *unc-93*-dependent activation. In addition, we have found that overexpression of *sup-18* in a *sup-10(gf)* but not in an *unc-93(gf)* mutant enhances the severity of the Unc defects. Together, these results support a model in which the *sup-10(gf)* mutation acts with the *sup-18* nitroreductase to activate the *sup-9* channel through a mechanism that is distinct from that of *unc-93(gf)* mutations.

**831575. LIN-5 and GoLoco domain proteins function in a complex to regulate spindle positioning in the early embryo**

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During cell division, the mitotic spindle segregates chromosomes to the daughter cells and specifies the location of the cleavage plane. In a multicellular organism, developmental cues can alter the position of the mitotic spindle to generate cells of different sizes and to place cells into different spatial contexts. The *Caenorhabditis elegans* gene *lin-5* is essential for both chromosome segregation and correct spindle positioning during mitosis. In the early embryo, *lin-5* activity is required for posterior migration of the mitotic spindle and rotation of the centrosomes, activities needed to determine the correct division axis. *lin-5* encodes a novel coiled-coil protein which localizes to spindle microtubules, spindle asters and the cell cortex.

To understand how developmental cues affect spindle position, we isolated LIN-5 protein complexes from *C. elegans* embryos. Gel filtration chromatography showed that LIN-5 protein is part of a complex of approximately 1 megadalton in size. Using LIN-5 specific monoclonal antibodies, we affinity purified endogenous LIN-5 from embryo lysates and analyzed these large-scale IPs by SDS/PAGE and silver staining. A specific 90 kDa band was identified conclusively as LIN-5 by tandem mass spectrometry. Peptides from a 60 kDa doublet were assigned to the product of either of two 98% identical genes located on chromosome III: C38C10.4 and F22B7.13 (here named *lap-1* and *lap-2* for LIN-5 associated proteins, also known as *ags-3.3* and *ags-3.2*, respectively).

RNAi analysis showed that *lap-1,2* are required for spindle migration and spindle rotations, similar to *lin-5*. LAP-2 protein coelutes with LIN-5 in gel filtration chromatography and colocalizes with LIN-5 to spindle asters, kinetochore microtubules and the cell cortex. Interestingly, LAP-2 localization but not protein stability requires *lin-5* activity. The predicted LAP-1,2 proteins each contain a GPR/GoLoco motif. This motif is thought to bind the GDP-bound  $G\alpha_{i/o}$  subunit of heterotrimeric G proteins and to affect the  $G\alpha$  guanine nucleotide exchange rates. Supporting the hypothesis that LAP-1,2 interact with  $G\alpha$ , combined RNAi of  $G\alpha$  subunits, *goa-1* and *gpa-16*, yielded embryonic spindle positioning defects, as previously reported, which were strikingly similar to the *lin-5* or *lap-1,2* loss of function phenotype. GOA-1/ $G\alpha_o$  and GPB-1/ $G\beta$  proteins colocalize with LIN-5 and LAP-2 at the cell cortex. Additionally, LAP-2 binds GOA-1 in a GDP-dependent manner suggesting LAP-1,2 may regulate  $G\alpha$  activity. Together, these results suggest that the LAP-1,2 GoLoco proteins function with LIN-5 in a complex that regulates spindle-cortex interactions in a signaling pathway involving heterotrimeric G proteins.

**650426. SQV-4 UDP-GLUCOSE DEHYDROGENASE IS TEMPORALLY AND SPATIALLY REGULATED TO CONTROL *C. ELEGANS* VULVAL MORPHOGENESIS**

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The development of the *C. elegans* vulva requires the involution of epithelial cells and provides a model for organ morphogenesis. Mutations in *sqv* genes affect both vulval morphogenesis and embryonic development. In early L4 larvae defective in the *sqv* genes, the vulval extracellular space that separates the anterior and posterior halves of the vulva is reduced in size. We found that *sqv-4* encodes a protein similar to UDP-glucose dehydrogenases and showed that the SQV-4 protein specifically catalyzes the conversion of UDP-glucose to UDP-glucuronic acid, which is essential for the biosynthesis of chondroitin and heparan sulfate glycosaminoglycans. SQV-4 is expressed in the vulva and oocytes, among many other cells, and SQV-4 levels are dramatically increased in a specific subset of vulval cells during vulval morphogenesis. The changes in the size and shape of the developing vulva coincide with specific changes in the number and positions of vulval cells with increased SQV-4 expression. Animals carrying multiple copies of the *sqv-4* transgene have enlarged vulval extracellular space. The number of SQV-4-expressing vulval cells is decreased in *lin-11(lf)* mutants, which have a reduced vulval extracellular space. By contrast, SQV-4 expression is increased in a subset of cells that form pseudovulvae in *lin-12(gf)* mutants. Thus, vulval morphogenesis, in particular expansion of the extracellular space of developing vulvae and pseudovulvae, may require SQV-4 function in a subset of cells that form those structures. Furthermore, there is a positive correlation between the size of the vulval extracellular space and the number of SQV-4-expressing vulval cells and possibly the level of SQV-4 expression in vulval cells. We propose that the regulation of UDP-glucuronic acid production in a specific subset of vulval cells drives aspects of vulval morphogenesis.



**999217. Computational Analysis of Putative Surface Protein Sequences with a Specific Six-Cysteine Pattern**

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Nematode surface proteins are thought to play a key role in the ability of nematodes' successful survival in diverse environments. It is possible that parasitic nematodes avoid attack by host immune responses by changing the proteins they display during infection (Politz and Philipp 1992; Blaxter et al 1992). Members of a specific class of mucin-like nematode surface proteins share a domain containing six cysteine residues arranged in a conserved spacing (referred to as the SXC domain, Gems and Maizels 1996, Blaxter 1998, Loukas et al 2000). Similar cysteine-rich sequences are found in many organisms; an important characteristic is the tendency of the cysteines to form disulfide bonds that help define the tertiary structure of the domains. To help understand evolutionary relationships between SXC domains of different species, an extensive set of SXC sequences was obtained by database searching, and similarities between amino acid sequences of the domains were determined.

BLAST searching identified over 300 SXC domains in three nematode species and two sea anemone species. One of the sea anemone domains was previously known to correspond to a potassium channel-blocking toxins consisting of a single SXC domain (Dauplais et al 1997, Loukas et al 2000). Sequence alignments, evolutionary trees, and gene structure analysis demonstrated that the SXC sequences can be categorized as members of distinct families. The results suggest that the individual families of SXC domains were present before the duplication and divergence events that accompanied speciation and created the separate genes that contain the SXC sequences. Many genes encode more than one SXC domain, and sequence similarities suggest that many of the genes encoding six cysteine sequences from *C. elegans* and *C. briggsae* are orthologous. This tends to support the hypothesis that these species diverged after the formation of six cysteine families. Without more complete genome sequence information (e.g., from the sea anemone species that constitute an outgroup) it cannot be determined how ancient these family relationships are.

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**490356. Check out my Profile! Isolation of mutants with altered *str-1* expression levels using automated, high-sensitivity fluorescence profiling**

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Autofluorescence background, the natural fluorescence from biological materials, reduces sensitivity of detection of localized fluorescence in multicellular organisms. For example in the case of a transgenic *C. elegans* that expresses GFP in two cells, that fluorescence can be masked by the autofluorescence of the remaining 1000 somatic cells. We have solved this problem with an add-on to our COPAS analysis and sorter technology that allows the instrument to measure and store the pattern of fluorescence along the length of the animal. That information was used to restrict fluorescence analysis to the region of the animal that contained the fluorescent cells thus improving the signal to autofluorescence ratio more than 20-fold. We used the system to perform a genetic screen in which we isolated mutants of PY1089 (kindly provided by Piali Sengupta), a transgenic *C. elegans* that expresses GFP in the AWB sensory neurons under control of the *str-1* promoter. Without the Profiler technology the COPAS system was unable to distinguish PY1089 from N2 wild type animals, and it could not be used in a genetic screen for mutants with reduced GFP expression. With the addition of Profiler we were able to clearly distinguish PY1089 from N2 and were further able to sort animals that had either reduced or increased GFP expression in the sensory neurons.

F2 progeny of mutagenized PY1089 were analyzed and sorted using the COPAS BIOSORT with prototype Profiler hardware. First, 100 animals were run and their peak fluorescence intensities were determined. That information was used to set a range of normal peak heights in the Profiler. The Profiler was instructed to dispense those animals whose peak heights were outside of the normal range, either higher or lower, one per well into 96-well plates. Mutants were confirmed both by regrowing the sorted animals and running them through the Profiler a second time and visually by microscopy. Approximately 50,000 animals from 8 separate pools were screened and 1,000 were dispensed to wells in the first pass. We have retested 96 lines from the sorted animals to date. We have isolated 4 independent mutant lines with decreased GFP expression, 3 independent lines with increased GFP expression and 2 lines with a mix of increased, wild-type, and decreased GFP expression. We are in the process of further characterizing the mutants by fluorescence microscopy and we will further characterize the mutants for their chemotactic response to 2-nonanone, which is dependent on proper AWB neuronal function.

**73613. Suppressor screen of the UNC-5 induced precocious migration of the hermaphrodite distal tip cells.**

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The hermaphrodite distal tip cells (DTCs) lead through their migration the extension of the hermaphrodite gonad arms. The DTCs undergo a 3-phase pattern of migration sequentially moving along the AP, DV and AP axes. The second migration phase, from the ventral to the dorsal side, requires the UNC-6/Netrin guidance cue and its receptors UNC-5 and UNC-40 (1). UNC-5, which is transcriptionally induced at the L3/L4 transition, is both necessary and sufficient to cause ventral to dorsal migration. *emb-9* promoter-driven UNC-5 in the DTCs causes a precocious ventral to dorsal migration (2). Analysis of the *emb-9::unc-5* strain led to two interesting observations. First, the *emb-9::unc-5* induced precocious migration was partially dependent upon the endogenous *unc-5* suggesting that *unc-5*, albeit undetected, is probably expressed and tightly regulated during the L3 stage. In addition, it was also suggested that some putative unknown negative regulation is relieved at the time of the turn (2). This prompted us to conduct modifier screens for both suppression and enhancement of this UNC-5 induced precocious migration phenotype. We believe that those screens will identify molecules that work upstream of *unc-5* to regulate its function or molecules that act downstream of *unc-5* to mediate its action. In addition, since the precocious migration is occurring prematurely under unfavorable conditions, it is reasonable to assume that it would be very sensitive to any changes with respect to the ability of the DTC to detach from the ventral side and reorient dorsally. This sensitivity, we believe, will allow us to identify molecules that participate in this process, but may act redundantly or have no marked phenotype when mutated on their own. As a starting strain for the screen we generated a strain that carries *emb-9::unc-5* along with *emb-9::gfp* (*evls129*). This strain has higher levels of the transgene compared to the original *emb-9::unc-5* and results in a more complex phenotype. The DTCs undergo precocious migrations at a higher penetrance (~90%) and in addition, once the DTCs reach the dorsal side, instead of reorienting towards the midbody, they migrate towards either the head or the tail. This sometimes involves extra turns of the DTC. This third phase migratory defect suggests that similar to the situation on the ventral side, in which *unc-5* must be tightly regulated for the normal process to occur, here the reduction in *unc-5* levels which occur as the DTC approach the dorsal muscle band (2) is necessary for the normal execution of the 3<sup>rd</sup> phase of migration. Apart from being informative, this phenotype allows the classification of the different suppressors with respect to their ability to suppress the 2<sup>nd</sup> or the 3<sup>rd</sup> or both migratory defects. We have conducted a clonal screen looking for mutations that would suppress *emb-9::unc-5* induced precocious migration. Out ~1000 genomes 5 suppressors were identified. All 5 are recessive and suppress the precocious migration from 80-90% to about 15-20%. (*ev768*)- has no phenotype on its own and suppresses the precocious migration as well as the 3<sup>rd</sup> phase migration defect. (*ev767*)- suppresses the precocious migration but, once the DTC approach the midbody, they turn and migrate away from it. (*ev764*)- suppress the precocious migration, but after the initiation of the 3<sup>rd</sup> migration phase the distal arm is frequently detached from the dorsal muscle band. This mutant might shed light on the cross talk between the migratory cell and the substratum it migrates upon. (*ev765*)- has a short gonad in 90% of the population on its own, as well as in the presence of *evls129*. The precocious migration is probably not suppressed in this mutant but the DTC do migrate to the midbody. We are in the process of mapping these mutants by using both genetic and snip-snip mapping. We are also generating a balanced *evls129* heterozygote strain that would serve as the starting strain for an enhancer screen.

1. Hedgecock et al. (1990). Neuron 4, 61-85.

2. Su et al. (2000). Development 127,585-594.

**989890. Does heritable interference induced by dsRNA involve transcriptional silencing in *C. elegans*?**

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In *C. elegans*, exposure to dsRNA triggers potent and specific genetic interference. Remarkably, interference can be transmitted to the progeny of exposed animals as well as to later generations. This spectacular and yet poorly explored phenomenon implies the transmission of an epigenetic determinant from the intestine of an exposed animal to its germline, and subsequently to the germline of its progeny. Although the transmitted agent has not been identified, siRNAs are likely candidates to be the inherited agent, since they are directly implicated in targeted destruction of mRNA during RNAi. Targeting two different germline-expressed genes, we have examined the transmission of interference, the steady state level of the targeted mRNA, and the accumulation of siRNA for several generations following exposure to dsRNA. In our hands, interference persisted until the F4 generation. Although the level of mRNA was dramatically reduced in F1 through F3 generations relative to control animals, the steady-state accumulation of mRNA later regained its original level. As postulated above, siRNAs were detected in the F1 larvae of exposed animals, indicating that siRNAs are indeed inherited. However, siRNAs were not detected in F1 adults or later generations, suggesting that the targeted degradation of mRNA mediated by siRNAs may not be solely responsible for long-term silencing. In lieu of these findings, one explanation for the long-term inheritance of interference is that RNA interference is coupled to transcriptional silencing in the germline of *C. elegans*. We are currently examining the transcriptional activity of the targeted germline genes to address this possibility.

**956316. RNAi based synthetic screens in *C. elegans***

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Genes that are involved in functionally redundant pathways may only display a phenotype when both pathways are rendered dysfunctional. Such interactions can be defined as synthetic phenotypes, and may not be predictable *a priori*. Our goal is to perform ORFeome wide RNAi screens to identify genes that are synthetic interactors of the *C. elegans* orthologs of human tumor suppressor genes. Identification of such genes may provide new insights into cellular homeostatic pathways.

The use of RNAi as a tool for systematic gene knock-down is significantly expedited by access to the *C. elegans* ORFeome, currently comprising ~12,000 of the ~19,000 estimated ORFs. We are currently analyzing the *lin-35* Rb, *cep-1* p53 and *vhl-1* VHL mutant worms using feeding RNAi. These mutants are viable and fertile, and each of these genes has orthology for important cellular regulatory functions of their mammalian homologs.

Briefly, the screen involves recombinational cloning of the ORFeome into RNAi feeding vectors and transformation into the HT115 *E.coli* feeding strain, in a 96 well microtiter plate format. Subsequently, parallel feeding RNAi analysis of N2 (wild type), *lin-35* Rb, *cep-1* p53 and *vhl-1* VHL mutant worms is performed in duplicate, in a 24 well format. RNAi clones resulting in synthetic phenotypes for *lin-35* Rb, *cep-1* p53 or *vhl-1* VHL mutants vis-à-vis the N2 controls will be further characterized. In parallel approaches we will screen for synthetic mutations obtained by EMS mutagenesis

### 677711. EGO-1 EXPRESSION

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*ego-1* functions in germline development and RNA interference (RNAi) and encodes a member of the RNA-directed RNA polymerase (RdRP) family (Qiao *et al.*, 1995; Smardon *et al.*, 2000). The gene was originally identified on the basis of interactions with *glp-1* and *lag-1*, components of the Notch-type signaling pathway that promotes germline proliferation (Qiao *et al.*, 1995; Smardon *et al.*, 2000). However, developmental studies indicate that *ego-1* activity is required for later aspects of germline development, as well.

In an effort to better understand the role of EGO-1 in development and RNAi, we have used anti-EGO-1 antibodies to characterize the EGO-1 expression pattern in protein extracts. In whole worm extracts, we detect a band of the predicted size, ~180 kilodaltons, that is absent from putative *ego-1(null)* strains. The developmental profile of EGO-1 expression mirrors the RNA expression pattern; we detect EGO-1 in L4 and adult animals. Comparison of cytoplasmic vs. nuclear protein extracts suggests that EGO-1 is a nuclear protein.

To complement these studies, we are (1) generating integrated *ego-1::gfp* transgenes, (2) screening for interactors via the yeast two-hybrid system, and (3) testing for genetic interactions between *ego-1* and components of the RNAi machinery. We will present an update of these studies.

**533797. TOWARD A MOLECULAR ANALYSIS OF *ego-3*, *ego-4*, AND *ego-5***

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GLP-1 is a Notch-type receptor that mediates various inductive signals during development. In the adult, it mediates a proliferative signal from the somatic distal tip cells to the germ line. In the absence of GLP-1 signaling, germ cells exit mitosis, enter meiosis, and differentiate. Mutations in *ego-3*, *ego-4*, and *ego-5* were isolated as recessive enhancers of *glp-1* in the germ line (Qiao *et al.*, 1995). In a *glp-1(+)* background, *ego-3*, *ego-4*, and *ego-5* mutants are sterile, with various defects in germline development. We have mapped them relative to visible genetic markers, but to refine their position in preparation for molecular studies, we have turned to SNP (single nucleotide polymorphism) mapping. *ego* mutations were induced in an N2 background, therefore we are mapping polymorphic sequences in the so-called "Hawaiian" strain, HA-8 (CB4856). When possible, we use "snip" SNPs that can be detected by restriction digest. Once each *ego* gene is mapped to a manageable region, we use RNAi to phenocopy predicted genes in the to see whether we can detect either the *ego* developmental phenotype or enhancement of *glp-1*.

Conventional mapping places *ego-3* to the left of *unc-61* (on Y50E8) and very close to *daf-21* (on C47E8) on chromosome V. In previous studies, we had attempted without success to identify *ego-3* by transformation rescue using cosmids from this region (L. Qiao and E. Maine, unpublished). As an alternative, we identified SNPs in the area, on C54G10, R08A2, Y50E8, and C48G7. Since genome-wide SNP screens have identified few SNPs here, ours may be useful for other researchers and will be included in a figure on the poster. We isolated a set of recombinants to the left and right of *ego-3*, and assayed the appropriate SNPs. This mapping allowed us to further delineate the *ego-3* region. We are now using RNAi to phenocopy genes in the region to see whether we can detect either the *ego-3* developmental phenotype or enhancement of *glp-1*.

Conventional mapping places *ego-4* and *ego-5* within a one map unit region to the right of *glp-1* on chromosome III. We are isolating recombinants to the right and left of each *ego* gene and assaying several known "snip" SNPs.

An update of the SNP mapping and RNAi assays will be presented.

**726386. EGL-26 Functions Involved in Vulva Morphogenesis**

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We identified two alleles of *egl-26* in a screen for mutants with vulval morphology defects. *egl-26* has a connection-of-gonad defective phenotype and encodes a novel protein. In *egl-26* mutants the most dorsal vulval cell, *vulF*, has an abnormally narrow apex. This results in a defective vulval-uterine passageway, resulting in an egg-laying defective phenotype. Moreover, a functional, translational fusion of EGL-26 and GFP reveals that EGL-26 is localized at the apical edge of the *vulE* cell. EGL-26::GFP expression is not detectable in *vulF*, suggesting a non cell-autonomous function.

In order to elucidate the function of EGL-26, we are examining its expression pattern in more detail and attempting to identify proteins that interact with EGL-26. We are generating antibodies and using other reporters to examine the expression and subcellular localization of EGL-26. To identify interacting proteins, we are conducting a yeast two hybrid assay using EGL-26 as bait. We will present results from the yeast two-hybrid experiment as well as progress towards identifying interacting proteins by co-immunoprecipitation.



**757291. *mat-1* Encodes the Anaphase Promoting Complex Sub-unit, CDC27**

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The Anaphase Promoting Complex or cyclosome (APC/C) is a multi-subunit E3 Ubiquitin Ligase that targets proteins for 26S proteasome-dependent proteolytic degradation. APC/C dependent degradation of key regulatory proteins promotes important transitions in M-phase of the cell cycle. Destruction of Pds1/Securin promotes chromosome separation at anaphase by facilitating the release of metaphase chromosome cohesion. Destruction of cyclin B promotes M-phase exit by turning off cyclin dependent kinase 1 activity.

An important regulatory subunit of the APC/C is CDC27. Studies of the *C. elegans* CDC27 ortholog (CeCDC27) indicate that CDC27 plays an essential role in promoting cell cycle progression during oocyte meiosis I. Like the majority of the other APC/C subunits, RNAi depletion of CeCDC27 in fertilized embryos prevents cell cycle progression past metaphase of meiosis I. These embryos arrest as meiotic one-cell embryos, and fail to complete zygotic development and initiate cleavage (Davis *et al.*, 2002).

We previously reported the isolation of several temperature sensitive mutations in the gene *mat-1* (Golden *et al.*, 2001). Sequencing of CeCdc27 from the various alleles revealed that *mat-1* encodes the *C. elegans* CDC27 ortholog. Shifting *mat-1* L4 larvae or young adult hermaphrodites to restrictive temperatures causes them to produce clutches of one-cell arrested embryos. Like the CeCdc27 RNAi depleted embryos, *mat-1* mutant embryos contain oocyte chromosomes that congress onto a metaphase plate within a barrel-shaped metaphase spindle. However, the first meiotic division fails to occur, anaphase is never observed, and polar bodies are never extruded. In addition, M-phase exit does not occur; pronuclei never form, sperm chromosomes remain condensed and the sperm centrosome remains quiescent. Consequently, the metaphase I arrested one-cell embryos fail to exit the meiotic cell cycle and complete zygotic development.

To broaden our understanding of APC/Cs role in the promotion of chromosome segregation and M-phase exit in *C. elegans*, we generated a temperature dependent allelic series for *mat-1*. The analysis of the various hypomorphic phenotypes seen in *mat-1* mutants confirms that CeCDC27 is required for cell cycle progression in somatic, germline and embryonic tissues. These studies also provide the first evidence that the APC/C promotes normal progression through oocyte meiosis II. Our phenotypic studies of the various *mat-1* strains reared under hypomorphic temperature conditions provide additional evidence of an interdependence between proper cell cycle progression and normal development in *C. elegans*.

**880440. Analysis of telomerase in *C. elegans***

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Telomeres, the ends of linear chromosomes, are composed of simple repetitive sequences, whose total length normally varies from 2 to 5kb in *C. elegans*. In most organisms, telomere length is maintained by telomerase, a ribonucleoprotein that reverse transcribes telomere repeats onto the ends of chromosomes. We have isolated several *C. elegans* mutants that are defective for telomere replication. One of these mutants, *trt-1*, is defective for an unusual reverse transcriptase that is the *C. elegans* catalytic subunit of telomerase. The *C. elegans* telomerase reverse transcriptase is substantially different from telomerase proteins found in other organisms. A second mutant, *mrt-1*, maps nearby *trt-1*. We have also taken a reverse genetic approach by silencing candidate subunits of telomerase. Phenotypic analysis of *C. elegans* strains with defects in telomere replication may help us to understand if and how alterations in telomere length affect biological processes in *C. elegans*.

**558872. *ego-2* FUNCTIONS IN GLP-1-MEDIATED SIGNALING IN THE GERM LINE AND EMBRYO.**

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GLP-1 is a Notch-type receptor that mediates a proliferative signal from the somatic distal tip cells to the germ line. In the absence of GLP-1 signaling, germ cells exit mitosis, enter meiosis, and differentiate. A single allele of *ego-2*, *om33*, was identified in screens for recessive enhancers of *glp-1(ts)* in the germ line and mapped between *dpy-24* and *unc-75* on chromosome I (Qiao *et al.*, 1995). Analysis of *ego-2* was confounded by the presence of linked mutations, including a *ts fer-6* allele. Recently, we recombined off the flanking mutations and characterized the *ego-2(om33)* phenotype. *ego-2(om33)* causes a *ts* germline proliferation defect. At 25°C, the *ego-2(om33ts)* germline "mitotic zone" is lost at a variable point during larval development or early adulthood. At 20°C, the *ego-2(om33ts)* mitotic zone persists well into adulthood but is often lost in older adults. Germ cells can enter and proceed through meiosis. This late-onset loss of germ cell proliferation resembles the phenotype of a weak *glp-1* mutation.

At 20°C, *ego-2(om33);glp-1(bn18ts)* animals typically have a severe Glp-1 germ line. The mitotic zone is typically lost in early larval development and all germ cells enter meiosis and form gametes. The phenotype is variable; the occasional animal has a much larger germ line and makes oocytes. In addition to its role in the germ line, GLP-1 mediates several inductive events in the embryo; this *glp-1* activity is maternally provided. We find that *ego-2(ts)* enhances the *glp-1* maternal effect lethality.

*ego-2* was previously mapped to the right of *dpy-24* (Qiao *et al.*, 1995). We have since obtained data that shuffle the position of *dpy-24* relative to other genes in the vicinity. In addition, *aph-2/Nicastrin* was identified as a component of at least some Notch-type signaling events in *C. elegans* and mapped to the vicinity of *ego-2* (Goutte *et al.*, 2000; Levitan *et al.*, 2001). We find that both *ego-2(om33)/aph-2(null);glp-1(bn18ts)* and *aph-2(null);glp-1(bn18ts)* animals have an enhanced Glp-1 phenotype. However, *ego-2/aph-2* transheterozygotes are enhanced to a lesser degree than either *ego-2* or *aph-2* homozygotes. DNA sequencing failed to detect a mutation in the *aph-2* gene isolated from the *ego-2(om33ts)* strain. Therefore, *ego-2* seems to be an independent gene and encode an additional component of the GLP-1-mediated signaling pathway. *aph-1* maps to the left of the *ego-2* region, however it functions in GLP-1-mediated signaling and interacts with *aph-2* (Goutte *et al.*, 2002). For completeness, we also sequenced the *aph-1* gene from our *ego-2* strain; no mutation was found.

We are currently using SNP mapping to localize *ego-2*.

**998474. The Function of Tax-4 in Surface Antigen Switching in *C. elegans***

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The *C. elegans* *srf-6* gene controls timing of expression of surface antigens displayed during the first larval stage. In wild-type, display of the antigen marker can be induced on the surface of stages L2-L4 by altering growth conditions (referred to as Inducible Larval Display or ILD). The *srf-6* mutant phenotype has been described as constitutive larval display (CLD), that is, the antigen marker is displayed constitutively on the surface of *srf-6* mutant worms through stage L4, regardless of growth conditions (Grenache et al 1996).

Previous studies of interactions between *srf-6* and genes controlling chemosensation in *C. elegans* suggest that *srf-6* controls surface antigen switching via a chemosensory response to changes in environmental conditions. Indeed, *srf-6* mutant worms are defective in chemotaxis to both volatile and non-volatile attractants (D. Phu and S. Politz, unpublished observations). To explain the details of the genetic interaction between *srf-6* and the cilium-structure mutant *che-3*, it is necessary to propose that the *srf-6* gene product functions by inhibiting an unknown component that activates display of the L1 marker antigen at later larval stages.

To test whether *tax-4*, a gene involved in both chemosensation and dauer formation (Komatsu et al 1996), encodes a component with these characteristics, *tax-4* mutants were tested after growth under different conditions and shown to be ILD-defective. To test a possible role for *tax-4* in the specific model proposed above, a *srf-6*; *tax-4* double mutant was constructed and tested for CLD. In the double mutant, a *tax-4* mutation suppresses the CLD phenotype of *srf-6*, suggesting that *tax-4* functions downstream of *srf-6* in controlling surface antigen switching.

Previous results suggested that *srf-6* functions downstream of *daf-11* (L. Miceli and S. Politz, unpublished), a gene involved in dauer formation and chemosensation that encodes a transmembrane guanylyl cyclase (Vowels and Thomas 1994; Birnby et al 2000). Together with the present results, this suggests that *srf-6* may function to mediate a signal between *daf-11* and the cyclic-nucleotide gated ion channel subunit encoded by *tax-4* (Komatsu et al 1996).

Birnby et al (2000) Genetics 155: 85-104.

Grenache et al (1996) PNAS 93: 12388-12393.

Komatsu et al (1996) Neuron 17: 707-718.

Vowels and Thomas (1994) Genetics 138: 303-316.

**456599. Functional Analysis of PAR-6**

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The PAR proteins are required to establish and maintain cellular polarity in the *C. elegans* embryo. PAR-3, PAR-6 and PKC-3 interact to form a complex, which, in response to a polarity cue from the sperm and the cortical spreading of PAR-2 in the posterior, becomes restricted to the anterior cortex. The PAR-6 protein is able to interact with both PAR-3 and PKC-3 as well as with the small GTPase CDC-42. In order to study the importance of these interactions for PAR-6 function, we are creating mutant forms of PAR-6 that disrupt interaction with PAR-3, PKC-3 and CDC-42 in vitro, and will then test these aberrant forms of PAR-6 in vivo using transgenesis and GFP tagging. We have introduced mutations in the semi-CRIB domain of PAR-6 that disrupt interaction with CDC-42 in yeast. Additionally, we have mapped the specific region of PAR-6 that is sufficient for interaction with PKC-3 to two well conserved N-terminal domains. We are currently testing mutations in these regions and in the PDZ domain for their ability to disrupt interactions of PAR-6 with PKC-3 and PAR-3. We have also developed a new assay for in vivo function of mutated transgenes using RNAi to specifically target the endogenous gene.

### **348426. Systemic RNAi: Form and Function**

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Three complementation groups, *sid-1*, *sid-2*, and *sid-3*, were identified in a screen for mutants defective in systemic RNAi (Winston *et al.*, 2002). The resultant mutants all retain *rde-1*-dependent cell autonomous RNAi function, suggesting that these loci contribute to the systemic nature of RNAi but do not interfere with RNAi processivity. We will discuss recent progress in mapping and/or characterization of the Sid loci.

1. SID-1 does not appear to be expressed in neurons, and neurons are frequently resistant to RNAi. Therefore, we expressed SID-1 in neurons to determine if RNAi of neuronal target genes could be enhanced. However, expression of a translational fusion between SID-1 and GFP did not provide sensitivity to RNAi of neuronal targets when dsRNA was introduced by injection or bacterial expression. We are developing a cell culture system in which to address the function of SID-1, a putative multipass transmembrane protein. Preliminary results indicate that *sid-1* mutant cells in culture are resistant to dsRNA in the medium. Additional current results will be presented.

2. *sid-2* RNAi resistance has been characterized, and *sid-2* worms appear to be resistant to bacterially-mediated RNAi, but not injected dsRNA. Current progress in mapping and cloning will be presented.

3. *sid-3* maps to the far "plus" end of LGX. We are in the process of cloning *sid-3* and hope to reveal its molecular identity at the meeting. *sid-3*, like *sid-1*, appears to be resistant to RNAi induced by both bacterially-mediated and injected dsRNA. Current phenotypic characterization results will be presented.

#### **References**

Winston W.M., Molodowitch C., Hunter C.P. (2002) Systemic RNAi in *C. elegans* Requires the Putative Transmembrane Protein SID-1. *Science* **295**(5564): 2456-9.

**604029. The Role of Phosphorylation by PKC-3 in PAR-3 Function**

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The development of many multicellular organisms is dependent upon the establishment and maintenance of cellular asymmetry and polarity. In *C. elegans*, polarity is established in the one-cell embryo and determines the anterior/posterior axis and the identity of the early blastomeres. The *par* genes (partitioning defective) are maternal-effect lethal mutations that disrupt the polarity of the one-cell embryo. PAR-3 and PAR-6 proteins colocalize to the anterior cortex of the embryo while PAR-1 and PAR-2 proteins localize to the posterior cortex. Evidence has accumulated over the past few years that the anterior proteins, PAR-3 and PAR-6, are part of a conserved protein complex that functions in similar ways in a variety of animals. A third component of this complex is an atypical protein kinase C (aPKC) named PKC-3 in *C. elegans*. Recent work by Lin et al. (Nature Cell Biology, Vol. 2, 540) in mammalian cells has shown that the mammalian aPKC can phosphorylate the PAR-3 homolog. My goal is to determine if this same kinase/substrate relationship holds for the *C. elegans* proteins and if so, to test its functional significance in vivo. I have obtained preliminary evidence that PKC-3 does phosphorylate PAR-3 in vitro in *C. elegans*. I have also made specific point mutations in the region of PAR-3 previously identified as the aPKC binding site, to test if this site is the target for phosphorylation in vitro. I am using these mutations to test for potential roles for phosphorylation in vivo.

**535451. PAR-1 is required for morphogenesis of the vulva**

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The vulva provides a simple model for the genetic analysis of pattern formation and organ morphogenesis during metazoan development. During morphogenesis a planar array of 22 columnar epithelial cells is transformed by shape changes, migrations and cell fusions into a stack of seven syncytial toroid-shaped cells (vulA-vulF) that create a central lumen (Sharma-Kishore et al., Development 126: 691). We have discovered a role for par-1 in the development of the vulva using post-embryonic RNAi to deplete larvae of PAR-1 protein. PAR-1 is a member of the EMK family of Ser/Thr kinases, which play roles in polarity in yeasts, insects and mammals. Reduced PAR-1 activity during development of the vulva has no effect on precursor proliferation and no obvious effect on fate specification but instead affects the process of morphogenesis. Using an adherens junction-associated GFP marker, we discovered that reduced PAR-1 activity leads to a failure to form the toroidal rings vulA-vulD. Normally these rings arise as a consequence of adhesion and fusion of certain mirror symmetric cells derived from P5.p (anterior) and P7.p (posterior). In PAR-1 depleted vulvae the cellular processes that normally mediate fusion between appropriate P5.p and P7.p progeny cells fail to establish or maintain contact. Despite these defects and the role of PAR-1 and its homologues in other contexts, all markers of cell polarity thus far examined appear normal after PAR-1 depletion. We also observed that during normal vulval development PAR-1 undergoes a redistribution from near adherens junctions to baso-lateral domains during morphogenesis. Given the morphogenetic defects and its subcellular localization, we propose that PAR-1 is acting at the cell cortex to transduce signals that direct the appropriate behavior of cell processes.



**622543. Towards the determination of the structure of the MEC-4 amino terminal intracellular domain**

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How do we feel touch? Elegant genetic and molecular studies by Chalfie and colleagues have identified several proteins thought to form a touch-transducing complex that mediates the response to body touch. At the core of this complex is a mechanically-gated ion channel made up of MEC-4 and MEC-10 protein subunits. MEC-4 and MEC-10 channel subunits are homologous to the epithelial amiloride-sensitive Na<sup>+</sup> channel (ENaCs) and the acid sensing channels (ASICs); the DEG/ENaC superfamily. The *C. elegans* channel subunits are called degenerins because specific mutant forms of the channel (MEC-(A713V, for example) can induce neurodegeneration. Recently, mammalian ASIC channels have been shown to be needed for normal mechanosensory perception in mouse hairy skin, suggesting that members of this channel class may play conserved roles in mechanotransduction.

Understanding how the MEC-4/MEC-10 channel functions in touch transduction is a major question in the field of mechanical signaling. These channel subunits are positioned in the plasma membrane with N and C terminal domains inside the cell and a large extracellular domain projecting into a specialized extracellular matrix. It is thought that specific proteins interact with the intracellular and extracellular domains to exert gating tension on the channel. We are currently focusing on the N-terminal domain to gain insight into mechanisms of channel function.

The MEC-4 N-terminal domain includes a highly conserved domain close to the first transmembrane domain (residues 87-95) that has been implicated in influencing channel open probability. Amino acids 1-86 are not highly conserved throughout the family, but this region is more closely related to MEC-10 and UNC-8 than other DEG/ENaCs. Both conserved and non-conserved regions are critical for MEC-4 function, and over-expression of just the N-terminal domain can have dominant negative effects on endogenous channel activity. In order to gain greater insight into structure/function relationships in the MEC-4 amino terminal domain and the nature of protein contacts it makes, we are working to solve the structure of MEC-4-N using NMR spectroscopy. Initial HSQC data reveals an encouraging preliminary structure. We will report on our progress at the meeting.

**926350. Reconstruction of connectivity in the *C. elegans* male nervous system**

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The original reconstruction of connectivity in the *C. elegans* hermaphrodite nervous system was mostly carried out by marking individual neuron profiles by hand on electron micrographic prints. This effort, undertaken primarily at the MRC Laboratory of Molecular Biology in Cambridge, England, involved a staff of 6-8 people and required 15 years. While some limited analysis of the developing nervous system and of mutants has been performed since that time, no further attempts have been made to define complete connectivity, which remains largely unknown in juvenile stages and in the adult male. To obtain further reconstructions, it is necessary to speed up the process of reconstruction many-fold. To achieve this, we have undertaken to develop computer aided methodology. Embedding, sectioning, and electron microscopy will be carried out as before. Digital analysis begins by scanning electron micrographic negatives. Neuron profiles are identified by an investigator and their positions marked by a single X,Y coordinate or their profiles may be traced. 3D reconstructions are then generated from the aligned series of images. Improved reconstruction methods will be applied to the original photographic images from Cambridge, which are now housed at the Albert Einstein College of Medicine, as well as to new images. On the Cambridge prints, which include a complete set of photographs of the adult male tail (1), as well as many valuable unpublished studies of mutants, corresponding profiles in adjacent sections are already marked by colored pen. For rapid analysis of new series, it will be essential to develop computer algorithms to aid the process of identifying correspondence between sections.

As an objective to test our methodology, we have chosen to reconstruct the preanal ganglion of the adult male, which contains the circuitry that processes inputs from a variety of specialized sensillae, including the rays, hook and post-cloacal sensilla (1). Nematode neuron processes are very small (0.1-0.2m diameter), similar in size to the finest terminal branches in mammalian dendrites. They are mostly unbranched, synapses being formed at en passant swellings. In the male, the preanal ganglion is a cylindrical neuropil some 20m in diameter and 60m in length. A typical transverse section contains the profiles of 100-200 neurites, which here, atypically, form a significant number of branches. Our progress in reconstructing this circuitry from the available series of several thousand electron micrographs from the MRC will be reported.

1. Sulston, J.E., Albertson, D.G., and Thomson, J.N. (1980) The *Caenorhabditis elegans* male: Postembryonic development of nongonadal structures. *Dev. Biol.* 78, 542-576.

### **347925. Dissecting the *C. elegans* ray developmental pathway with DNA microarrays**

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

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

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

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

**949808. IDENTIFICATION AND FUNCTIONAL ANALYSIS OF microRNA GENES IN *C. elegans* AND *D. melanogaster*.**

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*lin-4* and *let-7* are the founding members of a new family of regulatory RNAs, the microRNAs, many dozens of which have been identified in worms, flies and humans<sup>1-3</sup>. A total of 60 *C. elegans* microRNA genes have been published previously<sup>2,3</sup>. In order to develop a comprehensive catalog of *C. elegans* miRNAs, we are continuing to identify new miRNA sequences from *C. elegans* cDNA libraries and from genomic sequence conservation between *C. elegans* and *C. briggsae*. So far we have identified 65 distinct new microRNA genes, bringing the total to at least 125 in worms. We confirmed the expression of all of these new microRNA genes using Northern blot analysis and are characterizing their patterns of expression during development. 47 of the new miRNA sequences occur in single copy in the *C. elegans* genome, while the remaining 18 sequences are found in multiple loci. Some microRNA genes are clustered, suggesting coordinate regulation by a shared promoter. Using RNA folding programs, we find that approximately 70% of these new miRNAs are predicted to be processed from stem-loop hairpin structures, like *lin-4* and *let-7*. However, a significant portion of these miRNAs are not predicted to fold into a hairpin precursor. Interestingly, both hairpin and non-hairpin miRNAs exhibit Dicer-dependent production of the ~22nt mature miRNA. To aid in analyzing the whole family of microRNA genes, we have developed web interfaces to customized high-throughput Blast and mfold servers, and microViewer, a Java program tailored for microRNA annotation. In order to obtain mutants in microRNA genes, we are generating deletion mutations (for details, see abstract by Alvarez-Saavedra *et al.*) and analyzing candidate genetic loci that map in close proximity to cloned microRNA genes. Lastly, expression profiles of microRNAs conserved between flies and worms are being compared. For example, *mir-1*, 2 and 87 are expressed throughout development in both flies and worms whereas *mir-34* is enriched at later stages. These experiments should help elucidate the biological function of members of this new class of regulatory molecules.

<sup>1</sup> Lagos-Quintana M, Rauhut R, Lendeckel W, Tuschl T. (2001). Identification of novel genes coding for small expressed RNAs. *Science* 294, 853-8.

<sup>2</sup> 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.

<sup>3</sup> Lee RC, Ambros V. (2001). An extensive class of small RNAs in *Caenorhabditis elegans*. *Science*. 294, 862-4.

**342588. CHARACTERIZATION OF *lin-54* AND OTHER GENES THAT NEGATIVELY REGULATE *let-60* Ras SIGNALING DURING VULVAL DEVELOPMENT**

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The synthetic multivulva (synMuv) class A and class B genes act redundantly to negatively regulate Ras signaling during vulval development. The class B synMuv genes encode proteins that likely function to target the histone deacetylase HDA-1 to specific DNA sequences. Some of the proteins involved in this proposed targeting, including LIN-35 RB, DPL-1 DP and EFL-1 E2F, are conserved in mammals, and a similar targeting function has been described for their mammalian counterparts. To better understand this conserved pathway, we cloned the class B synMuv gene *lin-54* and identified new synMuv genes in a genetic screen.

*lin-54* was originally identified and mapped to a small region of LGIV by Jeff Thomas, a former graduate student in our laboratory. We cloned *lin-54* and found that it encodes a protein with two copies of a cysteine-rich domain. A similar domain is found in proteins in other species; however, the function of this domain is not understood. We observed partial maternal rescue of the Muv phenotype of *lin-54* mutants, indicating that maternally-provided *lin-54* can regulate the vulval cell fate specification decisions that occur during postembryonic development. However, the stage-specific ectopic expression of *lin-54* under the control of heat shock promoters indicated that *lin-54* need not function prior to vulval induction to regulate this process. In addition, we conducted in vitro protein interaction experiments using LIN-54 and other class B synMuv proteins. We will present preliminary data concerning these studies.

With graduate students Frank Stegmeier and Melissa Harrison, we screened approximately 6500 haploid genomes and recovered 95 new synMuv mutations. The mutations we assigned to complementation groups fall into three general classes: 1) mutations that affect new and previously characterized class B synMuv genes, 2) mutations that affect *ark-1*, *sli-1* and *gap-1*, genes that are thought to directly regulate Ras pathway components, and 3) mutations that synergize with class A and class B synMuv genes and define previously uncharacterized genes (see abstract by Ceol and Horvitz). We will present our characterization of these new mutations.

**352398. A possible role for the DNA damage checkpoint in aging**

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A possible role for the DNA damage checkpoint in aging

We have been studying possible effects of the DNA damage checkpoint response on aging of *C. elegans*. Clk-2 mutants grow slowly and have a long lifespan, as previously published [1].

Clk-2 is also defective for the DNA damage checkpoint and S-phase replication checkpoint. A second allele of clk-2, rad-5 (mn159), is also checkpoint defective, but grows at close to WT rate. Rad-5 mutants display the same lifespan as clk-2 worms, suggesting a role for the DNA damage checkpoint in aging of rad-5/clk-2 worms.

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

**727599. 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 alleles 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 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 orthologues 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.

## **748929. A NEW CLASS OF SYNMOV MUTATIONS IS PREDICTED TO DISRUPT A HISTONE ACETYLTRANSFERASE COMPLEX**

**Craig Ceol**, Bob Horvitz

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Elegant studies, primarily of budding yeast and mammalian cells in culture, have shown that covalent modifications of histones can regulate chromatin structure and accessibility. In multicellular organisms, mutations that affect proteins involved in chromatin modification often result in specific developmental defects. In *C. elegans*, mutations in the class B synthetic multivulva (*synMuv*) genes are proposed to disrupt histone deacetylation. Loss of zygotic function of these genes, together with loss of class A *synMuv* gene function, results in the specific transformation of P3.p, P4.p and P8.p from non-vulval to vulval cell fates. To further understand how these cell fates are specified, we screened for additional *synMuv* mutations. We uncovered a genetically distinct class of mutations that are predicted to disrupt a protein complex that, somewhat surprisingly, is implicated in histone acetylation.

We screened for mutations that cause a Muv phenotype in a class A *synMuv* background. As predicted, we recovered many mutations in known class B *synMuv* genes. In addition, we isolated mutations such as *n3712*, which differs from class B *synMuv* mutations in two important respects. First, unlike most class B *synMuv* mutations, *n3712* alone causes weakly penetrant ectopic vulval cell-fate transformations. Second, the penetrance of these cell fate transformations is enhanced in *n3712*; *synMuvB* double mutants, whereas synthetic interactions are generally not observed in *synMuvB*; *synMuvB* double mutants. Therefore, *n3712* and similar mutations define a new class of genes that synthetically interact with both class A and class B *synMuv* genes.

We cloned the gene defined by *n3712* and found that it encodes a protein similar to mammalian TRRAP (transcription/transformation domain-associated protein). *n3712* and five other allelic mutations introduce nonsense codons and are predicted to result in a loss of gene function. TRRAP and its yeast homolog Tra1p are proposed to bridge interactions between transcription factors and histone acetyltransferases. Using mutations and RNA-mediated interference we identified a histone acetyltransferase and other genes that show genetic interactions similar to those of *n3712* and are predicted to encode proteins that form a complex with the worm TRRAP homolog. We will discuss our characterization of these mutants and models of how complexes that mediate histone deacetylation and histone acetylation may cooperate to negatively regulate Ras signaling during vulval induction.



**844261. A large-scale screen for temperature sensitive embryonic lethal mutants.**

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<sup>2</sup>Howard Hughes Medical Institute

Genetic screens have identified numerous factors required for embryogenesis in *C. elegans* but these screens have begun to identify fewer and fewer new genetic loci. We know from RNAi studies, however, that hundreds of additional genes function in embryogenesis. Furthermore, we know that many genes whose products function in multiple capacities during development have been overlooked in past genetic screens because these genes would induce sterility or larval arrest phenotypes that preclude the production of embryos. As one solution to these problems we have undertaken a very large-scale screen for conditional embryonic lethal mutants. This screen is similar to strategies used in several other laboratories but includes some pre-selection steps that help to enrich for conditional embryonic lethal mutants (Ishidate *et al.*, 13th Annual International Worm Meeting, 2001). To facilitate our screen, we have recently resurrected an old protocol for growing worms on chicken egg food. We have found that a ratio of 10 parts whole blended and boiled chicken egg to 1 part concentrated OP50 can support the growth of at least three-times the number of animals that can be grown on the same volume of OP50 alone. Animals propagated on egg food grow in a tightly synchronous and healthy population, enhancing the yield of timed collections of progeny. Employing these improved culture techniques, we have currently screened the progeny of 20 million EMS-mutagenized P0 animals and have selected 15,000 candidates.

Briefly, each screen involves mutagenesis of roughly one million synchronous P0 *lin-11* egg-laying defective animals. After growth at permissive temperature (15°C in these screens) the F2 L4 stage animals are shifted to 25°C for 27 hours, then downshifted to 15° for 18 more hours. The population is then briefly hypochloritized to kill all hatched larvae and adults, but adult cuticles are not allowed to dissolve so that dead embryos made at 25° remain trapped together in the mothers uterus with viable eggs made after the shift down. The carcasses are then floated in a sucrose gradient, which allows most of the dead larvae and adults to sink to the bottom of the tube. Adults filled with inviable or unhatched eggs remain floating in a band at the top of the sucrose gradient. During the next three days these animals are maintained at 15°C in M9 solution while we screen through the sample under the dissecting scope looking for animals where a few viable larvae are trapped inside the same cuticle, bracketing numerous inviable eggs. Each such animal is a candidate for harboring a ts-allele and is picked to an individual plate for culture at 15°C for at least one more generation. Approximately one third of the candidates fail to have viable broods, and are discarded. The remainders (8109) are either leaky mutants that survived the selection process or ts alleles. Usually about 1/5 of the viable candidates prove to be bonafide ts-embryonic lethal mutants. So far 1753 ts mutant strains have been catalogued and frozen.

In order to measure the progress of the screen we have begun mapping mutants with recognizable phenotypes. Previous forward and reverse genetic screens have identified nine genes required for a signaling mechanism that specifies endoderm (Mom genes, for MOrse Mesoderm). In our collection we have identified 21 ts Mom alleles representing three genes: *lit-1* (11 alleles), *mom-4* (3 alleles), and *mom-2* (2 alleles) and not yet mapped (5 alleles). Among the 11 *lit-1* alleles there are 8 different residues altered in the protein, with two independent pairs of alleles that contain exactly the same lesion. It seems clear from this analysis that we are not recovering mutants with equal probability among the known Mom loci. To increase the repertoire and diversity of alleles we plan to increase the size of the collection by several fold and we have now switched to the more versatile mutagen ENU. We are encouraged by the identification of several mutants that map to novel genetic locations including several mutants with defects in early cell signaling and in morphogenesis (e.g. see abstract by Nakamura *et al.*). We are considering partnering with other labs to systematically map and/or more thoroughly characterize our mutants.

**527453. FUNCTIONAL ANALYSIS OF THE MICRORNA GENES OF *C. elegans***

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The heterochronic genes *lin-4* and *let-7* encode unusually small (21-22 nt) non-protein coding regulatory RNAs<sup>1,2</sup>. Strains carrying a mutation in either of these genes are heterochronic, displaying retarded development with some cell lineages having an altered temporal pattern of cell division and differentiation. *lin-4* and *let-7* normally inhibit translation of target genes that when mutated lead to an opposing phenotype: precocious development and early expression of certain paths of cell division and differentiation.

Recently, molecular and bioinformatic approaches have identified many genes encoding small RNAs in *C. elegans*, *Drosophila* and mammalian cell lines<sup>3,4,5,6</sup>. All of these genes encode 21-25 nt 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, some of which are conserved in *Drosophila* and mammals. To understand the biological functions of miRNAs, we are attempting to generate mutations in all of these genes. Using a library of *C. elegans* mutants and automated liquid handling, we are screening for deletion strains. In parallel, we hope to establish the temporal and spatial expression patterns of these genes.

<sup>1</sup>Lee RC, Feinbaum RL, Ambros V. Cell 75, 843-54 (1993).

<sup>2</sup>Reinhart BJ, Slack FJ, Basson M, Pasquinelli AE, Bettinger JC, Rougvie AE, Horvitz HR, Ruvkun G. Nature 403, 901-6 (2000).

<sup>3</sup>Lagos-Quintana M, Rauhut R, Lendeckel W, Tuschl T. Science 294, 853-8 (2001).

<sup>4</sup>Lau NC, Lim LP, Weinstein EG, Bartel DP. Science 294, 858-62 (2001).

<sup>5</sup>Lee RC, Ambros V. Science 294, 862-4 (2001).

<sup>6</sup>Mourelatos Z, Dostie J, Paushkin S, Sharma A, Charroux B, Abel L, Rappsilber J, Mann M, Dreyfuss G. Genes Dev. 16, 720-8 (2002).

**253033. Nematode embryonic cell lineages are computationally efficient**

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What is the minimal amount of information required to specify the cells of a metazoan? Based on ideas from algorithmic information theory and phylogenetics, we develop an algorithm for predicting the distribution of determination events in complete cell lineages. We assume that all such events are either cell autonomous or the outcome of permissive cell-cell interactions, and that the lineage is parsimoniously specified. Applying our algorithm to the complete embryonic lineage of *Caenorhabditis elegans*, we show that it predicts many known molecular events required to specify cell fates. We then show that less information is required to specify the actual *C. elegans* lineage than lineages simulated under null models. This is also true for two other species of rhabditid nematode, *Pellioditis marina* and *Rhabditophanes* sp., despite many interspecific differences in lineage topology and cell fate assignments.

Only one cell fate was found to be inefficiently specified in all species: programmed cell death. Unlike normal cells, most apoptotic cells appear to have no particular function during development. However, we show that the computational efficiency of embryonic development would be increased if cell deaths did not occur all. Thus, selection for increased computational efficiency should lead to a reduction in the number of programmed cell deaths in embryonic cell lineages. Although many programmed cell deaths occur in the *C. elegans* embryonic lineage (17% of all cells), all of them occur in single-cell monoclonal. This is a significantly higher proportion than that expected from permuted lineages and suggests that cell deaths have not accumulated neutrally in the cell lineages of the ancestors of *C. elegans*. That the absence of cell death monoclonal containing two or more cells is due to selection and not due to an intrinsic constraint is demonstrated by the observation that they have been found in other species. Such cases, we suggest, arise frequently, but are then eliminated by reprogramming. Indeed, the main function of somatic cell death in these nematodes might be to eliminate redundant cells over the course of evolution.

Our results strongly suggest that selection for computational efficiency moulds the evolution of nematode embryonic cell lineages. But even though nematode lineages are more efficient than random lineages, they are clearly not as efficient as they might be. Why not? The polyclonal origin of some cell fates might be due to the need to generate cells of the same type, such as neurons, in various parts of the embryo. This is supported by the observation that in all species studied here, the majority of cells are born close to their final position in the embryo. We speculate that, in *C. elegans*, *P. marina* and *Rhabditophanes* sp., the fitness cost of repeatedly specifying the same cell type may be less than the cost of additional cell migrations.

**394171. REGULATION OF *C. ELEGANS* MALE MATE-SEARCHING BY MONOAMINE NEUROTRANSMITTERS AND SIGNALS FROM THE GONAD**

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An adult *C. elegans* male when cultured alone tends to wander away from the food source. Because this behavior, which we call "leaving," is completely abrogated by the presence of a mate and is only expressed by sexually mature males, we have interpreted its expression as a mate-searching behavioral adaptation. We have exploited a quantitative assay for leaving behavior to explore the genetic underpinnings of a motivated sexual behavior.

The monoamine neurotransmitters dopamine and serotonin have been implicated in the regulation of motivational phenomena, reward, mood, and sexual behavior in humans. *C. elegans* male leaving is unaffected in a dopamine-deficient mutant, but is decreased in a serotonin-deficient mutant. Exogenous serotonin stimulates leaving behavior. We have identified single mutations in three genes, *las-1*, *unc-77*, and *mod-5*, that result in a loss of leaving behavior. *las-1* appears to be a novel locus. *unc-77* animals have loopy movement and have been reported to be serotonin hypersensitive in an egg-laying assay. *mod-5* mutations were originally identified by their hyper-slowness response on encountering a food source. *mod-5* encodes the *C. elegans* homolog of SERT, the serotonin reuptake transporter, which is the target of antidepressants such as fluoxetine (Prozac). Interestingly, loss of sex drive is a major side effect of fluoxetine administration in humans and has a similar effect in rodents. Effects on leaving behavior of mutations in *cat-1*, encoding a vesicular monoamine transporter, and in *goa-1*, encoding a G-protein subunit in the serotonergic signaling pathway, further implicate serotonin and possibly additional monoamine neurotransmitters in regulation of leaving behavior. *daf-16*, encoding a forkhead transcription factor, is required for many effects of serotonin deficiency. However, the leaving phenotype of the serotonin-deficient *tph-1 daf-16* double mutant is different from both single mutants, implicating a *daf-16*-independent pathway in serotonergic regulation of leaving behavior.

In vertebrates, signals from the gonad play a major role in regulating the expression of sexual behaviors. In both *C. elegans* males and hermaphrodites, the probability of wandering away from a food source is affected by gonad ablation--leaving rate is decreased in the case of the male and increased in the case of the hermaphrodite. After gonad ablation, males and hermaphrodites leave at similar rates, showing that sexual dimorphism of this behavior is dependent upon signals from the reproductive system. Two genes thought to function exclusively in germ cells affect leaving behavior: *fog-2* functional females (hermaphrodites lacking sperm) leave food, whereas *fog-1* males, containing oocytes instead of sperm, remain on food. These observations implicate *fog-1* and *fog-2* in pathways controlling expression of the gonadal signal.

**2727. Attempts to understand Semaphorins signaling pathway in *C.elegans*.**

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Throughout embryogenesis cell division is often followed by cell migration. The precise migration of motile cells is achieved by establishing transient contacts with the underlying extracellular matrix (ECM) and neighboring cells via membrane extensions. Axon migration is a specialized form of cell movement in which an axon can establish transient contacts with surrounding ECM via its motile organelle, the growth cone. The motility of the growth cone is modulated by changes in the cytoskeleton in response to attractive and repulsive cues in the ECM or on cell surfaces (Hedgecock et al., 1990). However, precisely how cues modulate cytoskeletal components that are required for cell guidance remains unclear. Also, little is known about the mechanisms used by motile cell extensions to make transient cell contacts with surrounding cells and the ECM during morphogenesis. In order to address these questions, I am using *C.elegans* for a genetic analysis of semaphorins, which are evolutionarily conserved guidance molecules. I have shown that Ce-Sema-1a (*smp-1*) and -1b (*smp-2*) deletion mutants exhibit mild axon guidance defects and other morphological defects of the hypodermis (Ginzburg, 2002). I plan to identify downstream and upstream components of the semaphorin signaling pathway by conducting a genetic screen for enhancers and suppressors of the phenotypic defects in these mutants.

In addition, to elucidate the functions of three Semaphorin genes in *C.elegans* I am conducting analysis of genetic interactions between them. Also, I am testing validity of putative PDZ binding motif and Furin like endoprotease site by testing for genetic interactions with genes encoding possible candidate such as Ce-SEMCAPs (Semaphorin F cytoplasmic domain-associated protein) that contain PDZ domains and AEX-5 ( a Furin-like endoprotease) (Ginzburg, 2002).

## 229812. Phytochelatin biosynthesis and transport are essential for heavy-metal detoxification in *C. elegans*

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Heavy-metal detoxification in *Caenorhabditis elegans* and other animals has almost exclusively been attributed to three classes of heavy-metal-binding molecules: the thiol tripeptide glutathione (GSH); a family of small (6-7 kDa) thiol-rich peptides, metallothioneins (MTs); and an assortment of structurally unrelated proteins. Here we present the results from our recent studies demonstrating that another class of heavy-metal-binding peptides, phytochelatins (PCs), formerly considered to be restricted to plants and some fungal species, play a critical, if not predominant, role in heavy-metal tolerance in *C. elegans*. PCs, ((gamma-Glu-Cys)<sub>n</sub>Gly polymers, where n = 2-11) derived from GSH by the action of PC synthases (gamma-glutamylcysteine dipeptidyl transferases), chelate heavy-metals with high affinity by thiol coordination and, in the case of plants and fungi, promote their sequestration in the vacuole, a lysosome-like compartment. In one of the better characterized systems, the fission yeast, *Schizosaccharomyces pombe*, the vacuolar sequestration of Cd.PCs is mediated by the half-molecule ATP-binding cassette (ABC) transporter, SpHMT1 (*alias* heavy metal tolerance factor 1). Previous investigations by our group and two others have resulted in the cloning of a novel cDNA (*AtPCS1*) from the model plant *Arabidopsis thaliana* encoding a 55 kDa soluble protein that is sufficient for metal-activated PC synthesis *in vitro*. Here we describe how we have determined that a homologous gene (*ce-pcs-1*) from *C. elegans* also encodes a functional PC synthase. Heterologous expression of *ce-pcs-1* in *Saccharomyces cerevisiae* promotes cadmium-elicited PC accumulation and yields cell-free extracts with PC synthetic activities comparable to those obtained from cells expressing *AtPCS1*. Crucially, RNA-mediated interference (RNAi) experiments demonstrate that the endogenous *ce-pcs-1* gene is needed for metal detoxification in the intact organism. The progeny of worms injected with ds*ce-pcs-1* RNA exhibit increased sensitivity to cadmium, manifest as irreversible larval arrest and extensive necrosis, at the concentrations tolerated by wild-type worms. Given this functional equivalence between *AtPCS1* and *ce-pcs-1*, we have gone on to explore the processes downstream of PC fabrication in *C. elegans*. In so doing we have isolated a cDNA encoding a 90.7 kDa half-molecule ABC transporter (CeHMT1), the only one of 58 ORFs in this organism that encode half-molecule ABC transporters that is topologically equivalent to and bears greater than 50% similarity to SpHMT1. Phenotypically, CeHMT1 satisfies all the requirements of a heavy-metal tolerance factor involved in the sequestration and/or elimination of heavy-metal-PC complexes. Heterologous expression of *ce-hmt-1* in *S. pombe* alleviates the Cd<sup>2+</sup>-hypersensitivity of *hmt1*<sup>-</sup> mutants and when worms are injected with ds*ce-hmt-1* their progeny acquire a cadmium-hypersensitive phenotype even more extreme than that exhibited by *ce-pcs-1*-deficient worms. Evidently, efficient heavy-metal detoxification in *C. elegans* is not only contingent on a functional *pcs-1* gene but also on a functional *hmt-1* gene. These results demonstrate for the first time that PC-dependent, HMT1-mediated heavy metal detoxification pathways are not restricted to plants and fungi but are also operative in some animals. This is a possibility that had not even been speculated previously. Given that *C. elegans* is only one of at least 100,000 nematode species, many of which are pathogenic, discovery of this pathway in *C. elegans* will likely prove to be of wide toxicological significance. The conditional lethality of the *ce-pcs-1* and *ce-hmt-1* mutations hand in hand with the identification of PC synthase homologs in the EST databases of other nematodes as well as a number of pathogenic trematodes and round worms reinforces this contention. This work was funded by NSF grant MCB-0077838 awarded to P.A.R.

**708159. Defining new components of TGF-beta signaling pathways in *C. elegans*.**

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Body size in multicellular animals is the combined result of total cell number and the size of each of those cells. The genetic contribution to cell number has long been accepted, but only recently has evidence shown that cell size is also genetically regulated.

Body size in *C. elegans* is regulated by a TGF $\beta$ -like signaling pathway that controls both animal length (Sma phenotype) and male tail development (Mab phenotype). The Sma/Mab pathway is activated when its ligand, *dbl-1*, binds to a heteromeric complex of transmembrane receptor serine kinases (RSKs), *daf-4* and *sma-6*, resulting in phosphorylation and nuclear accumulation of transcriptional regulators known as Smads, *sma-2*, *sma-3*, and *sma-4*. Initial mutants exhibiting the Sma phenotype were limited to a single RSK, *daf-4*, and each of the Smads. We reasoned, based on analogy with TGF $\beta$  signaling pathways in other organisms, that the missing molecular components of the Sma/Mab pathway could be identified in genetic screens along with novel mediators and modulators. Therefore, we performed a large scale F2 genetic screen for additional Sma mutants.

We screened 17,000 genomes and identified 33 Sma mutants that represent 18 complementation groups. The validity of the screening strategy was confirmed by the identification of expected TGF $\beta$  pathway components including *daf-4* and the Smads, along with a novel ligand, *dbl-1*, RSK, *sma-6*, and transcriptional regulator, *schnurri*. The remaining complementation groups represent novel Sma mutants. These alleles measure between 60 and 80% the length of wild type worms, consistent with previously identified Sma mutants. In addition, male tail defects are observed in less than half of the novel complementation groups; this is in contrast to the known Sma mutants which all have ray fusions. Finally, epistasis analyses indicate that all of the novel mutants lie downstream of the ligand, *dbl-1*, and 8 are upstream of a pathway target gene, *lon-1*; this suggests that at least these 8 may represent authentic modifiers of the Sma/Mab pathway. We expect that cloning and characterization of these genes will not only provide additional insights into TGF $\beta$ -like signaling pathways, but will also contribute to our understanding of genetic determination of cell size.

**302239. Characterization of GLC-4: a novel glutamate-gated chloride channel subunit**

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Glutamate-gated chloride channels (GluCl) constitute a relatively new family of ligand-gated ion channels that mediate fast inhibitory synaptic transmission. The GluCl s have important clinical relevance since they are the target of the anthelmintic drug ivermectin and are therefore involved in sensitivity to this drug. *Caenorhabditis elegans* has been used as a model organism to study GluCl function. Sequencing of the *C. elegans* genome has led to the prediction of approximately 40 ligand-gated anion channel subunits. Of these, five have been identified as belonging to the GluCl family. These subunits are categorized, primarily by their ligand affinities, into two classes: a and b.

Here we report a potential new GluCl subunit, GLC-4. Our RT-PCR clones indicate that *glc-4* may be the subject of alternative splicing yielding two transcripts, *glc-4A* and *glc-4B*. The *glc-4B* transcript differs by the presence of an unspliced intron that introduces a premature stop codon resulting in a slightly truncated protein at the carboxy end. We also present evidence supporting a possible association between GLC-4 and AVR-14/GluCl $\alpha$ 3. Specifically, we see similarities in phenotypes in the *glc-4(ok212)* and *avr-14(ad1302)* single mutants in terms of ivermectin hypersensitivity and hyperreversal behaviour. However, these phenotypes are suppressed in the *glc-4(ok212); avr-14(ad1302)* double mutant background. Also, expression studies using *glc-4* reporter gene constructs indicate possible co-expression with *avr-14* in neuronal cells. We suggest a direct interaction between GLC-4 and AVR-14 in which these subunits together form a functional heteromeric channel. In vitro expression and subsequent electrophysiological analysis of GLC-4 and AVR-14 is in progress to help support this model.



**562622. Analysis of Glutamatergic Neurotransmission by Knockout of Glutamate Transporter Genes.**

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Glutamate (Glu), the major excitatory neurotransmitter in the brain, is central to brain development, basic physiology and higher brain functions, but when in excess can cause neuronal cell death. In order to terminate Glu stimulation, separate Glu pulses and avoid buildup of toxic levels of this transmitter, Glu is rigorously pumped out of the synapse by specialized synaptic Glu Transporters (GluTs). Malfunction of GluTs, which leads to buildup of Glu concentration in the synapse, has been specifically identified as a key event in the initiation and/or progression of the neuronal damage seen in stroke and in ALS. The genes encoding many of the components of glutamatergic synapses are particularly conserved from nematodes to humans, making this model system particularly attractive for the study of Glu neurotransmission. We therefore use knockout of *C. elegans* GluT genes to study the consequences of Glu hyper-stimulation. We screened deletion libraries and isolated strains carrying a knockout in 3 of the 6 GluT genes, received one deletion strain from the Gene Knockout Consortium, and studied the expression pattern of these four genes. Analysis of the phenotypes of the deletion mutants by themselves and in combination with other Glu-related mutants suggests that GluTs are key regulators of synapses that control pharyngeal pumping and the responses to a range of chemical, osmotic and mechanical stimuli. Knockout strains are also defective in thermotaxis, a behavior that was suggested to involve learning and memory in the worm. GluTs appear to cooperate with other components of the synapse to maintain a functional balance of Glu neurotransmission. We are currently using genetic and pharmacological tools to analyze the possible involvement of nematode GluTs in neuronal cell death *in vivo* and *in vitro*. We are also expanding our analysis to investigate the role of the remaining two GluT genes in the nematode. We hope this information will bring us closer to understanding the function of GluTs in the nematode and how their function fits into the larger picture of normal and pathological Glu neurotransmission in *C. elegans*.

**813449. Mapping and characterization of the germ line desilencing mutation sig-7**

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The significance of the germ line lies in its totipotency: it retains the ability to not only produce the next generation, but also to produce all somatic lineages present in the offspring.

Transcriptional repression (silencing) of the embryonic germline appears to be essential for its development and maintenance in *C. elegans*. Mutations in the *pie-1* gene, for example, relieve transcriptional repression in the embryonic germ line and this results in transformation to soma. Repressive mechanisms are also important for maintaining the post-embryonic germline.

Repetitive transgenes are strongly silenced in the adult germline, and mutations that disrupt this silencing cause defects in fertility. Examples include the Maternal Effect Sterile genes (*mes*) identified and studied by Susan Strome's group, and several of the *rde* and *mut* genes (*rde-2*, *rde-3*, *mut-7*) studied by Craig Mello and Ronald Plasterk's groups. A screen for mutations that alleviate transgene desilencing recovered seven such mutations in loci termed Silencers In Germline (*sig*) genes. All *sig* mutants exhibit transgene silencing defects and fertility defects. Only one of the mutations, *sig-7*, exhibits observable somatic defects, suggesting little overlap between germline silencing mechanisms and essential somatic function.

*sig-7* is a fully recessive, zygotic sterile mutation whose phenotype is sterility, thin-body, and is often accompanied by the somatic defect of a protruding vulva. *sig-7* animals, like other *sig* mutants, also exhibit germ line desilencing. *sig-7* also exhibits a *mog* (masculinization of the germ line) phenotype in which hermaphrodites produce only sperm: the functional status of these sperm is unknown. The ultimate goal is to understand why silencing of its germ line is needed for its proper development and to understand how its totipotency is retained. *sig-7*'s role in this process will be investigated by identifying its molecular identity through mapping its genetic locus, and characterizing the nature of its silencing defect. We have genetically mapped *sig-7* to an interval on LG I, between *unc-29* and *fog-3*. Several *evl* mutants (*evl-9*, *-16*, *-17*) have been mapped to this region, and we are testing them for complementation by *sig-7*. Our progress in these studies will be presented.

### **976711. RME-6, A New Regulator of Early Endocytic Events**

**Barth D. Grant**

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The endocytic pathway is essential for the internalization and trafficking of macromolecules, fluid, membranes, and membrane proteins. The steps involved in uptake and endocytic trafficking within the endosomal system have been described, but many of the components mediating these steps at the molecular level remain to be identified. We have identified a group of genes required for endocytosis by developing oocytes, the *rme* genes. Many of these genes are required for endocytosis in multiple cell-types and have close relatives in humans that were not previously implicated in endocytosis. Recently we have focused on *rme-6*, an endocytosis gene represented by eleven viable alleles.

*rme-6* mutants accumulate yolk proteins in the pseudocoelom and have greatly reduced yolk accumulation by oocytes, indicating a defect in receptor-mediated endocytosis. *rme-6* mutants appear to have elevated levels of cell-surface yolk receptors, consistent with a defect in yolk receptor internalization. We also found that *rme-6* mutants display severe defects in the uptake of pseudocoelomic fluid-phase markers by coelomocytes, scavenger cells of the body cavity. We were able to visualize pre-lysosomal endosomes of the coelomocyte using an RME-8::GFP marker. Microinjection of Texas-Red BSA (TR-BSA) into the pseudocoelom allowed us to follow endocytosis and intracellular transport of TR-BSA within the coelomocytes over time. *rme-6* mutants displayed unusually small RME-8::GFP labeled endosomes. We also noted that accumulation of TR-BSA in these endosomes was delayed in *rme-6* mutants, but that TR-BSA accumulation in labeled endosomes did occur in *rme-6* mutants, and export of TR-BSA from the endosomes to presumed lysosomes also occurred. These results suggest a role for RME-6 early in endocytosis, either in internalization and/or endosome fusion events.

Molecular analysis of RME-6 indicates that it contains an N-terminal RAS-GAP-like domain and a C-terminal Rabex-5-like domain. Both of these motifs suggest that RME-6 exerts its influence on endocytosis by regulating the activity of small GTPases, perhaps RAS and/or RAB-5. This combination of domains also suggests the intriguing possibility that RME-6 links signal transduction processes with endocytic trafficking. In the coelomocyte an GFP::RME-6 fusion is cytoplasmic, and accumulates in zones near the plasma membrane, consistent with a direct role in early endocytic events.

**847276. Use of CeHR Axenic Medium for Exposure and Gene Expression Studies**

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An axenic liquid culture medium has been formulated that allows rapid development of *C. elegans* from the L1 to gravid adult stage. This medium, the *C. elegans* Habitation and Reproduction (CeHR) medium, uses a base medium similar to previously published axenic media such as CbMM, but includes refrigerated sterile ultra-pasteurized fat-free milk at 20% of the medium volume. The formula and directions for preparing CeHR medium are available at <http://usacehr.detrack.army.mil/clegglab.html>. The milk is obtained locally and is handled using sterile technique. Each batch of milk is checked thoroughly for sterility prior to use in the medium. The completed medium also is tested for sterility, and the worms added to the medium are either from existing microbiologically sterile cultures or from bleached embryos. Use of similar milk products containing milk fat results in a more opaque medium.

CeHR axenic liquid culture medium is being used to assess the physiologic, genomic and proteomic effects on worms of exposures to environmental chemicals under axenic conditions. Worms to be used in these experiments are maintained routinely in liquid culture as synchronized cultures. The protocol is begun with bleaching of gravid adult hermaphrodites. Surviving embryos are placed in M9 medium and held at 22C overnight to allow completion of hatching. The resulting synchronized larvae are placed into CeHR medium at a target concentration of 8,000 worms per ml; allowing development to proceed. Untreated worms require approximately 72 hours at 22C after release from the M9 to become gravid adults. The progress of development is monitored by differential interference contrast microscopy. Depending on the objective of the experiment, exogenous test chemical(s) may be added at any time during development. Experiments conducted in the Reproductive Hazards Laboratory to date are examining the effects of test chemical addition at the time of release from M9 on worms harvested as L4s or as gravid adults. Gene expression patterns are being evaluated using the Affymetrix *C. elegans* GeneChip.

The views and findings in this Abstract are those of the authors and should not be construed as official Department of the Army position or policy.

## 522847. A SCREEN FOR ESSENTIAL CELL-DEATH GENES

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Genes important for programmed cell death in *C. elegans* may remain unidentified because they are also essential for early embryogenesis. We are planning to perform a screen for such essential cell-death genes using the RNAi feeding library developed in the Ahringer laboratory (1). We plan to use two approaches. First, we will place wild-type eggs on bacteria expressing double-stranded RNA and score their phenotype later in development (postembryonic RNAi). This approach ensures that double-stranded RNA will be delivered only postembryonically, allowing animals to develop normally until hatching. Second, we will mate wild-type males with *rde-1* RNAi-deficient homozygotes that have been exposed to double-stranded RNA and score the phenotype of their progeny (2; zygotic RNAi). With this method, RNAi will be effective in cross progeny only after *rde-1* is expressed zygotically. In a pilot experiment, we tested the feasibility of using a *lin-11::gfp* reporter to assay cell death in RNAi experiments. In *ced-3(n717)* animals, *lin-11::gfp* allows the detection of five undead Pn.aap cells, because the deaths of these cells (normally in the L1 larval stage) are prevented (see abstract by Galvin, Reddien, and Horvitz; a sixth undead cell, P1.aap, is not reliably scored). Standard feeding of *ced-3* RNAi yielded an easily observable cell-death defect of, on average, three extra Pn.aap cells per animal. Postembryonic or zygotic RNAi resulted in a much weaker effect of, on average, fewer than one extra Pn.aap cell per animal. We will explore the use of different reporters and the RNAi-hypersensitive strain *rrf-3* (3) to increase the sensitivity of the proposed screen.

In cells that have initiated programmed cell death, CED-4 translocates from mitochondria to the perinuclear region, where it may bind and activate CED-3 (4). However, the localization of CED-3 in living or in dying cells is unknown. To address this issue, we are making polyclonal antibodies to CED-3.

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2 Tabara, H., Sarkissian, M., Kelly, W.G., Fleenor, J., Grishok, A., Timmons, L., Fire, A., and Mello, C.C. *Cell* **99**, 123-32 (1999)

3 Simmer, J., Ahringer, J., and Plasterk, R.H.A. European *C. elegans* Meeting 2002, Abstract 31

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**744618. Cell Fusion Defects in eff-1 Adults Seen by TEM**

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The gene eff-1 encodes a membrane glycoprotein and has been suggested to be necessary for lipid bilayer fusion (Mohler et al., 2002). The eff-1 gene is apparently expressed in many cells just prior to their fusion into syncytia. Cell fusions are an important mechanism in the morphogenesis of many tissues in the nematode, including the hypodermis, seam, vulva, uterus, and male tail (Podbilewicz and White, 1994; Podbilewicz, 2000; Nguyen et al., 1999).

We are using thin sections and TEM in hopes of collecting evidence for specific failures in tissue development, and for evidence of intermediate steps in the process of bilayer fusion. We will show current progress in examination of late larval and adult tissues which show excess membranes, retained adherens junctions, and fusion failures, including pharyngeal muscles, seam cells and hypodermis, and high power images of those membranes where fusion has apparently failed.

**913735. Post-embryonic Developmental Expression Chronograms: a new functional genomics data type generated using a nematode fluorescence sorting system**

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Using a microscope to chronicle the course of GFP reporter expression in post-embryonic development is not an approach well suited to high-throughput data acquisition or automated digital comparison/clustering of expression patterns for large numbers of genes. A recent software modification to the COPAS sorter systems now permits recording of fluorescence intensity along the length of the worm as it passes through the optical chamber. The output is in essence a 1-dimensional (long-axis) projection image of the 3-dimensional animal. Entire populations of worms can be passed through the sorter in a short time, and all the larvae in mixed-stage culture are profiled individually. The resulting traces can then be sorted by size (age) into a 2-dimensional image that clearly represents the expression localization/timecourse of the fluorescent marker during post-embryonic development. Other information readily extracted from these chronograms includes relative numbers of given larval stages and variability in expression within identically staged members of the population. Although intensity values are recorded at <sup>2</sup> 4  $\mu\text{m}$  increments along the body, true optical resolution for the system is probably  $>10\mu\text{m}$ . Nonetheless, even this level of spatial resolution might be sufficient in assigning expression to a portion of a tissue from (varying from 10 100 candidate cells). Temporal resolution is inversely proportional to population size in a mixed culture. These chronogram images should lend themselves particularly well to genomic comparisons via cluster/pattern recognition algorithms already in use in biology.

*ajm-1::gfp cdh-3::gfp*

Vertical axis is size(age) increasing from top to bottom.

Horizontal axis is anatomical position (head, left; tail, right).

**577894. Alae Mutant Defects Assessed by SEM and TEM**

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We have previously reported movement defects in adult C. elegans after RNAi (dsRNA feeding) with the gene PKC4, which encodes a novel Ser/Thr protein kinase (Ren et al., 2001). The PKC4 protein is expressed highly in the seam cells in late-stage embryos and again in late L4 stage, at the time of morphogenesis for seam-cell derived cuticular structures, the alae. It seemed likely that the movement defects, a sloppy zig-zag body motion of variable amplitude when placed on standard agar plates, were the result of absent or poorly formed alae. We have fixed adult animals for examination by scanning electron microscopy (SEM) and transmission electron microscopy (TEM) and compared these PKC4 RNAi animals to adult wild type. We will show SEM evidence for the normal extent of adult alae and several other hallmark features of the body surface, and TEM data for the corresponding seam cell cytology which underlies the alae.

As predicted, the "knockout" animals often show alae defects or complete loss of alae locally, with a penetrance which agrees well with the prevalence of movement defects. The most common defect is a merger of the tripartite ala structure into a large smooth ridge of cuticle, or less often, a zone of smooth cuticle with no ridge at all. In severely affected PKC4 RNAi animals, some tissue defects can be seen in seam cells, including cell swelling and general disorder in the cytoplasm.



**715333. Phenotypic characterization and initial mapping of *spe-42(eb5)*, a new fertilization defective mutant**

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The unusual reproductive physiology of the nematode *Caenorhabditis elegans* makes it an excellent model system in which to study the cell-cell interactions that occur during fertilization. This is not possible with any other commonly employed model organism, which all rely on a strictly dioecious mode of reproduction. The long term goal of our work is the identification and characterization of proteins involved in sperm-egg recognition during fertilization. A number of mutants (*spe-9*, *fer-14*, *spe-13*) produce cytologically normal spermatozoa which cannot fertilize oocytes. *spe-9*, the only member of the above set that has been cloned, encodes an integral membrane protein containing epidermal growth factor (EGF) repeat domains and is thought to function as a sperm surface ligand that interacts with an as-yet unknown receptor on the oocyte. Identification, cloning and analysis of genes for which the corresponding mutants phenocopy *spe-9* should reveal other members of the *spe-9* signal transduction pathway which will aid in our understanding of sperm-egg communication during fertilization and cell-cell signaling in general. Additionally, it is presently unclear if one or multiple genetic pathways regulate cell-cell interactions during *C. elegans* fertilization. The approach we have used is unbiased and will reveal new, interesting mutants without regard to their genetic relationship to other mutants that affect fertilization. Here we report the phenotypic characterization and initial mapping of *spe-42(eb5)*, a new mutant on chromosome V that, like *spe-9*, *fer-14* and *spe-13*, produces cytologically normal spermatozoa that are incapable of fertilizing oocytes. We found that *spe-42(eb5)* hermaphrodites produce almost no progeny at 25°C and < 5 progeny at 16°C. Through a sensitive mating assay, we also determined that infertility in males is not due to defective mating nor deficiency of spermatid activating factors in their seminal fluid. *spe-42(eb5)* was initially mapped between *dpy-11* and *unc-76* on chromosome V. Through a combination of three-factor mapping and deficiency complementation tests we mapped *spe-42(eb5)* to the interval bounded on the left by the right breakpoint of *sDf35* and on the right by the left breakpoint of *yDf12*, a distance of 0.653 map units.

**490819. Computational identification of regulatory elements involved in TGF $\beta$ -regulated dauer formation in *Caenorhabditis elegans*.**

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A TGF $\beta$  pathway plays a primary role in dauer formation. There are several genes already identified in this pathway: DAF-7 is a ligand, DAF-1 and DAF-4 are receptors, and DAF-8 and DAF-14 are SMAD transcription factors. Mutations in any of these genes cause a dauer constitutive phenotype suggesting that this pathway negatively regulates dauer formation. *daf-5* and *daf-3* suppress the dauer constitutive phenotype of the TGF $\beta$  genes. We want to know how TGF $\beta$  functions in dauer formation. Performing microarrays allow for whole genome gene expression analysis; however, there is a large amount of data which needs to be analyzed. We are utilizing several methods to simplify the data and make it more manageable for analysis for our purposes.

Microarray experiments were performed to identify novel genes and facilitate in identifying regulatory elements. We have performed 10 microarray experiments using *daf-7*, *daf-8*, and *daf-14* mutants. These experiments have identified a number of up and down-regulated genes (see abstract by Liu et. al). Since dauer formation causes physiological changes in the whole animal, we suspect that there are several transcriptional programs active that facilitate this transformation. Therefore we wished to identify groups of genes that might be part of a single transcriptional program. We are proceeding by using data from Kim, et al (2001, Science 293:2087). Kim gathered data from *C. elegans* microarray experiments involving many growth conditions, developmental stages, and varieties of mutants. He created a co-relational matrix and plotted the genes; the genes that tend to show similar expression through the variety of experiments form mountains in a three dimensional topographic map. We are grouping genes by identifying their positions on the topographic map and then using AlignACE to look for regulatory elements in these specified groups. AlignACE simply looks for sequence motifs that are found with a frequency higher than the random expectation. We hope that one or more of these groups will share a common transcriptional regulatory program in dauer. We expect that genes within a group will share other regulatory elements, because the groups are defined by co-regulation of expression. Therefore, we identify three categories of genes in each group: dauer up-regulated, dauer down-regulated, and dauer non-regulated. We want to find regulatory elements that are in common in the dauer up-regulated group, but not found in the dauer non-regulated group. We will present our progress using this approach and our plans to test the putative regulatory elements for function in dauer formation.

**70030. Evolutionary changes in stoma ultrastructure within the nematode taxon Diplogastridae**

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Most free living nematodes feed on bacteria. Within Diplogastridae, however, diet changed repeatedly, as did stoma morphology. The evolution of a large dorsal tooth probably allowed the evolution of carnivory and fungivory within this taxon. Our aim was to understand which changes occurred in the diplogastrid stoma at the ultrastructural level. Ultrastructure was already known for some bacterivorous species (e.g. De Ley, P et al. 1995, Nematologica 41: 153) and for the partly carnivorous *Diplogaster halicti* with its derived shortened stoma and large dorsal tooth (Baldwin, J et al. 1997, Can J Zool 75: 407). We used TEM to investigate *Diplogasteroides nasuensis*, a bacterivorous species with a tube-shaped stoma, probably representing an early branch of the diplogastrid clade.

The stoma of *D. nasuensis* is formed by cell processes whose number and arrangement corresponds to that observed in *Caenorhabditis elegans*, cephalobids and panagrolaimids. Some novelties evolved early in Diplogastridae: (1) The dorsal tooth is formed by two sets of cell processes (instead of one). (2) The channel of the dorsal pharynx gland lies between these cell processes, allowing the evolution of the tooth functioning as injection device. (3) Pharyngeal cell processes in the diplogastrid stoma are shortened and interlaced. (4) Some myofilaments are arranged longitudinally instead of radially, possibly allowing the kind of complex mobility of stoma parts observed in carnivorous species.

In the past it was debated how to homologize stoma parts in Secernentea. We reject the attempt to use cell lineage in *C. elegans* and *Cephalobus cubanensis* as a basis for homologization (Dolinski, C et al. 1998, Dev Genes Evol 208: 495), which disregards the importance of cell-cell signaling and positional information in cell fate determination. More importantly, this hypothesis leads to non-parsimonious assumptions for evolutionary events, involving a double gain and subsequent loss of cell processes in the lineage leading to *C. elegans*. However, stoma parts in Cephalobidae, Panagrolaimidae, *C. elegans* and *D. nasuensis* can be unambiguously homologized based on the conserved spatial arrangement of the cell processes by which they are formed.

We are currently using sequences of small subunit ribosomal RNA genes to independently test the phylogenetic relationships among these species and to trace evolutionary changes in stoma characters.

Supported by the Deutsche Forschungsgemeinschaft (Su 198/2-2)

**600906. New components of the RNAi pathway in *C. elegans***

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In a number of organisms, the introduction of double-stranded RNA into cells causes the post-transcriptional silencing of the corresponding gene. This experimental phenomenon is called RNA interference (RNAi). We are particularly interested in the molecular mechanism of this phenomenon. In order to study RNAi we screened for *rde* (RNAi deficient) strains and identified mutants that define at least seven complementation groups (*rde-1*, *-2*, *-3*, *-4*, *-5*, *-6*, *-7*). Among these genes *rde-1* (Tabara et al., 1999a) and *rde-4* have been cloned and analyzed further. RDE-1 encodes a novel protein with PAZ and PIWI domains found in numerous other proteins implicated in gene silencing and development. RDE-4 encodes a protein with two double-stranded RNA binding motifs. Genetic analysis by (Grishok et al., 2000) suggested that the functions of both *rde-1* and *rde-4* are required at an upstream step in the RNAi pathway. Recently we have shown through Immuno-precipitation experiments that RDE-4 associates with at least three other proteins *in vivo*, RDE-1, DCR-1 and DRH-1. DCR-1 is a conserved RNase III related protein implicated in RNAi in *Drosophila* and *C. elegans*. DRH-1 is a conserved DExH box helicase that appears to be required for RNAi in both the soma and the germline in *C. elegans*. Our data suggests that RDE-4 binds to foreign dsRNA and brings this dsRNA to DCR-1 to initiate RNAi.

RDE-1 appears to be necessary for RDE-4 to interact with the long-trigger dsRNA *in vivo*. However in other organisms RDE-1 homologs appear to interact with siRNA products that function downstream in mRNA destruction. We therefore decided to ask if other RDE-1 homologs in *C. elegans* could provide this more downstream activity. To date, we have identified a total 26 homologues of *rde-1* in *C. elegans* genome. Two of these, *alg-1* and *alg-2*, are implicated in the heterochronic pathway (Grishok et al. 2001). Interestingly, we have found that *alg-1* and *alg-2* are also required for RNAi (see the poster by Simard et al.). In order to analyze the potential function of other *rde-1* homologs in RNAi pathway, we have injected dsRNA targeting all 26 genes. We have found that in addition to *alg-1* and *alg-2*, three more subclasses within the *rde-1* gene family appear to be required for RNAi. We are currently investigating other cellular components that interact with these proteins and we are trying to determine if these RDE-1 homolog function redundantly or at distinct steps in the mechanism.

**627813. Characterization of *lon-2* and its suppressors**

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The TGF $\beta$  growth factor superfamily and its downstream core signaling components are found in all multicellular animal phyla studied, from nematodes to mammals, and are responsible for controlling several different developmental processes. Members of the superfamily are important for establishing the basic body plan. They regulate cell cycle progression, apoptosis and immune functions, and suppress some types of cell growth. Because of such roles, they are frequently mutated in metastases.

*C. elegans* has three characterized TGF $\beta$  genes, *dbl-1*, *daf-7*, *unc-129*, and each has a different signaling pathway, controlling body size/male tail development, dauer formation, and pioneer axon guidance, respectively. We focus on the *dbl-1* pathway.

*lon-2* acts genetically upstream of the ligand *dbl-1*. Based on genetic and sequence analysis, we predict that LON-2 acts at the extracellular surface to negatively regulate DBL-1 signaling. GFP expressed from the *lon-2* promoter is strongly expressed in the intestine, as are the TGF $\beta$  receptors *sma-6* and *daf-4*.

To identify novel components of the TGF $\beta$  signaling pathway, we screened for mutations that suppress the *lon-2(lf)* phenotype (see also abstract by Zimmerman et al.) Suppressors include 1) mutants of known TGF $\beta$  pathway genes, 2) the *C. elegans* homolog of *Drosophila* Schnurri, a transcription factor in the pathway, and 3) several novel alleles. These novel suppressors are currently being characterized and cloned (see also abstract by Maduzia et al.).

**881913. The enhancer-of-*akt-1* genes *eak-1* through *eak-5* may define novel components of DAF-2/insulin-like signaling**

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DAF-2 insulin-like signaling regulates development, longevity, and metabolism in *C. elegans*. This conserved signal transduction pathway consists of the DAF-2/insulin receptor; AGE-1/PI 3-kinase; PDK-1, AKT-1, and AKT-2 kinases; the DAF-18/PTEN tumor suppressor homolog; and the DAF-16/Forkhead transcription factor. Although genetic screens have identified multiple alleles of all of these genes, epistasis experiments suggest the existence of undiscovered DAF-2/insulin receptor outputs that are independent of AGE-1/PI 3-kinase.

To identify components of this parallel pathway, we screened for mutations that enhance the *akt-1* null phenotype (Eak mutants). Since *akt-1* null mutants are Daf-c at 27°C but not 25°C, we mutagenized *akt-1* mutant animals and isolated F<sub>2</sub> progeny that were now Daf-c at 25°C. We identified 30 independent mutants from a screen of approximately 21,000 haploid genomes.

26 mutants were suppressed by *daf-16* RNAi, suggesting that they are components of DAF-2 signaling. Among these, 13 had a true Eak phenotype; they form dauers at 25°C only in a homozygous *akt-1* null background. These 13 mutants define 5 complementation groups. Preliminary mapping analysis indicates that *eak-1* through *eak-5* do not correspond to known Daf or SynDaf genes. Therefore, the *eak* genes may define novel participants in DAF-2/insulin-like signaling. Fine mapping of these genes is ongoing.

#### **137144. CUL-2 regulates the level of multiple cellular proteins**

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Cullin/RING finger ubiquitin ligase complexes regulate a wide range of cellular processes by targeting protein degradation. In humans, the cullin CUL2 functions with the VHL tumor suppressor protein, Rbx1, and Elongin C/B in a CUL2/VCB complex. The only known substrates of the CUL2/VCB complex are the hypoxia-inducible transcription factors HIF-1 $\alpha$  and HIF-2 $\alpha$ .

In *C. elegans*, CUL-2 functions as a positive cell cycle regulator and is required for the G1-to-S phase cell cycle transition in germ cells by negatively regulating CKI-1 (a cyclin dependent kinase inhibitor). The three major phenotypes observed in *cul-2* mutant embryos are defects in chromosome condensation, delayed mitotic progression, and defective cytoskeletal dynamics. We propose to identify CUL-2 substrates and characterize their cellular functions using a proteomics approach, thereby unraveling the molecular pathways regulating these CUL-2-dependent processes.

Inactivation of a ubiquitin ligase is predicted to result in the accumulation of substrates that are no longer being degraded by the proteasome. CUL-2 is predicted to function as a ubiquitin ligase and the *cul-2* mutant phenotypes may therefore arise from an accumulation of substrates. A survey of the levels of all cellular proteins in *cul-2* mutants compared to wild type should enable us to identify *cul-2* substrates. This approach has been adopted by performing a proteomics screen using Two Dimensional Differential In Gel Electrophoresis (2D DIGE), in which mutant and wild type proteins are labeled with different Cy dyes and then run on the same gel to compare relative intensities of the dye in protein spots. Proteins whose levels change in *cul-2* RNAi animals relative to wild type will be identified by mass spectrometry. When proteins are run on pH 4-7 IPG strips, we observed that five proteins have increased levels in *cul-2* mutant lysates. Presently, we have identified two proteins from this screen: NADP dependent cytosolic Isocitrate Dehydrogenase and Vacuolar ATP synthase. With samples run on pH 5-6.7 IPG strips, we have observed increased levels of 11 proteins and decreased levels of 6 other proteins. We hypothesize that the decreased levels of certain proteins could be due to secondary effects resulting from altered levels of transcriptional regulators.

Identification of the other CUL-2 regulated proteins is currently in progress. We are also characterizing the role of Isocitrate Dehydrogenase and Vacuolar ATP synthase in CUL-2 mediated processes.

**371013. Toward Understanding Mechanotransducing Channel Function: *mec-6* Influences the Stability of the MEC-4 Subunit but Not the MEC-10 Subunit**

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Gentle touch to the nematode body is sensed by five mechanosensory neurons called touch receptors. A multimeric channel composed of MEC-4 and MEC-10 degenerin channel subunits is expressed in the touch neurons and is needed for touch sensation. These channel subunits are thought to be the core elements of a mechanotransducing complex that includes other MEC proteins needed for touch sensation. Understanding how these proteins interact to facilitate touch transduction is of major interest in the field of mechanical signaling.

Mutant, hyperactivated variants of MEC-4 (MEC-4(A713V) = *mec-4(d)*) can induce necrotic-like neurodegeneration of the touch receptor neurons, and thus *mec-4(d)* has been of use as an initiating event for studying genetic requirements for neurodegeneration. Analogously substituted MEC-10 can also induce touch receptor degeneration. Of the dozen or so identified *mec* genes, only one, *mec-6*, is essential for *mec-4(d)*-induced neurodegeneration. Previously, we had reported that *mec-6* influences the stability of MEC-4/LacZ and MEC-4/GFP fusion proteins, provided they include most of the channel coding region. In the absence of *mec-6*, very little if any tagged-MEC-4 protein is apparent. No effects on reporter activity are observed if transcriptional fusions that lack MEC-4 coding sequences are studied, suggesting that the loss of signal requires MEC-4 channel coding sequences. *mec-4* transcripts are not affected by *mec-6* mutations. We suggest that *mec-6* is needed for the stability of the MEC-4 protein and postulate that *mec-6* suppresses neurodegeneration by dramatically lowering the concentration of MEC-4(d), thereby eliminating toxic ion influx into the touch receptor neurons.

*mec-10(d)*-induced neurodegeneration requires both *mec-6(+)* and *mec-4(+)* activity. However, it appears that the mechanism of *mec-6* suppression of *mec-10(d)* is different from the case of *mec-4(d)*. We find that in the *mec-6* background, the expression levels of a MEC-10promoter/MEC-10/GFP (which encodes a chimeric protein including all but the last few MEC-10 amino acids followed by GFP) are not altered. Thus, *mec-6* does not appear to be required for MEC-10 stability although it is needed for MEC-4 stability. Since *mec-6* mutations appear to reduce/eliminate MEC-4 protein, we suggest that *mec-6* suppression of *mec-10*-induced neurodegeneration may occur via a mechanism analogous to suppression of *mec-10(d)* by *mec-4(null)* mutations. In this case, the MEC-10(d) subunit is still present, but cannot form a functional touch channel in the absence of sufficient MEC-4 subunits.



**565764. Cell cycle dependent localization of the *C. elegans* Myt1 ortholog wee-1.3**

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**Abstract**

All eukaryotes control mitotic timing through the activation of MPF, which is composed of a cyclin-dependent kinase and a regulatory cyclin subunit. MPF is negatively regulated by Wee1p kinases and positively regulated by Cdc25p phosphatases. These soluble proteins are actively localized to the nucleus or cytoplasm, respectively, during the cell cycle. Metazoans express another member of the Wee1p kinase family called Myt1, a type 2 transmembrane protein with an N-terminal Wee-1p kinase domain and a C-terminal regulatory domain. We have used a peptide antibody directed towards the C-terminal domain of WEE-1.3 to study its localization during *C. elegans* development. WEE-1.3 colocalizes with microtubules during sperm meiosis and blastomere mitosis, but is punctate throughout the cytoplasm or associated with the nuclear envelope during interphase and at the G2/M transition point, respectively. The *wee-1.3(q89 eb60)* mutant is hypomorphic and strongly reduces WEE-1.3 levels in early blastomeres, which frequently contain ectopic microtubule nucleation sites. Missense mutations in a four amino acid region within the WEE-1.3 C-terminal domain cause a dominant spermatogenesis defective (Spe) phenotype that arrests spermatocytes at the G2/M transition. WEE-1.3(gf) localizes to the nuclear envelope in arrested *wee-1.3(gf)* spermatocytes and this localization is indistinguishable from wild type spermatocytes at a similar stage of development, suggesting that the dominant Spe phenotype is not caused by abnormal protein localization or protein instability. In oocytes, WEE-1.3 is concentrated primarily in the nuclear envelope. *wee-1.3(q89 eb87)* mutants delete the transmembrane domain of WEE-1.3 and suppresses the dominant Spe phenotype. Phenotypically, these mutants are viable but recessive sterile, suggesting that the somatic functions that require WEE-1.3 are unaffected by the absence of the WEE-1.3 transmembrane domain. *wee-1.3(q89 eb87)* mutants result in the localized accumulation of WEE-1.3 within the oocyte nucleus. This suggests that the localization of WEE-1.3 to the oocyte nuclear envelope is important for some aspect of oocyte development.

**428127. Understanding the regulation of *C. elegans* Hox Gene *egl-5* through dissection of *cis*-regulatory elements**

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Hox genes are key transcription factors involved in patterning along the anterior-posterior body axis. Although the functions of Hox genes are generally conserved throughout the animal kingdom, in many cases the regulation of Hox genes is not well understood. We are using the male tail system to study the regulation of *C. elegans* Hox gene *egl-5*. In the seam cell V6 lineage, *egl-5* is expressed in a subgroup of precursor cells that give rise to sensory rays 3, 4, 5 and 6. This expression is male specific, restricted to the posterior branches of V6 lineage, and always starts at the V6.ppp stage. We are interested in finding out how the signals that account for the sexual, spatial, temporal and lineage specificity coordinate to activate *egl-5* expression in the V6 lineage.

To address this question, we have undertaken a PCR-based promoter dissection approach. A comparison of *C. elegans* and *C. briggsae* *egl-5* DNA sequence reveals perfectly conserved short DNA segments within the generally diversified intergenic region 5 of the *egl-5* gene. We have focused on these conserved sequences in our PCR deletion study, on the assumption that they may represent regulatory sequences for different lineages. We have mapped individual regulatory sequences that can drive expression of our *egl-5::gfp* reporter in rectal epithelial cells, tail hypodermis, seam cells and the touch cell PLM. We have also confirmed the function of some of these regulatory sequences by tissue-specific rescue experiments.

We have found a highly conserved 300 bp region, V6CRE (the V6 lineage *cis*regulatory element), which is not only necessary for the expression of *egl-5* in the V6 lineage, but is also able to replicate the wild type expression of *egl-5* in the V6 lineage when linked to a *pes-10::gfp* reporter (*V6CRE::gfp*). Further dissections of this region show recurring motifs and partial redundancy. In addition, certain reporter constructs are only expressed in the R5 sublineage but not in R3, R4 or R6, suggesting that there might be an R5-specific pathway. In an attempt to understand the lineage specificity of *egl-5* expression, we introduced *V6CRE::gfp* into *mab-5* and *pal-1* backgrounds, both of which are important regulators of the V6 lineage identity. In *mab-5* and *pal-1* mutants, the division pattern and the final fate of the V6 lineage is transformed to that of V1-V4. Surprisingly, *V6CRE::gfp* is still selectively expressed in V6.pppp and its descendants. This suggests that there is an as yet unknown V6 specific factor that acts upstream of, or in parallel to, PAL-1 and MAB-5 to regulate the expression of *V6CRE::gfp*. Another unexpected finding is that mutations in a motif within V6CRE that resembles a TRA-1 binding site cause ectopic expression of *V6RE::gfp* in anterior seam cells in both males and hermaphrodites at earlier stage than V6.ppp. This suggests a repressor may bind to this site in seam cells to prevent ectopic expression of the reporter. *V6CRE::gfp* does not show ectopic expression in a putative *tra-1* null allele, suggesting that TRA-1 alone does not account for the repression of *egl-5* expression in anterior seam cells. To identify the unknown factors that contribute to the expression of *egl-5* in the V6 lineage, we are now doing a yeast one-hybrid screen to search for transcription factors that bind to the V6CRE region.

**189201. Phagocytosis of necrotic corpses--a new gene involved in NCD corpse disposal**

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We are interested in the mechanisms by which "injured" cells that have died by necrotic cell death (for example, as induced by channel hyperactivating mutations in the *mec-4* touch channel) are recognized and removed by phagocytosis. There are seven known genes needed for phagocytic engulfment of both programmed cell death and necrotic cell death corpses. *ced-1*, *ced-6*, and *ced-7* act in one partially redundant pathway for corpse phagocytosis and *ced-2*, *ced-5*, *ced-10*, and *ced-12* function in another. In a screen designed to identify genes that might specifically function in the phagocytosis of necrotic cells, we identified allele *bz84*, which significantly enhances necrotic corpse persistence in a *ced-7* mutant background. *ced-7* encodes a member of the ABC transporter family that is required in both the engulfing and dying cell for removal of cells that have undergone programmed cell death. We are currently mapping and characterizing the genetic properties of *bz84*. In a complementary study, we are probing members of the CD36 scavenger receptor family to test their roles in necrotic corpse elimination since CD36 family members influence elimination of dead cells in both flies and mammals. Six CD36 *C. elegans* counterparts fall into two subfamilies. One branch consists of the ORFs, Y49E10.20, Y76A2B.6, and C03F11.3, all of which are represented by ESTs. Members of the second branch, F07A5.3, F11C1.3, and R07B1.3 are not represented by EST clones. We are using RNAi feeding to test the ability of scavenger receptors to act individually and in combination with known engulfment *ceds* to eliminate corpses.

**5991. Isolation and characterization of the *cks-1* mutant in *C. elegans***

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In wild-type embryos the 4-cell stage blastomere, EMS, undergoes an inductive interaction that specifies the endoderm and orients the division axis of the EMS cell onto the anterior/posterior (a/p) axis of the embryo. In our temperature-sensitive mutant screen, we isolated one recessive mutant, *ne549*, with a penetrant EMS division orientation defect and ectopic endoderm produced by the C blastomere. A mutant with a very similar phenotype, *cdk-1(ne236)* was identified in a previous screen by Martha Soto (See ECWM abstract). We mapped *ne549* to the right of *unc-24* on LGIV, into a region that contains a *C. elegans* homolog of the highly conserved CDK-1 interacting protein, CKS-1, which encodes a conserved 13 kD protein required for cyclin-dependent kinase activity. We obtained rescue of *ne549* with a single gene PCR product that contains the *cks-1(+)* allele, and we found a single point mutation in a highly conserved residue within the open reading of *cks-1*. Thus we conclude that *ne549* is an allele of *cks-1*.

RNAi of *cks-1* causes a drastic cell division defect during early embryogenesis, therefore *ne549* is clearly a special allele that alters but does not abolish the function of CKS-1. Given the similarity between *cks-1(ne549)* and *cdk-1(ne236)* phenotypes, we hypothesize that the two proteins function together in an activity that is required for endoderm specification and the control of division orientation. Although each single mutant exhibits wild-type cell-cycles we found that double mutants between *cks-1(ne549)* and *cdk-1(ne236)*, resemble the null (RNAi) phenotypes for the individual mutants. This finding suggests that both mutants impair a function(s) of the kinase complex that are required for the cell-cycle.

How do cell-cycle molecule(s) function to control both the orientation of the cell division and cell fate? This is a very interesting question but has yet to be answered in any organism. As one potential avenue to address the targets or interactors of CKS-1, we have started a suppressor screen for *cks-1* mutants. So far we have identified 50 dominant suppressors, which arise at knock-out frequency and define at least three loci. We will report on our progress toward mapping, and characterizing these suppressors, as well as our further analysis of the *cks-1(ne549)* mutant.

**475243. The putative RNA-binding proteins MEX-5, MEX-6, and SPN-4 regulate MEX-3 localization and activity to control PAL-1 spatial patterning**

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The spatial and temporal patterning of the homeodomain protein PAL-1 is dependant on the KH domain protein MEX-3 and the *pal-1* 3'-UTR. While *pal-1* mRNA is present throughout the oocyte and early embryo, PAL-1 protein is only detected in posterior blastomeres starting at the 4-cell stage. MEX-5, MEX-6 and SPN-4 are required for proper MEX-3 localization and activity in the embryo and subsequent spatial patterning of PAL-1. *mex-5* and *mex-6* encode homologous and functionally redundant CCCH zinc finger proteins that are localized to the anterior and are required to prevent premature degradation of anteriorly-localized MEX-3 protein and to maintain PAL-1 repression in the anterior. *spn-4* encodes an RNA recognition motif protein present throughout the early embryo that contributes to PAL-1 repression in the anterior and is required for the timely degradation of MEX-3.

MEX-6 and SPN-4 interact with MEX-3 in yeast, suggesting that these proteins may form a complex. Co-immunoprecipitation studies aim to verify and characterize these protein-protein interactions. Genetic analysis suggests that MEX-5, MEX-6, and SPN-4 may act in combination to link initial cell polarity to the asymmetric distribution of the cell fate determinant PAL-1 by controlling MEX-3 stability and activity. While SPN-4 is normally found throughout the early embryo, its activity is restricted to the posterior by anteriorly-localized MEX-5 and MEX-6. MEX-5 and MEX-6 are also required to restrict SPN-4 localization to the posterior as the embryo develops. As MEX-3, MEX-5, MEX-6 and SPN-4 all contain RNA binding motifs, an appealing hypothesis is that these proteins interact on the *pal-1* 3'-UTR. Ongoing genetic and biochemical studies aim to better understand how these putative RNA binding proteins regulate the spatial pattern of *pal-1* expression.

### 153148. CaMKI AND THERMOSENSATION

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Thermosensation is perhaps the most poorly understood of the sensory modalities, despite its critical role in modulating the behavior and metabolism of organisms. Worms respond to temperature using the AFD thermosensory and the AIY and AIZ interneurons. To investigate AFD neuron development and function, we isolated mutants with defects in AFD-specific gene expression. We showed that the *ttx-1* OTX/OTD-like homeodomain transcription factor is both necessary and sufficient to specify AFD fate (Satterlee et al, 2001). We also found that mutations in the *tax-2* and *tax-4* cGMP-gated channel genes and the *cmk-1* gene result in defects in expression of a subset of AFD-specific markers. *cmk-1* encodes the *C. elegans* ortholog of the calcium/calmodulin-dependent protein kinase I (CaMKI). Although CaMKI has been well characterized biochemically, the *in vivo* role of CaMKI is poorly understood.

Both *tax-2/4* and *cmk-1* mutants have reduced expression of the AFD-specific genes *gcy-8* and *nhr-38* encoding a receptor guanylate cyclase and a nuclear hormone receptor homolog respectively, although expression of the *ceh-14* LIM-homeobox gene is unaffected. While *tax-2/4* mutants exhibit athermotactic behaviors, *cmk-1* mutants are thermophilic, preferring temperatures warmer than their cultivation temperature. The *cmk-1* promoter drives GFP expression in many neurons, including the AFD neurons, and a GFP-tagged CMK-1 protein is cytosolic. The gene expression and thermotaxis defects of *cmk-1* mutants are rescued by AFD-specific expression of *cmk-1* indicating that *cmk-1* functions in a cell autonomous manner for these phenotypes.

*In vitro*, CaMKI enzymes can be activated by both calcium/calmodulin binding and by phosphorylation of threonine-179 by upstream kinases. To determine the relationship between CMK-1 and TAX-2/4 in the regulation of expression of AFD-specific genes, we generated a truncated CMK-1 mutant protein carrying a T179D substitution. Based on *in vitro* experiments (Eto et al, 1999), this mutant protein is predicted to be calcium-independent and constitutively active. We found that expression of this gain-of-function CMK-1 protein in the AFD neurons partly rescues the gene expression defects of *tax-4* mutants, suggesting that CMK-1 acts downstream of TAX-4.

Our working hypothesis is that *cmk-1* does not function directly in thermosensory signal transduction, but may instead play a role in modulating both long-term and short-term sensory plasticity. Preliminary experiments indicate that CMK-1 is also involved in adaptation to odorants sensed by the AWC olfactory neurons. We suggest that short-term sensory plasticity such as adaptation may occur via CMK-1 phosphorylation of AFD and AWC signal transduction components, whereas CMK-1 may regulate long-term changes via the modulation of the type and quantity of signaling molecules expressed. Current experiments are aimed at identifying both upstream regulators of CMK-1 function and downstream targets, utilizing genetic, genomic and biochemical methods.

**942098. The OTX-related homeodomain protein CEH-37 is required for the development of the AWB olfactory neurons.**

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*C. elegans* is equipped with highly specialized sensory neurons that enable the animal to accurately sense its environment. Our lab is interested in understanding how the unique functions of individual sensory neurons are specified during development. In order to identify genes required for the development of these diverse neuron types, we have taken the approach of isolating mutants that exhibit altered expression of sensory neuron-specific markers. This strategy has allowed the identification of cascades of transcription factors required to define the identities of specific sensory neuron types, including the AWA olfactory and the AFD thermosensory neurons. My goal is to understand the developmental mechanisms by which the AWB olfactory neurons are specified.

Previous work has demonstrated that expression of the LIM homeodomain transcription factor LIM-4 in the AWB neurons is crucial for their development. In *lim-4* mutants, the AWB neurons lose AWB-specific characteristics, and instead adopt a default sensory neuron fate (Sagasti et al. 1999). We isolated an allele of the gene *ceh-37* in a screen for mutants that fail to express an AWB-specific odorant receptor. *ceh-37* appears to act upstream of *lim-4*, since in *ceh-37* mutants, *lim-4* expression in the AWB neurons is variably lost. Interestingly, although in *lim-4* mutants the AWB neurons lose all neuron-specific characteristics including the ability to fill with DiO, the AWB neurons retain the ability to dye-fill in *ceh-37* null mutants. In addition, unlike *lim-4* mutants, the AWB neurons in *ceh-37* mutant animals do not adopt default olfactory neuron characteristics. This suggests that CEH-37 may act to regulate the expression of *lim-4* as well as additional factors required for AWB development.

*ceh-37* encodes an OTX-like homeodomain transcription factor. It is one of three similar *otx*-related genes encoded by the *C. elegans* genome. OTX-related proteins have been implicated in the patterning of anterior structures in vertebrates, *Drosophila* and also in the development of the *C. elegans* AFD thermosensory neurons. The *otx* homolog *ttx-1* has been shown to be both necessary and sufficient to specify the fate of the AFD thermosensory neurons in *C. elegans* (Satterlee et al. 2001). Thus, the development of both the AFD and the AWB sensory neurons require the function of closely related genes.

CEH-37 is expressed broadly in the early embryo although its expression is restricted to the excretory cell in larval and adult stages. Its embryonic expression implicates an early role for CEH-37 in the development of the AWB neurons. We have shown that expression of CEH-37 specifically in the amphid neurons is sufficient to rescue the AWB defects of *ceh-37* mutants. Surprisingly, we found that amphid-specific expression of any of the three *C. elegans* OTX-like genes is sufficient to rescue the AWB development defects of *ceh-37* mutants. While misexpression of CEH-37 is not sufficient to induce ectopic expression of AWB specific genes, *ceh-37* misexpression results in the ectopic expression of AFD-like characteristics in some neurons. The conservation of function between CEH-37 and TTX-1 suggests that precise regulation of the spatial and temporal expression of these *otx*-like genes during development is essential in order to ensure the correct development and function of the *C. elegans* sensory neurons.

**641409. exc Mutants Suppress *kal-1* Induced Axonal Branching and Misrouting**

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Mutations in the Kallmann Syndrome gene, *KAL-1*, are responsible for a subset of X-linked forms of KS, a neurological disorder characterized by axon and cell migration defects and frequently associated with unilateral renal agenesis. *KAL-1* encodes a cell surface protein that we and others have recently directly implicated in axon branching and pathfinding (2-4). In order to identify its molecular partners we have searched for modifier loci of *Cekal-1* induced neuronal branching of the AIY interneuron using both a candidate gene and a genetic screen approach (2).

Since the X-KS pathology includes kidney defects (5), we investigated whether mutations that affect the excretory system of *C. elegans*, namely the *exc* mutants (1) show a genetic interaction with our *Cekal-1* mis/over-expression transgenes. We found that members of a specific subset of the *exc* mutants significantly suppress *kal-1* induced axonal branching: *exc-2* and *exc-4* each reduce *kal-1* induced branching from 100% penetrance to 60%-65% penetrance. We have further investigated the specificity of suppression by *exc-4*. *exc-4* showed suppression of *kal-1* induced misrouting of the amphid commissure from 20% penetrance to 10% penetrance. Interestingly, *exc-4* had no effect on *kal-1* induced branching in the AFD sensory neuron, indicating cellular specificity. We found that *exc-4* could not suppress *ttx-3* induced branching in AIY, demonstrating specificity for *kal-1* induced branching.

We have SNP mapped *exc-4* to a 130 kb region on the right arm of Chromosome I and are in the process of rescuing and sequencing predicted genes in this region. We are also currently characterizing the neuroanatomy and behavior of *exc-4* mutants as well as investigating potential genetic interactions between *exc-4(lf)* and *kal-1(lf)* alleles. Both *exc-4* and our other suppressor loci may give insights into the molecular mechanisms of *KAL-1* protein function.

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**616232. The paired-like homeodomain protein SNS-10 is required for the development of the AWA olfactory neurons**

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*C. elegans* is attracted to the butter-like smell of diacetyl and almond-like odor of benzaldehyde, but is repelled by 2-nonanone. These odorants are sensed by the olfactory neuron pairs AWA, AWC and AWB respectively. All three neuron pairs respond to volatile chemicals but do so using distinctive specialized cilia, and different signaling pathways. How do the similarities and differences between these (and other) neurons arise? In particular, what molecular mechanisms are at play? By comparing and contrasting the developmental processes required in different sensory neurons we hope to understand how diversity of function is generated in the nervous system.

A number of factors have been identified as playing a role in specifying the fate of the AWA olfactory neurons. *odr-7*, a nuclear hormone receptor transcription factor, is expressed specifically in AWA and controls its specification, such that in *odr-7* mutants AWA-specific gene expression and function is lost (Sengupta et al, 1994). *lin-11*, a LIM HD transcription factor, is required to initiate *odr-7* expression (Sarafi-Reinach et al., 2001), and in *lin-11* mutants, the development of both the AWA neurons and their lineal sisters, the ASG neurons is affected. Restriction of *odr-7* expression to the AWA neurons is regulated by the forkhead transcription factor UNC-130 which is expressed in the AWA/ASG precursor cells (Sarafi-Reinach and Sengupta, 2000). In a screen aimed at identifying additional genes required for AWA specification, I isolated two alleles of the gene *sns-10*. In *sns-10* mutants, the expression of both AWA-specific genes, such as *odr-7*, and ASG-specific genes is lost. I also observe occasional ectopic expression of ODR-7. *sns-10* mutants exhibit various pleiotropies suggesting that *sns-10* is involved in the development and/or function of additional cells. *sns-10* encodes a paired-like homeodomain transcription factor with high homology to the *Drosophila* gene *Aristaless*. Interestingly, *Aristaless* is involved in the formation of the arista sensory organs (Schneitz et al. 1993).

How does *sns-10* regulate AWA development? A number of observations suggest that it acts prior to the birth of the AWA neurons. First, no expression of SNS-10 is seen in the AWA neurons once they are born. Second, I have shown that the phenotype of ODR-7 expression in *sns-10*; *lin-11* double mutants is similar to that of *lin-11* mutants alone, suggesting that SNS-10 and LIN-11 act in a linear pathway. Moreover, *lin-11* expression in the AWA and ASG neurons appears to be regulated by SNS-10. Third, *sns-10*; *unc-130* double mutants exhibit a synergistic effect on *odr-7* expression, suggesting that they act in parallel. Since work in our lab suggests that LIN-11 may act in the AWA neurons, while UNC-130 acts in their precursors, SNS-10 is likely to act early in AWA formation. Thus, a cascade of transcription factors acts to regulate the development of the AWA neurons. In addition, several of these factors are also involved in regulating the development of the ASG sister cells. Intriguingly, work by other people in the lab has shown that a homologous cascade of transcription factors, involving a paired-like homeodomain transcription factor regulating a LIM-homeobox gene, exists in the development of other sensory neurons such as the AFD and AWB neurons (Satterlee et al., 2001; see abstract by A. Lanjuin). In addition, the *Drosophila* homologs of SNS-10 and LIN-11 have also been shown to act in a similar regulatory cascade in the developing leg (Pueyo et al., 2000; Tsuji et al., 2000). Thus, understanding the developmental cascade of events that occurs in the AWA neurons may shed light not only on the development of other sensory neurons in *C. elegans* but also on similar processes in other organisms.

**316158. Transcriptional repression in the early *C.elegans* embryo by HDA-1**

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Cell-type and tissue specific patterns of gene expression during development are regulated by transcriptional activation and repression. The acetylation state of chromosomal histones plays an essential role in the developmental regulation of gene expression; while hyperacetylated chromatin is usually associated with active transcription units, hypoacetylated regions of chromatin frequently are transcriptionally silent. This acetylation status is enzymatically regulated by histone acetyltransferases (HATs) and by histone deacetylases (HDACs).

17 HDACs genes have been identified in humans so far, which fall into three categories based on their homology to yeast HDACs. *C.elegans* has 11 HDACs, and three of them belong to class I.

Histone Deacetylase 1 (*hda-1*) is a class I *C.elegans* HDAC gene that shows ubiquitous expression throughout development. The existing *hda-1* genetic mutant (*gon-10*, gonadogenesis defective-10) is not useful to analyze the role of *hda-1* in early embryogenesis since *gon-10* animals are sterile. However, depletion of both maternal and zygotic *hda-1* by RNAi results in embryonic lethality of variable penetrance. A high proportion of *hda-1* (RNAi) embryos display ectopic *end-1* expression in the early embryo. Since END-1 is sufficient to induce endoderm differentiation, one possibility is that these embryos are dying due to inappropriate specification of early endodermal tissue. In order to obtain fully-penetrant embryonic lethality we optimized the RNAi protocols and achieved 100% lethality.

To identify potential target genes of *hda-1*, we harvested *hda-1*(RNAi) and control treated embryos prior to the lethal stage, and performed DNA microarray analysis. We performed these experiments in triplicate, and averaged the resulting ratios. Our results show that, in agreement with a general role in transcriptional repression for histone deacetylases, lack of *hda-1* in the early embryo results in greater than three-fold upregulation of the expression of 95 genes. By contrast, only 10 genes are downregulated three fold or greater. Importantly, one of the most downregulated genes is *hda-1* itself, demonstrating that functional depletion of that gene product by RNAi was successful.

This genome-wide approach sheds light on the function of HDACs in multicellular organisms. For instance, it shows that a single predicted HDAC could be sufficient to determine gene activity by itself during development. In addition, it reveals that a specific histone deacetylase may have an effect on tissue-specific gene products, since many of the genes that are de-repressed in the *hda-1* (RNAi) animals appear to encode potential gut- and pharynx-specific genes.

**669075. A critical role for mitochondria in *C. elegans* lifespan regulation**

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To identify new components that regulate *C. elegans* lifespan, we performed a genetic screen for long-lived mutants. To eliminate the isolation of components of the well-characterized *daf-2* pathway, we screened for long life in a *daf-16* null mutant background. In a pilot screen of 2000 haploid genome, we isolated *mg312*, which exhibited a dramatic increase in lifespan, up to two-fold greater than controls. *mg312* also displayed other pleiotropies, including reduced pumping and defecation rate, slow growth and sterility. *mg312* is a mutation in the mitochondrial leucyl-tRNA synthetase gene *lrs-2*. *lrs-2(mg312)* is a probable null mutant because it is a nonsense mutation in the second exon of the gene. Using a rescuing *lrs-2::GFP* fusion gene, we showed that LRS-2 is localized to the mitochondria of many different tissues, including neurons, intestine and body-wall muscle. To assess mitochondrial morphology, we used a GFP marker that is specifically expressed in the body-wall muscle mitochondria. In wild-type animals, this marker exhibits highly organized rod-like staining, but in *lrs-2(mg312)* animals, it was found in contorted and swollen patterns, suggesting that mitochondria were somehow compromised in these mutants. The mechanism contributing to the long-lived phenotype of *lrs-2(mg312)* is unclear. However, a prediction based on the oxidative theory of aging is that reduced reactive oxygen species (ROS) production will lead to reduced cellular damage and longer lifespan. To test this hypothesis, we subjected animals to the oxidative damage-inducing agent paraquat. We found that *lrs-2(mg312)* animals were more sensitive to paraquat treatment, suggesting that these mutants may in fact produce more endogenous ROS, perhaps due to a block in electron transport chain activity.

We also utilized an RNA interference (RNAi) strategy to systematically analyze lifespan alterations following reduced expression of each of the predicted *C. elegans* gene. Inactivation of each gene of Chromosome I revealed that a large number (about 10%) of the RNAi candidates leading to a long-lived phenotype are annotated as mitochondrial genes. These results further indicate that mitochondria likely play an essential role in controlling *C. elegans* lifespan.

**747940. Poly(ADP-ribose) polymerases and poly(ADP-ribose) glycohydrolases in *C. elegans*: molecular characterization of the pme gene family members.**

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Poly(ADP-ribosyl)ation is a very important posttranslational modification for many important biological processes in mammals including apoptosis/programmed cell death and DNA repair. However, clear and unified roles for poly(ADP-ribose) polymerases (PARPs) and poly(ADP-ribose) glycohydrolases (PARGs) are not yet defined. In order to gain information on PARPs potential role(s) during development and programmed cell death, we used the model organism *Caenorhabditis elegans*. A computational analysis of the genome of *C. elegans* using human PARP-1 amino acid sequence revealed the presence of four loci encoding putative poly(ADP-ribose) metabolism enzymes or PME. Loci Y71F9AL.18, and E02H1.4 contain pme-1 and pme-2 genes respectively. PME-1 gene product has a highly similar structure to human PARP-1 and PME-2 protein is similar to PARP-2. The transcript of pme-1 was shown to be transspliced with splice leader SL1 and the transcript of pme-2 is transspliced with splice leader SL2. Both transcript are expressed throughout the life cycle of the worm, although mainly present in the embryos, suggesting a role during this stage of development. A third locus, AC8.1, contains an almost identical copy of pme-1. However, the putative transcript AC8.1 is shorter in size to PME-1 and was not detected on northern analysis, suggesting that AC8.1 may be a pseudogene. The recombinant enzymes rPME-1 and rPME-2 were shown to synthesize poly(ADP-ribose), confirming their identity as real PARP. A fourth locus, ZK1005.1 contains the gene pme-5. PME-5 is a homolog of tankyrase 2 in mammals, a novel PARP localized to telomeres. Crude protein extracts from *C. elegans* contain specific PARG activity as shown on TLC plate analyses. In order to link the enzyme activity to specific gene(s), we analyzed the genome of *C. elegans* using human PARG amino acid sequence. Two distinct loci, F20C5.1 and H23L24.5, were found encoding two putative PARGs. The genes were named pme-3 and pme-4. PME-3 and PME-4 share identity at 35% and 30% with human PARG respectively. We also determine that pme-3 encode two alternatively spliced transcripts, adding to the potential physiological role(s) for PARGs. We plan to address the role(s) of pme gene family members in development through a reverse genetics approach.

Funding for this research is provided by the Natural Sciences and Engineering Research Council of Canada and the Fonds de la Recherche en Santé du Québec.

**557051. The role of *C. elegans* autophagy genes in development**

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Autophagy is the bulk degradation of cytoplasmic components through an autophagosomic-lysosomal pathway. Under nutrient starvation, or some other forms of stress, mammalian cells as well as yeast cells, undergo bulk degradation of proteins and remodelling of cellular compartments. Autophagy is the major route of delivery of cytoplasmic proteins into lysosomes; it is important for cell survival, in growth control, and may be defective in tumour cells. However, little is known about the role of autophagy in the development of multicellular organisms. We are analyzing the role of evolutionarily conserved autophagy genes in *C. elegans* development, by studying the function of the worm *beclin* 1 homolog (Ce *bec-1*). The human *beclin* 1 gene is the first identified mammalian gene to mediate autophagy and also has tumour suppressor and antiviral function (Liang et al. Nature. 1999; 402:672-6). In yeast, the *beclin* 1 gene was isolated as an autophagy defective mutation, *apg6*, or as a vacuolar protein sorting mutation, *vps30*. Mammalian Beclin 1 promotes autophagy, but not vacuolar protein sorting in autophagy-defective yeast with a targeted disruption of the yeast ortholog, APG6/VPS30 (Liang et al., 1999). *C. elegans* BEC-1 shares 28% amino acid homology with the human Beclin 1 (T19E7.3; *C. elegans* genome sequencing project). We have inactivated the Ce *bec-1* homologue by injecting wild-type (N2) worms with *bec-1* dsRNA or by soaking worms with *bec-1* dsRNA. *bec-1* RNAi-soaked worms arrested at the L1 stage, with pronounced defects in the intestine and in the head. In contrast, the progeny of injected *bec-1* RNAi worms reached adulthood with varying defects in the intestine, characterized by the accumulation of large vacuolar structures. Ce *bec-1* RNAi also decreases the endocytosis of a YP170(yolk protein) ::GFP fusion by oocytes, an assay that has been used to isolate mutants in receptor mediated endocytosis (Grant and Hirsh Mol. Biol. Cell. 1999;10:4311-26).

In *C. elegans*, a developmental arrest, the dauer larva, occurs in response to high population density and limited food (Golden and Riddle Devel. Biol.. 1984; 102:368-78). As autophagy is induced by both of these stimuli in yeast and mammalian cells, we hypothesized that autophagy might be required for dauer formation or survival of the dauer larvae. To investigate this hypothesis, we injected *daf-2* mutants with Ce *bec-1* dsRNA. Mutations in the *daf-2* gene, encoding a member of the insulin receptor family, result in constitutive formation of dauer larvae at the restrictive temperature and increased lifespan at the permissive temperature. Ce *bec-1* (RNAi); *daf-2* double mutants arrest at different stages and do not complete dauer formation when grown at the restrictive temperature. Dauer formation is also affected in a *daf-2*; *unc-51* double mutant, when grown at the restrictive temperature (*unc-51* is the *C. elegans* orthologue of another autophagy gene in yeast, *apg1*). We are now using electron microscopy to determine if autophagy is increased upon dauer formation, and if autophagy is affected in the Ce *bec-1* RNAi mutants.

In summary, Ce *bec-1* RNAi-soaked worms have developmental defects and arrest at the L1 stage. Furthermore, inactivation of homologs of two different yeast *apg* genes in *C. elegans* has an effect on the *daf-2* constitutive dauer formation. While the molecular basis of these phenotypes is not yet known, these observations suggest a possible role for autophagy genes in *C. elegans* non-dauer development, as well as in the formation and/or survival of the *C. elegans* developmentally arrested dauer larva.

**62311. GLD-1 and the *pal-1* 3' UTR are implicated in the post-initiation translational repression of maternal *pal-1* mRNA in *C. elegans***

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The regulation of mRNA translation is a prominent, yet poorly understood mechanism of gene control. To gain insight into the molecular mechanisms of translational control, we are investigating the translational regulation of maternal *pal-1* mRNA, which encodes a conserved homeodomain protein involved in posterior patterning of the *C. elegans* embryo. We have focused on translational control in the distal gonad arms of adult hermaphrodites (where *pal-1* mRNA is present, yet no PAL-1 protein is detected), and have gained insight into 3' UTR regulatory elements, a *trans*-acting factor, and the level of the translational block.

To identify translational control elements in *pal-1* mRNA, we used a *lacZ* reporter RNA assay. We found that the *pal-1* 3' UTR is sufficient to repress the translation of a *lacZ* reporter RNA (*lacZ::pal-1* 3' UTR) in the distal gonad arms of adult hermaphrodites. Through deletion analysis of the *lacZ::pal-1* 3' UTR RNA, we identified two regions of approximately 180 nucleotides that repress distal germline expression. Subdivision of one of these regions identified a potential translational activation element as well as a 108-nucleotide germline repression element, the GRE, which is both necessary and sufficient for robust germline repression.

Through a candidate gene approach, we identified GLD-1, a maxi-KH domain protein, as a negative regulator of PAL-1 expression. In *gld-1* null mutants, ectopic PAL-1 immunofluorescence is detected in the distal gonad arms of adult hermaphrodites. In addition, *gld-1* can act through the GRE to repress distal germline expression; a *gfp* transgene under the control of the GRE is ectopically expressed in distal gonad arms when *gld-1* activity is reduced through RNAi.

Finally, to begin addressing the molecular mechanism of repression, we performed polysome analysis to determine where *pal-1* mRNA from the distal germline fractionates with respect to ribosomes. Multiple experiments suggest that *pal-1* translation in the distal germline may be blocked after *pal-1* mRNA is loaded on ribosomes. Since initiation is often the rate-limiting step in protein synthesis, the loading of *pal-1* mRNA on ribosomes in the germline may allow for the rapid accumulation of PAL-1 protein in the 4-cell embryo when the translational block is relieved.

**732462. SRC-1 acts in parallel with Wnt signaling to specify endoderm and to control cleavage orientation in early *C. elegans* embryos.**

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In early *C. elegans* embryos, signaling between a posterior cell, called P2, induces its anterior sister cell, called EMS, to divide A/P and to produce endoderm. Although Wnt signaling contributes to this polarizing interaction, no mutants identified to date abolish P2/EMS signaling. Here we show that SRC-1, a *C. elegans* homolog of c-SRCpp60, and MES-1, a protein with an overall similarity to receptor tyrosine kinases, are required for the intense phosphotyrosine accumulation at the P2-EMS junction. Double mutants between *src-1* or *mes-1* and any of 6 previously described components of the Wnt pathway including, *mom-1* (Porcupine), *mom-2* (Wnt/Wg), *mom-3* (uncloned), *mom-5* (Frizzled), *dsh-1*; *mig-5* (Disheveled), and *gsk-3*, abolish P2/EMS resulting in a left/right orientation to the EMS division axis and a complete lack of endoderm. These data suggest that *src-1* and *mes-1* function in parallel with Wnt signaling to control P2/EMS signaling. Our blastomere isolation experiments suggest that *mes-1* is required in both P2 and EMS while *src-1* is required cell autonomously in EMS. Interestingly, we have found that a similar genetic mechanism functions in another nematode species, *C. briggsae*, suggesting that there is some functional significance to the redundancy displayed between the Wnt and Src pathways in *C. elegans*.

**4837. Ubiquitin ligase CUL-2 is required for segregation of meiosis II sister chromatids and for establishing polarity**

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After fertilization, the *C. elegans* maternal chromosomes go through two rounds of meiotic divisions before the fusion of maternal and paternal pronuclei that initiates the first mitosis. The first meiotic division, meiosis I, separates chromosome homologs and generates a 2N polar body. The second division, meiosis II, separates sister chromatids and generates the second polar body.

The general machinery regulating chromosome separation is largely conserved among eukaryotes. Cohesin, a protein acting as a glue to hold chromosomes together, has to be cleaved at the metaphase-to-anaphase transition to allow chromosome separation. Cohesin is cleaved by separase, whose activity is indirectly controlled by the Anaphase Promoting Complex/Cyclosome, APC/C.

Currently all known proteins that regulate chromosome separation work through the separase pathway. The regulation of chromosome segregation is less well-understood. Here we report a novel player in regulating chromosome segregation, the ubiquitin ligase component CUL-2. In *cul-2* deletion mutants as well as *cul-2* RNAi animals, meiosis I is normal but meiosis II is abolished or extremely delayed. Histone::gfp movies show that these mutants have no obvious sister chromatid segregation at meiosis II. In a small percentage of the mutant embryos, sister chromatids separate during meiosis II but fail to segregate towards the spindle poles. This observation is strengthened by the finding that the meiotic-specific cohesin, REC-8, is degraded in the mutant with normal timing, suggesting that CUL-2 is essential for chromosome segregation, but not for chromosome separation. We conclude that: 1) CUL-2 is required specifically for meiosis II chromosome segregation, but not for major aspects of meiosis I; and 2) the regulation of chromosome segregation by CUL-2 is independent of the APC-separase-cohesin pathway, which appears to function normally.

The meiosis defect in *cul-2* mutant embryos leads to a prolonged duration of the meiotic spindle. As a result, the establishment of polarity is impaired in *cul-2* mutants, as shown by the initiation of a PAR-2 cortical patch at the anterior of the zygote, contrary to PAR2s wildtype posterior cortical pattern. This reversal of polarity is similar to that observed by Wallenfang et al, in which perdurance of the meiotic spindle led to reversed polarity. A further defect in *cul-2* mutants, extensive cytoplasmic flow, results in further distortions of polarity, via abnormal movement of the PAR2 patches along with the meiotic spindle and the sperm asters.



## 60721. Using *C. elegans* to model the peroxisome biogenesis disorders

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We have established the nematode, *Caenorhabditis elegans*, as a system for studying peroxisome biogenesis and modeling the associated human peroxisomal biogenesis disorders (PBDs). Peroxisomes are present in virtually all eukaryotic cells where they carry out a variety of essential metabolic functions including many components of lipid metabolism (such as beta-oxidation and ether phospholipid synthesis). The peroxisome is also involved in amino acid and purine metabolism. Peroxin proteins (called PEX genes in humans, PRX in *C. elegans*), are required for either the import of enzymes into the peroxisomal matrix, or are necessary for the division and growth of peroxisomes. Mutations in peroxin genes cause the devastating PBDs, such as neonatal adrenoleukodystrophy and Zellweger syndrome.

The genome of *C. elegans* encodes homologs for 10 of the 14 mammalian peroxins. We used dsRNA interference to determine the biological role of the peroxisome in nematodes. We targeted 5 nematode peroxin homologs with the highest degree of homology to PEX 5, PEX6, PEX12, PEX13 and PEX19. Using a GFP reporter targeted to the peroxisome, we show that peroxisome biogenesis is impaired in *prx-5(RNAi)* worms. In addition, inactivation of each of these 5 peroxin genes by RNAi results in an early larval arrest at the L1 stage. This implies that peroxisome function is necessary for the normal development of *C. elegans*. The L1 arrested worms are active and survive for up to 5 days, which is strikingly similar to starvation-arrested L1 worms. We hypothesize that a by-product of peroxisome metabolism may be necessary to signal the advance to post-embryonic development. Importantly, the L1 arrest phenotype suggests that genetic suppressor analysis might identify novel ways to bypass toxicity related to the PBDs.

Global developmental delay is a major hallmark of the PBDs, and is thought to be at least in part caused by defects in neuroblast migration during development. We have noticed no change in neuronal position in either *mec-4::GFP; prx-5(RNAi)* (touch neurons) or *unc129::GFP; prx-5(RNAi)* (DA and DB motor neurons) worms. In order to examine post-embryonic neuroblast migration in RNAi treated nematodes, we are now examining the Q neuroblasts which normally begin migration within the first hour after hatching.

To obtain a better understanding of the biochemical functions carried out by the nematode peroxisome, we took an *in silico* approach to identify all *C. elegans* proteins which contain a type I peroxisomal targeting signal (S/A/C-K/R/H-L/M/I). We identified more than 30 predicted open reading frames homologous to either human or yeast peroxisomal enzymes, including enzymes that participate in beta-oxidation and branched-chain fatty acid oxidation. We have also identified 15 novel PTS1-containing ORFs and are currently confirming the subcellular localization of these proteins with GFP fusion constructs.

**82045. Calcium/diacylglycerol-activated protein kinase C is essential for normal thermotaxis and responses to odorants.**

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In mammals, diacylglycerol (DAG),  $\text{Ca}^{2+}$  and phosphatidyl serine activate 10 protein kinase C (PKC) isoforms. PKC diversity is generated by transcription from 9 genes, alternative mRNA splicing and cell-specific patterns of PKC and target-effector protein expression. PKCs participate in regulation of many physiological processes including: differentiation, synaptic transmission, secretion, ion channel activation and gene transcription. Physiological roles of individual PKCs are ill-defined because of functional redundancies and variations in amounts and types of PKC isoforms in different cells.

*C. elegans* provides a complementary system for analysis of PKC functions *in vivo*. Four genes encode PKCs in *C. elegans*. Patterns of PKC isoform expression *in situ* suggest that individual phosphotransferases (PKCs1-3, TPA-1) govern properties and functions of discrete effector proteins in many cells. Molecular genetics, biochemical analysis and sensory assays are being used to determine physiological roles for *pkc-2* (*kin-11*) gene products. Three promoter/enhancer regions drive *kin-11* transcription and mRNAs are further diversified by alternative utilization of two terminal exons with divergent coding potential (50 or 52 amino acids in PKC2A or PKC2B). PKC2s encoded by *kin-11* are the only  $\text{Ca}^{2+}$ /DAG regulated phosphotransferases in the nematode. The A, B and C gene promoters target expression of PKC2 isoforms (~ 680 amino acids) to distinct subsets of neurons, intestinal, muscle and somatic gonad cells.

In collaboration with the *C. elegans* Gene Knockout Consortium (labs of Barstead and Coulson), we obtained animals carrying a disrupted *kin-11* gene. Sequencing revealed that 2312 bp of DNA were excised from the mutant kinase gene. As a result, exons (2-9) encoding amino acids 16 to 467 are deleted and a shifted reading frame encodes only 19 amino acids before encountering a termination codon. Anti-PKC2 IgGs detected a 77kDa PKC2 protein in wild type (WT) worms and confirmed the absence of the kinase in *pkc-2*<sup>-/-</sup> animals. Development, feeding, reproduction and movement are unaffected in *pkc-2* null worms. G protein coupled receptors mediate chemosensation in *C. elegans* by activating phospholipase C $\beta$ , thereby generating DAG and  $\text{Ca}^{2+}$  (via IP3). Since PKC2 is expressed in many sensory neurons, we tested responses of *pkc-2*<sup>-/-</sup> worms to odorants. PKC2-null animals are severely limited in their ability to detect odorants via AWA and AWC. Typically, the chemotaxis index for diacetyl was 0.17 in PKC2-null animals and 0.96 in WT N2. Although ASH-mediated osmotic avoidance behavior was normal, PKC2-null animals could not detect a water soluble attractant (0.2M NaCl) via ASE. Evidently, chemosensation mediated by AWA, AWC and ASE neurons is controlled (in part) by  $\text{Ca}^{2+}$ /DAG stimulated PKC2. Fed *Kin-11*<sup>-/-</sup> *C. elegans* that were grown at 20°C wandered randomly on plates subjected to a 16°C to 25°C temperature gradient (athermotactic); WT *C. elegans* readily located the 20°C zone. Thus, PKC2 plays a key role in thermotaxis.

PKCs regulate properties of effector proteins by phosphorylating Ser/Thr. Phosphorylated transcription factors or regulators of mRNA stability may alter target gene expression. Semi-quantitative RT-PCR was used to assess effects of a PKC2 deficit on accumulation of 5 mRNAs that encode proteins engaged in mediating thermotaxis. Levels of *tax-2*, *tax-4* and *ttx-1* mRNAs in PKC2 null worms were unchanged. However, WT nematodes contain 5-fold more *ttx-3* mRNA than *kin-11*<sup>-/-</sup> animals. Conversely, *tax-6* mRNA was 3-fold higher in PKC2-null *C. elegans*. Thus, PKC2 modulates expression of genes required for perception of a thermal gradient. PKC2 may also regulate thermotaxis via protein phosphorylation. Studies on this topic are in progress.

Individual PKC2 promoters (A, B or C) or a mixture of constructs, containing all 3 promoters, will drive WT PKC2 mRNA and protein synthesis in different cell types of *pkc-2*<sup>-/-</sup> *C. elegans*. Recent studies show that a *kin-11* B promoter:PKC2 cDNA construct rescues AWA odorant responses, but not the thermotaxis phenotype. Tests of responses of AWC to odorants, and ASE to NaCl, are in progress. The ability of engineered PKC2 mutants and other PKC isoforms to rescue discrete features of *kin-11*<sup>-/-</sup> mutants will advance our knowledge of physiological and structure/function relationships among PKC isoforms *in vivo*.

**201608. MOLECULAR AND PHENOTYPIC CHARACTERIZATION OF A *CAENORHABDITIS ELEGANS* MUTANT WITH ENHANCED SUSCEPTIBILITY TO *PSEUDOMONAS AERUGINOSA***

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The utilization of a host and a pathogen that are both easily genetically manipulated allows for the molecular dissection of host-pathogen interactions. As a first step in understanding the interplay between *P. aeruginosa* and its many hosts, researchers from the Ausubel laboratory identified a virulent clinical strain called UCBPP-PA14 (PA14 hereafter) that is pathogenic toward multiple hosts. In particular, PA14 is pathogenic to plants and a wide range of animal phyla including humans, mice, insects and the nematode, *Caenorhabditis elegans*. Due to the inherent limitations of mouse models that are used as hosts for *P. aeruginosa* infections, the Ausubel laboratory has used PA14 infection of non-vertebrate hosts as an adjunct to mammalian models to identify and study *P. aeruginosa* virulence-related genes. Using these systems, bacterial genes conferring virulence have been identified. This host-pathogen system can also be used to identify host genes involved in defense response to pathogen attack. Although host mutants conferring altered susceptibility to *P. aeruginosa* have been isolated, very few of the genes pertaining to these mutations have been identified. We have used the genetic approach of mutational analysis to uncover novel host defense mechanisms in *C. elegans*. Ten *C. elegans* mutants that confer enhanced susceptibility to *P. aeruginosa* (**Esp**) in infection-mediated killing have been isolated. This report focuses on one of these mutants. Esp-3 worms exhibit an extreme susceptibility to PA14. After 24-hour exposure to PA14, <10% of Esp-3 worms are alive (as compared to 100% survival of wild type). Moreover, this susceptibility phenotype is temperature sensitive. Esp-3 worms also exhibit heightened susceptibility to other Gram-negative (*Salmonella typhimurium*) and Gram-positive (*Enterococcus faecalis*) pathogens. We have taken a positional cloning approach to identify the gene corresponding to the Esp-3 mutation.

## **648545. Investigation of CUL-2 Complex Components**

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Our previous study (Feng et al, Nature cell Biology, Vol.1(8), 1999) described the function of the cullin CUL-2. CUL-2 is a positive cell cycle regulator that is required for the G1-to-S phase transition, functioning in part by negatively regulating CKI-1 (a cyclin-dependent kinase inhibitor). CUL-2 is also required for mitotic chromosome condensation. Finally, CUL-2 is essential for segregation of meiosis II sister chromatids and for establishing polarity in the early embryo. (J. Liu and E.T. Kipreos, personal communication).

By investigating CUL-2 associating proteins, we hope to gain understanding of how CUL-2 functions to regulate CKI-1 degradation, chromosome condensation, and meiotic chromatid segregation. The first described CUL-2/Ring finger complex was the human CUL2/VCB complex, containing a ring finger protein RBX1, a SKP1-like molecule Elongin C, and a substrate-binding subunit VHL (the von Hippel-Lindau tumor suppressor). VHL has a BC-box which is required for its interaction with Elongin C and B. However, VHL does not appear to function with CUL-2 in *C. elegans* to regulate the cell cycle since inactivation of the vhl ortholog either by RNAi or with a deletion mutant (obtained by Gary Moulder and Robert Barstead) does not phenocopy *cul-2*. We propose that there are substrate-binding components other than VHL that function in CUL-2 complexes to regulate the cell cycle.

We undertook two approaches to gain insights into the composition of the CUL-2 complexes that regulate cell cycle events. First, we tested if inactivation of potential components by RNAi phenocopies *cul-2*. RNAi of an Elongin C ortholog, Y82E9BR.15, gave both germ cell arrest phenotype and embryonic phenotypes of uncondensed DNA and multiple nuclei similar to *cul-2* mutants. Inactivation of the ubc5 homolog (*ubc2*) by RNAi phenocopies the *cul-2* embryonic phenotypes. Therefore, we think that it is likely that they function with CUL-2 in a complex to regulate the cell cycle. Second, we affinity-purified FLAG-tagged CUL-2 and its associating proteins from lysates of transgenic worms, using animals which do not contain the FLAG-tagged CUL-2 as a control. The purified complexes were separated by SDS PAGE/two dimensional gel. The protein bands which were present only in transgenic lysates and absent in control lysates were cut out and digested in-gel with trypsin. Proteins were identified by mass spectrometry. In addition to identifying CUL-2, we have successfully identified a transcription factor, The ortholog of which has been found in other organisms to associate with components of 19S proteasome subunit. This indicates that CUL-2 may function to regulate transcription. We also identified another ring finger protein, which suggests that CUL-2 may form additional cullin/RING finger complexes with ring finger proteins other than RBX1. Finally, we identified a protein containing a BC-box motif, suggesting the possibility that it functions as a substrate-binding subunit. We are currently checking this possibility by RNAi inactivation to determine if this gene shares any phenotype with *cul-2*.

**157276. At least two target-dependent events are required for RNA interference in *C. elegans***

**Darryl Conte, Jr**, Alla Grishok, Chun-Chieh Chen, Hiroaki Tabara, Craig C Mello  
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RNA interference (RNAi) is thought to be a type of sequence-directed immune system that protects *C. elegans* (as well as other plant and animal species) from invading nucleic acids, including viruses and transposable elements. A handful of components of the RNAi machinery have recently been identified by genetic and biochemical methods, and the function/activity of each component and how they fit into the RNAi pathway are active areas of investigation. The data overwhelmingly support the model that dsRNA is processed by Dicer into ~22 nt small interfering RNAs (siRNAs), which mediate mRNA destruction. siRNAs provide sequence specificity to an as yet uncharacterized nuclease complex (RNA-induced silencing complex, RISC). But how does the worm recognize [foreign] dsRNA and how is this signal converted into an agent for targeted destruction? Our analysis of the molecular requirements of RNAi suggests that RNAi involves at least two target-dependent steps: 1) retention of trigger dsRNA and 2) siRNA-mediated destruction of the target mRNA. We have found that an RNA species corresponding to the trigger dsRNA accumulates in worms that are undergoing RNAi. The retention of trigger dsRNA requires the presence of a target gene, suggesting that dsRNA taken up from the environment is actively and rapidly scanned for homology to endogenous sequences. Homologous sequences may then be selectively retained for further processing. The second target-dependent step is the accumulation of siRNA. Surprisingly, only antisense siRNAs are detected by Northern analysis in worms undergoing RNAi. This contrasts with the symmetric accumulation of sense and antisense siRNAs found in the plant and *Drosophila* systems and suggests that only active siRNAs that engage a target mRNA are retained or amplified during RNAi in *C. elegans*.

**906510. Wormatlas: A web-based behavioral and structural atlas of *C. elegans***

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

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

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

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

**150330. Towards the molecular identification of *srf-9*, *srf-8* and *srf-4***

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The body of *C.elegans* is surrounded by a complex cuticle that acts as a barrier to the environment and also is relevant in locomotion and maintenance of body morphology. Collagen-like proteins constitute the major components of the cuticle; mutations affecting collagen-like proteins sometimes produce striking body shape changes, such as Dpy or Rol. In contrast, little is known about other surface associated proteins, which have been identified by lectin or antibody binding to the surface of live nematodes and by differential solubility after radiolabeling in non-penetrating conditions.

*srf-9*, *srf-8* and *srf-4* (surface) mutants were isolated by Chris Link based on their ectopic surface binding to the lectin wheat germ agglutinin (WGA), which binds to N-acetylglucosamine. In contrast to *srf-2*, *srf-3*, *srf-5* and *srf-6* mutants, which appear grossly wild-type in morphology and movement, *srf-9*, *srf-8* and *srf-4* mutants have multiple defects, including uncoordinated movement, protruding vulva, abnormal egg laying, and defective copulatory bursae and gonad morphology, and therefore have been called "pleiotropic" *srf* mutants (1). Interestingly, the pleiotropic mutations interact with mutations in the *lin-12* gene, which plays a role in several different cell fate decisions requiring cell-cell interactions (1, 2). Therefore, the "pleiotropic" *srf* mutations may cause a broad underlying defect, which would interfere with the proper expression of one or more cuticle component and also affect other factors required for normal development. Glycosylation, protein targeting, sorting, secretion or assembly of extracellular matrices are among the potentially affected processes.

Prior genetic mapping located *srf-9*, *srf-8* and *srf-4* to specific intervals of LGV (1). In order to determine their molecular identity, we are using snip/SNP mapping (3) to narrow the physical intervals containing these genes. This will facilitate the subsequent identification of the genes by rescue experiments using cosmids within these regions. This strategy makes no assumptions about the nature of these genes.

Concomitantly, we have searched the physical intervals that contain each of the pleiotropic *srf* genes for ORFs encoding for products with homology to known components of glycosylation and secretion pathways in other organisms. We are performing dsRNAi experiments with these candidate genes in both N2 and *lin-12*(n302) strains and scoring for the phenotypes characteristic of the "pleiotropic" *srf* mutations.

- (1) Link C. et al (1992) Genetics 131: 867-881
- (2) Sundaram and Greenwald (1993) Genetics 135: 755-763.
- (3) Wicks S et al. (2001) Nature Genetics 28: 160-164

**589485. Biochemical and genetic analysis of RNA interference in *C. elegans***

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RNAi is a post-transcriptional gene-silencing phenomenon induced by double-stranded RNAs. During RNAi, small-interfering RNAs (siRNAs) are formed and are thought to guide a nuclease complex in the destruction of target mRNAs. Interestingly, in *C. elegans*, the presence of a complementary target gene is required for siRNA accumulation and only the antisense siRNA strand is detectable by Northern analysis (Alla Grishok unpublished). This asymmetric accumulation of siRNA differs from results observed in *Drosophila* and plants and suggests that RNAi in *C. elegans* may include a surveillance step that ensures that only "useful" siRNAs are retained or amplified. Interestingly, RDE-4 has recently been shown to interact with RDE-1, DCR-1 and the trigger dsRNA, presumably forming a complex that functions to detect the presence of target mRNA (H. Tabara et al.). To further understand the mechanism of the target-dependent asymmetric accumulation of siRNAs, we performed northern analysis of wild-type and RNAi-deficient mutant worms exposed to pos-1 dsRNAs by feeding. We found that no siRNAs accumulate in *dcr-1*, *rde-1* or *rde-4* mutants. The absence of siRNA in *rde-1* and *rde-4* mutant backgrounds is consistent with the finding that these two proteins are required for the initiation of RNAi. Our findings also demonstrate for the first time that DCR-1 activity is indeed required for siRNA production in vivo. Consistent with a block in dsRNA processing we found that long-trigger dsRNA molecules accumulate in the *dcr-1* mutants. Further biochemical tests are planned to examine the production of siRNAs by the DCR-1 complex. In addition, a new genetic screen will be carried out to identify additional *rde* mutants.



#### **413568. Investigation of CUL-2 Complex Components**

**Hui Feng**, Edward T. Kipreos

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Our previous study (Feng et al, Nature cell Biology, Vol.1(8), 1999) described the function of the cullin CUL-2. CUL-2 is a positive cell cycle regulator that is required for the G1-to-S phase transition, functioning in part by negatively regulating CKI-1 (a cyclin-dependent kinase inhibitor). CUL-2 is also required for mitotic chromosome condensation. Finally, CUL-2 is essential for segregation of meiosis II sister chromatids and for establishing polarity in the early embryo.(J. Liu and E.T. Kipreos, personal communication).

By investigating CUL-2 associating proteins, we hope to gain understanding of how CUL-2 functions to regulate CKI-1 degradation, chromosome condensation, and meiotic chromatid segregation. The first described CUL-2/Ring finger complex was the human CUL2/VCB complex, containing a ring finger protein RBX1, a SKP1-like molecule Elongin C, and a substrate-binding subunit VHL (the von Hippel-Lindau tumor suppressor). VHL has a BC-box which is required for its interaction with Elongin C and B. However, VHL does not appear to function with CUL-2 in *C. elegans* to regulate the cell cycle since inactivation of the *vhl* ortholog either by RNAi or with a deletion mutant (obtained by Gary Moulder and Robert Barstead) does not phenocopy *cul-2*. We propose that there are substrate-binding components other than VHL that function in CUL-2 complexes to regulate the cell cycle.

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**751922. Germline-Soma Distinction in *C. elegans* Required the Zinc-Finger Protein MEP-1 and LET-418/Mi2, a Conserved Component of the Nucleosome Remodeling and Histone Deacetylase Complex**

**Yingdee Unhavaithaya**<sup>1</sup>, Tae Ho Shin<sup>1,2</sup>, Nicholas miliaras<sup>1</sup>, Jong Soon Lee<sup>2</sup>, Craig Mello<sup>1,3</sup>

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Among the earliest and most fundamental of differentiation events in many species is the distinction between the somatic cells which make up the body and the germline cells which will produce future generations. In the present study we describe MEP-1, a conserved Krüppel-type zinc-finger protein that is broadly expressed in all nuclei throughout *C. elegans* development. We identified the MEP-1 protein as a PIE-1 binding protein in a yeast two-hybrid screen. Interestingly, embryos lacking MEP-1 exhibit a developmental phenotype nearly opposite to that of *pie-1* mutants. Whereas *pie-1* mutant embryos exhibit somatic differentiation in the germline, *mep-1* mutant embryos arrest development shortly after hatching with evidence of ectopic germline differentiation in the somatic cells. We show that MEP-1 protein functions along with and forms a complex with the chromo-/helicase-domain protein, LET-418. LET-418 is a close homolog of Mi-2/CHD3, which in *Drosophila* and vertebrates is a core component of the nucleosome remodeling deacetylase (NURD) complex (von Zelewsky et al., 2000; Zhang et al., 1998). Developmental defects associated with the inactivation of *mep-1* and *let-418* are suppressed by mutations in *mes-2*, *mes-6*, and *mes-4*, which are worm homologs of proteins implicated in transcriptional maintenance in the *Drosophila*. These findings suggest a model in which chromatin remodeling by MEP-1/LET-418 is necessary to prevent somatic cells from reverting to germline transcriptional programs. In the germline cells, PIE-1 transiently inhibits MEP-1 in early embryos to maintain the pluripotency of germ cells, while at later times, and in other lineages, chromatin remodeling by MEP-1 and LET-418 is required to erase existing stable transcriptional competency and to allow the establishment of new differentiation programs.

Von Zelewsky, T., Palladino, F., Brunschwig, K., Tobler, H., Hajnal, A., and Muller, F. (2000). *Development* 127, 5277-5284.

Zhang, Y., LeRoy, G., Seelig, H. P., Lane, W. S., and Reinberg, D. (1998). *Cell* 95, 279-289.

**919883. Multi-parameter & Dual Color Fluorescence Analysis and Flow Sorting of *C. elegans*.**

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Union Biometrica, Inc., Somerville, MA 02143

We have previously reported single color applications for the COPAS *BIOSORT*, such as live-from-dead assays (using propidium iodide or Sytox Green) or male-from-hermaphrodite separation (using wheat germ agglutinin-phycoerythrin), as well as protocols to isolate transgenic animals with predefined levels of expression of a fluorescent protein from specific promoters of interest. We now demonstrate multi-parameter and dual color analysis and how this is accomplished on the *BIOSORT*.

The COPAS *BIOSORT* is a continuous flow sorter that analyzes, dispenses and sorts *C. elegans*. The system is composed of two lasers, which are coaxially focused to a horizontal line at the center of the flow cell to form an optical analysis zone. Objects in the flow then intersect with the optical analysis zone. A red diode laser is used to measure both the object length and optical attenuation, which is dependent on the thickness and the scattering properties of the object of interest. A multi-line argon laser (488 and 514 nm) excites user-selected fluorophores in the organism, or simply the autofluorescence of the animal. Fluorescence emission is detected by a series of optics and detectors. The real time-analysis of these parameters (size, optical density and fluorescence) can be used to analyze, sort and dispense animals of interest into multiwell plates or stationary receptacles.

Four parameters are recorded for each object of interest: Extinction (EXT), Time of Flight (TOF), and two fluorescence parameters (FLU). EXT is related to the optical density of the object of interest and TOF is related to the axial length of the object. TOF and EXT allow selective sorting of embryos, larval stages or adults from a wild type population as well as a number of mutants with affected body length or opacity of internal structures. FLU measurement is the amount of fluorescence from the analyzed animals and can be autofluorescence, fluorescent protein expression or fluorescent binding markers.

The optics and fluorescence detection on the COPAS *BIOSORT* allow for multiple excitation wavelengths and simultaneous fluorescence measurements for any two of three different color ranges. The instrument has fluorescence detectors for the green, yellow and red regions of the spectrum. This allows for the use of transgenic animals expressing GFP (Green Fluorescent Protein) and variations of the fluorescent protein from *Aquoria victoria* (EGFP, YFP), as well as other fluorescent proteins such as DsRed or various other reef coral proteins (BD Biosciences Clontech, Palo Alto, CA). Fluorescence can also be measured from *C. elegans* treated with certain molecular stains, such as Sytox Green, propidium iodide (PI) or phycoerythrin (PE)-tagged lectins (Molecular Probes Inc., Eugene, OR). This allows for the design of experiments where fluorophores with emissions in different parts of the spectrum can be combined in one experiment.

Mixed populations of wild type and several transgenic strains expressing different combinations of myo-2::ZsGreen, myo-2::ZsYellow, myo-2::DsRed2 (pharyngeal expression), myo-3::ZsYellow (body wall muscle expression), and ZK813.3::ZsGreen were tested on the *BIOSORT*. We show that the *BIOSORT* can selectively identify and sort animals with one fluorescent pattern from a mixed population of patterns. Accuracy of sorting was confirmed by microscopy of the sorted animals. The results show the *BIOSORT*'s ability to discriminate between transgenic strains with various color combinations. This technology allows for the design and implementation of biological assays that utilize two colors in their analysis.

**23187. Identification and characterization of enhanced RNAi mutants**

**Scott Kennedy**, Gary Ruvkun

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In *C. elegans* RNAi is relatively ineffective in neurons. We have undertaken a genetic screen to identify mutants that are capable of undergoing RNAi within neurons. We hope to identify endogenous inhibitors of RNAi and possibly to further our understanding of why neurons are refractory to RNAi. Animals carrying an integrated *unc-47::GFP* transgene, which expresses within the GABAergic neurons, fail to undergo RNAi against GFP following exposure to GFP dsRNA. *unc-47::GFP* animals were mutagenized and screened for loss of GFP expression following exposure to GFP dsRNA. Candidates were then screened for a wild-type GFP pattern in the absence of GFP dsRNA. From an initial screen of 5,000 haploid genomes we have identified ten mutations that satisfy these criteria. We have begun the characterization and molecular identification of two of these mutants: *mg366* and *mg377*. Both *mg366* and *mg367* exhibit a GFP dsRNA induced loss of GFP in 70% of GABAergic neurons. Utilizing amphid neuron specific GFP constructs we have found that *mg366* and *mg377* show enhanced sensitivity to GFP RNAi within the amphid neurons as well. Both mutants also exhibit enhanced sensitivity to dsRNAi targeted against numerous endogenous genes. Both mutants complement *rrf-3* (*pk1426*), the only other previously identified enhanced RNAi mutant in *C. elegans*. Progress on the phenotypic and molecular characterization of *mg366* and *mg367* will be reported.

**385087. A Gene targeting strategy using the yeast endonuclease I-SceI in *C.elegans***

**Masaki Shirayama**, Jennifer A. McCullough, Craig C. Mello

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Although the nematode, *C. elegans* has proven to be powerful model organism to study a variety of biological problems, it is still not possible to (routinely) induce homologous recombination between introduced DNA and genomic loci.

In an effort to solve this problem we are developing a method similar to that recently described for *Drosophila* (Rong and Golic, *Science*, **288**, 2013, 2000). The plan is to initiate gene targeting by introducing double strand breaks into extrachromosomal transgenes, *in vivo*, in germline nuclei. To do this we have expressed the yeast endonuclease, *I-SCE1*, that recognizes an 18 bp target sequence absent in the worm genome but present in flanking regions of homology within a transgene. To express the *I-SCEI* gene in the germline, we subcloned the *mes-6/cks-1* operon and replaced the *mes-6* gene with the *I-SCEI* gene. Since the *mes-6* and *cks-1* genes are in the same operon, both mRNAs are simultaneously processed from a single polycistronic preRNA and expressed in the germline of larvae and adults. We injected the *I-SCEI/cks-1* construct into *cks-1* maternal effect temperature sensitive mutants and were able to rescue the ts phenotype of *cks-1*. *I-SCEI* expressing lines can now be maintained by keeping *cks-1* rescued animals at 25 °C for subsequent generations.

We have also designed transgenic target constructs that contain either the entire sequence or a portion of the *pha-1* gene flanked by *I-SCEI* restriction sites. We are now preparing to introduce these constructs together with a counter-selectable gene, *avr-15*, into the *I-SCEI* expressing strain that also contains a *pha-1* ts lethal mutation. The extrachromosomal array will thus contain both *pha-1*(+) rescue activity and a counter-selectable *avr-15* gene that renders the strain sensitive to the drug ivermectin. In animals that carry this extrachromosome, expression of *I-SCEI* should lead to excision of the *pha-1* gene and integration into the genome, thus separating *pha-1*(+) activity from the *avr-15* gene. Then we can screen for homologous integration by scoring for ts(+), ivermectin resistant animals. Of course we expect pitfalls but see no reason why this basic idea should not work. We are open to any form of collaboration or suggestion as we simply are hoping to be able to use this important tool as soon as possible.

**198777. Regulation and function of DAF-9 cytochrome P450 in the dauer pathway**

**Ho Yi Mak**, Gary Ruvkun

Department of Molecular Biology, Massachusetts General Hospital and Department of Genetics, Harvard Medical School, Boston, MA 02114

The decision to proceed to dauer stage requires the integration of multiple sensory inputs such as food, pheromone and temperature. The sum of these inputs triggers the execution of a remodelling program which affects the anatomy and physiology of the animal. Molecular genetic analysis has identified a TGF-beta like (DAF-7) pathway and an insulin-like (DAF-2) pathway which are thought to couple sensory signals to the subsequent animal remodelling through transcriptional regulation of target genes. Notably, downregulation of DAF-7 and DAF-2 leads to activation of DAF-3 Smad protein and DAF-16 Forkhead transcription factor, respectively. In contrast to the detailed knowledge of the DAF-2 and DAF-7 signalling cascades, only a handful of DAF-3 and DAF-16 target genes are currently known. Using a candidate gene approach, we focused on the function and transcriptional regulation of *daf-9* which is genetically downstream of *daf-3* and *daf-16*.

We generated a *daf-9::gfp* fusion gene which rescues the strong loss of function *daf-9 (e1406)* mutation. Consistent with Gerisch et al, 2001 and Jia et al, 2002, we detected *daf-9::gfp* expression in a pair of inner labial (IL1) neurons in L1 larvae which persists in all larval stages and in adults, in the hypodermis from mid-L2 stage and in the spermatheca of adult animals. No dramatic change was observed in *daf-9::gfp* expression in animals where DAF-2 or DAF-7 signalling pathway was attenuated. In contrast, hypodermal *daf-9::gfp* is eliminated in *daf-12(lf)* mutant animals. Hence, DAF-12 appears to activate *daf-9* gene expression in the hypodermis and this suggests an auto-inhibition mechanism by which DAF-12 modulates its own activity by upregulating *daf-9*.

We investigated the site of action of DAF-9 by generating transgenes which direct *daf-9* expression in a tissue-specific manner. *daf-9 (e1406)* mutant animals can be fully rescued and reach adulthood by pan-neuronal expression of *daf-9* using the *unc-14* promoter or hypodermal expression of *daf-9* using the *dpy-7* promoter. This indicates that either neuronal or hypodermal *daf-9* expression is sufficient for reproductive development and that DAF-9 is likely to act in a cell non-autonomous manner. Furthermore, *unc-14promoter::daf-9::gfp* or *dpy-7promoter::daf-9::gfp* is able to suppress dauer arrest of *daf-7 (e1372)* mutant animals, reinforcing the notion that the hormonal signalling pathway of DAF-9 acts either downstream or in parallel to the DAF-7 pathway.

**226533. Regulation and function of DAF-9 cytochrome P450 in the dauer pathway**

**Ho Yi Mak**, Gary Ruvkun

Department of Molecular Biology, Massachusetts General Hospital and Department of Genetics, Harvard Medical School, Boston, MA 02114

The decision to proceed to dauer stage requires the integration of multiple sensory inputs such as food, pheromone and temperature. The sum of these inputs triggers the execution of a remodelling program which affects the anatomy and physiology of the animal. Molecular genetic analysis has identified a TGF-beta like (DAF-7) pathway and an insulin-like (DAF-2) pathway which are thought to couple sensory signals to the subsequent animal remodelling through transcriptional regulation of target genes. Notably, downregulation of DAF-7 and DAF-2 leads to activation of DAF-3 Smad protein and DAF-16 Forkhead transcription factor, respectively. In contrast to the detailed knowledge of the DAF-2 and DAF-7 signalling cascades, only a handful of DAF-3 and DAF-16 target genes are currently known. Using a candidate gene approach, we focused on the function and transcriptional regulation of *daf-9* which is genetically downstream of *daf-3* and *daf-16*.

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**881887. FUNCTIONAL GENOMIC ANALYSIS OF SIGNALING PATHWAYS INVOLVED IN C. ELEGANS DAUER FORMATION**

**Muneesh Tewari**<sup>1</sup>, Patrick Hu<sup>2</sup>, Svetlana Busiguina<sup>1</sup>, Sabrina Chaklos<sup>1</sup>, Jin Sook Ahn<sup>1</sup>, Gary Ruvkun<sup>2</sup>, Marc Vidal<sup>1</sup>

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Classical genetic analysis has identified about 25 genes involved in a *C. elegans* developmental pathway known as dauer formation. The gene products are homologous to components of human signaling pathways including the insulin signaling pathway and the transforming growth factor-beta signaling pathway. Despite vigorous genetic analysis, however, it is likely that many proteins that regulate dauer formation remain to be discovered. We are combining protein interaction mapping, large scale RNAi screening and classical genetics to identify new genes involved in dauer formation.

We have undertaken yeast two-hybrid-based protein interaction mapping using all of the known dauer open reading frames (ORFs) as baits. The dauer ORFs were cloned into a yeast two-hybrid bait vector by recombination-based cloning. Each bait was used to screen a yeast two-hybrid *C. elegans* cDNA library. The screens identified approximately fifty novel interacting proteins. These were subjected to in vivo analysis using RNA interference (RNAi). Preliminary RNAi experiments have demonstrated that several of the novel interactors play a role in dauer formation.



**623959. A *mod-5* SUPPRESSION SCREEN FOR GENES INVOLVED IN SEROTONERGIC NEUROTRANSMISSION**

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Wild-type animals that have been food deprived slow their locomotory rate upon encountering bacteria (the enhanced slowing response). *mod-5* mutants slow even more than wild-type animals (the hyperenhanced slowing response). *mod-5* mutants were originally identified as defective in serotonin (5-HT) reuptake, and the *mod-5* gene encodes a 5-HT reuptake transporter. To identify additional genes involved in 5-HT signaling, we designed a screen for suppressors of *mod-5*. The screen takes advantage of a second characteristic of *mod-5* mutants: hypersensitivity to exogenous 5-HT. When placed in M9 containing 5-HT, *mod-5* mutants become immobilized sooner than wild-type animals. Screening for animals that continue to move after *mod-5* mutants would have stopped allows us to identify suppressors of *mod-5*.

We previously screened 22,000 EMS-mutagenized haploid genomes and obtained 13 independent *mod-5(n3314)* suppressors. At least five of these suppressors also suppress the hyperenhanced slowing response exhibited by *mod-5(n3314)* mutants. All 13 mutants confer semidominant suppression of the 5-HT hypersensitivity of *mod-5(n3314)* animals. This semidominance and the variability of this suppression have made mapping difficult. We have employed two strategies to circumvent this problem. First, by further characterizing the phenotypes of *mod-5(n3314)* suppressors, we have identified other defects that they exhibit. If these defects are caused by the same mutation that suppresses the 5-HT hypersensitivity of *mod-5(n3314)*, then mapping experiments may be performed by following those defects. Second, we have begun a screen using the Mos1 transposon as a mutagen (Bessereau, J. L. et. al, (2001) *Nature*, 413: 70-74). To date, ~8,000 haploid genomes have been screened and three suppressors identified. One suppressor is an allele of *mod-1*, which encodes a 5-HT-gated Cl<sup>-</sup> channel. Mutations in *mod-1* were previously known to partially suppress *mod-5*. We will continue to screen Mos1-mutagenized animals to identify additional suppressors. We will also characterize the existing mutants and identify the genes disrupted by the Mos1 transposon.

The suppressors may define genes that act downstream of the synapses at which *mod-5* acts, i.e., genes with products that are involved in transducing the signal in postsynaptic neurons or muscle cells responsible for slowing the locomotory rate of the animal. We will focus on genes that mediate the enhanced slowing response and hope to define both the molecular signals that elicit this behavior as well as the neural circuit(s) through which these signals act.

**51849. Genetic Analysis of Abl in Regulating *C. elegans* Germ cell Apoptosis**

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The mammalian c-Abl exhibits structural conservation with that of the sea urchin, fruit fly and *C. elegans*. Although mammalian c-Abl elicits various biological responses including cell proliferation and apoptosis, the molecular mechanism(s) and the signaling events underlying its action remain largely unknown. We have recently characterized a deletion allele for *C. elegans abl-1*, *abl-1(ok171)*, and demonstrated that *abl-1(ok171)* displayed hypersensitivity to radiation-induced germ cell apoptosis mediated by the classical cell death pathway. To investigate involvement of cell cycle checkpoints in radiation-induced germ cell apoptosis, we constructed double mutants containing *abl-1(ok171)* with the *lf* alleles *hus-1(op241)*, *rad-5(mn159)* and *mrt-2(e2663)*, and irradiated using gamma rays from a <sup>137</sup>Cs source. Results from these studies indicate that all three check point genes were required for radiation-induced germ cell apoptosis in *abl-1(ok171)*.

*ena* was first identified as a genetic modifier of *abl* in *Drosophila*. *lf* mutations of *ena* suppressed *lf abl-1* mediated neuronal defects in a dose-dependent manner, indicating *ena* as a potential downstream effector of *abl*. *unc-34* is a *C. elegans* homolog of *Drosophila ena* and it is required for cell and neuronal growth cone migration. We have found that three mutants of the *C. elegans ena* homolog *unc-34* suppress the *abl-1(ok171)* phenotype. This result is consistent with the genetic evidence from *Drosophila* showing these proteins function in the same pathway, and identify *ena* as a new mediator of apoptosis.

### 916454. Regulation of Touch Cell Functional Genes

Ji Wu, Martin Chalfie

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We have previously shown that *egl-44* and *egl-46* are two transcription regulators involved in the development of several types of neurons in *C. elegans*, with significant homology in other organisms, and that they repress the expression of touch cell-specific features in FLP neurons (Wu et al., 2001, *Genes. Dev.*, 15: 789).

In this work, we looked at the molecular level into the mechanisms of the combinatorial control of touch cell specification. We found that EGL-44 directly bound to the promoters of touch cell-specific genes, such as *mec-4*, *mec-7*, *mec-17* and *mec-18* in gel shift assays, suggesting that these genes are direct targets of EGL-44. Consistent with this, like *mec-4::gfp* and *mec-7::gfp*, *mec-17::gfp* and *mec-18::gfp* were expressed ectopically in FLP cells in *egl-44* animals. With *mec-4* promoter, we also found that the DNA binding of EGL-44 was enhanced by EGL-46. The EGL-44 binding sites were near but different from those of UNC-86 and MEC-3, and the binding of these latter proteins was prevented by the presence of EGL-44 and EGL-46. When the EGL-44 binding site was mutagenized in *mec-4* promoter, EGL-44 no longer bound to the mutant promoters *in vitro*. Furthermore, these mutant *mec-4* promoters led to ectopic expression of *mec-4::yfp* in FLP neurons *in vivo*, mimicking the *Egl-44* phenotype.

We also found that EGL-44 and EGL-46 bound to each other in *in vitro* S-Tag pulldown assays. We examined the roles of different domains of EGL-44 and EGL-46. The first zinc finger of EGL-46 was essential and sufficient for this interaction, and interestingly, the other two zinc fingers somehow weakened its EGL-44 binding, suggesting some fine regulation in this protein-protein interaction. Neither the N-terminal half of EGL-44 (containing the TEA/ATTS domain) nor its C-terminal half (containing the putative transcription regulatory domain) alone showed binding activity to EGL-46, suggesting that the function of the TEA/ATTS domain is not limited to DNA binding. EGL-44 also bound to MEC-3 and UNC-86 *in vitro* with or without the *mec* gene promoters.

We propose that EGL-44 and EGL-46 repress the transcription of the touch cell functional genes by two mechanisms in FLP cells. First, they compete with UNC-86 and MEC-3 directly for DNA binding. Second, they interact with UNC-86 and MEC-3 physically and prevent their binding to the promoters, possibly through motif masking or conformational changes.

**606692. Evidence that the Heterochronic Gene *lin-58* is a MicroRNA Homologous to the *let-7* Small Temporal RNA**

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The heterochronic genes of *C. elegans* are global temporal regulators that control the relative timing and sequence of diverse events during post-embryonic development. One of these events is the terminal differentiation of the lateral hypodermal "seam" cells, an event that occurs during the final (L4) molt in wild-type animals. During the L4 molt, seam cells exit the cell cycle, fuse and synthesize a morphologically distinct adult cuticle. Mutations in heterochronic genes advance or retard the timing of hypodermal terminal differentiation, resulting in larvae with adult-type hypodermis or adults with larval-type hypodermis. Hypomorphic mutations of *lin-57*, as well as post-embryonic RNAi depletion, cause seam cell fusion and adult cuticle synthesis one stage early, during the L3 molt. Cloning of *lin-57* revealed that it is allelic to *hbl-1*, a gene previously identified by Fay et al. (1999 Developmental Biology 205: 240-253) based on its sequence homology to *Drosophila hunchback*. *lin-57::gfp* fusions are down-regulated during the L4 stage and this down-regulation is mediated by the *lin-57* 3'UTR. Examination of the *lin-57* 3'UTR reveals binding sites for multiple microRNAs, including *let-7*, and the temporal expression pattern of the *let-7* microRNA (late L3 through adult) is consistent with it playing a role in *lin-57* down-regulation. However, *let-7* is not absolutely required for *lin-57* inactivation as *lin-57::gfp* fusions are still down-regulated in a *let-7(null)* background. One possibility is that other gene products function redundantly with *let-7* to ensure proper *lin-57* expression.

The recent identification of a large family of microRNAs in *C. elegans* (Lau et al. 2001 Science 294:858-862, Lee and Ambros, 2001 Science 294:862-864) provides a possible explanation for these observations. Three potential *let-7* paralogs have been found (Lau et al. 2001 and these abstracts). All three have sequence similarity to *let-7* and are expressed with the same temporal specificity as *let-7*. Conceivably, some or all of these *let-7* homologues could participate in *lin-57* down-regulation. Examination of the *lin-57* 3'UTR suggests that it might serve as a landing pad for multiple miRNAs that act in concert to inactivate *lin-57* expression during the late L3-L4 stage. Mutations in these miRNAs are needed in order to understand their role in *lin-57* regulation and other developmental processes. Two *let-7* homologues are situated in tandem (~1.7 kb apart) on chromosome V in the interval where *lin-58*, a heterochronic gene identified as a *lin-4* suppressor, maps. *lin-58* has a weak precocious phenotype and interacts genetically with the heterochronic gene *lin-42*. To test whether one of these miRNAs is allelic to *lin-57*, we sequenced in the region of the microRNAs. Each *lin-58* allele contained a single, independent, point mutation in a palindromic sequence in the 1.7 kb region between these two miRNAs (~200 bp 5' to the downstream RNA). One possible scenario is that these mutations disrupt a repressor binding site such that one or both miRNAs are expressed prematurely, resulting in precocious inactivation of target genes. Consistent with this idea, one *lin-58* allele is weakly dominant. Northern blot analysis of RNA from synchronized populations of *lin-58* mutants is underway to further test this hypothesis. When expressed from an extrachromosomal array, these RNAs cause diverse developmental defects, including hypodermal abnormalities. Preliminary analysis indicates that transgenic arrays can be used to recapitulate the *lin-42* genetic interaction.

**584160. Confocal and whole-genome-microarray analysis of STAT ortholog deletion mutant in *C. elegans***

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STAT proteins are latent transcription factors activated by tyrosine phosphorylation in response to extracellular signals and have been identified in a variety of organisms, including a single orthologue in *C. elegans*.

We cloned the nematode ortholog of mammalian STAT and characterized its expression pattern. It encodes a protein of 82KD that contains a DNA binding domain, SH2 domain, and site for tyrosine phosphorylation conserved with its mammalian counterparts. Surprisingly, ceSTAT does not contain an N-terminal domain that is highly conserved among mammalian STATs as well as in *Drosophila* STAT. Using Tc1 transposon mediated reverse genetics, we isolated a ceSTAT mutant that has a 2.1kb deletion in the gene, resulting in complete loss of full length protein expression. The genetic background of the mutant was cleaned by 10 to 12 outcrossings to Bristol N2 strain.

Immunohistochemistry using affinity-purified rabbit antibody against the endogenous protein showed a staining pattern similar to the expression of a promoter-GFP transgenic line. Confocal microscopy revealed constitutive nuclear staining pattern in a few neuronal cells in the posterior head ganglia in wildtype N2 strain cultured under normal laboratory condition. This pattern suggests that ceSTAT is constitutively active in a subset of amphid neurons, that was lost in mutant worms.

Independent mRNA samples were prepared from synchronized young adult wildtype and ceSTAT null worms, growing under normal laboratory conditions. Genome wide gene expression patterns were compared using the Stanford DNA chip, which contains 17,871 genes, representing about 94% of the 18,967 genes currently annotated in the *C. elegans* genome. Data sets from six chips were analyzed. With  $p < 0.05$ , there are about 60 genes which are downregulated more than two fold in ceSTAT knockout worms compared to wildtype worms, whereas there are about 110 genes which are upregulated more than two fold in the same comparison. The fact that there are twice more upregulated as downregulated genes is surprising, since STATs are generally considered to transcription activator, not inhibitors. Further studies using *C. elegans* model system should provide more evidence on the role of STAT in gene regulation.

## 648701. Mechanosensory Channel Complex Interactions with the Extracellular Matrix

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*C. elegans* have six neurons dedicated to sensing gentle touch along the body. These neurons contain channels necessary for touch sensitivity that are proposed to be integral components of a mechanosensing apparatus. We have found that the mechanosensory channel complexes (MCCs) are associated with hemi-desmosome-like attachment structures that appear at one  $\mu$ m intervals along the length of the axon, and which couple the touch neuron to the cuticle through the hypodermis at sites underlying the annuli. The hypodermally-derived collagen encoded by *mec-5* was tagged with GFP and exhibits two patterns of binding to the touch neurons. On the neurons of the ventral cord, MEC-5::GFP binds in a punctate pattern in which the puncta are about a micron apart; along the lateral touch neurons, MEC-5::GFP appears as two parallel lines punctuated by points of higher intensity. This touch cell binding is abolished in *mec-1* mutants that fail to attach the touch cell processes to the cuticle. Many of the ventral cord puncta co-localize with both MEC-2, a member of the MCCs, and MH4, an antibody which stains trans-hypodermal attachment structures [1].

Although MCCs co-localize with touch cell attachment complexes, attachment, per se, is not necessary for touch sensitivity. The touch neurons of *him-4* mutants are not attached to the cuticle, yet they are touch sensitive [2]. HIM-4 localization on the lateral touch neurons is very similar to the parallel lines of MEC-5::GFP. In a *him-4* mutant background, the parallel lines of MEC-5::GFP on the lateral touch neurons disappear, leaving the points of higher intensity, which form a punctate pattern like the pattern of MEC-5::GFP binding to the ventral cord. The MEC-2 distribution remains normal in the attachment-defective *him-4* worms.

The extracellular matrix protein MEC-1 appears to be a part of a complex that is necessary both for touch neuron attachment and for the localization of MEC-5. The MEC-1 N-terminal half is sufficient to restore wild-type touch cell attachment and to mediate MEC5::GFP binding to the touch cell axon. The C-terminal half of MEC-1 is necessary but not sufficient for touch sensitivity and preliminary evidence suggests that it preferentially localizes to the sites of MCCs, even in attachment-defective mutants. This observation, along with the MEC-5::GFP binding pattern in *him-4* animals, together demonstrate that a touch cell-hypodermal interaction occurs at the presumptive attachment sites underlying the annuli independent of whether the complex at the attachment site has the mechanical strength necessary to hold the touch cell close to the cuticle.

Touch cell attachment complexes are morphologically very like the muscle dense bodies, which serve an analogous function in attaching the muscle to the cuticle via the hypodermis. The known hypodermal components of the touch cell and muscle attachment structures are identical. We would like to fully characterize the composition of the MCCs and are working on defining which dense body proteins are also present in the touch cell attachment complexes and therefore might be a part of the touch sensing complex.

1. Francis, R., and Waterston, R. H. (1991). *Journal of Cell Biology* 114, 465-479.

2. Vogel, B. E., and Hedgecock, E. M. (2001). *Development* 128, 883-894.

**266719. MEC-2 interacts with MEC-4 via stomatin-like region**

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The response to gentle body touch in *C. elegans* is mediated by a set of six mechanosensory neurons. On the basis of genetic interactions, electrophysiology, and molecular studies of the *mec* genes and their products, we have proposed that a mechanosensory complex converts mechanical stimuli into electrical signals. Two degenerins, MEC-4 and MEC-10, which produce amiloride-sensitive ionic currents in *Xenopus* oocytes, are believed to conduct the mechanosensitive currents in vivo. MEC-2, which interacts and regulates the MEC-4/10 channels in oocytes, forms a complex with them. Whole-mount immunohistochemistry using rabbit polyclonal antibodies against MEC-2 reveals that MEC-2 is localized to regular puncta along the entire length of the touch cell processes. Indirect immunofluorescence of MEC-2 is colocalized with full-length MEC-4::GFP, also suggesting that MEC-2 and the degenerins form a complex.

To study further the punctate pattern of MEC-2, we stained different *mec* mutants with the MEC-2 antibodies. Normally touch cells are distinguished by their bundle of 15-protofilament microtubules and their prominent extracellular matrix. *mec-7* and *mec-12*, which encode beta- and alpha-tubulins, respectively, are required for these microtubules; *mec-1* is needed for the extracellular matrix. The axonal distribution of MEC-2 is almost completely abolished in *mec-7* and *mec-12* mutants. Cell body staining of MEC-2, however, is noticeably elevated. Therefore, the touch cell-specific microtubules are involved in the axonal distribution of MEC-2. In contrast, the *mec-1*-dependent extracellular matrix is not required to localize MEC-2, since the MEC-2 punctate pattern is unchanged in *mec-1* mutants. MEC-4, MEC-10 and MEC-6, as well as MEC-2, are essential components of the mechanosensory channel complex. MEC-2 staining becomes diffuse in *mec-4*, *mec-10* and *mec-6* mutants, suggesting that the integrity of the channel complex is important for the localization of MEC-2. MEC-4::GFP expression, however, remains punctate in *mec-2* animals. Therefore, MEC-2 is not required for the localization of mechanosensory complexes.

MEC-2 interacts with the cytoplasmic N-terminus of MEC-4 in GST pull-down experiments. By deleting various regions, we find that the stomatin-like region of MEC-2 is needed for this binding. MEC-2 antibody staining becomes diffuse in alleles that have missense mutations in stomatin-like region but remains punctate in alleles that have missense mutations outside this region. Therefore, the stomatin-like region is required for MEC-2 to interact with the mechanosensory channel complexes in vivo.

**357282. Screens to identify longevity-control genes in *C. elegans***

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Lifespan in *C. elegans* is controlled by an insulin-like signaling pathway that includes DAF-2, an insulin-receptor like protein, and AGE-1, a homolog of the PI3K p110 catalytic subunit.

Reduction-of-function mutations in *daf-2* or *age-1* extend adult lifespan, while loss-of-function mutations result in constitutive dauer arrest. Signaling via the DAF-2/AGE-1 pathway antagonizes the DAF-16/forkhead transcription factor, which is required for increased longevity and dauer arrest in *daf-2* and *age-1* mutants. A working hypothesis is that, in animals lacking DAF-2 signaling, DAF-16 is active and directs the expression of target genes that confer long lifespan and control dauer arrest.

Why do animals lacking DAF-2/AGE-1 signaling live longer than wild-type? To answer this question, we are screening for mutations that alter lifespan in *daf-2* mutants. Because lifespan is a post-reproductive phenotype, we are conducting clonal screens for altered lifespan in the F2 generation after mutagenesis.

Two genes, *ctl-1* and *sod-3*, may confer increased longevity to *daf-2* mutants (1,2). Therefore, we expect to recover *ctl-1* and *sod-3* mutations in a screen for reduced lifespan in *daf-2(e1370)* animals. Two pilot screens revealed a high level of mid-lifespan lethality in *daf-2(e1370)* animals following mutagenesis. This suggests that many genes are required for increased adult longevity and not all will be specific DAF-16 targets. However, this approach will be useful for identifying mutations that enhance lifespan in *daf-2(e1370)* mutants. In addition, we are performing screens to identify genes that limit adult longevity when DAF-2/AGE-1 signaling is active.

1) Taub et al. 1999. 2) Honda, Y. and S. Honda, 1999.



**146395. Characterization of Genes Required for Fertilization in *C. elegans***

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The primary goal of our lab is to identify and characterize molecules involved specifically in the process of fertilization. Towards this end, we study mutants collectively referred to as the *spe-9*-class (named after the first member of this family to be cloned). *spe-9*-class mutants produce sperm that are morphologically and functionally indistinguishable from wild-type sperm, except that they are incapable of fertilizing oocytes. This suggests that these gene products are required specifically during the process of fertilization, rather than for some other aspect of sperm development. *spe-13*, *spe-19* and *spe-36* are three such *spe-9*-class genes that we are currently investigating.

We present our functional analysis of *spe-13* that confirms it to be in the *spe-9*-class. Two-point, three-point and SNP-mapping data localize *spe-13* to within 1 Mb of the left telomere of chromosome I. We will use a combination of YAC rescue and SNP-mapping to clone this gene.

Two-point, three-point, and deficiency mapping data have positioned *spe-19* to the right arm of chromosome V, approximately 11.5 map units from *dpy-21*. Further mapping is underway to narrow down the interval within which *spe-19* lies. Again, we will use a combination of SNP mapping and subsequent transgenic rescue via YAC injection to clone this gene.

While the functional analysis of *spe-36* is incomplete, the available data strongly suggests that it, too, is a *spe-9*-class mutant. We are in the preliminary stages of mapping this gene, and have roughly localized it to position +8 on chromosome IV using a combination of two- and three-point mapping.

**842211. Identification and Analysis of mutations affecting fertilization in *C. elegans*.**

**Indrani Chatterjee**, Emily Putiri, Pavan Kadandale, Andrew Singson  
Waksman Institute, Rutgers University, Piscataway, NJ

The nematode *Caenorhabditis elegans* provides a practical model system for identifying genes required by oocytes or sperm at fertilization. We are conducting a conditional egg sterile screen for mutations that define functions required by the egg. Additionally, we are analyzing a group of mutations required for sperm function during fertilization. These mutants produce morphologically normal sperm that cannot fertilize oocytes even after contact between gametes. These genes will encode functions required for sperm-egg recognition, adhesion, signaling or fusion. We will summarize our progress on the phenotypic and molecular analysis of these genes. The study of these genes will help us gain an understanding of fertilization in the worm and provide insight into fertilization in other organisms.

**248400. Composition and Dynamics of the *C. elegans* Early Embryonic Transcriptome**

**Ryan Baugh**<sup>1</sup>, Andrew Hill<sup>2</sup>, Donna Slonim<sup>2</sup>, Gene Brown<sup>2</sup>, Craig P. Hunter<sup>1</sup>

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Understanding the system-wide relationship of genotype and phenotype during animal development requires global identification and characterization of embryonic regulatory networks. To obtain temporal profiles of transcript abundance during embryonic development we have isolated RNA from precisely staged *C. elegans* embryos and then amplified this RNA in order to perform whole-genome microarray analysis. The result is a highly resolved time course that commences with the zygote and extends into mid-gastrulation, spanning the transition from maternal to embryonic control of development and including the presumptive specification of most major cell fates. Transcripts for approximately 6,000 genes are detected at any given time point and nearly half (8,890) of the predicted open reading frames are ever detected. The expression level for the majority of expressed genes (~70%) is temporally modulated. However, rates of synthesis and degradation are matched such that the transcriptome maintains a steady-state frequency distribution. Two complementary approaches are used to group genes by expression pattern: cluster analysis of similarly modulated genes and a classification scheme based on developmental genetic concepts. The resulting gene sets provide a versatile platform for bioinformatic analysis. In summary, these data comprise a comprehensive, quantitative description of mRNA levels during *C. elegans* early embryogenesis and a baseline for future perturbation experiments intended to delineate specific regulatory network architectures.

**302019. Preparation of Nematode, Bacteria, and Yeast Nucleic Acids and Proteins by Pressure Cycling Technology (PCT) Preparation of Nematode, Bacteria, and Yeast Nucleic Acids and Proteins by Pressure Cycling Technology (PCT) Preparation of Nematode, Bacteria, and Yeast Nucleic Acids and Proteins by Pressure Cycling Technology (PCT)**  
**Richard Schumacher**

Boston Biomedica, 375 West Street, West Bridgewater, MA

Poster Abstract for The C. elegans Conference, June 14, 2002

Title: Preparation of Nematode, Bacteria, and Yeast Nucleic Acids and Proteins by Pressure Cycling Technology (PCT)

Authors: Richard T. Schumacher, Feng Tao, Nathan P. Lawrence, Chunqin Li, Wayne W. Miller, and Mark M. Manak. Boston Biomedica, Inc., 375 West Street, West Bridgewater, MA 02379 The extraction of cellular components, such as nucleic acids and proteins from cells and tissues, is the critical first step in biological analysis and applications. This often requires the addition of chemicals or multiple processing steps. It is crucial that with any sample preparation system that the integrity of nucleic acids and proteins be preserved for downstream applications and analysis. Extraction of biomolecules from nematodes, yeast, and bacteria, as well as other organisms such as plants and animals, present particular problems, for current sample preparation protocols. To meet the challenges of sample preparation, Boston Biomedica, Inc. has developed an automated sample preparation system based on pressure cycling technology (PCT) for the release of nucleic acids and proteins from a wide variety of organisms. The system uses an instrument; the Barocycler™ 2017 programmed to generate alternating hydrostatic pressure pulses, and specially designed tubes (PULSE™ Tubes) to homogenize cells or tissue. The cellular components are released within minutes and can be further purified or used directly for numerous downstream applications. In this presentation we will be describing in detail the PCT Sample Preparation System and presenting data showing the release of DNA, RNA, and proteins from nematodes, yeast and bacteria and other organism. We will also be showing data using the released biomolecules in downstream applications. METHODS: The PCT Sample Preparation System includes an instrument, the Barocycler™ NEP2017, and specially designed PULSE™ Tubes. PULSE Tubes are used to store and process samples, such as cultured cells and tissue. Specialized lysis buffers, or simple buffered solutions (such as TE or PBS) are placed in the PULSE Tubes and mixed with the sample during the PCT extraction process. The Barocycler NEP2017 can be programmed to expose samples to multiple pressure cycles between atmospheric pressure and 35,000 psi, or 235 MPa. The PCT homogenized lysate can then be either withdrawn for further downstream processing, such as nucleic acid purification and amplification, or stored in the PULSE Tube for future use. RESULTS: Diverse sample types including 'hard-to-lyse' nematodes, bacteria, and yeast were processed by PCT. Efficient release of nucleic acids and protein from these samples was achieved. The quantity and quality of the PCT extracts were comparable or superior to those obtained by conventional methods, such as mortar and pestle for tissues or sonication for cultured cells. DNA, RNA and protein from samples prepared by PCT exhibited excellent quality and yields, and were suitable for a variety of downstream processes. The PCT Sample Preparation System offers simple-to-use, rapid and effective processing of a wide range of specimens, and should have broad utility in clinical, agricultural, pharmaceutical, environmental and forensic applications.

**551689. APH-1 and PEN-2 are Required for Notch Pathway Signaling, gamma-Secretase Cleavage of betaAPP, and Presenilin Protein Accumulation**

Ross Francis<sup>1</sup>, **Garth McGrath**<sup>1</sup>, Jianhuan Zhang<sup>1</sup>, David A. Ruddy<sup>1</sup>, Mary Sym<sup>1</sup>, Javier Apfeld<sup>1</sup>, Monique Nicoll<sup>1</sup>, Mark Maxwell<sup>1</sup>, Bing Hai<sup>1</sup>, Michael C. Ellis<sup>1</sup>, Annette L. Parks<sup>1</sup>, Wei Xu<sup>1</sup>, Jinhe Li<sup>2</sup>, Mark Gurney<sup>2</sup>, Richard L. Myers<sup>2</sup>, Carol S. Himes<sup>2</sup>, Ronald Hieschsch<sup>2</sup>, Cara Ruble<sup>2</sup>, Jeffrey S. Nye<sup>2</sup>, Daniel Curtis<sup>1</sup>

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Presenilins are components of the gamma-secretase protein complex that mediates intramembranous cleavage of betaAPP and Notch proteins. A *C. elegans* genetic enhancer screen revealed two genes, *aph-1* and *pen-2*, encoding conserved multipass transmembrane proteins, that share several properties. Both genes exhibit strong genetic interactions with *sel-12*/presenilin and *aph-2*/nicastrin, share the same subset of *glp-1/lin-12* pathway phenotypes, and act at or upstream of LIN-12 S3 cleavage. Conserved human APH-1 and PEN-2 proteins are able to partially rescue the *C. elegans* mutant phenotypes, but only when the two are provided together. Rescue is enhanced when human presenilin 1 is also provided, suggesting that APH-1 and PEN-2 cooperate functionally with one another and with presenilin. Using cultured *Drosophila* cells, we show that RNAi-mediated inactivation of fly *aph-1*, *pen-2*, or nicastrin strongly reduces gamma-secretase cleavage of betaAPP and Notch substrates while also dramatically reducing the accumulation of processed presenilin. These data indicate that APH-1 and PEN-2, like nicastrin, are required for the activity and accumulation of the gamma-secretase complex.

**657837. SERPIN FUNCTION IN *C. ELEGANS*: A GENETIC AND BIOCHEMICAL ANALYSIS**

**Stephen C. Pak**, Cliff J. Luke, Yuko S. Askew, David J. Askew, David R. Mills, Christopher Tsu, Vasantha Kumar, Sule Cataltepe, Gary A. Silverman

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To better understand the biology of ov-serpins, we undertook a comparative genomics study using *C. elegans* as a model organism. A screen of the *C. elegans* database (ACeDB) using the human serpin, SCCA2, amino acid sequence identified the presence of 9-10 serpin genes (*srp-1-10*). These genes are arranged in tandem and are distributed throughout the length of chromosome V. By a combination of cDNA cloning and RT-PCR, cDNAs corresponding to 6 of these genes were isolated. Like the human ov-serpins, no typical N-terminal signal peptides were found suggesting that the worm serpins may also reside intracellularly. Each serpin contains different residues in the reactive site loop suggesting that they have distinct proteinase targets. Interestingly and reminiscent of the *Manduca sexta* serpin gene-1, *srp-7* was found to have 3 distinct reactive site loops resulting from alternative splicing of exon 5. *In vitro* kinetic analysis, using recombinant proteins expressed in *E. coli*, showed that *srp-2*, *srp-3* and *srp-6* are inhibitors ( $k_{ass-1} \times 10^4 \text{ M}^{-1} \text{ sec}^{-1}$ ) of granzyme B, chymotrypsin-like serine proteinases, and papain-like cysteine proteinases, respectively. Expression studies using promoter::GFP-fusions indicate that serpins are present in the hypoderm, intestine, neurons and excretory glands. Null deletion mutants of *srp-1*, *srp-2*, *srp-6*, *srp-7(a-c)* and *srp-8* have been isolated and initial characterizations suggest that serpins play important roles in osmoregulation and host defense against bacterial infection.

**471013. Bioinformatic analysis of the leucine-rich repeat protein family in *Caenorhabditis elegans***

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Leucine-rich repeats (LRRs) are short, amino-terminal sequence motifs that characterize a superfamily of proteins. Although the individual members of this protein family vary in function and cellular localization, most seem to be involved in protein-protein interactions. *Caenorhabditis elegans* possess 23 genes that appear to encode proteins containing an extracellular amino-terminal LRR domain. Some of these are apparent orthologs of molecules implicated in activities such as neuronal development. However, the functional significance and expression patterns for most of the predicted LRR gene products have not yet been determined. The simple multicellular architecture of the *C. elegans* nematode will provide an ideal context in which to designate roles for the putative LRR gene products and, additionally, to characterize the apparent orthologs more completely.