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10534. Release of the Major Sperm Protein from Spermatids and Spermatozoa in vivo

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Oocyte meiotic maturation is an essential biological process required to prepare the oocyte for fertilization and embryonic development. During meiotic maturation, *C. elegans* oocytes undergo nuclear envelope breakdown, cortical rearrangement, and meiotic spindle assembly in an assembly line-like manner such that these events are spatially restricted to the most proximal (-1) oocyte, which is ovulated and fertilized. Recently, we found that the major sperm protein (MSP) signals oocyte meiotic maturation and gonadal sheath cell contraction (*1*). Previous studies have shown that MSP functions as the main cytoskeletal element of amoeboid nematode spermatozoa (*2*). Thus, the discovery of MSP's signaling role raised the question of how sperm release a cytoskeletal protein to signal oocytes and sheath cells at a distance. Previously, we found that spermatids release a small fraction of their MSP upon incubation *in vitro* and this release is not due to lysis (*1*). MSP release from sperm might occur through a novel mechanism because MSP lacks a hydrophobic leader sequence and many standard secretory components, such as ribosomes, endoplasm reticulum, and Golgi.

To better understand MSP signaling mechanisms, we asked if we could detect MSP release *in vivo*. We stained mated and unmated *fog-2(q71*) females with anti-peptide antibodies generated to the N- and C- terminal regions of MSP, as well as previously described antibodies (*3*). Unmated females do not stain with these antibodies. In mated females, we observe extracellular MSP localized in a gradient fashion, with the highest levels of MSP within the spermatheca and lower levels in the proximal gonad arm, in addition to intracellular staining within spermatozoa. This gradient is observed to extend up to 30-90 μm from the position of sperm in the spermatheca. A time course of MSP staining in wild-type hermphrodites and the spermiogenesis-defective *spe-8* mutant also suggests that MSP is released from non-motile spermatids. To address the specificity of MSP release, we stained mated females with the sperm-specific antibody 1CB4 (kindly provided by S. L'Hernault), which detects components of the fibrous body-membranous organelle. We found that 1CB4 staining is largely restricted to spermatozoa in the mated females. In addition, we labeled sperm mitichondria using Mitotracker, and found that active mitochondria are not released from spermatozoa, as expected. These results suggest that non-motile spermatids and motile spermatozoa possess the ability to release a small fraction of their MSP to signal oocytes and sheath cells We are currently conducting immunoEM studies to examine MSP release at an ultrastructural level.

Previous results suggested that MSP is a dose-dependent signal for meiotic maturation (*1*). We analyzed the time course of meiotic maturation and MAPK activation in hermaphrodites and correlated these responses with the levels of extracellular MSP detected. In addition, we examined histone H3 phosphorylation, which depends on AIR-2 kinase function (*4*). We found that histone H3 phosphorylation also depends on the presence of sperm in the gonad and that *in utero* MSP injection results in histone H3 phosphorylation in oocytes from *fog-2(q71)* females. When sperm levels are high (>~75 per spermatheca), oocytes up to the -3 position exhibit activated MAPK and histone H3 phosphorylation and oocyte maturation rates are high (~1.5-2.5 maturations per gonad arm per hr). As sperm are depleted and extracellular MSP levels decline, so does the number of responding oocytes in the gonad arm and the maturation rate. We propose that MSP directly signals the -3 and -2 oocytes, in addition to the -1 oocyte, to promote responses required for cell cycle progression in order to achieve maximal maturation rates.

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12473. A New C. elegans Mutant Affecting Pharyngeal Morphogenesis

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A New C. elegans Mutant Affecting Pharyngeal Morphogenesis.

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Organ formation requires the production of appropriate cells and their organization into a functioning structure. The C. elegans pharynx is a neuromuscular organ consisting of five different cell types, including muscles, neurons, epithelial cells, gland cells, and marginal cells. It initially forms during mid-embryogenesis as a primordium of undifferentiated cells that subsequently undergoes differentiation and dramatic shape changes. The result is a bilobed structure consisting of morphologically distinct subsections called the procorpus, metacorpus, isthmus, and terminal bulb. We have identified a spontaneous maternal effect lethal mutant, cu8, with defective pharyngeal morphogenesis. Approximately 95% of self progeny from cu8 homozygous hermaphrodites arrest as late stage embryos, and many of these animals exhibit defects in pharyngeal morphogenesis. The pharyngeal primordium appears normal until the 2-fold stage of embryogenesis, however it fails to undergo the final shape changes to form the mature bilobed organ. Many of theses embryos also exhibit defects in body elongation. The 5% of animals that hatch can grow to fertile adults, but they exhibit short, thick pharyngeal isthmuses. The cu8 maternal effect lethal phenotype is paternally rescued indicating maternal or zygotic expression is sufficient for viability. cu8 is unlikely to be a null allele because it is weakly temperature sensitive and it exhibits partially penetrant zygotic lethality in trans to a deficiency. Recombinational and deficiency mapping have localized cu8 between zyg-9 and rol-6 on LG II and it complements existing partial mel mutations in this region. We have rescued cu8 by transformation with the cosmid D2085 and are currently attempting to identify the locus affected by cu8 by transformation with D2085 fragments and RNAi.

47229. Identification and Comparison of *C. briggsae* and *C. remanei* homologs of *C. elegans daz-1*

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DAZ family proteins play important roles in meiosis and gametogenesis, and all family members carry of a well-conserved RRM (RNA Recognition Motif) in their N-terminus followed by a highly diverged C-terminal tract. We have previously characterized the *C. elegans daz-1* gene, which belongs to the Boule subtype of this family. The *daz-1* gene is necessary for oogenesis but not for spermatogenesis. Immunohistochemical analysis has shown that the DAZ-1 protein is mainly localized in the mitotic and early meiotic regions in hermaphrodite gonads.

Here we report an initial characterization of the *daz-1* homologs in two nematode species closely related to *C. elegans*, namely *C. briggsae* and *C. remanei*. We isolated a full-length *C. briggsae daz-1* (*Cb-daz-1*) cDNA, based on the EST sequence reported by P. Kuwabara, using the RACE PCR technique. A full-length *C. remanei daz-1* (*Cr-daz-1*) cDNA was also isolated by RACE, using degenerate PCR primers prepared according to a sequence alignment of *Ce-daz-1* and *Cb-daz-1*. The deduced amino acid sequences indicated that the N-terminal RRM region was highly conserved among the three species (79% identity), implicating a possible conservation of their target RNA molecules. However, the C-terminal region following the RRM was diverged among them. Ce-DAZ-1 (499 a.a.) had a long C-terminus (349 a.a.), which was unique compared to the DAZ family proteins in other species. Cr-DAZ-1 (452 a.a.) also had a long C-terminus (297 a.a.), but fewer amino acid residue matched in this region than in the RRM region. Cb-DAZ-1 was the smallest among the three (224 a.a.), with only 61 amino acid residues in the C-terminal region.

Possible function of *Cb-daz-1* and *Cr-daz-1* was investigated by RNAi. Interestingly, RNAi of *Cb-daz-1* in *C. briggsae* caused a Mog phenotype in F1 hermaphrodites. In mature P0 animals, *Cb-daz-1* RNAi "switched off" oogenesis that had already started, and induced re-initiation of spermatogenesis. In contrast, trials to block *Cr-daz-1* function by RNAi in *C. remanei* have generated no obvious phenotype so far, the reason for which is under investigation. Comparison of the structure and function of the three nematode DAZ-1 proteins will be important in elucidation of the molecular function of the DAZ family proteins.

80592. The Role of Dynactin Complex in Spindle Alignment in *C elegans* Early Embryo

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Positioning of the mitotic spindle within dividing polarized cells is crucial for the proper inheritance of segregated cytoplasmic determinants to daughter cells. The orientation of the spindle determines the cell division plane. The cleavage furrow bisects the spindle during telophase, partitioning localized components to specific daughter cells. In C. elegans, the nucleus-centrosome complex rotates in germline lineage to ensue the proper segregation of P granules. Studies by Skop and White (Curr Biol. 1998), Gonczy et al. (J Cell Biol. 1999) have shown that knockout of dynein and dynactin function by RNAi abolishes spindle rotation in both P0 and P1 cells. Immunoflorescent staining with p150glued, the largest subunit of dynactin, revealed the accumulation of dynactin to the cell division remnant. It is hypothesized that this cortex spot may serve to tether the plus-ends of astral microtubules from one spindle pole and rotate the mitotic spindle towards this spot.

In order to study in more detail the dynamic distribution of dynactin in C. elegans early embryo, stable transgenic GFP fusion lines with p150 glued, dynamitin and arp-1 were constructed. Two-photon microscopy showed that these three dynactin components co-localized to meiotic and mitotic spindles, centrosomes, nuclear envelope and adjacent membranes between cells. Arp-1, which is also called centractin, has a prominent localization to centrosome compared to the other two components. Although the accumulation at the cell division remnant is not quite obvious in the living cell images, antibody staining of GFP in these transgenic lines confirmed its presence. We are now working on improving the sensitivity of two-photon imaging.

Mutants that have altered spindle alignment in either AB, P1 or both, such as par-3, par-2, gpb-1, ooc-3, ooc-5 and let-99 are being tested to see whether or not the dynactin complex localizes to cortical sites in cells that rotate their spindle and are missing in those that do not. In par-3 RNAi embryos, where spindles in both AB and P1 rotate, dynactin accumulates at the cell division remnant. While the accumulation of dynactin at the cell division remnant is not present in par-2 RNAi embryos, where neither the AB nor the P1 spindle rotates. These results indicate that accumulation of dynactin to the cortex is either required for or is the consequence of spindle rotation. We are now looking at dynactin localization in other mutants and have initiated a yeast-two hybrid screen to check for possible interactions between dynactin and these proteins.

102296. A Role for Inositol 1, 4, 5-Triphosphate Receptor During Epithelial Migration

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During embryogenesis, epithelial cells migrate and change shape in order to generate three dimensional structure. During the process of ventral enclosure in *C. elegans* , epithelial cells that originate on the dorsal surface of the embryo migrate to the ventral midline, forming adhesive junctions. *jc5* is a cold sensitive maternal mutant that displays defects during varying stages of embryogenesis, including ventral enclosure. 20% of embryos arrest because migration of epithelial cells from the dorsal side never occurs. Approximately 20% die during enclosure. In these embryos, the epithelial sheet initiates migration towards the ventral midline, fails to seal, and eventually retracts back to the dorsal surface. Others display partial enclosure phenotypes. Approximately 40% of dead embryos enclose, but develop severe body shape defects during elongation.

Using RNA interference, we phenocopied the *jc5* phenotypes with RNA corresponding to the inositol 1, 4, 5-triphosphate receptor-1 gene, *itr-1*. We have obtained rescue of the *jc5* phenotype by injection of genomic *itr-1* DNA. In addition, *jc5* fails to complement another loss of function allele of *itr-1*, *n2559*.

It is known that ITRs control intracellular calcium levels, and a recent paper has shown that calcium regulates actin in growth cone turning in another system (Gallo et al, Current Biology 9, 490). We hypothesize that ITR-1 regulates cytoskeletal rearrangement during ventral enclosure. Consistent with this model, preliminary data using alpha-catenin-GFP suggest that in some cases filopodia are misdirected, and we are continuing to study filopodial dynamics using an epithelial specific cytoplasmic GFP. Moreover, phalloidin staining suggests that actin is disorganized in epithelial cells that have halted migration in mutants. In order to show a specific role for calcium during ventral enclosure, we have phenocopied *jc5 (itr-1)* in N2 using two drug treatments: BAPTA-AM to chelate calcium during ventral enclosure, and, xestospongin in order to inhibit ITR-1. We are currently addressing if ITR-1 is exclusively needed in epithelial cells during their migration by constructing a tissue specific system to drive an IP3 sponge (Walker et al, Mol. Biol. Cell 13, 1329).

110676. The *C elegans* hook protein, ZYG-12, mediates an essential association between the centrosome and nucleus

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The centrosome and nucleus are intimately associated during interphase in a variety of organisms and cell types, yet the role of the association in cell division and development, as well as the molecular mechanism of the association, are unknown. Mutations in *zyg-12* specifically affect the association of centrosomes and nuclei. Analysis of the mutant phenotype demonstrates for the first time that this association is essential for normal mitosis, pronuclear migration and nuclear anchoring. Molecular characterization of *zyg-12* reveals that it encodes multiple isoforms of hook-like proteins. Hook proteins have been shown to mediate the interaction of membraneous organelles such as the Golgi with the microtubule cytoskeleton. We observe that functional GFP::ZYG-12 isoforms localize to both nuclei and centrosomes, depending on whether they include a transmembrane domain. Two-hybrid analysis indicates that ZYG-12 can dimerize through a coiled-coil motif. We propose that ZYG-12 connects nuclei with centrosomes through dimerization of ZYG-12 isoforms that independently localize to the nuclear envelope and centrosome.

140746. *daf-28* encodes an insulin-like ligand and can mutate to affect dauer formation and longevity in *C. elegans*

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Dauer formation in *C. elegans* is controlled by the *daf-2* (insulin receptor-like) signal, in conjunction with the *daf-7* (TGF-beta-like) and the *daf-11* (guanylyl cyclase) signals. Although multiple components of the DAF-2/IR pathway have been identified in various Daf-c (dauer-formation constitutive) and Daf-c suppressor screens using *daf-2* and *age-1* (PI3 kinase) mutants, no loss-of-function *(lf)* mutation has been found in an insulin-like molecule, a presumed ligand for DAF-2/IR. Failure to isolate such a *lf* mutation may be attributed to the presence of 37 *ins* (insulin-like) genes in the *C. elegans* genome (Pierce *et al*., 2001), some of which may serve redundant functions in dauer formation. We have determined that the Daf-c gene *daf-28,* defined by the dominant-negative mutation *sa191,* encodes an insulin-like molecule. Given that *daf-28(sa191)* also shares additional phenotypic features with mutants of *daf-2* and other components in the insulin-like pathway, such as extended life span and interaction with *daf-16* (fork head transcription factor) (Malone and Thomas, 1993; Malone *et al.,* 1996), we propose that DAF-28 exerts its function as a ligand of DAF-2/IR.

We cloned *daf-28* by SNP mapping in conjunction with traditional 3-factor mapping. We have also found that high-copy transgenes of *daf-28* can effectively suppress the Daf-c phenotype associated with *daf-28(sa191).* This is consistent with previous genetic evidence showing that *daf-28 (sa191)* was a semi-dominant allele and that its product may poison the process in which wild-type DAF-28 functions (Malone and Thomas, 1993). Since increasing the dose of the wild-type DAF-28 reduces the Daf-c effect resulted from the mutant product, we predict that the wild-type function of DAF-28 is to inhibit dauer arrest.

This hypothesis is further supported by evidence on how *daf-28* expression is regulated. A *daf-28::gfp* transcriptional fusion is expressed in two pairs of sensory neurons and the hind gut from late L1 to adult. The sensory neurons are ASI and ASJ, which have previously been shown to be important for regulation of dauer formation. *daf-28::gfp* expression in the sensory neurons is down-regulated in natural dauers, pheromone induced dauers and *daf-11(lf)* dauers. These results suggest that in response to environmental cues and the *daf-11* signal, *daf-28* functions in sensory neurons to inhibit dauer arrest.

*daf-28(sa191)*is an arginine to cysteine alteration at position 37. One possibility is that creation of an extra cysteine in DAF-28 results in the formation of inappropriate inter-molecular disulfide bonds that cause improper folding and association of DAF-28 with other insulins. Given the possible redundant function among the *ins* genes, it makes sense that it would take such a dominant-negative mutation to reveal functional involvement of insulins in dauer formation. Consistent with redundancy, transgenes encoding the wild-type INS-4 protein, a close relative of DAF-28, can also suppress the Daf-c phenotype associated with *daf-28(sa191)*.

161188. Regulation of the different chromatin states of autosomes and X chromosomes in the germ line of C. elegans.

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Recent observations support the hypothesis that in the *C. elegans* germ line, the X chromosomes differ in chromatin state from the autosomes and are globally repressed during most of germline development. Specifically, microarray analysis of genes whose expression is enriched in the germ line revealed that genes involved in germline proliferation, meiosis, and spermatogenesis are virtually absent from the X chromosome (1). Also, histone modifications correlated with active gene expression are present on the autosomes but not detected on the Xs in the germ line (2,3).

The Maternal-Effect Sterile (MES) proteins in *C. elegans* appear to be involved in regulation of chromatin structure in the germ line, at least in part by affecting histone modification. Mutation of any of the four *mes* genes (*mes-2*, *mes-3*, *mes-4* and *mes-6*) results in germline degeneration and sterility in the XX progeny of *mes/mes* mothers; XO progeny are less severely affected and are usually fertile. MES-4, a SET domain protein, binds specifically to autosomes but not detectably to the X chromosomes. The Polycomb group proteins, MES-2 and MES-6, and the novel protein MES-3, participate in regulating the chromatin state of the X in the germ line; in *mes-2*, *mes-3*, or *mes-6* mutants, the X chromosomes acquire autosomal features in a generation-specific fashion. In the absence of zygotically-synthesized MES-2, MES-3, or MES-6, but with maternally-provided product ($M^{+}Z^{-}$ *mes* mutants), MES-4 spreads onto the X chromosomes beginning in late pachytene. In the absence of maternal MES-2, MES-3, or MES-6 (M^{-} *mes* mutants, which have moderately to severely degenerated germ lines), histone modifications correlated with active gene expression spread onto the X chromosomes. These findings strengthen the view that the X chromosomes are silenced in the germ line in wild type, and predict that the MES proteins participate in this silencing.

MES-4 shows a dynamic distribution in the germ line: abundant in the distal, mitotic region, diminishing to barely detectable in the transition zone through mid-pachytene, and then up-regulated in later pachytene and in oocytes. Although, as stated above, MES-4 is aberrantly on the Xs in $M^{+}Z^{-}$ *mes* mutants in late pachytene through diakinesis, it is off the Xs in the distal germ line. This normal distal distribution may result from epigenetic control of chromatin organization by maternally-provided *mes-2*, *mes-3*, and *mes-6* products. Current studies with leaky and TS alleles of *mes* genes may shed light on when during germ line development these *mes* products are required to properly model chromatin.

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166201. Characterization of *odd-skipped homologs in worms*

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The *odd-skipped (odd) gene of Drosophila was the first identified member of a small subfamily of C2H2 zinc finger genes. By characterizing the developmental functions of various odd homologues, we hope to understand the evolution of this family of transcription factors. In Drosophila, odd and its paralogs show divergent roles during embryonic pattern formation: odd has a genetically-unique role as a pair-rule segmentation gene and is also expressed in numerous tissues during organogenesis, both odd and the very closely-linked gene sob are expressed in a segment-polarity like pattern, and a third linked paralog (bowel) is regulated by the terminal hierarchy and plays a key role in gut development. The worm genome sequence includes two homologues, which, unlike the fly genes, are not linked. As with the Drosophila paralogs, sequence homology is limited to the zinc finger regions, and potential orthologous relationships are not apparent from sequence data. Results of our investigation into the functions and expression of these worm genes will be presented.*

176386. LIN-44 misexpresion confers defects in the development of the vulval secondary lineage.

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The Wnt pathway is a conserved signaling pathway that regulates many developmental processes in animals. Our research group is investigating the possibility that the Wnt pathway regulates the development of the *C. elegans* vulva. The vulva develops from precursors that adopt either a primary or secondary fate. These precursors then divide to produce descendants that differentiate into one of seven distinct vulval cell types. Several lines of evidence suggest that the Wnt signaling pathway could regulate the cell type decisions made by these descendants. First, mutants in the gene *lin-17*, which encodes a putative Wnt receptor, display possible cell type transformations among the descendants of the vulval precursor P7.p. Second, these descendant cells express POP-1, a transcription factor that functions in the Wnt signaling pathway, in a manner that correlates with their cell type (Hill *et al*., I.W.M., 1999).

We have been using a genetic, gain-of-function approach to determine if any of the *C. elegans* Wnt signaling molecules are capable of regulating the cell type decisions made by these descendant cells. Transgenes have been produced that should express *C. elegans* Wnt signals under the control of the inducible *hsp16-2* or *hsp16-41* promoters. We observe that expression of high levels of the Wnt signal LIN-44 during early vulval development results in defects in vulval anatomy during the mid-L4 stage. By examining the pattern of cell fusions that occur among the vulval cells, we have obtained preliminary evidence that at least some of these anatomical defects are due to cell fate transformations among the descendants of P5.p and P7.p (the approach of using cell fusions to detect possible cell fate transformations is described in an abstract by Browning and Hill, this meeting). An alternative interpretation of these results is that ectopic expression of LIN-44 alters the cell fusion behavior of the vulval cells. We intend to examine the expression of cell-type specific markers in the LIN-44 transgenic animals to help distinguish between these two interpretations.

177767. GLD-2 is a novel cytoplasmic poly(A) polymerase

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The *gld-2*gene was originally identified as critical for progression through meiosis and, together with *gld-1,*for commitment to the meiotic cell cycle (Kadyk and Kimble, 1998). We have cloned *gld-2*, and find that the GLD-2 protein is a member of the DNA polβsuperfamily of nucleotidyl transferases (Aravind and Koonin, 1999), which includes classical eukarotic PAPs. GLD-2 protein is predominantly cytoplasmic in the germ line and co-localizes with P granules in early embryos. Two predicted RNA binding proteins interact with GLD-2: GLD-3 (see abstract by C. Eckmann et al.) and GIP-1. *In vitro*, GLD-2 has little PAP activity on its own but has readily detectable PAP activity when associated with GLD-3. Mutational analyses suggest that the GLD-2 PAP activity requires both its nucleotidyltransferase active site and its interaction with GLD-3. Therefore, GLD-2 is an unconventional cytoplasmic PAP that appears to act as a heterodimer. We speculate that GLD-2 PAP activity is targeted to specific mRNAs via its interaction with GLD-3, and perhaps with other RNA binding proteins.

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Aravind, L. & Koonin, E. V. (1999) *Nucleic Acids Res* **27,** 1609-18.

180510. CHARACTERIZING AND CLONING *pat-12*, AN ATYPICAL Pat GENE THAT MAY BE REQUIRED FOR MUSCLE ATTACHMENT

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To effectively locomote, *C. elegans* needs to transduce muscle contraction through the hypodermis to the cuticle. To date, many mutants having a breakdown of the adapters in this chain have been identified and have provided insight into the formation and attachment of muscles during development. Our lab is studying a class of these mutants that display the Pat phenotype (**P**aralyzed and **A**rrested elongation at **T**wo-fold). One mild Pat mutant, *pat-12*, appears to assemble functional body wall muscles that subsequently detach from the hypodermis.

Previous three-factor cross analysis placed *pat-12* on linkage group *III* between *unc-45* and *daf-7*. To narrow this area, we examined RNAi screen data posted on Wormbase corresponding to this region. In experiments done by Pierre Gonczy in the Hyman lab, the RNAi from one predicted gene, T17H7.4 gave 100% embryonic lethal phenotype. We duplicated this experiment and found that 96.2% of the progeny displayed the Pat phenotype. To obtain direct evidence that T17H7.4 corresponds to *pat-12*, we performed a complete DNA sequence analysis of the entire predicted coding region isolated by PCR from a *pat-12(st430)* homozygote. We discovered only a single base pair change relative to wildtype (G to A) in the exon 10 splice acceptor site. If exon 10 is skipped due to this change, the resulting mutant transcripts will be frameshifted at this point and a premature stop codon will be introduced just downstream. To conclusively prove that T17H7.4 is *pat-12*, we are applying standard transformation rescue techniques.

The predicted gene T17H7.4 is large (~20 Kb) and complex with as many as 23 predicted exons and 5 proposed splice isoforms. Blast searches of the sequence of T17H7.4 have identified interesting homologies to both a hypodermal protein found in the parasitic nematode *Onchocerca volvulus* (Taylor et al.) and human adenomatous polyposis coli (APC) tumor suppressor gene.

We generated an antibody to a region of the proposed T17H7.4 protein common to many of the predicted splice isoforms and used it to stain wild-type worms. Our preliminary results reveal a hypodermal circumferential ring-like banding pattern restricted to an area over the body-wall muscles similar to that reported by the Bucher lab for the MUP-4 protein and are consistent with the *pat-12* phenotype.

189833. Analysis of a Proposed Cell Binding Site on *C. elegans* Collagen IV.

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Type IV collagen, one of the most abundant extracellular matrix proteins, provides structural support, developmental cues, and contains cell binding sites (CBS) for cellular receptors. Most collagen IV molecules are composed of a heterotrimer formed by two alpha 1 chains (*emb-9*) and one alpha 2 chain (*let-2*), with CBS domains in the triple helix region. The human collagen IV molecule contains a CBS composed of aspartate (D) on each alpha 1 chain and arginine (R) on the alpha 2 chain. These form a three-dimensional D-R-D sequence that is recognized by the cell surface receptor, integrin alpha 1 beta 1. Amino acid sequence alignment has revealed that the D-R-D sequence is conserved on the collagen IV molecule of *C. elegans*. This proposed CBS sequence is being analyzed to determine its function. Previous data suggest this site has potential importance during *C. elegans* development.

Transgenic techniques have been used to produce worm strains carrying a mutation Asp(480) to Glu on the alpha 1 chain in the putative D-R-D site. Control strains were also produced using a control mutation of Asp(472) to Glu on the alpha 1 chain in a Gly-X-Y repeat upstream of the proposed CBS (Asp480). The transgenic strains were produced in an *emb-9* null background with the alpha 1 gene CBS and control mutations in combination with marker genes *rol-6(su1006)* and a GFP marker construct that localizes specifically to the muscle plasma membrane (pPD122.36, Andy Fire). The Asp(480)-Glu mutation does not rescue homozygous null animals. These transgenic animals arrest during embryogenesis as do the *emb-9* null mutants with both showing degeneration of muscle structure. The control mutation rescues the *emb-9* null homozygote. These transgenic mutants, both rescued and non-rescued, identified by the muscle plasma membrane specific localization of GFP have allowed further characterization of the Asp(480) mutation in the putative D-R-D sequence on the functioning of the collagen IV molecule. Immunohistochemical techniques are being used with antibodies to GFP protein, the collagen IV alpha 2 chain, and myosin heavy chain to characterize the physical defects of body wall muscle structure and function exhibited by animals with a CBS mutation. These data, in addition to previous data, show the proposed CBS in *C. elegans* collagen IV has functional importance.

206356. SPD-1, a putative microtubule associated protein required for the formation of the spindle midzone and involved in the completion of cytokinesisK

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Several mutants that disrupt the spindle midzone, including *zen-4* and *air-2*, indicate that this structure is required for the completion of cytokinesis in *C. elegans*. The midzone may be required for regulating the various events required for sealing the two daughter cells, including the removal of the acto-myosin ring and the trafficking and fusion of membranes. We are studying a temperature sensitive mutant *spd-1(oj5)* in which the spindle midzone is disrupted in all cell cycles but cytokinesis fails only in EMS.

We have cloned *spd-1* and identified it as the homolog of human PRC1(1) and tobacco MAP-65(2) and S. *cerevisiae* Ase1p(3). These proteins localize to the spindle midzone, or its equivalent, and are involved in cytokinesis in HeLa cells and spindle elongation in yeast. SPD-1 antibodies also recognize the spindle midzone in *C. elegans* embryos.

Live imaging of β -tubulin::GFP in *spd-1* mutant embryos showed that the bundled microtubules of the spindle midzone are absent or much reduced during anaphase in all cell divisions. However, preliminary results indicate that ZEN-4 appears to localize correctly to the midzone of the one cell embryo. We are in the process of determining whether ZEN-4 and other known spindle midzone proteins localize correctly at all cell divisions of *spd-1(oj5)* embryos. We are also trying to determine why the EMS cell fails by determining the involvement of the wnt signalling pathway and the early polarity determining pathway.

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3. D. Pellman *et. al. Journal of Cell Biology* **130**, 1373-1385. (1995).

215932. Putting *exc-5* into Those Blasted Worms

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The EXC-5 guanine exchange factor regulates the amount of cytoskeleton devoted to the apical vs. basolateral surface in the excretory canal. In null mutants, the apical cytoskeleton is disrupted and the apical surface swells into large fluid-filled cysts. When *exc-5* is overexpressed, however, the lumen retains its normal diameter, while canal extension fails. We are currently performing a noncomplementation screen to isolate other point mutations in the *exc-5* gene. In addition, we would like to perform enhancer-suppressor screens to isolate other members in this signal transduction cascade. In order to perform this screen, we are trying to integrate a rescuing *exc-5::gfp* construct into the chromosome in a single copy.

In order to place genes into recipient worms, we have adapted the Biollistic "GeneGun" technique as pioneered by Judith Austin's lab. This technique has been reported to be effective at getting genes into worms more quickly, and in lower copy numbers, than are obtained through microinjection. In addition to trying to rescue *exc-5* mutants with the *exc-5::gfp* transgene, we have successfully placed varying copy numbers of a *vha-1::gfp* transgene (*vha-1* encodes a vacuolar ATPase, the gene was graciously supplied by M. Futai and T. Oka), into wild-type animals, to create worm lines in which the excretory canal lumen is fluorescently labeled. We are currently determining how many of these transgenic worms have incorporated the transgene into a chromosome.

We are also trying to blast larger pieces of DNA, such as cosmids, into worms, as an alternative to microinjection. We are using the *vha-1::gfp* transgene as a selectable marker to indicate expression of cosmid DNA in the correct cell.

227326. Systemic RNAi

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Early experiments revealed that the response to dsRNA has a "systemic" character in that silencing of gene expression is observed in cells distal from the initial site of dsRNA delivery. The mobile nature of the dsRNA signal is a subject of study in this lab. In particular, we are interested in the mechanims(s) by which dsRNA traverses the organism and trafficks in cells. We demonstrate that dsRNA not only has the ability to enter cells, but also to exit cells. We will demonstrate that the cellular uptake mechanism for two different cell types can accommodate large dsRNA molecules. A genetic mutant defective the uptake mechanism for one of these cell types will be described.

229358. PROTEIN INTERACTIONS IN *C. ELEGANS* MUSCLE ATTACHMENT STRUCTURES STUDIED *IN VIVO* BY FLUORESCENCE RESONANCE ENERGY TRANSFER (FRET)

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Dense bodies and M-lines are highly ordered, multiprotein attachment structures that span the plasma membrane and function to link the myofilaments found in body-wall muscle cells to the underlying ECM. Assembly of these structures is essential for the viability of the organism, and recent work in a number of labs has identified several new dense body and M-line components that are essential for this process. A crucial step towards understanding the assembly process is the characterization of the many protein-protein interactions that occur within attachment structures. Biochemical analysis identified protein-protein interactions between PAT-4 and UNC-112, PAT-4 and PAT-6, and PAT-4 and UNC-97 *in vitro*, and currently we are attempting to measure and characterize these interactions *in vivo*. Toward this goal, we created transgenic animals in which different dense body and M-line attachment proteins are fused to either CFP (cyan) or YFP (yellow) and are analyzing Fluorescence Resonance Energy Transfer (FRET) between the CFP- and YFP-labeled proteins *in vivo*. A significant FRET will only occur if the CFP and YFP tags are less than ~8 nM apart, suggesting that a direct intermolecular interaction between the labeled host proteins exists. We are using several different methods to measure FRET *in vivo* including analysis of donor fluorescence life-time changes via photon counting, donor fluorescence recovery after acceptor photobleaching, and the more standard donor/acceptor ratio-imaging technique. Our results indicate that PAT-4, a protein homologous to integrin-linked kinase, interacts with itself in an as yet unknown stoichiometry. To evaluate the accuracy of our *in vivo* FRET measurements, we performed control measurements on CFP-YFP fusion constructs expressed in *C. elegans*, as well as on a purified, bacterially expressed Ca^{2+}-sensitive chameleon construct in solution (plasmids proved by R. Tsein). We will describe the measurements we have made to date, as well as particular considerations for in vivo experiments.

233062. Analysis of *fbf-2* and its role in controlling germline stem cells in *C. elegans*

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Germ line stem cells are defined by their unique ability to maintain an undetermined population of cells and by their ability to generate differentiated gametes (Watt and Hogan, 2000). In the *C. elegans* germ line, the conserved GLP-1 (Notch) signaling pathway promotes mitotic proliferation at the expense of entry into meiosis. Our long-term aim is to identify genes that are regulated by GLP-1 signaling and that control the mitotic/meiotic decision. An excellent candidate is the *fbf-2* gene, one of two nearly identical genes that encode proteins collectively known as FBF (Zhang et al., 1997). An *fbf-1 fbf-2* double mutant is sterile, with defects in both germline stem cells and the sperm/oocyte switch (Crittenden et al., 2002). By contrast, an *fbf-1* single mutant is virtually wild-type, suggesting that *fbf-1* and *fbf-2* are largely redundant in their control of germline stem cells. The *fbf-2* gene (but not *fbf-1*) possesses binding sites in its upstream flanking sequence that suggest control by GLP-1 signaling. We have begun to characterize deletion mutants in the *fbf-2* gene. In addition, we plan to examine the expression pattern of FBF-2 protein. We will present progress in characterizing *fbf-2* as well as progress characterizing other potential GLP-1 targets.

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242121. The *C. elegans* eIF-5A homologue, IFF-1, functions in germ cell proliferation and affects localization of P granule components.

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We have previously identified a gene, *iff-1*, encoding a *Caenorhabditis elegans* eIF-5A homologue in a reverse genetic screen as one of the genes that function in germline development (Hanazawa *et al.*, 2001). A further analysis was made on its role in germline development.

We constructed a deletion mutant of *iff-1*, *iff-1(tm483)*, which hatches and develops into sterile adults similar to the RNAi-affected worms; small gonads with few nuclei with an appearance of mitotic interphase nuclei were observed. The fact that no excess dead cell was observed in the gonad shows that the reduction in the number of germ cells is not caused by ectopic cell deaths in the *iff-1(tm483)* mutant. To assess the possible role of *iff-1* for germline proliferation, we tested the effect of *iff-1* knock-down in the *gld-2(q497) gld-1(q485)* double mutant. The germline-tumorigenesis of the *gld-2 gld-1* mutant was suppressed by RNAi against *iff-1*, supporting the idea that *iff-1* is essential for the germ cells to proliferate. Localization of *iff-1* mRNA and protein, as detected by *in situ* hybridization, Northern analysis and Western analysis using the anti-IFF-1 antibodies, also supported this idea. *iff-1* mRNA and protein could not be detected in the *glp-4* mutant, which has few germ cells. This is consistent with our original assumption that IFF-1 is expressed in a germline-specific manner. Upon *in situ* hybridization, *iff-1* mRNA was detected in the distal region of the gonads where germ cells actively proliferate. Although eIF-5A was originally isolated as a candidate translation initiation factor, recent studies have suggested roles of eIF-5A in many aspects of RNA metabolism, including nuclear export, cytoplasmic degradation and translation. In the *C. elegans* gonads, many RNA binding proteins are known to play various key roles in regulating germ cell development. Many of them are localized on P granules, which are located close to the nuclear pore complex. Because the eIF-5A homologue in Xenopus was reported to localize on the filaments extending from the nuclear pore, it is of interest to test the possibility that IFF-1 collaborates with these RNA binding proteins that function on P granules. As one way of tesing the interplay of IFF-1 and P granule components, we examined the localizatioin of PGL-1 and GLH-1 in the *iff-1* mutant by immunostaining. In the *iff-1* mutant, unlike in wild type, these P granule components did not localize at the nuclear pore but were dispersed in the cytoplasm. These results suggest that IFF-1 is necessary for some P granule components to localize normally, and point to a possible functional link among IFF-1, P granules, and RNA metabolism.

There is another gene, *iff-2*, encoding an eIF-5A homologue in *C. elegans*. The *iff-2(tm393)* deletion mutant grows slowly during larval stages and becomes sterile adults lacking a part of the somatic gonad structure. This phenotype and the results of expression analysis indicated that two eIF-5A homologues might take charge of part of roles: one for germ cells and the other for somatic cells.

We are now working to clarify the targets of IFF-1 and the functional differences between these two homologues.

250069. Transgenic animals containing multiple copy arrays of *egl-13* upstream regulatory sequences have developmental and egg-laying behavioral defects similar to those of *egl-13* mutants

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The specification of the uterine pi cell fate during C. elegans gonadogenesis is an excellent cellular model for studying Notch-mediated genetic regulation of functional organ development. During larval development, six uterine intermediate precursor cells adopt the pi cell fate as a result of LIN-12/Notch signaling induced by the gonadal anchor cell (AC). Once specified, the pi cells undergo a well-characterized lineage distinct from the default rho cell fate. Eight of the pi cell daughters arising from one round of division along the dorso-ventral axis eventually differentiate and fuse along with the single AC to form the syncytial uterine seam cell (utse). The utse normally has a thin laminar morphology that permits passage of eggs from the uterus out through the vulva. We have found that the egl-13/cog-2 gene, which encodes a SOX domain transcription factor(1), is required for the maintenance of the pi cell fate. In egl-13 mutants, although the pi cell fate is initially specified properly, the presumptive pi cells undergo additional rounds of division and instead of a thin laminar utse, a thick, obstructive connection between the uterus and vulva is observed. Thus the cell fate maintenance defect observed in egl-13 mutants translates into the more apparent phenotype of the inability to lay eggs.

We are utilizing a transgenic approach to define potential cis-regulatory regions upstream of egl-13 that are recognized by transcription factors activated through Notch signaling. We have generated transgenic lines carrying integrated chromosomal arrays of an egl-13::gfp transcriptional fusion that show earlier utse development defects and later egg laying defects resembling the egl-13 mutant phenotype. Since these arrays contain multiple copies of the transgene, we are interested in determining if the observed defects are due to titration of regulatory factors required at the level of the egl-13 promoter thus preventing the transcription of endogenous egl-13. Such titration effects by upstream regulatory sequences have been previously described in other, unrelated C. elegans studies(2,3,4). We have observed that transgenic animals that are not completely egg-laying defective can be enhanced for both earlier utse defects and egg-laying defective phenotypes upon transformation with additional copies egl-13 regulatory sequences present in extrachromosomal arrays. By conducting such transformation experiments with an egl-13::sel-12 fusion construct and with several deletion constructs generated from the egl-13 upstream regulatory region of egl-13::gfp, we are taking advantage of this transgenic-based, feasible assay as a means to resolve the exact cis-regulatory sequence(s) that accomplishes the observed dominant negative effects. Lines transformed with extrachromosomal egl-13::sel-12 demonstrate a marked enhancement of the utse and egg laying defect, suggesting that over-expression of GFP is not the causative factor. This conclusion can be further tested by similar transformations with more 3'deletion constructs that abolish transcriptional activity of extrachromosomal egl-13::gfp. In addition, deletion constructs that retain transcriptional activity could assist in determining sequences that are necessary and sufficient to drive pi cell-specific expression. These sequences can then serve as bait to isolate the factors that specifically recognize and bind to the egl-13 5' regulatory region to activate egl-13 transcription via the Notch pathway.

(1)Hanna-Rose et al., 1999. Development 126: 169-179.
(2)Li et al., 1999. Genetics 152: 237-248.
(3)Toms et al., 2001. BMC Developmental Biology 1:2.
(4)Nicoll et al., 1997. Nature 388: 200-204.

269525. CLUSTERING PARASITIC NEMATODE ESTS USING PHRAP/CONSED

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In order to condense EST data from our parasitic nematode project and to provide complete information about the full length mRNA transcripts, we have devised a method of clustering ESTs using the Phrap assembler. We use locally produced trace data with Phred sequence quality values to generate high quality clusters. We also can add new data to existing clusters. Initially, no commercial clustering program met our needs. The clustering method we have developed uses Phred/Phrap to build initial contigs of overlapping ESTs. The resulting contigs are screened for misassemblies using Consed tools and newly written scripts. Misassemblies are recognized by: 1) regions of high quality unaligned sequence; 2) multiple runs of poly-A's and/or poly-T's; 3) internal poly-A and/or poly-T runs; 4) internal stretches of low consensus quality. Contigs flagged for possible misassembly are manually edited in Consed, where potentially chimeric ESTs and other suspect ESTs are identified and removed from the pool of traces. The edited reads are reassembled with phrap and screened again. The resulting contigs are compared to each other using WU-BLASTN and grouped based on similarity, forming clusters of related contigs. When new ESTs are added to the clustered data set, the old contig and cluster names are retained, even though the sequence may have changed with the addition of the new data. Any new contigs and clusters are named sequentially relative to the existing contig names. We provide our EST cluster information for the parasitic nematode project on our web site, www.nematode.net. Improved commercial clustering methods are becoming available. Since our clustering method is labor intensive, we would prefer to find a less cumbersome clustering program that generates equally high quality clusters. Therefore, we compared our clustering method to a commercially available software package by clustering a common set of human ESTs. The quality of the resultant clusters was ascertained by aligning the clustering outputs from both assemblers to finished human sequence. We will show the results of these comparisons.

278511. The role of SPE-15 in asymmetric organelle segregation during spermatogenesis.

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The cytoskeleton plays a critical role in many developmental processes, from cell migration to asymmetric segregation of cellular components. Isolation of the C. elegans mutant spe-15(ok153) lacking a functional class VI myosin highlights the importance of motor proteins in development. Deletion of a myosin VI (SPE-15) results in defective asymmetric segregation of organelles and cytoskeletal components required for the formation of motile sperm that occurs during spermatogenesis (Kelleher et al. 2000. Curr. Biol. 10:1489-1496), and also causes a Spe phenotype (sterility that can be rescued by wild-type sperm). The potential role of SPE-15 in organelle transport was addressed by real-time imaging of organelle movements during spermatid budding. Secondary spermatocytes (SS) undergoing the process of spermatid budding were obtained either from adult wild-type or mutant males lacking SPE-15. Budding was observed by DIC microscopy over the course of 90 minutes. As the spermatids bud, organelles are moved into the growing spermatid and the remaining residual body (RB) appears transparent. However, in spe-15(ok153) SS, organelles appear to be segregated into the spermatids but are then released back into the RB. Subsequently, the spermatids undergo abnormal contractions. The overall time required for spermatid budding also appears to be decreased by about half in the mutant SS. These results suggest that SPE-15 is not required for the translocation of cellular components, but rather for the restriction of their localization to the budding spermatid. SPE-15 may also provide an opposing force to prevent premature budding and spermatid deformation during the final stages of budding. We are currently addressing this hypothesis by SPE-15 localization studies, including microparticle bombardment of a spe-15::GFP construct and immunofluorescent staining of fixed spermatocytes with an anti-SPE-15 polyclonal antibody. An ongoing analysis of spermatogenesis will allow us to further define the role of myosin VI during this process.

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286365. Serotonin promotes Go-dependent neuronal migration in Caenorhabditis elegans

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The directed migration of neurons during development requires attractive and repulsive cues, which control the direction of migration, as well as permissive cues that potentiate cell motility and responsiveness to guidance molecules. We have found that the neurotransmitter serotonin functions as a permissive signal for embryonic and post-embryonic neuronal migration in the nematode C. elegans. In serotonin-deficient mutants (tph-1), the migrations of the ALM, BDU, SDQR and AVM neurons were often foreshortened or misdirected, indicating a serotonin requirement for normal migration. Moreover, exogenous serotonin could restore motility to AVM neurons in serotonin-deficient mutants as well as induce AVM-like migrations in the normally non-motile neuron PVM, indicating that serotonin was functioning as a permissive cure to enable neuronal motility. The migration defects of serotonin deficient mutants were mimicked by ablations of serotonergic neuroendocrine cells, implicating humoral release of serotonin in these processes. Mutants defective in Gq and Go signaling, or in N-type voltage-gated calcium channels, showed migration phenotypes similar to serotonin-deficient mutants, and these molecules appeared to genetically function downstream of serotonin in the control of neuronal migration. Thus, serotonin is important for promoting directed neuronal migration in the developing C. elegans nervous system. We hypothesize that serotonin may promote cell motility through G-protein-dependent modulation of voltage-gated calcium channels in the migrating cell.

290696. UNC-44 Protein Interaction

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The correct connection of neurons is a critical part of neural development. The growth of neurons to their targets has been shown to require dozens of genes in the nematode, *Caenorhabditis elegans*. One of those genes is *uncoordinated-44* (*unc-44*) that encodes a series of ankyrin proteins. Previous studies showed that the large AO13 ankyrin isoform, rather than the other isoforms is required for proper axonal guidance. To determine which molecules interact with UNC-44 and whether they form a signal transduction complex, the yeast two-hybrid system has been used. One bait, pDD5, was constructed by inserting the DNA coding for the carboxyl domain of AO13 ankyrin into plasmid pAS2-1. Transformation of pDD5 into yeast cells containing *C. elegans* library plasmids yielded positive colonies that were picked and DNA sequenced. Four of ten clones analyzed encode W08G11.4 which is related to the B56 or B' regulatory subunit of phosphatase 2A (PP2A). PP2A has been shown to be involved in many cellular processes by combining a limited number of catalytic (C) and adapter (A) subunits with a variety of regulatory (B, B', B'', and B''') subunits. The B-type subunits of PP2A determine the substrate specificity of PP2A phosphatase. PP2A containing the B56 regulatory subunit has been reported to be involved in axonal guidance in *Drosophila*.

313951. Mechanisms Regulating Zn^{2+} Homeostasis in *C. elegans*

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Zinc is an essential trace element that organisms must extract from nutrient sources. Since high or low concentrations of Zn^{2+} can be toxic, homeostatic mechanisms are required to maintain optimal cytosolic Zn^{2+} levels, regardless of dietary availability. Recently, proteins encoding Zn^{2+} transporters belonging to two families have been identified. Cation diffusion facilitator (CDF) proteins and ZRT/IRT-related proteins (ZIP) function to decrease or increase cytosolic Zn^{2+} levels, respectively. However, the biochemical mechanisms controlling Zn2+ transport by these proteins have not been elucidated nor is it known how their activity is coordinately regulated with the amount of nutritionally accessible Zn^{2+}. Furthermore, proteins involved in sensing cytosolic Zn^{2+} levels and relaying the information to the appropriate Zn2+ transporters have yet to be identified. Thus, the mechanisms controlling Zn2+ homeostasis remain poorly characterized.

In a genetic screen for suppressors of constitutively active LET-60 Ras, we isolated a loss-of-function mutation in the gene encoding the *C. elegans* CDF protein CDF-1. CDF-1 is most similar to mammalian ZnT-1; ZnT-1 activity increases Zn2+ efflux in cultured cells. *cdf-1(lf)* mutants are hypersensitive to supplemental Zn^{2+} but not Cd^{2+}, Co^{2+} or Cu^{2+}, suggesting that CDF-1 is specifically involved in Zn^{2+} metabolism and that the concentration of cytosolic Zn2+ is elevated to toxic levels in the mutants. This hypersensitivity is fully rescued by overexpression of mammalian ZnT-1, demonstrating that these proteins are functionally interchangeable. CDF-1 appears to be localized to the plasma membrane, consistent with the prediction that CDF-1 also functions in Zn^{2+}-efflux. These studies suggest that at least some aspects governing Zn^{2+} homeostasis have been conserved between nematodes and mammals. Other mutants that affect Zn^{2+} homeostasis in *C. elegans* have not been reported. To further investigate the mechanisms regulating Zn^{2+} homeostasis, we are conducting genetic screens to isolate mutants that are resistant to defects caused by culturing worms in either high- or low-Zn^{2+} environments. We will present our progress in establishing Zn^{2+}-limited media using the Zn^{2+}-specific chelator TPEN (*N, N, N', N'*-tetrakis(2-pyridylmethyl)ethylenediame). Additionally, we will provide preliminary data describing candidate suppressors of the *cdf-1(lf)* hypersensitivity to supplemental Zn^{2+}. These mutations may reduce the concentration of cytosolic Zn^{2+} and affect genes involved in cytosolic Zn^{2+} sensing or influx.

316846. CeGSTP2-2, an invertebrate glutathione S-transferase with activity for 4-hydroxynonenal: possible roles in stress resistance and aging

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Reactive oxygen species (ROS) and oxidative stress are the inevitable consequence of aerobic metabolism. Enhancing organismal defenses that confer resistance to ROS, the primary initiators of the oxidative stress cascade, have been shown to play a pivotal role in extending the life span of invertebrates. In Drosophila, overexpression in motor neurons of superoxide dismutase-1 (SOD-1) can extend longevity by up to 40%. Similarly, characterization of the age-1 mutation in the nematode Caenorhabditis elegans demonstrated that the long-lived mutants have increased late life expression of catalase and SOD. These enzymes are thought to attenuate oxidative damage by lowering the level of initiating ROS. The mechanism by which ROS exerts damaging effects that ultimately lead to a shortened life span is not completely understood. The ·OH radicals can react with proteins and DNA directly, although more detrimental maybe their ability to initiate the lipid peroxidation cascade. This occurs when ROS react with poly-unsaturated fatty acids on the membrane in a self-propagating mechanism that can amplify the original insult 100-1000 fold. The resulting lipid hydroperoxides are oxidants themselves but they can decompose to form toxic electrophiles such as 4-hydroxynonenal (4HNE). 4HNE has a relatively long half-life, is diffusible, and can react with proteins and DNA leading to cellular dysfunction. Glutathione S-transferases are a multifunctional family of phase II detoxification enzymes that catalyze the conjugation of glutathione to toxins in order to make them more soluble and consequently less toxic. We have recently cloned and characterized a pi-class (P) C. elegans GST with a high level of activity for 4-HNE. Although this enzyme accounts for less than 20% of the total 4-HNE activity of worm homogenate, it appears to be highly expressed in the neurons (figure 1). It therefore may play a significant role in neuronal defense against toxic electrophiles. Because of the electrophilic protection afforded to this organism by the 4-HNE-conjugating activity of CeGSTP2-2, it is our hypothesis that nematodes with increased levels of CeGSTP2-2 may have increased resistance to stress and aging. Consistent with this hypothesis, we found that long-lived daf-2 mutants show increased expression of CeGSTP2-2.

328991. A forkhead encoding gene directs male development in the *C. elegans* somatic gonad

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In *C.elegans*, most tissues are sexually dimorphic between hermaphrodites and males. Notable examples include the musculature, the nervous system, the germline, and the somatic gonad. The somatic gonads of the two sexes arise from two precursor cells, Z1 and Z4, which undergo different lineages, migration, and morphogenetic movements to generate very different organs. The adult hermaphrodite somatic gonad is two-armed and symmetrical, with each arm comprised of a uterus, spermatheca, and sheath cells, while the male somatic gonad is single-armed and asymmetrical with a seminal vesicle and vas deferens. To identify genes that control sex-specific gonad development, we have performed a genetic screen. We used a GFP marker expressed in the male vas deferens and seminal vesicle (see Thoemke et al abstract), and examined F2 males for abnormal expression or gonad morphology (see abstract by Illi et al). We isolated a number of mutants, including one we have tentatively named *tog-1* (***t****ransformer* ***o****f* ***g****onad*). The *tog-1(ez16)* mutant male gonad develops into a mass of disorganized tissue and about 25% of the mutant males have hermaphrodite vulval structures. The tail and other structures of the *tog-1* males are normal. Most mutant males express *lim-7::gfp*, a hermaphrodite sheath cell marker, and *cdh-3::gfp*, an anchor cell marker. *tog-1* males express male-specific GFP markers for valve cells, seminal vesicle, and vas deferens, indicating that feminization of the male gonad is not complete. *tog-1* hermaphrodite gonads have normal overall morphology with two symmetrical arms, although the spermatheca appears abnormal and does not function properly. We have mapped the *tog-1* locus to chromosome II and found that *tog-1* encodes a novel forkhead transcription factor. The *ez16* allele appears to be null: the phenotype is the same in trans to a deficiency, and the *ez16* mutation changes the start AUG to AUA. Lineage analysis (described in Tilmann et al abstract) showed that *tog-1* is required for male-specific early asymmetrical cell division and cell migration. The global sex determining gene *tra-1* also regulates the symmetry of early cell division and migration, and we are currently analyzing how *tra-1* and *tog-1* function together to direct sexually dimorphic gonadogenesis.

329511. Wormatlas: A web-based behavioral and structural atlas of *C. elegans*

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

Wormatlas is designed to have two main sections, Index and Guides, with multiple chapters within each section. The Index section will contain the Handbook, Slidable Worm, Literature Archive, Cell Identifications, Neuron Data, Glossary, and Methods. The main goal of the Handbook is to provide a relatively simplified, image-supported and curated information about the general and specific anatomy of *C. elegans.*The images included in the Handbook will be annotated scanning and transmission electron (TEM) micrographs, computer-drawn images as well as DIC and fluorescent micrographs. The Slidable Worm is designed to provide 600-1200 annotated and nonannotated versions of TEM cross-sections of the animal available for viewing by the users with the help of a newly designed JAVA applet interface. The images will come from the original images from the MRC/LMB archive, from the MIT archive (courtesy of E. Hartwieg 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.

337517. VAB-9 is a claudin-like adherens junction protein that regulates hypodermal morphology and adhesion

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To understand how hypodermal cell shape and adhesion regulate organismal morphology in *C. elegans,* I am analyzing the gene *vab-9. vab-9* mutants have tail and body shape defects resulting from defects in either the attachment or distribution of circumferential actin filaments at the adherens junctions. In the normal embryo, circumferential actin filament bundles are evenly spaced along the anterior-posterior axis; their contraction results in the elongation of the embryo. VAB-9 is a putative four-pass transmembrane protein homologous to canine BCMP1 (Brain cell membrane protein 1) and similar to the PMP22/Claudin family of proteins. Claudins are the major protein components of tight junctions and mediate calcium independent cell adhesion. Despite the similarity of VAB-9 to the tight junction claudin proteins, VAB-9 localizes to the adherens junctions of all *C. elegans* epithelia. VAB-9 localization is dependent on other adherens junction components. In the absence of HMR-1 (cadherin), VAB-9 localization at the adherens junction is completely missing, whereas in the absence of HMP-1 (a -catenin) or HMP-2 (b -catenin), VAB-9 localizes at junctions, but the distribution about the periphery of the junction is disrupted. 3D time-lapse analysis of VAB-9-GFP localization in *hmr-1(RNAi)* and *hmp-1(RNAi)* animals using multi-photon excitation microscopy was carried out by Mathias Köppen and Jeff Hardin at the University of Wisconsin. In *hmr-1(RNAi)* animals, VAB-9-GFP apparently fails to reach the plasma membrane, whereas in *hmp-1(RNAi)* animals, initial VAB-9-GFP junctional localization is normal, but the distribution about the periphery of adherens junctions is rapidly lost. Ultimately, in *hmp-1(RNAi)* animals VAB-9-GFP becomes concentrated into small, isolated puncta at the junction. Interestingly, HMR-1 localization is almost identical to VAB-9 in *hmp-1* and *hmp-2* mutants. In contrast to these findings, mutations in *vab-9* do not obviously disrupt the localization of HMR-1, HMP-1, or HMP-2. Together, these results suggest that VAB-9 is closely associated with the adherens junction and that VAB-9 affects actin organization by regulating the interaction between actin and the cadherin-catenin complex. Alternatively, VAB-9 may mediate interactions with the actin cytoskeleton distinct from those with cadherin and catenins. VAB-9 similarity to PMP22/Claudin proteins suggests that VAB-9 may have a role in cell adhesion, however, *vab-9* mutants alone have no obvious cell adhesion defects. Nevertheless, *vab-9* mutations enhance the cell adhesion defects of *ajm-1* mutants and *dlg-1(RNAi)* animals. Transmitting electron microscopy (carried out by Paul Sims and Jeff Hardin, University of Wisconsin) revealed that large gaps are present between hypodermal cells of *vab-9; ajm-1* animals. These gaps extend along the entire apical-basal axis of the hypodermal cells and are more extensive than the gaps in the apical junctions of *ajm-1* mutants alone. Since VAB-9 is localized apical to DLG-1 and AJM-1, one possibility is that VAB-9 and DLG-1/AJM-1 independently regulate hypodermal cell adhesion. To determine how VAB-9 is associated with the cytoskeleton, I am currently searching for VAB-9-interacting proteins. To test directly for the ability of VAB-9 to mediate cell-cell adhesion, I am expressingVAB-9 in L fibroblasts. I thank the Hardin Lab (experiments), the Priess Lab (lab space, reagents, advice), John White (multi-photon excitation microscopy) and David Hall (TEM advice).

341753. Characterization of a New Gene Involved in Ras-mediated Vulval Induction

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The development of the *C. elegans* vulva requires the activity of the Ras signaling pathway. To identify components of this pathway, we screened for mutations that suppress the multivulva phenotype caused by *let-60(n1046gf)*, a mutation that constitutively activates the *ras* gene. Forty-three alleles comprising 20 complementation groups were identified. These genes include conserved members of the Ras signaling cascade, such as Raf *(lin-45)*, KSR *(ksr-1)*, MEK *(mek-2)*, MAPK *(mpk-1)*, and an ETS transcription factor *(lin-1)*. We are now analyzing *n2528*, an allele that defines a new complementation group. The *n2528* mutation strongly suppresses the *let-60(n1046gf)* Muv phenotype and partially suppresses the *lin-1(e1275)* Muv phenotype, suggesting that the affected gene may act downstream of or in parallel to the transcription factor *lin-1*. In a wild-type genetic background, the *n2528* mutation does not dramatically affect vulval development. *n2528* mutants also display a cold-sensitive larval lethal phenotype that can be partially rescued by *let-60(n1046gf)*. Thus, the gene affected by *n2528* may contribute to the function of the Ras pathway at multiple times during development. We used single nucleotide polymorphisms in the Hawaiian strain, CB4856, to map the *n2528* mutation to a 17.5 kilobase interval on LGX. We sequenced this interval to identify the molecular lesion and confirmed the identity of the gene by transformation rescue. The affected gene shares about 23% similarity with sec14, the yeast phosphatidylinositol transfer protein, mammalian alpha-tocopherol associated proteins, and squid cytosolic retinal binding protein, suggesting that proteins which function to bind small hydrophobic molecules may play an important role in Ras signaling.

368409. A role for Wnt signaling in morphogenesis

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Two major processes that occur during hypodermal morphogenesis in *C. elegans* are intercalation of the dorsal hypodermis and enclosure of the embryo by the ventral hypodermal cells. A deletion of *dsh-2 (or302)* , one of three dishevelleds in *C. elegans*, results in defects in morphogenesis. *dsh-2* mutants display 70% embryonic lethality due to hypodermal ruptures shortly after enclosure. Multiphoton imaging with *ajm-1*::GFP shows that leading cells lag behind the pocket cells during enclosure and failure of ventral cells to meet and join completely prior to the beginning of elongation, resulting in ruptures. Multiphoton analysis also reveals defects in dorsal intercalation in some embryos.

Dishevelleds are members of the Wnt signaling pathway. They transduce signals when a Wnt ligand binds to a Frizzled receptor. Depending on the Wnt and the Frizzled, Dishevelled can pass the signal through one of at least two pathways: the canonical wingless (wg) pathway or the planar cell polarity pathway (PCP). The canonical pathway signals primarily through the conserved DIX and PDZ domains of dishevelled, while the PCP signals through the conserved DEP domain. Both pathways have been shown to play a role in cell migrations and cell fate decisions. To determine which pathway causes the defects seen in hypodermal morphogenesis in *C. elegans*, I have made deletion constructs that lack different domains of *dsh-2*.. Transformation of mutants is underway to determine which constructs will rescue the hypodermal defects. Knowing which pathway is involved will allow us to investigate other members of the pathway that may also play a role in hypodermal morphogenesis.

369975. Analysis of loss-of-function *lin-1* mutations that identify functionally significant residues of the ETS DNA-binding Domain.

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A highly conserved RTK/Ras/MAP kinase pathway promotes the primary vulval cell fate by negatively regulating the ETS transcription factor LIN-1. Although LIN-1 plays a critical role in vulval precursor cell (VPC) fate determination, the regulatory mechanisms that control LIN-1 activity and the mechanism of action of LIN-1 have not been extensively characterized.

Fifty alleles of *lin-1* have been isolated in a variety of screens for vulval defects conducted in several laboratories. These alleles include loss-of-function and gain-of-function mutations of different severities. To identify residues and domains that are important for LIN-1 function, we used DNA sequencing to determine the molecular lesions of these alleles. The loss-of-function mutations include twenty-five nonsense mutations that are predicted to encode a series of truncated LIN-1 proteins. There is not a simple relationship between the severity of the loss-of-function phenotype and the position of the nonsense mutations, suggesting that LIN-1 contains domains that negatively affect its function or stability. The loss-of-function alleles form a series of nonsense mutations which are under current investigation.

The loss-of-function mutations also include eleven missense mutations that affect nine conserved residues in the ETS domain. Most of these mutations cause a severe loss-of-function phenotype although one allele causes a partial loss-of-function. We examined the DNA-binding properties of the wild-type and mutant ETS domains using purified extracts. The wild-type LIN-1 ETS domain displays sequence specific binding to a consensus ETS binding site. These mutant proteins are severely defective or absent in DNA binding. These finding indicate that the DNA binding activity of LIN-1 is essential for function *in vivo*.

378007. Ultrastructural analysis of *C. elegans* embryos using correlative light and electron microscopy

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Ultrastructural analysis of *C. elegans* mutant embryos requires determining both the genotype and the developmental stage of the embryos. We have developed a method allowing correlative light and electron microscopy of embryos of identified genotypes, and we have applied this method to the analysis of *ajm-1* mutants. AJM-1 is a novel coiled-coil protein expressed in all epithelial cells of *C. elegans. ajm-1(ok160)* mutants show fully penetrant embryonic lethality, arresting at about the 2- fold stage. To study the defects of *(ok160)* mutants at the ultrastructural level, we used SU159, a strain homozygous for *ajm-1(ok160),* carrying a rescuing *ajm-1::gfp* array. Mutant embryos which lack the *ajm-1::gfp* array were identified by fluorescent imaging on a confocal microscope. Simultaneous imaging with Nomarski optics allowed us to determine the developmental stage of the embryos. Embryos were then processed by high pressure freezing (Balzers HPM 010) and fixed and dehydrated by freeze substitution in acetone/ methanol with OsO4 and uranyl acetate. Using this technique we identified ring-shaped separations along apical epithelial junctions in *ajm-1* mutants, suggesting that AJM-1 is required for maintaining junctional integrity in elongating embryos (Koppen et.al., 2001.). This correlative technique may be useful for the examination of other mutants and could be modified for immuno-electron microscopy.

1. Koppen et.al., (2001). Nature Cell Biology. 3:983-91.

382344. Disruption of epithelial morphogenesis by perturbation of molecules affecting actin dynamics

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During *C. elegans* embryogenesis, the hypodermis undergoes three major morphogenetic processes that lead to formation of the worm's tubular shape: dorsal intercalation, ventral enclosure, and elongation. It has previously been demonstrated that proper completion of all three of these events requires an intact actin cytoskeleton in the hypodermis, but the mechanisms involved in regulation of actin during epithelial morphogenesis are not yet entirely clear. We have taken a candidate gene approach to examine whether molecules known to be involved in actin dynamics have roles in epithelial morphogenesis.

Members of the WASP family have been shown *in vitro* to act downstream of CDC-42 as activators of the Arp2/3 complex to promote actin branching during cell locomotion. Surprisingly, animals null for *was-1*, a *C. elegans* homolog of the WASP family, undergo normal embryogenesis. Likewise, *Drosphila* Enabled is known to affect epithelial morphogenesis and the vertebrate Ena family members have been implicated in actin remodeling. *unc-34* encodes an ortholog of Ena and has been shown to function in cell migration in *C. elegans*[1]. As with *was-1*, a null allele of *unc-34* results in virtually no defects in embryogenesis. However, RNAi of *was-1* in *unc-34(gm104)* animals yields embryonic lethality[2]. These embryos appear to be specifically defective for certain hypodermal cell migrations that occur during ventral enclosure, but not those during dorsal intercalation. In addition to its synergy with *was-1*, *unc-34* has other morphogenetic roles. RNAi for a homolog of the WASP relative SCAR/WAVE generates hypodermal defects and lethality, and performing this experiment in an *unc-34(gm104)* background enhances this phenotype to yield embryos that are completely defective in morphogenesis. Finally, *unc-34* enhances morphogenetic phenotypes associated with mutations in the cadherin/catenin complex[3] similar to those seen in *Drosophila*. Experiments are underway to establish the localization of UNC-34 throughout embryogenesis, the specific causes of the various *unc-34* synthetic phenotypes, and to establish which additional molecules are involved in these processes.

1. Dell et al., 1998 West Coast Worm Meeting abstract 109 2. Dell et al., 2000 West Coast Worm Meeting abstract 119 3. Pettitt and Broadbent, 2002 European Worm Meeting abstract 12

391731. PAT-6/Actopaxin mediates integrin signaling during body wall muscle development in *C. elegans*

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Dense bodies and M-lines are related cellular structures that organize the assembly of the myofilament lattice in body-wall muscle cells, as well as physically anchor the mature lattice to the basal sarcolemma. Both dense bodies and M-lines contain the transmembrane protein integrin and many other proteins also found in focal adhesions, the tissue culture cell attachments that have been used extensively to study integrin. We are using genetic approaches to investigate dense bodies and M-lines and are focusing on a subset of the *pat* (**P**aralyzed, **A**rrested elongation at **T**wo-fold) genes that are necessary for their proper assembly. A partial list of these genes includes *pat-2* and *pat-3*, which are integrin subunit genes (Williams and Waterston, unpublished; Gettner et al., 1995, JCB 129:1127), *unc-112*, a novel dense body/M-line component (Rogalski et al, 2000, JCB 150:235), *pat-4*, which encodes integrin-linked kinase (Mackinnon et al., 2002, Curr Biol., 14:787), and *pat-6*, which we focus on here.

We have molecularly isolated *pat-6* gene and have found that it encodes the worm homologue of a recently identified mammalian focal adhesion protein Actopaxin/CH-ILKBP/Affixin/β-Parvin. PAT-6/actopaxin localizes to dense bodies and M-lines in *C. elegans* body wall muscle cells. Using the existing collection of *pat* mutants in combination with antisera to various dense body and M-line components, we have found that PAT-6/actopaxin functions downstream of integrin in initiating the membrane-proximal assembly of dense bodies and M-lines.

PAT-6/actopaxin contains a tandem repeat of Calponin Homology (CH) domains in its C-terminal half. We have found no conserved motif in the N-terminal half of the protein. To understand the function of CH domains *in vivo*, we made a set of constructs encoding truncated versions of PAT-6::GFP and assayed their localization and function *in vivo*. Our results suggest that: 1) the C-terminal CH domain is necessary and sufficient for the proper localization to dense bodies and M-lines; 2) both CH domains are needed to rescue *pat-6* null animals; and 3) the unique N-terminal sequence of PAT-6 appears to be dispensable for both localization and full rescue of *pat-6* null mutants.

We are interested in the interaction between PAT-6/actopaxin and PAT-4/ILK due to the unexpected finding that PAT-6/actopaxin is not detectable in body-wall muscle cells when PAT-4/ILK has been removed by either mutation or RNAi. Yeast two-hybrid experiments show that PAT-6/actopaxin binds directly to PAT-4/ILK. The C-terminal CH domain of PAT-6/actopaxin and the kinase domain of PAT-4/ILK are both necessary and sufficient for this binding.

Based on the above results, we are currently testing the following hypotheses: 1) PAT-6/actopaxin is brought to nascent dense bodies and M-lines through direct interaction with PAT-4/ILK; 2) any PAT-6/actopaxin molecules failing to bind PAT-4/ILK are degraded in the cytoplasm; and 3) PAT-6/actopaxin regulates dense body and M-lines assembly by recruiting other necessary component(s)to the nascent attachment sites.

394581. Cloning Characterization of *pat-9*: A Gene Required For The Assembly Of Thin Filaments Into The Myofilament Lattice

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The related dense body and M-line structures that are found in *C. elegans* body wall muscle cells are integrin-based transmembrane attachments that couple the basal lamina to the muscle cell cytoskeleton. In an effort to genetically dissect the assembly of dense body and M-lines, we have been studying mutants with the Pat (Paralyzed, Arrested elongation at Two-fold) phenotype. To date all of the molecularly isolated genes associated with the Pat phenotype are known to encode dense body components, including*unc-52* (UNC-52/perlecan) (1), *pat-2* (integrin alpha subunit) (Williams and Waterston, unpubl.), *pat-3* (integrin beta subunit) (2), *unc-112* (UNC-112) (3), *deb-1* (DEB-1/vinculin) (4), *unc-97* (UNC-97/PINCH) (5), *pat-4* (PAT-4/integrinl-linked kinase) (6), and *pat-6* (PAT-6/actopaxin) (Lin and Williams, unpubl.). These mutants, analyzed with antibodies to specific attachment proteins and/or functional Green Fluorescent Protein fusions, have produced an ¡°assembly dependence¡± pathway for dense bodies and M-lines (6, 7).

Here we focus on the *pat-9* gene, which has not yet been molecularly cloned. *pat-9* mutants have defects in body wall muscle assembly that most closely resemble those found in *deb-1*vinculin mutants. In particular, the recruitment of actin thin filaments to the muscle cell membrane appears to be completely disrupted, while deficits in the recruitment of myosin thick filaments are less severe. We predict that *pat-9* encodes a dense body protein.

To positionally clone *pat-9*, we are currently refining its location on the genetic map. Previously we mapped *pat-9* to the interval defined by the right end points of deficiencies *mnDf8* and *mnDf1* on the right end of the *X* linkage group. We are now using single nucleotide polymorphisms between N2 and the *wild-type* Hawaiian *C. elegans* strain CB4856 to further refine the *pat-9* map location. We are currently selecting Unc recombinants from *unc-3 pat-9*/ + + heterozygotes, and then assaying them for several different SNPs that span the right end of *X*.

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398734. Cytoskeletal defects associated with NUD-1 knockdown in early embryogenesis of *C. elegans*

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nud-1 is the *C. elegans* ortholog of the *Aspergillus nidulans* gene *nudC*, originally identified in a screen for proteins affecting nuclear positioning within the hyphae. Our lab has previously shown functional conservation between *nudC* and *nud-1* (Dawe *et al.* 2001). Results presented in this work also include expression in the neurons, gonad, gut, vulva, hypodermal seam cells and the embryo. The mammalian ortholog of *nudC* encodes a protein that interacts with Lis1, a gene essential for neuronal migration in the developing mammalian brain. Mammalian NudC has also been shown to interact with dynein subunits and to colocalize with p150dynactin and with gamma tubulin at the MTOC. This protein is also highly expressed in bone marrow cells from patients with leukemia, suggesting a role in hematopoietic cell growth.

We have previously described the phenotypes resulting from injection of *nud-1* dsRNA. Injected animals exhibit significantly reduced fecundity. Initially, fertilized embryos are formed; phenotypes associated with these F1 escaper animals include oogenesis defects that lead to sterility and uncoordinated movements. Subsequent to the first wave of escapers, embryonic lethality occurs at approximately the comma stage. The phenotype of the one-celled embryo is limited to a defect in pronuclear rotation. Following pronuclear meeting, rotation onto the longitudinal axis does not occur, reminiscent of the p150glued phenotypes observed by Skop and White (1998). Oogenesis fails rapidly after injection of *nud-1* dsRNA, preventing an examination of potentially stronger embryonic phenotypes by this method.

We have utilized the technique of RNAi feeding to fine tune and more thoroughly examine the embryonic phenotypes associated with NUD-1 knockdown. *nud-1* (RNAi) feeding yields not only the phenotypes discussed above, but also additional defects in the one-celled embryo. Centrosome overreplication occurs during pronuclear migration, resulting in multipolar spindles. Each MTOC nucleates microtubules, resulting in multiple cytokinetic furrows, some of which regress before the next cell division. Additionally, chromosomes are missegregated following these events. These results indicate an important function for NUD-1 in centrosome duplication and cytokinesis during *C. elegans* early embryonic development, a case strengthened by the mammalian data demonstrating both interaction and localization with spindle components.

Dawe *et al.* Dev Genes Evol. (2001) 211:434-441.
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404966. Defining Regulatory Regions In Nematode Muscle Genes By Computational And Transgenic Studies.

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We have initiated experiments designed to understand the regulatory regions of *C. elegans* genes using known muscle genes of *C. elegans* as a model(1). Our primary approach is to use a combination of computational methods to identify potential muscle specific regulatory elements from the known set of *C. elegans* muscle genes. Local multiple sequence alignment methods like Consensus(1), Ann-Spec(2), and Co-Bind(3) are being used to identify these potential regulatory elements. Using the above method we have already identified several potential regulatory elements that show high degree of specificity for the muscle genes. The regulatory elements that these computational methods predict can then be used to screen the *C. elegans*genome for new genes that are expressed in muscle cells.

To test our results we have developed a method to examine the expression patterns of genes in *C. elegans* using gfp promoter fusions. We are including in our promoter fusions 6,000 nucleotides upstream of the start methionine, all of the first exon and all the first intron. In our initial experiments, known muscle genes tested in this manner show muscle-like expression. We can now use this method to test the requirement for regulatory regions predicted by the computational work to determine if they convey muscle specific expression. In addition, we can use this method to test genes we predict to be, but not previously known to be expressed in muscle. Furthermore, we are developing these methods to allow for the rapid production of these promoter fusions so that ultimately, a genome wide program to categorize all *C. elegans* genes by gfp and automated lineaging can be done.

For the purpose of predicting muscle cell expression, we can rank the genes of the *C. elegans* genome by summing the scores of our three best putative regulator elements. When we look at the top scoring 25 genes to see how accurate our computation methods are at predicting muscle cell expression, 18 genes are known to be expressed in muscle cells or are shown to be by gfp expression, two genes are not expressed in muscle cells, two genes have no gfp, and one gene has not been done.

We hope to improve our ability to predict muscle cell expression by modification of the computional scores based on the grouping of the regulatory motifs. We also hope to take advantage of the comparisons of the regulatory regions of *C. elegans* and *C. briggsae*orthologs. We are presently using site-directed mutagenesis to alter the putative regulatory sites in a subset of known muscle genes.

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406949. *tcl-2* encodes a novel protein that acts synergistically with Wnt signaling pathways in *C. elegans*

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During the development of multicellular organisms asymmetric cell divisions are required to increase the cell numbers and generate diverse cell types. In *C. elegans*, as in other animals, the conserved Wnt signaling pathway controls asymmetric cell divisions and cell fate specifications. For example, in the *C. elegans* tail, *lin-44/Wnt* and *lin-17/frizzled* control the asymmetric division of the TL and TR cells (collectively referred to as T cells). In wild-type animals, POP-1, a TCF-4/LEF-1 homolog, is distributed asymmetrically to the nuclei of the T cell daughters: the anterior daughter, T.a, has a higher level of POP-1 than does the posterior daughter, T.p. Mutations in *lin-44*cause a reversal of T cell polarity and a corresponding reversal in the level of POP-1: T.a has a lower level of POP-1 and T.p. has a higher level. Mutations in *lin-17* cause a loss of T cell polarity and equally high level of POP-1 in both T.a and T.p. This suggests that *pop-1* is downstream of *lin-44* and *lin-17*. We have isolated several mutations defining the new genes that interact with *lin-44* and *lin-17* in the control of asymmetric division of the T cell. One of these is *tcl-2* (T cell lineage defective). Cell lineage analysis has revealed that *tcl-2* mutations cause defects in the pattern and asymmetry of the T cell divisions. Furthermore, both T.a and T.p had equally high levels of POP-1 in *tcl-2* mutants, as we observed in *lin-17* mutants, suggesting that *tcl-2* also functions before *pop-1* in the control of the T cell division. We cloned *tcl-2* by transformation rescue and found sequence changes in four sequenced *tcl-2* mutant alleles. No related proteins have been found in a variety of database searches, suggesting that *tcl-2* encodes a novel protein. A *tcl-2::gfp*translational fusion construct rescued the T cell division defect and was weakly expressed in the nuclei of the T cells and certain T cell descendants. In addition, we found that *tcl-2*mutants also have defects in the development of gonadal axes and the specification of the P11/P12 cell fate, both of which involve the Wnt signaling pathway. Double mutant analyses revealed that *tcl-2* acts synergistically with *lin-44* and *lin-17* in the control of gonad development and P11/P12 cell fate specification. A role for LIN-44 in the development of the gonadal axes was unexpected, as neither *lin-44* nor any other *C. elegans Wnt* gene has been shown to be involved in gonad development. Our data suggest that *tcl-2* may either act in a parallel pathway or to modulate the Wnt signaling pathway in the control of the asymmetric T cell divisions, gonad development and P11/P12 fate specification.

417288. Structure function analysis of DLG-1 and AJM-1 in apical junction formation and function.

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We are interested in the genes involved with formation and function of epithelial junctions. Recently two proteins have been identified which localize and act at the *C. elegans* septate like junction. AJM-1, a novel coiled coil protein, and DLG-1, a homologue of *Drosophila* Discs large, have been shown to colocalize to a domain of apical junctions distinct from the catenin-cadherin complex. Loss of function studies with these genes demonstrate their requirement for proper junctional integrity. Individuals deficient for either protein arrest during elongation and have disrupted apical junctions at the level of electron microscopy. In addition, these two genes have been shown to interact both genetically and physically. A genetic interaction is seen by disruption of AJM-1 localization along the apical junction in the absence of DLG-1. A physical interaction is seen by the binding of AJM-1 to the N-terminus of DLG-1 in yeast 2-hybrid and GST pull down experiments.

In order to further characterize the formation and function of the DLG-1/AJM-1 domain we are undertaking a series of structure function experiments. A *dlg-1* deletion mutant provided by the *C. elegans* knockout consortium confers a loss of function background in which rescue with constructs deleted for domains of *dlg-1* can be performed. Constructs are being tested for their ability to rescue *dlg-1* mutant phenotypes with respect to lethality and AJM-1 localization. In parallel we are performing directed 2-hybrid experiments with deletion constructs of *dlg-1* and *ajm-1* to further map the physical interaction domain. Initial results of these structure function experiments will be presented.

430903. The *Caenorhabditis elegans Skp1*-related (*skr*) gene family: diverse functions in cell proliferation, morphogenesis, and meiosis

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The SCF complex is a multi-subunit ubiquitin-ligase (E3) that mediates the degradation of a variety of cellular proteins such as cell cycle regulators and transcription factors. There are four SCF subunits: Skp1, a cullin (orthologs of metazoan CUL-1 or yeast Cdc53), Rbx1/Roc1/Hrt1, and an F-box protein. The cullin subunit acts as a scaffold to link the E2 (Ubc3/Cdc34) to the E3 complex and this association is facilitated by the Rbx1/Roc1/Hrt1 subunit. The cullin scaffold also binds to Skp1, and Skp1 binds to the F-box protein through a direct interaction with the F-box motif. In the SCF complex, the F-box protein selectively binds phosphorylated proteins to bring them into close proximity to the associated E2 that covalently transfers ubiquitin to the substrate. Poly-ubiquitinated substrates are then subsequently degraded by the 26S proteasome.

Here we present data indicating that the cullin (cul), F-box, and *Skp1*-related (*skr*) gene families have expanded in *C. elegans*. However, in contrast with the relatively ancient expansion of the cullin genes, the *skr* and F-box gene families have expanded more recently and to a greater extent in the *C. elegans* genome. We identified 21 *skr* genes in the *C. elegans* genome compared to a single *SKP1* gene in fission yeast, budding yeast, and humans. Similarly, numerous F-box proteins have been identified primarily based on gene predictions including 11 in budding yeast, 22 in *Drosophila*, at least 38 in humans, and a remarkable 325-350 in *C. elegans*; however, the vast majority have not been characterized. In the context of the current SCF paradigm, any given cullin can form the base to an SCF with unique substrate specificity conferred by the selection of a Skp1 family member and an F-box. The staggering potential for combinatorial interactions between core components is believed to increase the repertoire of substrates that can be recognized by the core complex. We probed potential interactions with cullins using the two-hybrid system and found that multiple *C. elegans* SKRs have the ability to interact with CUL-1 and at least one SKR is able to interact with CUL-6, a close paralog of CUL-1. Thus, there is the potential to generate many distinct SCF and SCF-like complexes from combinatorial SKR/CUL interactions. Finally, using RNAi we have identified roles of the *skr* gene family in regulating multiple cellular processes including restricting cell proliferation, morphogenesis, and the pachytene stage of meiosis. From comparisons of *skr* RNAi phenotypes with *cul-1* mutant and RNAi phenotypes we conclude that the *skr* gene family has *cul-1* dependent and *cul-1* independent functions.

435099. Regulation of the *C. elegans* posterior *Hox* paralogs *nob-1* and *php-3*

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During metazoan embryonic development, regional differences along the anterior-posterior axis are specified by Hox gene products, which are highly conserved homeodomain transcription factors. We are studying how the expression of two of the three C. elegans posterior group Hox genes, nob-1 and php-3, is established in the early embryo. nob-1 and php-3 are separated by only 232bp, with nob-1 upstream, and may have resulted from a gene duplication. A genetic null allele, ct223, does not affect the coding region of either gene but removes about 15kb of sequence upstream of both genes, indicating that this upstream sequence is important for the regulation of both genes. A nob-1::gfp reporter construct rescues ct223 and shows expression in the tail region of the embryo. Expression begins at about 100 cells and is restricted to the ABp and E lineages. Early embryonic expression in the Ep cells and in some of the Abp cells overlaps with that of ceh-13, the C. elegans anterior group Hox gene, while later expression overlaps with that of egl-5, the third C. elegans posterior group Hox gene. We have also constructed two php-3::gfp reporter constructs and are in the process of characterizing them. We have identified C. briggsae nob-1, aligned it with C. elegans nob-1 and analyzed conserved noncoding regions for predicted transcription factor binding sites; we will test the effect of RNAi or mutations of likely candidates on the nob-1 and php-3 gfp reporters. Preliminary results suggest that nob-1 is temporally regulated similarly to ceh-13: both are expressed in the early embryo and required for embryogenesis, while the other three C. elegans Hox genes, egl-5, lin-39, and mab-5 are not required for embryonic survival and are only expressed in later embryos.

435620. A CaM-kinase cascade activates CRE-mediated transcription in neurons of *Caenorhabditis elegans*

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Calcium (Ca^{2+}) signals regulate a diverse set of cellular responses, from proliferation to muscular contraction and neuro-endocrine secretion. The ubiquitous Ca^{2+} sensor, calmodulin (CaM), translates changes in local intracellular Ca^{2+} concentrations into changes in enzyme activities. Among its targets, the Ca^{2+}/CaM-dependent protein kinases I and IV (CaM-Ks) are capable of transducing intraneuronal signals, and these kinases are implicated in neuronal gene regulation that mediates synaptic plasticity in mammals. Recently, the cyclic AMP-response element binding protein (CREB) has been proposed as a target for a CaM-K cascade involving not only CaM-KI or IV, but also an upstream kinase kinase that is also CaM-regulated (CaM-KK).

We previously identified *C.elegans* orthologue of CaM-KI(*cmk-1*), CaM-KK(*ckk-1*) and CREB (*crh-1*) which constituted a signaling cadcade analogous to mammalian system. In order to examine the function of this signaling cascade *in vivo*, we generated transgenic worms carrying p*CRE::GFP* monitoring vector. Whereas, worms carrying only this vector showed weak fluorescence in small number of neurons, transgenic worms expressing either constitutively-active or Ca^{2+}-independent form of *cmk-1* showed enhanced *CRE-GFP* expression. Furthermore, *crh-1*-deficeient worms exhibited extremely reduced GFP fluorescence, suggesting that *crh-1* is a main activator of CRE-genes in *C. elegans.* Introduction Ca^{2+}-independent form of *cmk-1* with T179A mutation did not induce the *CRE::GFP* fluorescence and also *ckk-1*-deficient worm expressing Ca^{2+}-independent form of *cmk-1* did not show the enhanced GFP-expression, indicating that *ckk-1* is absolutely required for *cmk-1*-mediated transcriptional activation through phosphorylation of *crh-1*. These results indicate that CaM-KK/CaM-K/CREB cascade is conserved and operated in the several neurons of *C. elegans* and plays an important role for regulation of Ca^{2+}-dependent gene expression in this organism.

443982. Mis-regualtion of mRNA targets of GLD-1 and tumor formation

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During germ line development, translational control emerges as a heavily utilized mechanism by which gene expression is regulated. The germ line thus provides an excellent model for studying translational regulation. Recently, many RNA binding proteins have been identified as essential components governing germ line development and early embryogenesis. A comprehensive understanding of how these RNA binding proteins control development remains largely unknown. Identification of their RNA targets is a necessary first step. Subsequent work can provide information on the normal function of each target and the consequences of its mis-regulation in the mutant/disease state.

GLD-1 is a germ line specific, maxi-KH motif containing RNA binding protein that acts as a tumor suppressor and regulates multiple aspects of *C. elegans* germ cell development, suggesting that it regulates multiple RNAs. GLD-1 is a member of a family of proteins, including mouse/human Quaking and *Drosophila* How, that share not only a maxi-KH motif but sequences surrounding the maxi-KH motif. At the distal end of *C. elegans* germ line, there are proliferating germ cells that, as they move proximally, initiate meiotic development and then undergo gametogenesis. GLD-1 is abundant in the cytoplasm of early meiotic prophase germ cells but absent in developing oocytes. GLD-1 was proposed to spatially restrict the accumulation of certain proteins by regulating the translation and/or the stability of a subset of maternal mRNAs that function in oocyte differentiation, maturation/ovulation and/or early embryogenesis. The tumorous mutant phenotype could result from mis-regulation of mRNA targets during early meiotic prophase.

We have identified multiple *in vivo* mRNA targets of GLD-1 by their ability to interact with GLD-1 in cytosol extracts. These target mRNAs are preferentially expressed in the germline and as expected, several of them have essential functions in oocyte differentiation, maturation/ovulation and early embryogenesis. Analysis of three mRNA targets (*rme-2, oma-1 and oma-2*), reveals that GLD-1 acts as a translational repressor. Antibody staining of wild-type hermaphrodite germlines show that the corresponding proteins for these three mRNA targets are absent from the distal region where GLD-1 is abundant, while they increase in abundance in growing oocytes in the proximal region where GLD-1 levels fall precipitously. Consistent with GLD-1 functioning as a translational repressor, they prematurely accumulate in the distal region of *gld-1* null hermaphrodites. These data, as well as RNA in situ analysis, imply that GLD-1 is likely acting as a translational repressor for most mRNA targets.

lin-45 RAF was identified as one of the mRNA targets, suggesting that GLD-1 may modulate the activation of RAS/MAP kinase pathway and mis-activation of this pathway may contribute to the tumor formation in *gld-1* mutant animals. Consistent with this idea, we have found that the *gld-1* tumorous phenotype is partly suppressed by loss of *lin-45*, as well as downstream components *mek-2* and *mpk-1*. In addition, MPK-1 is ectopically activated in transition zone and early pachytene stage germ cells in *gld-1* null germlines. This suggests that mis-regulation of the *lin-45* RAF kinase mRNA leads to the mis-activation of MPK-1, which partly contributes to tumor formation in *gld-1* null germlines.

446341. MPK-1 ERK signaling is necessary for male germline sex determination

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MPK-1 ERK signaling is necessary for pachytene progression and oocyte meiotic maturation (Church et al., 1994; Lee et al., 13th International C. elegans Meeting, 2001). Strong loss-of-function/null mutations in *lin-45* RAF, *mek-2* MAPKK and *mpk-1* ERK result in hermaphrodites that fail to produce either sperm or oocytes and instead germline nuclei are arrested in pachytene. In the distal meiotic prophase germline of wild-type animals, germ cells (nucleus with surrounding plasma membranes except on the side facing the central cytoplasmic core) are packed in a hexagonal pattern on the surface of the gonadal tube. In *lin-45*, *mek-2* and *mpk-1* mutants, the hexagonal germ cell packing is disrupted and instead pachytene arrested nuclei and surrounding plasma membranes are found in clumps, often in the center of the gonadal tube. We call this a Pac phenotype, for *pa*chytene arrest and *c*lumped nuclei and membranes. A similar Pac phenotype is observed in mutant males.

Under certain partial loss-of-function conditions, where pachytene progression and oocyte differentiation is normal, we observed hermaphrodites with feminized germlines (proximal germ cells developing as oocytes instead of sperm). The partial loss-of-function conditions were: a) viable escapers from *lin-45* or *mpk-1* RNAi; b) *mek-2(n1859)* (Kornfeld et al., 1995); and c) trans-heterozygotes of *mpk-1* null and *mpk-1(ts)* (Lackner and Kim, 1998) at the permissive temperature. These results raise the possibility that the Pac phenotype observed in males and in the proximal germline of hermaphrodites for *lin-45*, *mek-2* and *mpk-1* strong loss-of-function/null mutants is the result of the combined effect of a sexual fate transformation followed by pachytene arrest during oogenesis. *In situ* hybridization using a probe to *rme-2* mRNA, which encodes the oogenesis specific yolk receptor, was used to test this possibility. In wild-type L4 hermaphrodites, germ cells in the proximal gonad, most of which are in pachytene, are undergoing spermatogenesis and lack *rme-2* mRNA while germ cells in the proximal third of the distal gonad contain *rme-2* mRNA. By contrast, germ cells throughout the proximal gonad and into the distal gonad have *rme-2* mRNA in L4 *mpk-1* null hermaphrodites. In *mpk-1* null males, *rme-2* mRNA is found throughout the region showing the Pac phenotype, unlike in wild-type males. Thus, *mpk-1* is required for the male germ cell fate in both hermaphrodites and males. Additional experiments indicate that *mpk-1* is functioning in the germline, not in the soma, for male germline sex determination and that *mpk-1* activity is required continuously. Epistasis experiments indicate that *mpk-1* is acting downstream or in parallel to *tra-2* in the sex determination pathway, as *tra-2* null; *mpk-1* null double mutant germlines are feminized. *mpk-1* may not be required for male somatic sex determination as sexual transformation in the adult soma (yolk synthesis) has not been observed.

Accumulation of MPK-1 and the appearance of activated MPK-1 is sexually dimorphic. An affinity purified C-terminal fraction of the SC94 (Santa Cruz) antisera is specific for germline MPK-1 as germ cells do not stain in the null mutant. MPK-1 is found at high levels throughout the germline of wild-type adult hermaphrodites. By contrast, in wild-type males, MPK-1 is found at significantly lower levels and only in the distal most germ cells, up to about the distal third of the pachytene region. ERK MAP kinases are activated by phosphorylation on a specific Thr and Tyr motif, which can be detected by the MAPKYT mAb (Sigma; Miller et al., 2000). In hermaphrodites, activated MPK-1 is found at high levels in the 2 to 3 most proximal oocytes and at moderate levels in the proximal half of pachytene. In males, activated MPK-1 is only found at very low levels in the transition zone and the first few pachytene germ cells, a region where activated MPK-1 is not observed in adult hermaphrodites. Thus, MPK-1 is activated sex specifically and to different extents in three spatially distinct regions of the *C. elegans* germline.

455348. Closing in on Cloning *exc*

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We are continuing to map and microinject cosmids in order to clone several *exc* genes. Mutants in these genes exhibit excretory canals that swell into large fluid-filled cysts, rather than maintain the normal narrow tubular shape. Other cloned exc genes (*exc-4*, *exc-5*, *exc-7*, *sma-1*, and *let-653*) encode proteins that regulate the diameter of the excretory canals, and determine the cytoskeletal structure of the apical surface of the canal and other epithelial tissues.

At present we are focusing our efforts on the *exc-1*, *exc-2*, *exc-3*, and *exc-9* genes. The first three of these have been genetically mapped to narrow regions, and microinjection has begun to attempt to rescue the mutant phenotypes. We narrowed the position of *exc-1* on LGX via 3-factor cross between *eat-20* and *lin-15*, a region covered by just 7 cosmids. At present, *exc-1* appears to be rescued by cosmid C02C6, which encodes three novel genes and *dyn-1* (encoding dynamin).

exc-2 is located between *lon-2* and *mec-2* on the left end of LGX a region encompassed by 27 cosmids. We have prepared most of these and have injected several to attempt rescue of this gene. Similarly, *exc-3* maps between *him-4* and *unc-115*, an area encompassed by 14 cosmids. About half of these cosmids have yielded no rescue via microinjection.

Finally, *exc-9* maps on LGIV near *unc-24*. We are using SNP mapping to narrow the position of this gene prior to attempting rescue.

456065. 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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458071. Evolution of sex determination mechanisms in nematodes

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We are studying the evolution of genetic pathways, using sex determination in nematodes as a model. The hallmark of sex determination is that the final result of the pathway is the same throughout evolution: the production of males and females. Detailed genetic and molecular analysis in Drosophila and C. elegans indicate that this pathway evolves very rapidly, making it an excellent model to study how developmental mechanisms and pathways evolve.

Although there is low structural conservation for many sex determination genes in the Rhabditidae familiy, recent studies suggest that the function of some these genes in somatic sex determination is conserved. We are taking a genetic approach to study the sex determination pathway in a nematode of the Diplogasteridae family, Pristionchus pacificus. We would like to know whether in this more distantly related nematode there is conservation in the mode of sex determination. We performed a pilot screen of 1500 gametes and isolated 8 putative transformers. Although the soma is almost completely transformed to a male phenotype (development of spicules, rays and bursa in the tail and an one-armed gonad), using molecular markers we have determined that two of them have a XX genotype. These transformers show no mating behaviour and the germ line may exhibit increased cell death. Both transformers map to P. pacificus linkage group III, which show regions of synteny with C. elegans chromosome III. tra-1, the terminal sex determination gene of C. elegans is located on C. elegans chromosome III. We cloned Ppa-tra-1 and generated a polymorphic marker for this gene. There is strong linkage between the Ppa-tra-1 marker and the two transformers. Further molecular characterization is in progress.

461800. GLD-3, a KH protein and Bicaudal C homolog, antagonizes FBF and controls germline sex determination

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The FBF RNA binding protein controls germline sex determination in the nematode *C. elegans* (Zhang et al. 1997). Normally, XX hermaphrodites make sperm and then oocytes, while XO males make sperm continuously. FBF, a 3UTR-binding repressor in the PUF family, is essential for the hermaphrodite switch from sperm to oocytes. Here we report identification of GLD-3, a protein discovered in a two-hybrid screen for FBF interactors. GLD-3s motif architecture places it in the Bicaudal-C family of RNA binding proteins. It possesses five tandemly repeated, imperfect KH repeats. GLD3 is cytoplasmic and associated with P granules during germline development and in the early embryo. The *gld-3* gene is required maternally for embryogenesis and germline survival and zygotically for spermatogenesis: *gld-3* is critical not only for progression through spermatogenesis, but also for making the correct number of sperm.

Genetically, GLD-3 antagonizes FBF: in *fbf* mutants, sperm are made continuously, whereas in *gld-3* mutants, germ cells switch aberrantly from spermatogenesis to oogenesis. To explore the molecular basis of this antagonism, we have tested FBF binding in a three-hybrid system. Without GLD-3, FBF binds to the *fem-3* PME regulatory element. When GLD-3 is introduced, FBF binding is markedly reduced. Controls indicate that this interference with FBF binding is specific for FBF and for GLD-3. For example, the binding of other PUF proteins to their targets is unaffected by GLD-3. We conclude that GLD-3 antagonizes FBF function and that a balance between FBF and GLD-3 activities is required to control the sperm/oocyte switch and specify the correct number of sperm.

486214. GLD-2 and NOS-3 Act in Opposition to GLP-1/Notch Signaling in Regulating the Accumulation of GLD-1 for Meiotic Development

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The distal ends of both male and hermaphrodite germlines are populated by proliferating stem cells that initiate meiotic development as they move proximally, away from the distal tip cell (DTC). We are interested in how the distal germ cells exit from a mitotic proliferative state to meiosis and differentiation. Previous work has shown that a GLP-1/Notch signaling pathway, with the LAG-2 ligand expressed by the DTC and the GLP-1 receptor expressed by the germ cells, functions to promote proliferation (Austin and Kimble, 1987; Lambie and Kimble, 1991). We have shown genetically that gld-1, encoding a KH domain containing RNA binding protein, promotes entry into meiosis or inhibits proliferation (Francis et al., 1995). Kadyk and Kimble (1998) further demonstrated that gld-1functions redundantly with gld-2 for this role such that loss of the activities of both genes results in a germ line tumor of proliferating cells in males and hermaphrodites, similar to that seen when glp-1 is constitutively activated (Berry et al., 1997). Kadyk and Kimble (1998) also demonstrated that the gld-2(0) gld-1(0) tumor is epistatic to glp-1(0), suggesting that GLP-1/Notch signaling inhibits the activities of gld-1 and gld-2. GLD-1 levels are low in the distal end but increase proximally until reaching maximum levels around 21 cell diameters from the DTC, approximately where germ cells appear to initiate meiotic development. gld-1(oz10gf) single mutants, which have increased GLD-1 accumulation in the distal end, have a smaller proliferative zone, and gld-1(oz10) enhances the weak glp-1(bn18) allele, both supporting that GLD-1 spatial regulation is important in regulating the entry into meiosis decision. Crittenden et al. (2002) have also suggested that GLD-1 levels in the distal end are important for regulating entry into meiosis based on analysis of FBF regulation of GLD-1 accumulation. Three lines of evidence suggest that the GLP-1/Notch signaling pathway is involved in inhibiting GLD-1 accumulation in the distal end. First, in the absence of GLP-1 activity GLD-1 levels are increased in the distal end. The lack of GLP-1 activity normally results in a very small germline, therefore we looked at GLD-1 accumulation in gld-2(0) gld-1(q361) mutants with and without GLP-1 activity (in the gld-1(q361) allele protein is made but is non functional). In gld-2(0) gld-1(q361) animals GLD-1 protein levels are low in the distal end but increase gradually (same as wild-type), however in gld-2(0) gld-1(q361); glp-1(0) animals, GLD-1 accumulation is dramatically increased in the distal end. Second, the rise in GLD-1 level is delayed (more proximal) in animals with increased GLP-1 activity in the distal end. Third, GLD-1 levels are low or absent in animals with tumorous germlines due to constitutively activated GLP-1.

In order to identify other genes that regulate the entry into meiosis decision, possibly through regulating GLD-1 levels, we conducted a genetic screen looking for mutations that are synthetic tumorous with gld-2(0). From this, we identified five loss-of-function mutations in a Drosophila Nanos homologue, nos-3, which was previously identified for its ability to bind FBF (Kraemer et al., 1999). While GLD-1 levels are roughly equivalent to wild-type in single nos-3(0) and gld-2(0) mutants, they are dramatically reduced in gld-2(0); nos-3(0) double mutants suggesting that gld-2 and nos-3 function redundantly to promote GLD-1 accumulation. Therefore gld-2 and nos-3 function in opposition to Glp-1/Notch signaling in regulating GLD-1 accumulation in the distal germline.

488313. MOLECULAR ANALYSIS OF HSP90, A MULTI-FACETED GENE INVOLVED IN THE GROWTH AND DEVELOPMENT OF FREE-LIVING AND PLANT PARASITIC NEMATODES. KELI K. AGAMA*, Lynn K. Carta, and Andrea M. Skantar USDA-ARS Nematology Laboratory, PSI, Beltsville, MD 20705

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Plant parasitic nematodes cause in excess of $100 billion of global crop losses each year. Cyst nematodes (Heterodera spp.) are endoparasitic root-feeding nematodes, and the soybean cyst nematode (SCN), Heterodera glycines, causes substantial losses in soybean yield in the U.S.A. as well as throughout the world. The most successful method for controlling SCN infestation involves the use of resistant cultivars, but because soybean populations are constantly changing, the durability of this strategy may be threatened. Crop rotation may be economically unfavorable to growers, and several nematicides have been or soon will be banned from use. Therefore, an urgent need exists for the development of novel, biologically based control strategies. The developmentally arrested juvenile (J2) stage of SCN comprises a vulnerable point in the nematode life cycle, and the genes that control nematode development in response to environmental changes provide attractive targets for disruption. One such target is the hsp90 gene. In the free-living nematode Caenorhabditis elegans, the hsp90 gene known as daf-21 is involved in the dauer pathway, an alternative developmental pathway that occurs as a result of extreme environmental conditions such as starvation and overcrowding. HSP90 molecular chaperones regulate the correct folding, activation and assembly of specific target proteins that control normal cellular development and metabolism. The objective of this study is to investigate the role of HSP90 in the growth and development of SCN and to determine the effect of HSP90 disruption on nematode development. We are using the yeast two-hybrid system to characterize the interactions between H. glycines HSP90 and other known members of the C. elegans dauer pathway. In addition we are studying interactions between HSP90 and HCH-1, a putative HSP90 co-chaperone that is involved in nematode hatching. Finally, we are also investigating the effects of geldanamycin, a naturally occurring compound that binds to and inhibits HSP90, on the growth and development of C. elegans as a model for later studies on H. glycines

490192. Using RNAi to search for enzyme interactions in the ubiquitination pathway

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Within the cell, protein degradation is primarily accomplished through the ubiquitin-proteasome pathway. The proteasome degrades proteins that are covalently linked with ubiquitin, a 76 amino acid protein that attaches to the terminal amino group of lysine side chains. Ubiquitination occurs via E2 ubiquitin-conjugating enzymes and E3 ubiquitin ligases that work together to attach ubiquitin to a specific protein substrate, thereby condemning the substrate to degradation by the proteasome.

E3 ubiquitin ligases recognize target proteins for ubiquitination. Known E3s contain either a HECT or RING finger domain. Our previous studies identified four RING finger E3s that are required for embryogenesis (IWM 2001, #560). In addition, RNAi of another RING finger E3, C32D5.10, leads to early larval arrest.

Since RING finger E3s must partner with E2 ubiquitin-conjugating enzymes, we reasoned that RNAi of corresponding E2s would phenocopy the RNAi phenotype of essential E3s. For example the RNAi phenotype of the E2 which partners with PAR-2 should exhibit a Par-2 phenotype. We have identified 17 potential E2s within the C. elegans genome. None of the 13 E2s we have tested show considerable embryonic lethality, suggesting that there may be functional overlap. These results are similar to those reported by Jones et.al. (Genome Biology, 2001), who found that few E2s are required for embryogenesis.

In order to examine further the embryonic roles of E2s we are using combinatorial RNAi by feeding. Combined RNAi of putative UBCs F52C6.12 and M7.1 produces an Egl phenotype. Interestingly, RNAi of the putative E2 F52C6.12 produces a larval arrest phenotype similar to that produced by RNAi of the RING finger protein C32D5.10. We hope to explore the possible E2-RING finger interaction between F52C6.12 and C32D5.10 and will continue combinatorial RNAi screening of E2s.

496373. Check out my Profile! Isolation of chemotaxis defective mutants with altered *str-1* expression levels using automated, high-sensitivity fluorescence profiling

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

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

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

503314. The dystroglycan-like protein DGN-1 is required for multiple epithelial and neural functions.

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The vertebrate protein dystroglycan provides a transmembrane linkage between basement membrane components and intracellular signaling and cytoskeletal networks. While research has focused on the function of dystroglycan in muscle, the wide expression of the protein in epithelial and neural tissue suggests important roles in mediating cell-ECM interactions in other cell types. Three predicted genes in *C. elegans* show sequence similarity to dystroglycan. Based on comparison of amino acid sequence and domain organization of the predicted products, the most similar of these is *dgn-1* (LG X, T21B6.1). We have begun to characterize the protein product and biological roles of *dgn-1*.

Analysis of *dgn-1*::GFP reporter constructs reveals expression in numerous epithelial and neural cells throughout embryogenesis. In early larvae, prominent expression occurs in the excretory cell, somatic gonad precursors, epithelial cells of the pharynx and rectum, hypodermis, and ventral cord neurons. In later larvae, expression is retained in the excretory cell, rectal epithelia and ventral cord neurons, and is also seen in the distal tip cells, distal sheath, and spermatheca of the gonad. Immunostaining with specific antisera confirms the promoter expression results and reveals that DGN-1 is concentrated on basement membrane-associated surfaces of expressing cells. Neither GFP reporter studies nor immunostaining showed expression of DGN-1 in muscle, in striking contrast to the prominent muscle function of vertebrate dystroglycan. DGN-1 is not detectably cleaved into separate alpha and beta subunits like the vertebrate protein, and is a glycoprotein that undergoes differential glycosylation throughout development.

A deletion of *dgn-1*, *cg121*, is a molecular null with numerous epithelial and neural defects. Homozygotes are viable but sterile due to the failure of morphogenesis of the somatic gonad, while heterozygotes are fertile with well-organized gonads that show low penetrance migration defects. Disorganization and eversion of the vulval epithelium and multiple vulvae are frequently seen in the mutant. The tubular processes of the excretory cell are often missing or aberrantly short in *cg121* animals. Mutant animals also display a variety of defects in the targeting of commissural axons of motor neurons, although gross impairment in muscle function or movement is not observed.

Homozygous *cg121* is synthetically lethal with loss-of-function alleles of *nid-1*, which encodes the basement membrane component nidogen. Double homozygous mutant animals arrest in embryonic or early larval stages with defects in epithelial integrity, such as detachment of the pharynx from the body wall and extrusion of the excretory cell through the hypodermis. Significant early lethality is also observed in *cg121* heterozygotes that are homozygous for *nid-1* alleles; animals surviving to adulthood display defects in gonad morphology and migration and impaired fecundity. Thus NID-1 and DGN-1 may interact directly or indirectly, possibly through their common binding partner, laminin. Future studies of DGN-1 should help to elucidate the roles of dystroglycan in the organization and attachment of basement membranes and in the morphogenesis and function of neural and epithelial tissues.

506947. Setting up to identify FBF target mRNAs in *C. elegans*

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FBF is an RNA binding protein that is essential for regulation of multiple germline fates. Two nearly identical genes, *fbf-1* and*fbf-2,* code for the largely redundant FBF-1 and FBF-2 proteins. An *fbf-1 fbf-2* double mutant makes only sperm and lacks germline stem cells in late development (Crittenden et al., 2002). To date, only two target mRNAs are well established (Zhang et al., 1997; Crittenden et al., 2002). To identify additional targets, we will use the approach successfully pioneered by Lee and Schedl (2001) to find GLD-1 target mRNAs. Specifically, we will generate transgenic worms expressing epitope-tagged versions of FBF-1 and FBF-2 to co-precipitate FBF associated mRNAs. At the current time, we have obtained rescue of *fbf-1 fbf-2* double mutants with a genomic fragment of *fbf-1* and a distinct genomic fragment bearing *fbf-2.* The line bearing the *fbf*-2 transgene has been stable for over 45 generations. Approximately 510% of the animals are fertile, and remaining animals are sterile. We are currently making the epitope-tagged versions, and will report progress at the meeting.

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512758. Behavioral Effects of laughing gas (nitrous oxide, N_2O) in *C. elegans*

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The molecular mechanism of action of the general anesthetic nitrous oxide (N_2O) is unknown. However, recent results indicate that the N-methyl-D-aspartate (NMDA) glutamate subtype receptor might be a potential target for nitrous oxide. In this study we first wanted to see if N_2O has behavioral effects in *C. elegans* and, if so, were the effects consistent with antagonism of NMDA receptors. Recently, a worm NMDA-receptor knock-out strain *nmr-1(ak4)* was isolated and found to confer a distinctive behavioral phenotype, namely a marked increase in the time spent moving forward and, of course, a decreased amount of reverse movement. To test the effects of nitrous oxide on locomotion, worms were picked to unseeded agar plates and put into gas-tight glass chambers containing either room air or a mix of 70% nitrous oxide/30% oxygen. After a 30-min equilibration, we scored the rates of forward and backward movement during a 7-minute period for each worm. Wild-type worms had an average duration of forward movement of 78.5+/-33.1 s (mean+/-SD) in air and 213.7+/-110.9 s in 70% nitrous oxide, $p < 0.01$. The average rate of reversal during the observation period was 3.2+/-0.5 per minute in air and 0.6+/-0.3 per minute in nitrous oxide. $p < 0.01$. We are currently testing *nmr-1(ak4)*. Unlike for volatile anesthetics, nitrous oxide had no effect on the overall locomotion rate (ie, body bends/min). Our results show that nitrous oxide does produce a behavioral effect in *C. elegans*. Moreover, the effect is similar to that produced by *nmr-1(ak4)*, consistent with an NMDA antagonism mechanism for nitrous oxide. Finally, the different behavioral effects of volatile anesthetics (drugs like ether and halothane) and nitrous oxide suggests that the mechanism of action of these two classes of general anesthetics differ.

514707. 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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517641. ALP/Enigma Proteins Play a Critical Role in Muscle Stabilization in *C. elegans*

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Proper muscle function is absolutely vital and yet our understanding of how the muscle cytoskeleton is organized and how this organization is maintained during contraction is incomplete. Recently, members of the ALP/Enigma family of proteins have been postulated to contribute to muscle stability. In vertebrates, the ALP/Enigma family is comprised of at least 6 proteins which all contain N-terminal PDZ domains and either a single C-terminal LIM domain (ALP) or three tandem LIM domains (Enigma). Four members of this protein family have been shown to interact with alpha-actinin via their PDZ domains and the ALP protein enhances the ability of alpha-actinin to cross-link actin filaments. We hypothesize that the ALP/Enigma family of proteins plays a critical role in stabilizing the muscle cytoskeleton during contraction. In an effort to dissect genetically the roles of this large protein family, we turned to the nematode *Caenorhabditis elegans*. *C. elegans* contains a single gene that encodes both ALP and Enigma isoforms through alternate splicing. We cloned the *C. elegans* ALP/Enigma gene and demonstrated that alterations in the gene result in the *eat-1* mutant phenotype. Furthermore, germline introduction of ALP/Enigma DNA fully rescues the *eat-1* mutants. We have generated GFP-tagged versions of the proteins and have determined that the Enigma protein is localized to all muscle types and more specifically to the dense bodies and to sites of cell-cell contact in body wall muscle. *eat-1* mutants have a noticeable pharyngeal pumping defect, but also display defects in locomotion. In an effort to understand the role of the *eat-1* gene products in body wall muscle, we have begun a high resolution analysis using both immunohistochemistry and TEM. *eat-1* mutants display a range of body wall muscle defects including dense body and muscle cell attachment irregularities. We propose that *eat-1* plays both a role in dense body stability via its alpha-actinin interaction and a role in muscle-muscle cell attachments at the dense plaques in an alpha-actinin independent manner. We postulate that the ALP/Enigma family of proteins plays an essential role at sites of actin-membrane anchorage in muscle. The identification of *C. elegans* mutants provides an excellent opportunity for further analysis of these proteins and their role in muscle stabilization.

524424. Genetic Studies of the Proliferation versus Meiotic Development Decision

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GLP-1 is a member of the Notch family of transmembrane receptors. Expressed in the germline of *C. elegans*, it functions to promote proliferation of germ cells. Spatial regulation of GLP-1 receptor activation is controlled, at least in part, by localizing the transmembrane ligand, LAG-2, to the distal tip cell, which caps the distal end of the germline. Binding of LAG-2 to GLP-1 induces cleavage of the intracellular domain of GLP-1, where the activated GLP-1(INTRA) is presumed to translocate to the nucleus of the germ cell and bind LAG-1, a Notch downstream effector and putative transcriptional regulator. These three components of the GLP-1 pathway work together to produce a population of proliferating germ cells that extends ~20 cell diameters from the distal tip. Disruption of this pathway, through a loss of function *glp-1* mutation, for example, causes nearly all proliferating germ cells to prematurely enter meiosis. Constitutive activation by the *glp-1(oz112 gf)* mutation, conversely, results in the formation of a germline tumor where proliferative cells are found throughout the gonad. Weaker *glp-1(gf)* alleles (eg, ar202) also cause an over-proliferation phenotype, characterized by proximal proliferation and the formation of a late onset tumor (Pepper et al, 13th Int. *C. elegans* Meeting). These results indicate that the activity state of the GLP-1 signaling pathway determines whether germ cells remain in the proliferative state or enter meiotic development.

We have taken a genetic approach to identify genes that regulate or are targets of GLP-1 signaling. A number of genes have been isolated that enhance the *glp-1(gf)* phenotype. *teg-1*, for tumorous enhancer of *glp-1*, enhances a very weak *glp-1(gf)* allele by causing the formation of a late onset tumor. This result suggests that *teg-1* is a negative regulator of *glp-1* signaling (Hansen et al, 2000 Midwest Worm Meeting). However, to date, little is know about the specific function of TEG-1.

In a genetic screen to find enhancers of *teg-1*, we have isolated a *glp-1* double mutant, *oz264oz270*, that forms a tumor with *teg-1*. In a *teg-1(+)* background, the double mutant displays a weak over-proliferation phenotype. Another weak *glp-1 (gf)* allele, *ar202*, also displays a weak over-proliferation phenotype, however it is stronger than that of *glp-1(oz264oz270)*. The *glp-1(oz264)* lesion lies in a residue adjacent to *glp-1(ar202)* (Hubbard et al, personal communication), suggesting that *glp-1(oz264)* may also act as a *glp-1(gf)* mutant by causing some level of constitutive activation. But in the *teg-1(-)* background, the tumor formed with *glp-1(ar202)* is apparently weaker than that with *glp-1(oz264oz270)*. These conflicting results could be explained by the other lesion, *glp-1(oz270)*, acting with both partial loss and partial gain of function characteristics. Experiments are in progress to try to understand the nature of the *glp-1(oz264oz270)* double mutant.

We are also conducting other genetic screens to identify novel interactors in the GLP-1 pathway. One mutation, *oz272*, causes the formation of a germline tumor, but does not behave like *glp-1(gf)* alleles, or the synthetic tumorous alleles previously isolated, suggesting that it is either a mutation in a novel gene, or a new allele in a previously identified gene involved in the proliferation versus meiotic development decision.

547196. HERMAPHRODITE OR FEMALE? THE EVOLUTION OF NEMATODE MATING SYSTEMS

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In animals, sexual traits evolve rapidly. To learn why, we are studying nematodes with two different mating systems. Caenorhabditis elegans and C. briggsae have male and hermaphrodite sexes, whereas C. remanei has male and female sexes. We want to learn why XX animals become self-fertile hermaphrodites in some species, but females in others.

The essential difference between self-fertile hermaphrodites and females is that the former produce sperm and oocytes, but the latter only make ooctytes. In C. elegans, we have shown that FOG-3 and the CPEB protein FOG-1 are required for germ cells to initiate spermatogenesis. To learn how the control of germ cell fate differs among the caenorhabditids, we cloned the homologs of these genes from C. briggsae and C. remanei.

We found that each species has four CPEB genes, just like C. elegans. In all three species, RNA-mediated interference (RNAi) against the fog-1 homolog causes germ cells to become oocytes rather than sperm. The requirement for cpb-1 in early spermatogenesis has been conserved as well, but in C. briggsae, cpb-1 also plays a novel role in the development of the male tail. Thus, the divergence of these proteins pre-dates the origin of the caenorhabditids, but their specialization is still continuing.

All three species have a single fog-3 gene, which is required for germ cells to become sperm rather than oocytes. Since the levels of fog-3 transcripts are highly correlated with spermatogenesis, the control of fog-3 expression could be responsible for determining if XX animals become females or hermaphrodites. How might this work? Experiments with chimeric transgenes show that the fog-3 promoters from all three species can drive expression of fog-3 in C. elegans XX larvae. Furthermore, these promoters each contain multiple binding sites for the sex-determination protein TRA-1A. Thus, we propose that fog-3 controls germ cell fate in all caenorhabditids, and that the activity of TRA-1A is modulated in hermaphrodite species to allow fog-3 expression in XX larvae. Because the fog-1 and fog-3 promoters are interchangeable in C. elegans, this regulation probably affects both genes. The activity of TRA-1A could be modulated by an upstream factor like FOG-2. A single mutation in such a factor could change a male/hermaphrodite species into a male/female one.

550634. Calcium-activated Potassium Channels in C. elegans chemosensory neurons

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Calcium-activated potassium channels are present in the C. elegans nervous system, including many neurons of the nerve ring. Properties of these channels have been reported in a heterologous expression system; however, it was of interest to determine whether channels of similar properties could be recorded from C. elegans neurons.

We recorded single channels in excised patches from the chemosensory neuron AWA, which were labeled with GFP. Neurons were exposed by cutting the worm with a scalpel blade just behind the terminal bulb. Excised patches were formed in conventional manner.

Channels corresponding to BK potassium channels were present in AWA neurons. In patches containing the channels, channels were absent or only activated at high positive potentials (+100 mV). Addition of 100 mM calcium markedly shifted the activation of the channels toward more negative potentials, with channel openings visible at 0 mV and more positive potentials. Mean conductance of these channels was about 65 pS, with a reversal potential of -50 mV.

551586. The tog-1 gene controls male gonadogenesis

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Hermaphrodite gonads have two symmetrical arms, and male gonads have a single asymmetric arm. We have sought genes that regulate early gonadogenesis specifically in one of the two sexes. Here, we describe identification of a locus required for early male gonadogenesis, which we tentatively call 'tog-1'. The tog-1 male gonad develops as an amorphous mass, whereas the tog-1 hermaphrodite gonad has two arms and appears nearly normal. The tog-1 locus maps to chromosome II and encodes a forkhead transcription factor, as described in the abstract by Weiru Chang et al. Our focus has been on understanding early defects in tog-1 mutants and assessing the relationship between tog-1 and tra-1. The somatic gonad is generated from two precursor cells, called Z1 and Z4. In wild-type hermaphrodites, Z1 and Z4 generate daughters of roughly equal size that remain in the positions of their birth. In wild-type males, Z1 and Z4 generate daughters of different size that become rearranged shortly after their birth. We have examined the early Z1/Z4 divisions and migrations in tog-1 and tra-1 single mutants as well as tog-1 tra-1 double mutants. We find that tog-1 gene is required for the male-specific Z1/Z4 lineage, but not for the hermaphrodite-specific lineage; we also find that tra-1 is critical for the hermaphrodite pattern. The double mutant results are preliminary and will be discussed at the meeting. We conclude that tog-1 controls the male-specific gonadal lineage.

555330. Yet Another Germline Helicase

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RNA helicase A (RHA) is conserved in many organisms and has numerous cellular functions. Human RHA is required for RNA export from the nucleus (Tang *et al.* (1999) *MCB 19,* 3540) and the *Drosophila* homolog, MLE, is required for dosage compensation in male flies (Kuroda *et al.* (1991) *Cell 66,* 935). We are characterizing *tm329*, a strain containing a deletion in *rha-1*, the *C. elegans* homolog of RHA. These worms develop normally between 17-20°C, but above 23°C hermaphrodites accumulate oocytes in the uterus or have atrophied gonads (small, few germ cells). In males, sperm development occurs at lower temperatures but appears to be defective at 25°C. Double-mutant worms, *gld-1 (q266); rha-1 (tm329)*, were constructed since human RHA and the human homologue of GLD-1 (Sam68) interact (Reddy *et al.* (2000) *Oncogene 19,* 3570), but no epistasis or enhancement was observed in hermaphrodites between 17-25 °C. We are continuing experiments to identify the relationship between RHA-1 and other known germline and spermatogenesis proteins. We are grateful to the *C. elegans* Gene Knockout Consortium for the *tm329* strain and to T. Schedl for the *rha-1 (q266)* strain and for helpful advice.

580840. DAF-21 (HSP90) function is required for germline development in *Caenorhabditis elegans*

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The *daf-21 (p673)* gene has been reported to encode the HSP90 homologue in *C. elegans* (Birnby DA et al., 2000). To study DAF-21 functions in *C. elegans* development, we used three different approaches of immunohistology, transgenic examination, and loss-of-function analysis. Immunohistological studies showed that DAF-21 was distributed in germ cells throughout the life cycle at 20. When worms were heat-shocked at 32 for two hours, in addition to the distribution in germ cells, DAF-21 was also localized distinctly in the perinuclear region of somatic cells. To define further the role of DAF-21 in germline development, we performed two different types of gloss-of-functionh analyses by using the RNAi method and the mutant Daf-21 (p673).

The RNAi experiment revealed several phenotypes in F1 animals born of the *daf-21* dsRNA-injected parental animal, which showed reduced fertility. Some F1 showed embryonic lethality and growth arrest at L1 and L2 stages, whereas some others that grew to be adults displayed defects in germline development, such as egg-lying abnormality, small brood size, and sterility. Using sterile F1 animals, we performed paternal rescue experiments by crossing them with wild-type males, and observed them to produce very few eggs. These results indicate that oocyte production in the F1 animals was suppressed or caused to be defective by *daf-21* dsRNA, suggesting an essential role of DAF-21 for *C. elegans* oogenesis.

In another loss-of-function study with *daf-21 (p673)*, we again performed paternal rescue of its reduced brood size by crossing the daf-21 hermaphrodites with wild-type males and found that the reduced brood size was not rescued. Considering this together with the RNAi results, we interpret that DAF-21 plays a critical role in *C. elegans* germline development.

To examine the roles of two 5f-UTR in the *daf-21* promoter region, we constructed transgenic animals wherein a GFP reporter gene was fused with various sites in the *daf-21* promoter region. The observation of the DAF-21 expression indicated that the second 5f-UTR is indispensable for both normal constitutive and heat-induced expression.

582781. General anesthetic binding sites in *C. elegans*: Presynaptic SNARE proteins

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The formation of a ternary complex (SNARE complex) by three presynaptic SNARE proteins - syntaxin, SNAP-25 and VAMP - is a fundamental requirement for neurotransmitter release. Its formation is thought to overcome the repulsive forces between the membranes of the presynaptic nerve terminal and the synaptic vesicle, thereby facilitating membrane fusion. Based on our previous genetic work in *C. elegans*, which found that a neomorphic mutation *unc-64(md130)* (*unc-64* codes for the *C. elegans* neuronal syntaxin) caused high-level resistance to volatile anesthetics (PNAS 96: 2479,'99), we hypothesized that a single SNARE protein or the ternary SNARE complex are molecular targets for volatile anesthetics. To test this hypothesis, we produced recombinant SNARE proteins and the SNARE complex and measured their abilities to bind volatile anesthetics by ^{19}F-NMR. The NMR technique makes use of the fact that when small molecules (the volatile anesthetic) bind to bigger ones (the protein) the movement of the smaller molecule is restricted and can be measured by a drop in what is called the transverse relaxation rate (T2) of the smaller molecule. As measured by a marked drop in T2, the SNARE complex bound volatile anesthetics saturably at clinical concentrations as did SNAP-25 multimers. Syntaxin appears to bind volatile anesthetics in a non-dose-dependent manner over a wide concentration range (0.06 - 6.0 mM). VAMP and monomeric SNAP-25 did not bind volatile anesthetics. To examine if two different volatile anesthetics compete for similar binding sites on syntaxin, we incubated syntaxin at different concentrations of halothane and isoflurane. Addition of isoflurane markedly increased the T2 time of halothane and vice versa, indicating competition between the two anesthetics. Our results show that volatile anesthetics bind to two presynaptic SNARE proteins (syntaxin and SNAP-25 multimers) and the SNARE complex. These proteins share the common structural feature of a 4-alpha-helical bundle, a synthetic form of which has been shown to bind volatile anesthetics. Based on our genetic and biochemical results, SNARE proteins and ternary SNARE complex are plausible presynaptic targets for volatile anesthetic action.

611595. Analyzing MUA-1 function by altering its expression patterns

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The *C. elegans* gene *mua-1* encodes a member of the xKLF family of transcriptional regulatory proteins and plays a crucial role in maintaining cell attachments. In the nematode, as in other organisms, cell matrix attachments are essential for the development of body morphology, the maintenance of tissue integrity and organ positioning, and the transmission of force across tissue boundaries. Mutations in *mua-1* disrupt two classes of attachments: those involved in the transmission of muscle contractions to the cuticle and those that attach the uterus to the body wall, resulting in paralysis of movement and prolapse of the uterus through the vulval opening. Although *mua-1*mutants appear essentially wild type as L1 larvae, progressive detachment of the body wall muscles from each other as well as from the cuticle is seen in later developmental stages. All animals homozygous for strong alleles die prior to adulthood as do most weak allele homozygotes. Adult escapers commonly have an everted uterus. A *mua-1*::GFP fusion gene shows that *mua-1* is expressed in the uterus, anterior pharynx and hypodermis (excepting the seam cells). In the uterus, *mua-1* expression is seen in the uv1, uv2, uv3 and the utse cells. Utse is directly involved in attachment of the uterus to the seam cells of the hypodermis. To determine whether prolapse of the uterus in *mua-1* mutants is due to loss of MUA-1 in the hypodermal or the uterine cells, we are driving MUA-1 expression using the *dpy-18* hypodermal specific promoter in*mua-1* (*null*) animals. In addition, we are also driving *mua-1* expression with the inducible hsp-16-2 promoter in these same animals to investigate the effects of temporally varying the time of expression. Animals in which this latter construct is expressed are also being examined for possible ectopic or overexpression phenotypes caused by inappropriate *mua-1* expression in tissues that do not usually express it. We expect that these studies will allow us to experimentally separate the hypodermal and uterine phenotypes of *mua-1*, with the eventual goal of using genetics to define other genes specifically involved in each of these separate aspects of *mua-1* function.

624538. Two GLH interactors, critical for meiosis, are not necessary for P granule assembly.

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Using two-hybrid screens with the GLH proteins as bait, we have identified several GLH-interacting proteins. Their physical interactions with GLH proteins have also been shown by pull-down assays. For two of these, CSN-5 and KGB-1, loss of function results in complete sterility. CSN-5 encodes the subunit 5 of the COP-9 signalosome, a protein complex conserved from plants to man that has been implicated in many cellular functions, including protein degradation. KGB-1, a kinase that GLHs bind, is a predicted MAP serine/threonine kinase in the JNK sub-family. With the loss of either CSN-5 or KBG-1 there is a lack of mature oocytes, fertilized eggs and embryos, with sperm produced. Their phenotypes also differ. *csn-5(RNAi)* results in little or no oogenesis, severe reductions in mitotic germ cells and tiny gonads, a phenotype essentially identical to that of the combined loss of *glh-1* and *glh-4* (Kuznicki et al. Development 2000 127:2907-16). For the *kgb-1(um3)* deletion strain, a presumed null, the sterility is temperature sensitive; at 25-26C oocytes undergo endomitosis, an Emo phenotype, while mitotic germ cells and pachytene nuclei are not reduced and gonads are of normal size. Anti-CSN-5 antibodies reveal CSN-5 protein is found in all cells of the worm, in the cytoplasm and concentrated in the nucleus; thus it may be surprising that *csn-5(RNAi)* appears to affect only germline function. Like the loss of any of the *glhs*, when either *csn-5* or *kgb-1* is reduced or missing, P granules still assemble; however, P granules are missing (and therefore unable to carry out their unknown function) in the proximal gonad. The genetic relationships between the GLH proteins and these binding partners are being pursued with combinatorial RNAi and with the construction of double mutant strains.

626415. Suppressors of *gon-1*

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The *gon-1* gene is required for distal tip cell (DTC) migration and gonadal morphogenesis. *gon-1*encodes a secreted metalloprotease of the ADAMTS family (Blelloch and Kimble, 1999). Previous work identified an allelic series of *gon-1* alleles, although all alleles cause sterility (Blelloch et al., 1999). We are concentrating on the null allele, *gon-1(q518),* and a weaker allele, *gon-1(e1254)*. To identify targets or regulators of *gon-1*, we have conducted suppressor screens for mutations permitting DTC migration in the absence of GON-1. As a pilot screen, GFP was employed under control of the *lag-2*promoter as a DTC marker. Specifically, we mutagenized a balanced*gon-1(q518); lag-2::GFP* strain and scored F2/F3 progeny for DTC migration. From 660 F1 animals, two mutations were found that suppressed a *gon-1(0)* mutant to fertility. Linkage tests are in progress. Given the finding that fertile suppressors could be obtained, we have used the COPAS Biosort (Union Biometrica) to isolate 25,000 mutagenized F1 *gon-1(e1254)* animals and 50,000 F1/F2/F3 *gon-1(q518)* animals. These were plated in pools of 100-400 animals and screened for fertility. So far, 140 individual suppressors have been isolated. Progress with these screens will be reported.

Blelloch and Kimble (1999) Control of organ shape by a secreted metalloprotease in the nematode *Caenorhabditis elegans. Nature* 399(6736):586-90.

Blelloch et al (1999) The *gon-1* gene is required for gonadal morphogenesis in *Caenorhabditis elegans*. *Dev. Biol*. 216(1):382-93.

635045. Pharmacological Analysis of Aging in *C. elegans*

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Several pharmacological compounds have been reported to extend lifespan in *C. elegans* and/or *Drosophila*. The identification of additional pharmacological compounds that can extend *C. elegans* lifespan may provide insights into the mechanisms that regulate aging, and such compounds represent potential therapies for age-related illnesses such as Alzheimer's disease. We have initiated a screen for pharmacological compounds with known mechanisms of action that extend lifespan in *C. elegans*. Compounds are dissolved in NGM at a variety of concentrations, and the lifespan of worms raised on dishes containing the compound is compared to worms raised on untreated NGM. After identifying drugs that positively influence life span, we will determine the optimum dosage and time of administration. In addition, we will examine currently available *C. elegans* strains with mutations in the pathway(s) affected by each drug to determine if these mutations also affect aging.

The temporal and spatial control of gene expression is an important technique for analyzing development and aging. Inducible systems that rely on pharmacological compounds to increase transcription of target genes have been developed in vertebrates and *Drosophila*. These include systems that use ecdysone analogs to induce the activity of the ecdysone receptor, estrogen to induce the activity of the estrogen receptor, mifepristone to induce the activity of a modified progesterone receptor, rapamycin analogs to dimerize DNA binding and activation domains, or tetracycline to inhibit the association between the tetracycline repressor and its operator. We have begun to modify several of these systems for use in *C. elegans*.

644614. Student Directed *C. elegans* Research in the Physiology Laboratory

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Faced with task of designing a semester's worth of comparative physiology laboratory exercises in my first semester as an assistant professor, I turned to my research and found what I had always suspected: worms are as wonderful in the teaching laboratory as they are in the research laboratory. As a supplement to several traditional physiology laboratory exercises, this past Spring semester, I required my physiology students to design and implement physiology research projects involving *C. elegans*. Although I tended to guide the student's projects as little as possible, the level of instructor involvement can be quite variable. By requiring students to write a research proposal prior to initiating their experiments, they can be directed towards realistic projects likely to succeed. The only stipulations were that 1. the research investigate some area of physiology discussed in lecture and 2. they not reproduce published data or experiments. At the first laboratory meeting, I introduced *C. elegans* as a research organism and asked them to consider their favorite topic from lecture. From this topic, I guided them towards the relevant *C. elegans*literature and ultimately to a research plan. Prior to spring break, the students had solidified a research plan that included a hypothesis, a detailed experimental design, and identification of the relevant controls. This way, during the break, I was able to gather the required reagents and supplies. As the groups begin their experimentation, it is important to regularly check their progress in case experimental design troubleshooting is required. Ultimately, the students were responsible for initiating the experiments, limited troubleshooting, data collection, and assembly of the data into a research paper. I required that the research papers adhere to a specific journal format, typically one that is readily available for examples such as *Current Biology*. In this way, they experience the many steps in the process of converting great research ideas into meaningful publications. In the future, I plan to incorporate peer review into the process by having their papers reviewed by their student peers and other biology faculty prior to completion of the project. At the end of the semester, I required the students to present their hypothesis, experimental design, data, and conclusions to their colleagues in order to gain experience in oral research presentations and expose all the students to the various research projects. At the meeting, I will present the data collected by four of my undergraduate research teams who investigated many areas of physiology including aging, chemosensory adaptation, neurotransmitters, and serotonin modulated behavior.

652371. A novel plant-like nematode chitinase potentially involved in hatching, molting, or fungal resistance

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The early development of the parasitic nematode, *Ascaris suum*, occurs within a highly resistant chitinous eggshell, but little is known about the chitinase
responsible for hatching. Using 2D gel electrophoresis and NH2 terminal sequencing, an abundant chitinase (As-p50) has been identified in the perivitelline fluid surrounding infective larva prior to hatching. A cDNA encoding As-p50 was cloned, sequenced and the protein expressed in *Escherichia coli*. As-p50 is a member of family 19 glycosyl hydrolases, previously identified only in plants and a few bacteria, making the characterization of As-p50 the first family19 glycosyl hydrolase from any animal species. Homology modeling indicates that two glutamate residues (Glu119 and Glu 151) in As-p50 are spatially aligned with the two conserved glutamate signature residues in the *Hordeum vulgare* structure, confirming the assignment of As-p50 to family19. As-p50 statement was developmentally regulated. As-p50 mRNA appeared between days 5 and 8 of early embryonic development prior to the formation of the first-stage larva (L1). As-p50 protein and its associated chitinase activity began to appear between days 8 and 15 of embryonic development and remained at near constant levels until hatching. To further characterize the statement and secretion of these novel chitinases, GFP-putative promoter constructs of C08B6.4, the most closely related *Caenorhabditis elegans* As-p50 homologue, were transiently expressed in *C. elegans*. C08B6.4 was expressed in hypodermal cells of three-fold stage larva and hatched L1s with a timing similar to that of As-p50 and the GFP fusion protein appeared to be secreted into the space between the hypodermis and the cuticle. C08B6.4 also was expressed in four anterior amphidial neurons in all stages and the GFP-fusion protein appeared to be secreted and accumulated within the pharynx. Taken together, these results suggest that this novel chitinase may be involved in the formation of the L1 cuticle and/or the initial molt. However, it may be multifunctional and also responsible for the digestion of the eggshell during hatching, or involved in fungal resistance, as observed for its plant homologues.

657420. The C. elegans Knockout Consortium: Interim Report 2002

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

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

In the past three years, the consortium has received 1437 requests for targeted gene disruptions. From this request list, consortium labs have eliminated the function of approximately 500 genes using a chemical mutagenesis approach [as of April, 2002]. All mutants and data provided by consortium laboratories are in the public domain and freely available to all researchers.

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

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

671655. Membrane Localization and Serine Phosphorylation Might Regulate the Activity of the Actin-Binding Protein UNC-115 During Neuronal Morphogenesis

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UNC-115 is an actin-binding protein that acts in axon pathfinding : UNC-115 has three N-terminal LIM domains and a C-terminal actin-binding villin headpiece domain (VHD), and *unc-115* mutants display axon pathfinding defects. Loss-of-function studies and epistasis experiments indicate that UNC-115 acts downstream of the RAC-2 Rac GTPase in axon pathfinding and neuronal morphogenesis.

We have begun to characterize the mechanism of UNC-115 regulation by RAC-2 . RAC-2, like all GTPases, contains a membrane-targeting CAAX motif and is membrane-localized. Possibly, UNC-115 is activated by translocation to the plasma membrane from the cytoplasm in response to RAC-2 signal. To test this idea, we constructed an *unc-115* transgene that encodes full-length UNC-115 with a membrane targeting myristylation (myr) sequence at the N-terminus. MYR::UNC-115::GFP was relocalized to the plasma membrane and caused striking dominant defects in neurons, including ectopic axon and dentrite branching and lamellipodia-like membrane ruffling. These effects resembled the those of constitutively active RAC-2, consistent with the idea that RAC-2 signaling recruits UNC-115 to the plasma membrane where UNC-115 is then activated.

There is a conserved serine phosphorylation site in the VHD of UNC-115. To investigate the role of phosphorylation at this site in the regulation of UNC-115 function, we changed the Ser to Ala or Asp to mimic dephosphorylated state and constitutively phosphorylated state respectively. MYR::UNC-115(S617A) caused more severe neuronal defects than MYR::UNC-115 did, while MYR::UNC-115 (S617D) caused less severe neuronal defects than MYR::UNC-115 did. These results suggest that phosphorylation at Ser617 might repress UNC-115 activity.

To determine the importance of actin-binding in UNC-115 function, we constructed transgenes consisting of *myr::unc-115* or *myr::unc-115(S617A*) with point mutations that eliminate actin-binding activity of the VHD. These mutant transgenes caused less severe defects than both MYR::UNC-115 and MYR::UNC-115(S617A) did, suggesting that actin-binding is necessary for the morphogenetic defects caused by MYR::UNC-115 and MYR::UNC-115(S617A) in neurons.

684244. Identification of *C. elegans rab-8* as an essential gene involved in vulval development

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The conserved Ras signaling pathway regulates multiple cell fates during *Caenorhabditis elegans* development. Genetic analyses suggest that a major function of this pathway during vulval development is to negatively regulate *lin-1*. *lin-1* encodes a 441 amino acid protein with a C-terminal ETS domain and N-terminal docking sites and phosphorylation sites for ERK MAP kinase. ETS-domain proteins typically function as transcription factors and are conserved mediators of Ras signaling. Many genes have been identified that act upstream of *lin-1*, however little is known about genes that act downstream of *lin-1* during vulval development. To identify genes that interact with *lin-1*, we screened for suppressors of the Muv phenotype caused by *lin-1 (n383)*, a non-null but nonetheless strong loss-of-function allele. After screening about 20,000 haploid genomes, we identified 14 suppressor mutations. One mutation caused fully penetrant recessive lethality; mutants display a rigid, rod-like morphology. The same phenotype is caused by mutations that completely inactivate the Ras pathway, indicating that the gene identified by this mutation may play a crucial role in Ras signaling during early development. Animals that are heterozygous for this mutation display partial suppression of the Muv phenotypes caused by *let-60 (n1046)*, an activating mutation in the *C. elegans* Ras gene, and *lin-1(e1275)*, a weak loss-of-function allele.

This mutation was mapped using genetic and polymorphic markers to a small region in the center of chromosome I. Transformation rescue experiments have identified a minimal construct that contains only one predicted open reading frame and is sufficient for rescue of the lethal phenotype; this construct also causes a partially penetrant Muv phenotype. This open reading frame encodes the *C. elegans* homologue of the Ras-like molecule, Rab-8. We have not yet identified the molecular lesion associated with the mutation, and thus, the gene assignment remains tentative.

Rab proteins are small GTPases that regulate vesicular transport during exocytosis and endocytosis, and Rab-8 proteins are implicated in transport between the trans-Golgi network and the plasma membrane. Rab-8 proteins have not been implicated in the regulation of Ras signaling or vulval development.

710701. A role for the Polycomb Group in the development of the *C. elegans* male nervous system

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Development of the *C. elegans* male-specific nervous system depends on interplay between two regulatory pathways, one involving the *Hox* genes *mab-5* and *egl-5*, and the other involving the conserved sexual regulator *mab-3*. Males harboring *Hox* or *mab-3* mutations lack V rays, sensory structures required for mating. The *Hox* proteins activate the bHLH gene *lin-32* to specify the V rays, while the *doublesex* homolog MAB-3 synergizes with the *Hox*/*lin-32* pathway to promote V ray differentiation. V rays can be restored in *mab-3(-)* males by overexpression of *lin-32*, suggesting that LIN-32 acts as a primary determinant of V ray fate, while MAB-3 potentiates LIN-32 activity.

To better understand the mechanism by which the *mab-3* and *Hox/lin-32* pathways converge to direct V ray development, we performed a screen to identify mutations that suppress the Mab-3 V ray defect. Three mutations identified in this screen are alleles of *mes-3*, a novel gene that functions with Polycomb group (PcG) homologs *mes-2* (*enhancer of zeste*) and *mes-6* (*extra sex combs*) to maintain silencing in the *C. elegans* germline.

In flies and vertebrates, the PcG of chromatin-interacting proteins maintains domains of *Hox* repression during development. To determine whether MES proteins also regulate *Hox* expression in the *C. elegans* male soma, we tested whether *mes* mutations interact with mutations in the *Hox/lin-32* pathway. Mutations in *mes-2*, *mes-3*, and *mes-6* suppress the ray defect caused by a regulatory mutation in the *mab-5* activator *pal-1*, but do not suppress the *mab-5(0)* ray defect. *mes-2*, *mes-3*, and *mes-6* thus act upstream of the *Hox* gene *mab-5*.

mes-2, *mes-3*, and *mes-6* mutant males display ectopic V rays and occasional V ray fusions, providing further evidence that the MES proteins modulate *Hox* expression in the male soma. Consistent with this hypothesis, the expression domain of the *Hox* target *lin-32::GFP* is expanded anteriorly in *mes* mutant males. Experiments to examine *Hox* expression in *mes* mutants are in progress. The ectopic V rays and ray fusions of Mes males do not require wild-type *mab-3*, suggesting that *mes* mutations suppress *mab-3* by increasing *Hox/lin-32* activity. Thus in nematodes, as in other phyla, the PcG functions in somatic cells to regulate *Hox* gene activity.

720432. Examining the Role of Candidate Calmodulins in the Nematode *Caenorhabditis elegans*.

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Several observations suggest that calmodulin plays an important regulatory role in cytokinesis. Calmodulin has been localized to the cleavage furrow and/or mitotic apparatus in different organisms and inhibition of calmodulin has been shown to disrupt cytokinesis and DNA segregation in some systems. Further, calmodulin is a known component of many calcium dependent pathways.

Our work has focused on identifying a possible role for calmodulin in regulating cytokinesis in *C. elegans*. There are several candidate calmodulins in the *C. elegans* genome, the most conserved of which is CMD-1. Surprisingly, RNA mediated interference (RNAi) on *cmd-1* did not give rise to cytokinesis or any other early embryonic defects, although a dramatic embryonic arrest phenotype at the time of morphogenesis was seen. A second candidate, CAL-1 (for calmodulin-like) is also well conserved but is longer than typical calmodulins by 13 amino acids. Inhibiting expression of *cal-1* by RNAi resulted in low penetrant early embryonic defects including defects in DNA segregation, furrowing and possibly polarity. RNAi-mediated inhibition of other calmodulin-like genes gave no obvious phenotypes. It therefore appears that the while the prime candidate calmodulin CMD-1 does not pay a role in *C. elegans* cytokinesis, a less conserved candidate, CAL-1 may be required for execution of several aspects of cell division in the early embryo.

725004. EGL-27 MTA-interacting proteins are components of the CCR4-NOT complex and synMuv B pathway

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Mutations in *egl-27* cause defects in cell migrations, cell fate specification, cell fusion and morphogenesis. The amino terminal region of EGL-27 is similiar to a human metastasis-associated factor (MTA), recently identified as a co-purifying member of a human complex having ATP-dependent nucleosome-remodeling and histone deacetylase (NURD) function. Other *C. elegans* homologs of human NURD components--specifically, *let-418* Mi-2, *lin-53* RbAp48 and *hda-1* HDAC--have been shown to act as synthetic multivulva (synMuv) class B genes. The synMuv class B genes function redundantly with synMuv class A genes to antagonize Ras signaling-mediated vulval induction in the vulval precursor cells. Several class B synMuv members have been identified, including those similar to the human tumor suppressor, pRB, and its human binding partners, DP and E2F. Despite a ubiquitous nuclear expression pattern that overlaps with other NURD and synMuv B members, the apparently obscure role of EGL-27 MTA in vulval induction suggests a lack of functional conservation with its human counterpart. In an effort to better understand the function of EGL-27, a two-hybrid screen was performed in yeast (two-hybrid library kindly provided by Robert Barstead).

Multiple library clones were retrieved that corresponded to the *mep-1* locus and two genes having similarity to components of the highly conserved CCR4-NOT complex, *Cenot-2* and *Cenot-3*. MEP-1 is a large C2H2 zinc-finger protein with two potential homologs in *Drosophila*. *Ce*NOT-2 and *Ce*NOT-3 contain a previously unidentified domain of similarity located near their respective carboxy termini. Bacterially-expressed GST::EGL-27 interacts *in vitro* with *Ce*NOT-2 and *Ce*NOT-3 fragments corresponding to the two-hybrid library clones, thus verifying the two-hybrid interactions.

mep-1 and *Cenot-2* give an identical RNAi phenotype. P0 eggs layed 18 hours post-dsRNA injection hatch and arrest as L1 larvae. A reduced-function RNAi phenotype was achieved by analyzing the development of eggs layed as early as 4 hours post-injection. These latter *mep-1* and *Cenot-2* RNAi animals display sterility (Ste) and everted vulvae (Evl) at high penetrance, and a low incidence of single and multiple pseudovulvae. We obtained a *mep-1* deletion allele, *ok421*, from the *C. elegans* Gene Knockout Consortium. Homozygous *ok421* worms derived from a heterozygous mother approximately recapitulate the *mep-1* reduced-function RNAi phenotype. *Cenot-3(RNAi)* causes embryonic lethality. The reduced-function *Cenot-3* RNAi phenotype is a low penetrance of ectopic vulval induction and morphologically abnormal animals that are reminiscent of *egl-27* alleles, *mn585* and *ok151,* that affect the function of all three *egl-27* transcripts.

The Ste, Evl, Muv and Lethal character of *mep-1* and *Cenot-2* mirrors the descriptions of other synMuv B genes. Thus, *mep-1*, *Cenot-2* and *Cenot-3* were assayed for synthetic enhancement of ectopic vulval induction in synMuv A genetic backgrounds. *mep-1(ok421)*; *synMuvA* double mutants are synergistically Muv. Likewise, reduced function *mep-1* RNAi using synMuv A mothers results in a highly penetrant Muv phenotype. *Cenot-2* RNAi in synMuv A mothers yields identical results. This suggests that *mep-1* and *Cenot-2* are synMuv B genes. However, *Cenot-3* does not display a synMuv B phenotype.

That EGL-27 MTA physically interacts with at least two synMuv B proteins supports the hypothesis that, like other NURD components, it has a conserved function in *C. elegans*. Our results suggest that EGL-27 MTA is a molecular link between CCR4-NOT complexes, NURD complexes and MEP-1 proteins. Recent studies in yeast indicate that the CCR4-NOT complex regulates mRNA degradation and transcription via a deadenylase activity in the cytoplasm and physical interaction with transcription factors in the nucleus. A simple model for the function of MEP-1 and these complexes during vulval induction is that they cooperate with LIN-35 Rb and its binding partners to antagonize Ras signaling by both degrading transcripts and repressing the transcription of genes necessary for vulval cell fates.

732787. 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.

751934. Characterizing PKD-2 of *C. elegans* and Vertebrates

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We are studying the *C. elegans* homologue of the human PKD2 gene, which has been characterized as a relatively nonspecific divalent cation channel located in the endoplasmic reticulum of ciliated neurons. The *C. elegans* PKD-2 protein regulates neural function in the male reproductive system. Mutant males exhibit impaired abilities to recognize hermaphrodites and to mate. We are currently determining whether the cloned human PKD2 cDNA (graciously supplied by the Somlo laboratory) is capable of rescuing this mutant phenotype. We have also cloned the nematode *pkd-2* cDNA, and are attempting to place it into both human kidney cell (HEK293) and insect cell (Sf21) lines, in order to determine if the single-channel electrophysiological properties of this protein are similar to those of the human protein. The vertebrate PKD2 proteins share a common core region with the *C. elegans* PKD-2 protein, but in addition have an EF-hand region in the C-terminus that is expected to alter the Calcium conductance of this protein. The nematode protein has no such EF-hand, but does contain an acidic region that could serve a similar function.

Interestingly, nematode *pkd-2* cloned into an overexpressing plasmid was lethal to *E. coli*, unless expression was downregulated by growing the bacteria in the presence of glucose and the absence of IPTG (overexpression was controlled by the *lac* promoter). The colonies obtained when the gene was overexpressed in bacteria all contained deletions or multiple point mutations in the *pkd-2* gene. We are investigating whether this lethality arose through excess Calcium conductance expressed in these cells. If this is the case, a screen for bacterial revertants could be used to indicate which areas of the PKD-2 protein are essential for proper gating and conductance of this channel.

We are also attempting to rescue the nematode *pkd-2* mutant with the human PKD2 gene, and investigating whether any fluorescent Calcium indicators can be used to visualize activity of the PKD-2 protein in *C. elegans*.

755093. QTL Mapping for Longevity: Segmental Association with Stress Resistance Phenotypes in *C. elegans*.

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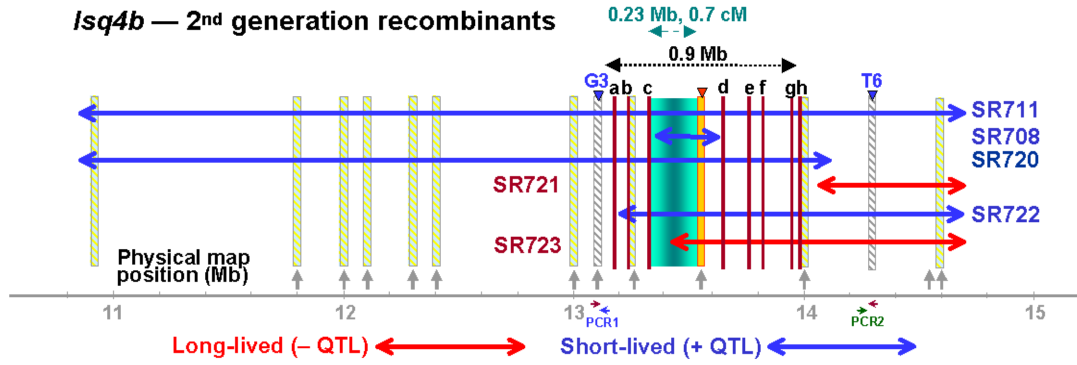
We performed QTL mapping for loci governing *C. elegans* longevity, in 4 interstrain crosses (Bristol-N2, RC301, and CL2a ´Bergerac-BO, and Bristol-N2´DH424) ¾ revealing 4- 11 significant QTLs each, and a total of 13 life-span loci (each $p<0.01$; most LOD scores >8). Four of these longevity QTLs were backcrossed 20 generations to introgress the Bergerac-BO allele into the other parental background. Recombinants arising in the last two generations were rendered homozygous for the introgressed region and were tested for longevity and stress resistance. Each QTL showed retention of the longevity phenotype -- conferring allelic effects on median life-span of 1.5-3.5 days at 20° C; three of these were also backcrossed 3 generations in the opposite direction, with similar allelic trait effects. Each QTL tested also produced marked allelic differences for two or three of the four stress-response traits tested: resistance to thermal stress, hydrogen peroxide, paraquat, or ultraviolet irradiation. Of these stresses, thermotolerance correlated best with longevity ($r = 0.91$), followed by peroxide resistance ($r= 0.72$), paraquat resistance ($r= 0.67$) and UV resistance ($r= 0.19$). The QTL on chromosome IV, *lsq4b,* is associated with thermotolerance and paraquat resistance (each showing a 1.7-fold allelic effect on median survival), as well as longevity (1.27-fold effect on median life span) -- all traits mapping within the same 0.9-Mb region -- whereas this locus affects peroxide tolerance only weakly, and UV resistance not at all. Segmental stress-resistance, as observed, contradicts the "general stress resistance" phenotype proposed previously for several long-lived mutants.[1] The longevity QTL has now been narrowed to 0.23 Mb, positioned on the physical map by a combination of Anchor-PCR Display[2] and SNP typing; it comprises 22 known protein-coding genes, 6 snRNA genes, and 19 predicted genes. Further partitioning of this QTL interval is underway.

[1]Morris, Tissenbaum & Ruvkun, *Nature* **382**:536-539, 1996; Lin, Seroude & Benzer, *Science* **282**:943-946, 1998; Lithgow *et al., Proc. Natl. Acad. Sci. U.S.A*. **92**: 7540-7544, 1998; Cypser & Johnson, *Neurobiol. Aging* **20**:503-512, 1999; Honda & Honda, *FASEB J*. **13**:1385-1393, 1999; Fabrizio *et al*., *Science* **292**:288-290, 2001. [2]Ayyadevara *et al., Anal. Biochem*. **284**:19-28, 2000.



lsq4b — 2nd generation recombinants
0.23 Mb, 0.7 cM
0.9 Mb
G3
a b
c
d
e f
g h
T6
SR711
SR708
SR720
SR721
SR722
SR723
Physical map
position (Mb)
11
12
13
PCR1
14
PCR2
15
Long-lived (– QTL)
Short-lived (+ QTL)

757356. *daf-2*(rf) protects from hypoxic cell death in *C. elegans*

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We have previously reported that hypoxic exposure kills *C. elegans* and that mutations in the insulin/IGF receptor homolog *daf-2* and its downstream signaling pathway prevent hypoxia-induced organismal death. Here, we examined cell as opposed to organismal death. L1 larvae of various genotypes were exposed to hypoxia for 18 hrs, allowed to recover for 24 hrs, and the remaining live animals examined. The majority of wild type animals after hypoxic exposure contained one or more markedly swollen cells resembling cells undergoing necrotic death. *daf-2(e1370)* greatly reduced the number of necrotic cells (0.4+/-0.2 necrotic cells/animal vs 13.2+/-1.3 in wild type, p << 0.01, n=20/strain); other weaker hypoxia resistant *daf-2(*rf) alleles also blocked necrosis although less well. As for organismal death, this protection from necrosis was dependent on the forkhead transcription factor DAF-16 as *daf-16(*null);*daf-2(e1370)* animals had wt levels of necrotic cells (14.7+/-1.8). To examine neuronal and myocyte death, we tested strains expressing GFP in various neuronal subtypes and in body wall muscle. In all neuronal subtypes examined, hypoxia induced axonal blebbing resembling that described for traumatic and ischemia-induced axonal injury in vertebrate brain and spinal cord. Moreover, a subset of hypoxic neurons were GFP-negative, consistent with cell death. *daf-2(e1370)* significantly reduced both the axonal defects (86% wt animals with blebs, 15% *daf-2* mutants with blebs, p <<0.01, n=91) and presumptive neuronal death (0 +/- 1% vs 61.9+/- 4.3 % cell loss by GFP, p << 0.01, n=113). The muscle GFP expression vector contained the SV40 nuclear-localization signal allowing observation of nuclear morphology. Hypoxia induced a fragmentation of the nuclear GFP expression that mirrored that seen by Normarski, suggesting that nuclei were being fragmented after hypoxia. As in neurons significant numbers of myocytes were lost after hypoxia as monitored by GFP expression and this presumptive cell death was prevented by *daf-2(e1370).* Thus, hypoxia does appear to produce cell death in *C. elegans*. The death has the morphological characteristics of both necrosis (cell swelling) and apoptosis (nuclear fragmentation). *daf-2(*rf*)* prevents both hypoxia-induced morphological defects and cell death. The insulin/IGF receptor and its signal transduction pathway represent a novel mechanism for modulating hypoxic cell death.

757761. POP-1 function within the vulval secondary lineage.

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The *C. elegans* vulva is produced from the descendants of the primary and secondary vulval lineages. These descendant cells differentiate into distinct vulval cell types in a precise pattern. The mechanisms which regulate the cell type decisions occurring among these descendant cells is currently not well understood. The transcription factor POP-1 is a component of the *C. elegans* Wnt pathway and is expressed during vulval development in a pattern that correlates with the cell type decisions made by these descendants (Hill *et al.*, I.W.M., 1999). For example, after the precursor P7.p has undergone two rounds of cell division (P7.pxx), its descendants are of cell types D, C, B, and A (from anterior to posterior). These descendant cells express POP-1 in the pattern Low, High, Low, High (from anterior to posterior). Thus, each P7.pxx cell expresses a different level of POP-1 than its sister cell and adopts a cell type distinct from its sister. Therefore, we tested whether sister P7.pxx cells would produce the same cell type if they had the same level of POP-1 activity.

Because the gene *lin-17* encodes a Wnt receptor and is known to regulate the development of P7.p, we examined POP-1 expression in *lin-17* mutants. In *lin-17(n671)* mutants, P7.pxx sister cells often express equal levels of POP-1. To determine if these animals have cell type transformations among the P7.pxx cells, we examined the cell fusion behavior of the P7.pxx descendants. In wild-type development, the vulval descendants undergo cell type-specific fusion events. For example, all descendants of cell type C fuse together to form a multi-nucleated cell (Sharma-Kishore et al., Development 126: 691-9. (1999)). We reasoned that if sister PN.pxx cells adopted the same cell type in a mutant, then their descendant cells would undergo ectopic cell fusions. We observe ectopic cell fusions among the descendants of P7.p in 75% of *lin-17(n671)* animals (n = 81). To alter POP-1 activity in an animal that is wild-type for *lin-17*, we used the transgene *huIs4* to express a dominant negative version of the POP-1 protein during vulval development. We observe that these animals also display ectopic fusions among the descendants of P7.p. We propose to use additional methods to confirm that alterations in POP-1 activity result in cell type transformations among PN.pxx cells. Our current results support the model that POP-1 regulates cell type decisions that occur during the execution of the 2° lineage.

759533. Genetic control of male-specific events in early gonadogenesis

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Hermaphrodite gonads have two symmetrical arms, whereas male gonads have a single asymmetric arm. We have sought genes that regulate gonadogenesis specifically in one of the two sexes. Here, we describe identification of a locus that has a strong, fully penetrant effect on male gonadogenesis, but a much weaker effect on hermaphrodite gonadogenesis. We tentatively call this locus *tog-1,* for ***t****ransformer* ***o****f* ***g****onad.* The *tog-1* male gonad develops as an amorphous mass, whereas the *tog-1* hermaphrodite gonad usually has two arms and appears nearly normal. The *tog-1* males are unaffected in non-gonadal tissues except for an occasional vulva, which is likely to rely on the defective gonad. The *tog-1* locus maps to chromosome *II* and encodes a forkhead transcription factor, as described in the abstract by Weiru Chang et al. Our focus has been on understanding the lineage defects in *tog-1*gonadogenesis and assessing the relationship between *tog-1* and *tra-1.* The somatic gonad is generated from two precursor cells, called Z1 and Z4. In wild-type hermaphrodites, Z1 and Z4 generate daughters of roughly equal size that remain in the positions of their birth. In wild-type males, Z1 and Z4 generate daughters of different size that become rearranged shortly after their birth to establish the asymmetry of the male gonad. We have examined the early Z1/Z4 divisions and migrations in *tog-1* and*tra-1* single mutants as well as *tog-1 tra-1* double mutants. In *tog-1* mutant males, Z1 and Z4 divide to generate daughters of equal size that do not migrate from their birthplace. Therefore, the *tog-1* male gonadal lineage resembles that of wild-type hermaphrodites. We find that *tra-1*is critical for the early Z1/Z4 lineage in both hermaphrodites and males. The*tog-1 tra-1* double mutant results are preliminary and will be discussed at the meeting. We conclude that *tog-1* controls the male-specific gonadal lineage.

767509. Characterization of Reproductive Senescence in C.elegans Hermaphrodites

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Caenorhabditis elegans hermaphrodites display an age-related decline in fertility. Self-fertilized hermaphrodites display a sharp decrease in progeny production around day 8 of adulthood. This is caused by an exhaustion of sperm, since mated hermaphrodites that have an abundance of sperm produce additional progeny for several additional days. However, mated hermaphrodites display a sharp decrease in progeny production around day 10, indicating that hermaphrodites undergo reproductive senescence despite ample sperm availability. We are using two approaches to analyze reproductive senescence. First, we are trying to identify morphological, molecular, and progeny production markers that correlate with reproductive senescence in C.elegans hermaphrodites. These markers will assist in the identification and analysis of mutations that delay reproductive senescence and they may be a cause of the functional decline. Second, we are searching for genes that affect reproductive senescence. Four mutant strains that increase lifespan have been analyzed for effects on reproductive senescence. While *daf-2(e1370)* displayed a modest delay in reproductive senescence for one prodgeny production marker, the other longevity alleles displayed wild-type degrees of reproductive senescence and *daf-2(e1370)* was wild-type for another progeny production marker. The absence of a change in the onset of reproductive decline in some longevity mutants suggests that the mechanisms and genetic pathways that specifically control reproductive senescence are at least partially distinct from the pathways that control lifespan. We have initiated F2 clonal screens for mutations that delay reproductive senescence. One approach that we have used is to mate hermaphrodites on day two of adulthood and then screen for additional progeny production on day 10 and beyond. A second approach is to mate hermaphrodites on day 10 and screen for greater than wild-type progeny production. Almost 1500 haploid genomes have been screened using these two strategies. Four candidate strains have been identified and are in the early stages of genetic analysis. By characterizing markers of reproductive senescence in *C. elegans* hermaphrodites and identifying mutations that delay this process the genetic and physical mechanisms that have evolved to control this process may be elucidated.

776861. UAP56, A DEAD-box helicase involved in both splicing and mRNA transport

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How is mRNA transport out of the nucleus prevented until splicing is complete? Recent data has implicated UAP56, a member of the DEAD-box family of ATPases, in both splicing and transport. UAP56 was discovered as a splicing factor associated with U2AF65 in mammalian cells. Its homolog in yeast, SUB2, has been shown to act to counter U2AF, an essential protein that recognizes the 3' splice site. It may physically remove U2AF from the 3' splice site to allow binding of the U2snRNP. More recently, UAP56 has been implicated in RNA transport in both mammalian cells and yeast. It has been found in association with Ref/Aly, a protein that marks the exon/exon junction left after splicing as a prerequisite to mRNA transport.

We have expressed GFP from a vit promoter, and RNAi'd various RNA processing proteins that we expected to be required for its expression. Removing UAP56 in adults is lethal to the progeny of injected worms. It also reduces expression of GFP in the injected animals, and it does so in an interesting way: it results in a much more severe reduction than RNAi of other essential splicing factors such as U2AF and PUF60 (another U2AF-associated protein) but much less severe than removal of NXF-1 (TAP), known to be required for mRNA transport. RNAi of UAP56 does interfere with mRNA transport: in situ hybridization of the RNAi'd worms demonstrates that the gfp mRNA is more nuclear-localized in UAP56 RNAi'd worms than in controls. Furthermore, double RNAi experiments with UAP56 and either PUF60 or U2AF65 have less severe effects on GFP levels than RNAi of UAP56 alone, suggesting that PUF60 and U2AF do indeed act antagonistically to UAP56. These results are consistent with models that suggest UAP56 acts to remove U2AF prior to mRNA transport.

We have also RNAi'd other factors reported to be involved in tagging exon/exon junctions in mammalian cells, namely Mago and 3 homologs of Ref/Aly. Surprisingly, none of these affects the level of GFP expression. RNAi of mago is embryonic lethal but even a triple RNAi of all three REF homologs is without apparent effect.

We have made a flag-tagged version of UAP56 under heat-shock control and shown that UAP56 overexpression results in lethality and sterility. We are investigating the effects of this overexpression on GFP activity and RNA localization by in situ hybridization.

799610. Signal transduction in *C. elegans* male mating behaviors

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The nervous system of the adult *C. elegans* male possesses 381 neurons to the hermaphrodites's 302. These additional sex-specific neurons are required for male mating behaviors, namely, response to hermaphrodite contact, backing, turning, location of vulva, spicule insertion, and sperm transfer (1). We are specifically studying the molecular basis of male sensory behaviors. We ask the questions: what are the receptors of the sensory neurons that initiate the signal cascade? What molecules act during synaptic transmission?

G-protein coupled receptors (GPCR) are a large family of seven-transmembrane spanning proteins involved in signal transduction in response to hormones, neurotransmitters, light, and chemicals. The *C. elegans* genome possesses over 1000 GPCRs with expression of 10 candidate GPCRs being highly enriched in adult males (2). Characterization of these sex-regulated GPCR genes is essential in determining a role in sex-specific sensory behaviors. To characterize the expression pattern and subcellular localization of candidate GPCRs in male nervous system, GPCR-X::GFP reporters will be constructed and expression patterns analyzed. To characterize the role(s) of candidate GPCRs that are expressed in male sensory neurons, we will attempt to demonstrate that GPCR candidates encode functional chemoreceptors. We will isolate deletions in candidate genes and perform behavioral and phenotypic analysis on mutants.

Neuropeptides are non-classical neurotransmitters and play critical roles in synaptic signaling in the nervous system. 21 putative *neuropeptide like protein* (*nlp*) genes and 23 *FMRFamide-like peptide* (*flp*) genes (personal communication) have been identified in *C. elegans* (3, 4). We are characterizing their expression patterns in adult males and analyzing the mutant phenotypes with respect to male mating behaviors.

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811911. Deciphering the role of AHR-1 in neuronal development

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The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor. In mammals, AHR mediates the toxic effects of dioxin and related environmental pollutants. Exposure to dioxin can cause cognitive deficiencies, but the neuronal function of AHR during normal development is not understood. We are investigating the function and regulation of *ahr-1*, the *C. elegans* homolog of the aryl hydrocarbon receptor (AHR), during neural development. We have isolated a deletion mutation in *ahr-1*, and *ahr-1*-deficient animals have neuronal defects (1). Here we describe a requirement for *ahr-1* during the development of the SDQR interneuron.

In wild-type animals, SDQR migrates dorsally to a position on the ALMR-associated nerve, and its axon projects dorsally. These migrations are disrupted in *ahr-1 (ia03)* mutants. Others have demonstrated that dorsal migration of the SDQR cell and axon is mediated by UNC-6/Netrin and the UNC-5 receptor (2). To understand potential interactions between *ahr-1* and Netrin signaling, we constructed and analyzed double mutants. *ahr-1 (ia03)* is a putative null mutation, and it enhances the SDQR cell migration defects in *unc-5*or *unc-6* mutants. This suggests that *ahr-1* has a role in SDQR migration that is independent of *unc-5*and *unc-6*. We examined the dorsal-ventral position of SDQR in animals lacking other guidance molecules. Our data demonstrate that *sax-3*/Robo and *unc*-129/TGFbeta both contribute to the dorsal migration of the SDQR cell. Further, the *ahr-1*null mutation enhances the SDQR migration defects in *sax-3*- or *unc-129*-deficient animals. We propose that *ahr-1* is required for the function and/or integration of more than one dorsal-ventral guidance pathway in SDQR. An alternative hypothesis is that *ahr-1* defines a previously undiscovered mechanism of dorsal-ventral pathfinding that is independent of *unc-6*, *sax-3*, or *unc-129*.

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827632. AXM-1, a New Negative Regulator of RAC GTPase Activity in Neuronal Morphogenesis.

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We are interested in the molecular mechanisms of neuronal morphogenesis, particularly the signal transduction pathways that mediate growth cone actin cytoskeleton rearrangement during axon pathfinding. Loss-of-function studies and epistasis analysis indicates that the actin-binding protein UNC-115 acts downstream of the RAC-2 RAC GTPase signaling during neuronal morphogenesis and axon pathfinding. UNC-115 might modulate the actin cytoskeleton of the growth cone in response to RAC-2 signaling. UNC-115 has three N-terminal protein-binding LIM domains and a C-terminal actin-binding villin headpiece domain. We can detect no direct binding of UNC-115 to RAC-2, indicating that other molecules might bridge this interaction. To identify other components of RAC-2 and UNC-115 signal transduction, we screened for molecules that bind to the UNC-115 LIM domains in the yeast two-hybrid system. This screen yielded AXM-1, a novel 7-WD repeat-containing molecule that defines a protein family of unknown function present in fungi, plants and animals. We found that in addition to binding to the UNC-115 LIM domains, AXM-1 interacts directly with RAC-2. Thus, AXM-1 is a molecular adapter that links UNC-115 to RAC-2.

To investigate the role of *axm-1* in neuronal morphogenesis and *rac* signaling, we obtained a deletion mutation in the *axm-1* locus from the *C. elegans* Gene Knockout Consortium (B. Barstead and G. Moulder). We found that *axm-1(ok267)* enhanced the neuronal morphogenesis defects caused by dominant, constitutively-active *rac-2(G12V),* indicating that the normal role of *axm-1* is to repress *rac-2* activity. Consistent with this idea, we found that overexpression of wild-type *axm-1* suppressed the morphogenetic effects of *rac-2(G12V)*. These experiments indicate that AXM-1 is a new negative regulator of RAC GTPase activity in neuronal morphogenesis.

837997. Structural failure of the *mua* mutants and cloning

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In *Caenorhabditis elegans*, locomotion depends on the transmission of contractile force from skeletal muscles to the cuticle through an intervening basal lamina and hypodermis. This mechanical pathway is composed of various cytoskeletal proteins, transmembrane linkers, and matrix components that are essential for its assembly and maintenance. Mutated genes of the *mua* (*m*uscle *a*ttachment) class result in a progressive paralysis due to post-embryonic failure of these structural linkages during normal use. Immunofluorescent and GFP reporter analysis suggest that many of these genes affect hypodermal components. Electron microscopy of *mua-3* mutants confirms this, showing clear disruption of attachment between hypodermis and cuticle (1). Currently, we are examining mutants of *mua-10*, *mua-6*, *mua-1* (*nc11*, *rh160*), and *mua-5*.

Mutations in the *mua-5* gene result in animals that hatch and move normally as L1 larvae. However, muscle detachment from the body wall during growth rapidly ensues with most animals dying very young. Since all *mua-5* alleles have the same penetrance, it is unclear whether they are null. *mua-5* maps between *unc-44* and *lin-45* on chromosome IV. One candidate gene in this region is a cytoskeleton-like protein with homology to *Drosophila melanogaster* Bicaudal-D (Bic-D). In *Drosophila* oogenesis, Bicaudal-D is required for the establishment and maintenance of the polarized microtubule network as well as the transport of determinant mRNAs and proteins to their appropriate locations.

Knock out of *Ce*Bic-D by RNAi, resulted in many animals that were slow-moving, sick, and necrotic. In rare instances, animals were observed to be stuck within an unshed cuticle. Rare animals were also observed with muscle detachment. Although *Ce*Bic-D (RNAi) has some phenotypic similarities to the *mua-5* mutant phenotype, they are not identical. To conclusively identify *mua-5*, rescue analysis is being done using *mua-5* mutants and genomic DNA microinjection.

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838246. Characterization of two LIN-1-associated proteins involved in SUMOylation.

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During the development of the *C. elegans* hermaphrodite vulva, activation of the evolutionarily-conserved Ras/MAPK pathway results in phosphorylation of the MPK-1 MAPK and inhibition of the LIN-1 ETS transcription factor, the critical negative regulator of the primary vulval cell fate. The mechanism by which LIN-1 regulates cell fates remains enigmatic, as all the molecularly-characterized genes involved in this pathway function upstream of *lin-1*, and LIN-1 target genes have yet to be identified. We conducted a yeast two-hybrid screen to identify proteins that associate with LIN-1 and may regulate vulval cell fates. We identified two enzymes, UBC-9 and GEI-17, that have homologues that mediate the covalent attachment of a small ubiquitin-related modifier (SUMO) to proteins. SUMOylation of proteins is likely to have different effects depending on the target protein; for example, SUMOylation has been shown to antagonize ubiquitin-mediated degradation and to modulate transcriptional activity. We used mutational analyses to demonstrate that UBC-9 binds two separable domains within the amino-terminus of LIN-1, and each domain contains a consensus SUMOylation site. These UBC-9-interaction domains are distinct from the three PEST domains present in LIN-1, which may promote ubiquitin-mediated degradation. Experiments are in progress to determine if LIN-1 is SUMOylated and to monitor the effect of such post-translational modification on LIN-1 function and vulval cell fate specification.

842767. RNAi Analysis of Regulatory Particle Subunits of the Proteasome

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The 26S proteasome is a large protein complex that is responsible for the degradation of cellular proteins. The ubiquitin-proteasome pathway is the primary means of protein degradation. Our lab is using RNA-mediated-interference (RNAi) to determine the function of the regulatory particle protein subunits within the 26S proteasome.

The 26S proteasome consists of a core particle (alpha- and beta- subunits) and either 19S or 11S particles flanking one or both ends of the core particle. The catalytic portion of the proteasome is within the core particle. RNAi of five gene products corresponding to a respective core particle subunit resulted in 100% lethality.

The 19S regulatory particle consists of 6 ATPase subunits (RPT's) and 12-13 non-ATPase subunits (RPN's). Using Wormbase, a list of *C. elegans* ORF's corresponding to yeast regulatory particle subunits was created. A total of 19 putative genes were found including three RPT6's and two RPN2 splice variants. RNAi has been performed on 15 of the 19 genes. Seven of the 15 showed significant embryonic lethality. Continued investigation will include combinatorial RNAi on the three RPT6's and all the subunits that showed no significant embryonic lethality. Phenotype analysis of embryos is underway.

859912. Hypoxia signaling and response

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C. elegans is emerging as a powerful system for studies of oxygen signaling and response to hypoxia (low oxygen). When environmental oxygen levels are low, individual cells and tissues must adapt by executing the appropriate changes in gene expression. Many of the critical cellular and systemic responses to hypoxia are implemented by hypoxia inducible factors, heterodimeric DNA binding complexes consisting of alpha and beta subunits. The *C. elegans* homologs of HIFα and HIFβ are encoded by *hif-1* and *aha-1*, respectively (1, 2). *C. elegans hif-1* mutants do not exhibit severe defects under standard laboratory conditions, but they are unable to adapt to hypoxia. While wild type animals can survive and reproduce in 1% oxygen, the majority of *hif-1*-defective animals die in these conditions (1). Under normoxic conditions, HIF-1 binds VHL-1, which is encoded by the ortholog of the von Hippel-Lindau tumor-suppressor protein. VHL-1 targets HIF-1 for degradation via a ubiquitin-proteasome pathway (3, 4). To characterize hypoxia-dependent changes in gene expression and to further define the requirements for *hif-1* and *vhl-1*, we performed microarray analyses in collaboration with Stuart Kim and his colleagues at Stanford. We assayed the mRNA expression levels for 17,871 individual genes (representing 94% of the *C. elegans* genome) under normoxic and hypoxic conditions. As expected, some transcriptional targets of HIF-1 are expressed at decreased levels in *hif-1* mutants and are expressed at constitutively high levels in *vhl-1* mutants. We also identified genes that are induced by hypoxia in a manner that is independent of *hif-1*. At the meeting, we will present these and other microarray data, and we will discuss plans for future studies.

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861630. Identification and characterization of male sexual regulators and TRA-1 target genes

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C. elegans is highly sexually dimorphic, with about 30% of the somatic cells in the adult hermaphrodite and 40% of the somatic cells in the adult male being sexually specialized. All aspects of somatic sexual development are controlled by TRA-1A, a zinc finger transcription factor that is active in hermaphrodites and can repress transcription. TRA-1A probably directly regulates multiple genes involved in restricted aspects of sexual differentiation, and an important goal is to identify these genes. We have identified a large number of sex-enriched genes, mostly male-enriched, using microarray analysis. We compared N2 XX hermaphrodites and tra-2(ts) XX pseudomales from the L2 through the L4 stages to generate a developmental profile of sex-specific gene expression. Many of these genes likely function as male-specific sexual regulators. We have also identified potential TRA-1A binding sites in the C. elegans genome using a hidden Markov model (HMM) computer algorithm and weight matrix searches. Combining these two approaches, we seek to identify and characterize male-specific TRA-1A target genes in an effort to understand the mechanisms by which C. elegans achieves sexual dimorphism. We are currently conducting functional analysis of these putative male sexual regulators using RNAi and GFP reporter analysis.

862113. Fine mapping of introgressed regions for longevity QTLs in *C. elegans*

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QTL mapping for longevity in *C. elegans*, in 4 interstrain crosses, implicated a total of 13 life-span loci (each p<0.01 by permutation; 6 of the peak LOD scores were >8, 2 were > 20). Based on recapture statistics, the total number of loci of comparable effect is 13-30. For 4 longevity QTLs, the BO allele was backcrossed 20 generations into the other parental background. These, and late-arising recombinants, were used to create homozygous stocks, for which the varying introgressed regions were fine-mapped by Anchor-PCR Display ***(1)*** and SNP typing. We also attempted mapping by microarray analysis of allelic variants differing markedly in expression levels; this has proven less useful than SNP mapping at present. We assessed 5 "snip-SNPs" defined by the Plasterk group as dimorphic between Bristol-N2 and Hawaiian strain CB4856. Two of these loci, at 13.18 and 13.95 Mb on chromosome IV, were dimorphic at the same positions between strains CL2a (=N2) and Bergerac-BO. We then sequenced intermittent regions across one QTL interval, *lsq4b,* from parental strains Bergerac-BO, CL2a, and congenic-recombinant line SR708 -- using an ABI Prism 3100 capillary sequencer. We have found and confirmed 8 SNPs distinguishing Bergerac-BO from CL2a in the vicinity of *lsq4b* (see **Figure 1**), out of a total sequenced expanse of 7.3 kbp, averaging 1.1 SNP per kbp. This level is higher than the range (0.11-0.69 SNP per kbp) reported for four other strains relative to Bristol-N2 ***(1)***, suggesting that CL2a and Bergerac-BO may be more diverged (more distantly related to each other) than Bristol-N2 is diverged from the four strains evaluated, although regional variation in SNP density across the genome could also contribute ***(2)***. In our calculation of SNP frequency, we excluded 4 changes clustered inside a span of 8 nucleotides; with these included, the SNP frequency is 1.35 per kbp. We also used NCBI's BLASTN server (BLAST 2, ver. 2.2.3) to align Bristol-N2 sequences, ascertained by The *C. elegans* Sequencing Consortium, with the Bergerac-BO coding sequences determined for *unc-22* ***(3)*** and *unc-54* ***(4)*** genes following their identification by transposon-tagging mutagenesis ***(3,5)***. For *unc-54*, there were 4 nucleotide changes in 4,394 nucleotides (AT® TA, T® G, G® -), a frequency of 0.91 SNPs per kbp, whereas more than 39 kbp of *unc-22* aligned sequence revealed only 2 single-nucleotide deletions, both within oligo(A) runs (A_3 ®A_2, A_5 ®A_4). These, and the single-base deletion (G ® -) noted above, are likely to be artefactual since they would disrupt the open reading frame. The agreement between strains with respect to *unc-22* sequence is particularly remarkable in that this alignment included >13 kbp of codon-position-3 nucleotides, positions shown to diverge almost as rapidly as the bulk of intronic and intergenic sequences ***(2)***. These combined data support the presence of striking regional variation in the frequency of interstrain sequence polymorphisms.

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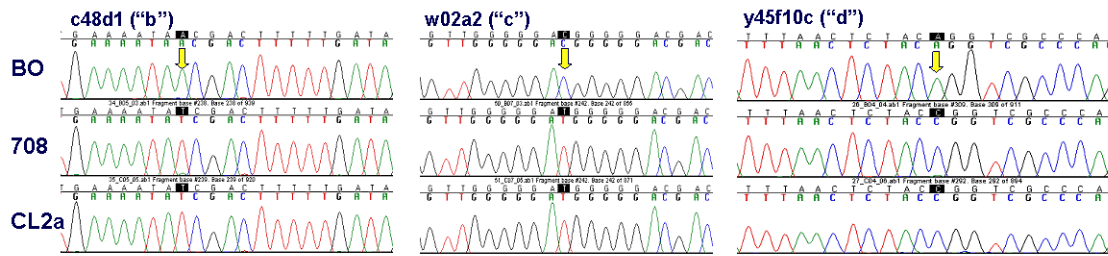

Figure 1. **Dimorphic SNPs used in mapping.** Sequences were determined for PCR products amplified from genomic DNAs of strains Bergerac-BO, SR708, and CL2a. Regions harboring SNPs (yellow arrows) are shown; clone names indicate loci at 13.23 Mb (c48d1), 13.34 Mb (w02a2), and 13.65 Mb (y45f10c) on chromosome 4, labeled **b - d** respectively in Fig. 2. Similar results were seen at 0.25, 0.5, or 1.0x recommended inputs of ABI BigDye(TM) fluors, scaling down all reagents relative to DNA.

869031. Genetic control of POP-1 expression in the vulva.

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The *C. elegans* Wnt signaling pathway has been shown to regulate cell fate decisions during embryonic and post-embryonic development. An important component of this pathway is the transcription factor POP-1, a homolog of the vertebrate proteins TCF-1 and LEF1. During *C. elegans* development, most cells express different levels of POP-1 than their sisters. An example of this pattern can be seen during vulval development where each granddaughter (PN.pxx) of the vulval precursors expresses a different level of POP-1 than its sister. Since each of the PN.ppx cells produces different cell types than it sister, it is possible that POP-1 directs sister PN.ppx cells to become different cell types. This model is supported by studies on the gene *lin-17*, which encodes a putative Wnt receptor. *lin-17* mutants are known to display defects in the cell lineage of P7.p. We find that *lin-17* mutants have defects in POP-1 expression at multiple stages of the P7.p lineage. Normally, the posterior daughter of P7.p can express a higher level of POP-1 than its sister. In *lin-17(n671)* mutants, the anterior daughter can express a higher level of POP-1 than its sister. In wild type, P7.pap and P7.ppp express higher levels of POP-1 than their respective sisters. In *lin-17* mutants, P7.pap and P7.ppp can have the same or lower levels of POP-1 than their respective sisters. These defects in POP-1 expression could account for the defects in the cell lineage of P7.p in *lin-17* mutants. We are extending this analysis to include additional mutations in the Wnt and other pathways to determine if there are other genetic inputs that regulate POP-1 expression in the vulva. As part of these efforts, we are developing improved techniques to examine POP-1 expression during vulval development. We are building transgenes that express POP-1 in temporally and spatially-specific manners. This will allow the directed expression of wild-type and mutant forms of POP-1 to test the function of POP-1 at specific times and in specific cells.

881628. Expression and Function of Type XVIII Collagen in *C. elegans*

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Basement membranes are specialized extracellular matrices that provide structural support, tissue separation, and have essential signaling roles during development. Vertebrate type XVIII collagen is a homotrimeric basement membrane molecule whose functions are largely unknown. Its carboxyl terminal fragment, endostatin (ES), inhibits endothelial cell proliferation and migration, and angiogenesis. The *C. elegans* homologue, *cle-1*, is being characterised in our lab. An internal deletion allele, *cg120*, that removes the ES/NC1 domain, was shown to cause multiple cell migration and axon guidance defects. Rescue of some of these defects are obtained by expressing transgenically, in *cg120*, the shorter *cle-1* isoform, containing just the NC1 domain.

In *cg120* mutants truncated CLE-1 is localized in a nearly wild-type pattern, but at reduced level. A second deletion allele, *cg122* (see below), which causes embryonic and larval arrest, severely reduces *cle-1* mRNA and protein. RNAi against *cle-1* by injection of double-stranded RNA results in some embryonic and larval lethality. These results suggested that a *cle-1* null mutant might exhibit some degree of lethality. Most forms of *cle-1* are mainly expressed in neurons, which are refractory to RNAi. We tried to overcome this problem by transgenically producing double-stranded RNA against *cle*-1 in neurons using a heat shock vector. However, the effect using this method was not more severe than using microinjections. We examined the persistence of CLE-1C::GFP derived fluorescence after heat shock treatment, and found no detectable reduction in fluorescence.

The allele *cg122*, removes part of the NC1 domain plus 350 bp of 3' UTR, including the putative polyadenylation signal. Most *cg122* mutants arrest as embryos or L1s, 15% become small, Muv, sterile adults. Deletion of the 3'UTR might destabilize the *cle-1* mRNA and these phenotypes could represent the near null state for *cle-1*. However, the deletion also appears to remove 160 bp of the 3'UTR of the adjacent gene, F39H11.3, a putative CDK8 protein kinase. The F39H11 cosmid transgenically rescues the *cg122* lethality. To determine whether one or both of these genes are responsible for the phenotype we have tested subclones containing only *cle-1* or F39H11.3. Unfortunately, for our purposes, the F39H11.3 subclone completely rescues the lethality, while the *cle-1* subclone does not. So the *cg122* lethality appears to result from affects on the adjacent kinase.

The *cle-1* gene produces at least three isoforms, *cle-1A-C*, expressed from different promoters, and with different temporal and spatial expression. These isoforms are being tagged with a c-myc epitope tag to further investigate the localization to function of the expressed proteins.

896313. Roles of L1 cell adhesion molecule in *C elegans*

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L1CAMs, cell adhesion molecules that belong to the Ig superfamily, have been shown to be essential for nervous system development. Mutations in the human L1 gene result in several neurological disorders, whose symptoms include mental retardation, spastic paraplegia, and hydrocephalus. L1CAMs is conserved in *C elegans* and is encoded by a single gene, *lad-1*. LAD-1 is expressed in all cells throughout development, and is localized to the plasma membrane at sites of cellular contact. LAD-1 expression suggests function in both the nervous system and non-neuronal tissues. In addition, LAD-1 is also subjected to tyrosine phosphorylation by the FGFR pathway. Interestingly, phosphorylated LAD-1 is localized to epithelial adherens and axon-muscle junctions that are free of non-phosphorylated LAD-1, suggesting distinct functions for phosphorylated LAD-1. We recently identified a *lad-1* genetic mutant. We will report our findings from our ongoing characterization of the mutant.

898908. Differential requirement for the myosin rod and nonhelical tailpiece in body wall muscle

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Myosin heavy chain (MHC) is a large, multi-domain protein important for both cellular structure and contraction. To examine the functional role of two C-terminal domains, the end of the coiled-coil rod and the nonhelical tailpiece, we have generated C-terminally truncated MHC constructs and examined their behavior in C. elegans striated muscle. Genetic tests demonstrate that MHC lacking only tailpiece residues is competent to support the timely onset of embryonic contractions, and therefore viability, in animals lacking full-length MHC. Antibody staining experiments show that this truncated molecule localizes as wild type in early stages of development, but may be defective in processes important for thick filament organization later in embryogenesis. Ultrastructural analysis reveals thick filaments of normal morphology in disorganized arrangement, as well as occasional abnormal assemblages. In contrast, truncated molecules lacking both the tailpiece and four residues of coiled coil fail to rescue animals lacking endogenous MHC, and exhibit delayed localization and delayed function during early embryogenesis. Thus, even small deletions within the rod C-terminus severely disrupt early assembly events in vivo. Our results suggest that these two MHC domains, the rod and the tailpiece, are required for distinct steps during muscle development.

903845. An essential function for AHA-1: Evidence that AHA-1 dimerizes with CKY-1 in the pharynx

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The basic-helix-loop-helix-PAS (bHLH-PAS) proteins are a family of transcription factors that mediate diverse processes, including cellular adaptations to environmental signals and developmental cell fate decisions. The *C.elegans* genome encodes 5 bHLH-PAS proteins. These include AHR-1, HIF-1, and AHA-1. The AHA-1 protein dimerizes with multiple bHLH-PAS partners and is expressed in most, if not all, somatic cells. Although *ahr-1*, *hif-1* double mutants are viable, animals homozygous for null mutations in *aha-1*arrest development as young larvae. To understand the essential functions of *aha-1*, we examined the expression of the two remaining bHLH-PAS genes, T01D3.2 and *cky-1*/C15C8.2. A T01D3.2:GFP reporter is expressed in two neurons. CKY-1:GFP is expressed in most non-neuronal pharyngeal cells. These data suggested two non-exclusive models to explain the essential developmental function of AHA-1: i) AHA-1 might form a complex with CKY-1 in pharyngeal cells; or ii) AHA-1 might function as a homodimer or independently of other bHLH-PAS proteins. In support of the first model, AHA-1 and CKY-1 form a DNA-binding complex *in vitro*, and AHA-1 is localized to the nucleus of cells that express CKY-1:GFP. To determine whether expression of AHA-1 in the pharynx could rescue *aha-1*-deficient animals, we constructed a *cky-1:aha-1* chimeric gene, in which *cky-1* 5' regulatory sequences directed the expression of the *aha-1* transcript. We injected *cky-1:aha-1* into the *aha-1*mutant background, thereby creating animals mosaic for AHA-1 expression. The transgenic animals were viable and fertile. We conclude that AHA-1 function is essential, and we propose that AHA-1 and CKY-1 have a role in pharyngeal function.

908445. A Screen for Mutations that Affect *Caenorhabditis elegans* Aging

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How aging is controlled is a fundamental biological question that remains largely unanswered. *C. elegans* promises to be a powerful model system for studying the genetic basis of aging, as it ages rapidly and mutations in genes such as *age-1* and *daf-2* have been shown to significantly extend the lifespan. We have initiated a screen for mutations that delay the aging process. We characterized the average time for N2 worms to cease egg-laying, display significantly reduced body movement and pharyngeal pumping, and die. Using EMS mutagenesis, we are conducting an F2 clonal screen for mutants with a persistence of the youthful condition for one or more of these processes. We have screened about 2000 (haploid) genomes and identified several mutations that significantly delay one or more of these senescence traits. We are now backcrossing and mapping several of these mutations in preparation for more extensive phenotypic analysis.

920733. Identification of genes regulating sex-specific gonad development in C. elegans

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Title: Identification of genes regulating sex-specific gonad development in *C. elegans*

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The *C. elegans* gonad undergoes highly dimorphic development in the two sexes, resulting in very different structures. One example of this is observed in the divergent properties of the somatic gonad. In both sexes, the gonad arises from a morphologically identical 4-cell symmetrical primordium consisting of the two somatic precursor cells, Z1 and Z4, and the two germline precursor cells, Z2 and Z3. The adult hermaphrodite gonad maintains this symmetry and develops into a two armed structure consisting of uterus, spermatheca, and sheath cells. In contrast, the adult male gonad is a single armed, asymmetrical structure made up of vas deferens and seminal vesicle. We would like to understand how the sex determination pathway causes these different developmental programs to occur, and are seeking genes required sex-specifically for gonad development.

We performed a F2 screen for male specific gonad mutants using a GFP marker expressed in the male vas deferens and seminal vesicle (see Thoemke *et al* abstract). From approximately 2700 haploid genomes screened we have isolated 25 mutant alleles. The mutants fall into three broad phenotypic classes: 1) gonad with normal male morphology but abnormal differentiation of specific cells, 2) grossly abnormal male morphology but no signs of sex reversal, and 3) abnormal morphology with hermaphrodite cell types present (see Chang *et al* abstract). We are currently mapping the mutants and determining the number of complementation groups in each class. Representative phenotypes of the different classes will be presented.

927884. Vesicular fusion protein NSF is required for fusion of the uterine anchor cell with the utse syncytium

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Cell fusion plays key roles in various aspects of animal development. A gene required for epidermal cell fusions in C. elegans has recently been identified (1). However, in general, the molecular mechanisms underlying the fusion between cells are largely unknown. Also, it is unclear whether the well-characterized molecules mediating membrane fusion during vesicular transport within cells function in the fusion between cells as well.

The anchor cell of the C. elegans hermaphrodite uterus induces the two cell types necessary for formation of the uterine-vulval connection. The anchor cell later fuses with the utse (uterine-seam cell) syncytium, which is formed by fusion of eight daughters of the uterine pi cells that are induced by the anchor cell. The utse syncytium forms a thin process dorsal to the vulva that can be broken, enabling eggs in the uterus to enter the vulval passageway to the outside. If the anchor cell fails to fuse with the utse, it obstructs this passageway, and eggs cannot be laid.

In ty10 mutants, the thin process of the utse appears normal, but the anchor cell fails to fuse with it. Subsequently, the unfused anchor cell either detaches from the adjacent tissue and floats free in the uterine lumen or remains dorsal to the vulva and degenerates. We observed that the ty10 mutant defect was rescued by cosmid H15N14 in transgenic animals. We found that the ty10 allele was a missense mutation in a conserved residue of H15N14.2, which encodes C. elegans N-ethylmaleimide sensitive factor (NSF). NSF has a conserved and essential role in intracellular membrane fusion. Specifically, NSF appears to be required to disassemble complexes of the membrane proteins (SNAREs) that mediate fusion so that they can be re-utilized. We found that elimination of H15N14.2 function by RNAi resulted in lethality at various embryonic and larval stages, depending on the time of administration. This is consistent with previous results (2, 3), and presumably reflects the basic cellular requirement for NSF. In addition, we found that animals containing ty10 in trans to a deficiency died as embryos or larvae. Future experiments will be aimed at determining how the ty10 mutation (which is in the D2 ATP binding domain of NSF) interferes specifically with anchor cell fusion without significantly affecting viability.

The finding that NSF, a key mediator of intracellular fusion, is also required for the fusion between cells, provides an important link between these two processes. Future studies of the fusion between the anchor cell and the utse should further delineate similarities and differences in the genetic requirements for this event and for intracellular membrane fusion.

(1) Mohler et al., 2002. Developmental Cell 2: 355-362.
(2) Fraser et al., 2000. Nature 408: 325-330.
(3) Maeda et al., 2001. Current Biology 11: 171-176.

942006. WormBase; An Update

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WormBase is an international consortium of biologists and computer scientists dedicated to providing the research community with accurate, current, and easily accessible genetic, sequence and phenotypic information for C. elegans and a few closely-related nematodes. This information is accessed through our website (www.wormbase.org). The database is also freely available with new releases being made every two weeks.

The database is constantly improving through the curation of existing data and the addition of new datasets. With the goal of making all the data easily available, improvements to the website and the addition of data mining tools are continuely being made. Recent improvements and ongoing projects will be presented.

Feedback, question and contributions from the C. elegans, as well as the broader biomedical, community are welcome and encouraged and can be made by emailing to wormbase-help@wormbase.org

950793. Suppression of Polyglutamine Induced Protein Aggregation in *C. elegans* by Torsin Proteins

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Torsion dystonia is a movement disorder characterized by involuntary and repetitive muscle contractions. The most severe form of dystonia (early-onset) has been linked to a mutation in a human gene named *TOR1A (DYT-1)* encoding a protein termed torsinA. While a causative genetic mutation has been identified, the cellular and molecular mechanisms underlying this disorder remain unknown. We are applying the advantages of *Caenorhabditis elegans,* towards a detailed analysis of torsin-like gene products *(tor-1, tor-2 and ooc-5)* in this nematode. Preliminary studies utilizing reporter gene fusions to GFP indicate *tor-1* and *tor-2* act neuronally and may represent a functionally co-expressed operon unit. We have isolated a cDNA encoding *C. elegans* TOR-2, and created a deletion in the C-terminal region of high sequence homology designed to mimic the dominant human defect in this protein. We are studying the effect of overexpression of these constructs in transgenic animals.

Phylogenetic analysis of the torsin family indicates these proteins share distant sequence similarity with the functionally diverse AAA+/HSP/Clp-ATPase family that includes a variety of molecules including molecular chaperones. To determine if torsins function in the capacity of molecular chaperones, we adopted an elegant *in vivo* assay for examining states of intracellular protein aggregation (PNAS, 97:5750-55). In this regard, we have determined that ectopic overexpression of either *C. elegans tor-2* and *ooc-5*, or human *TOR1A* results in a reduction of polyglutamine repeat-induced protein aggregation in a manner similar to that previously reported for molecular chaperones. Interestingly, the co-overexpression of *tor-2* and *ooc-5* results in a more marked reduction of aggregates. Moreover, site-directed mutagenesis studies of mutant forms of TOR-2 show an inability to ameliorate aggregation. Furthermore, immunolocalization experiments indicate that TOR-2 and ubiquitin are highly co-localized at sites of protein aggregation. These data directly imply a normal role for torsin proteins in cells is to effectively manage protein folding, or perhaps as components in the cellular response to protein aggregation.

958888. The *sys-4* gene and gonadogenesis in *C. elegans*

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Normally, the two somatic gonadal precursor cells, Z1 and Z4, divide asymmetrically to generate distal and proximal daughters with distinct fates. In *sys-1* and *sys-2* mutants, Z1 and Z4 divide symmetrically: each daughter has a proximal fate such that it has potential to generate an AC/VU precursor, and DTCs are not formed[1,2]. The Z1/Z4 division is controlled, at least in part, by the Wnt pathway (*e.g. sys-2* is allelic to *pop-1*[2]).

Here we report progress characterizing the *sys-4* locus. We have identified six *sys-4* alleles with a range of phenotypic strengths. The most severe alleles have gonadogenesis defects similar to those of *sys-1* and *sys-2*. Genetic interactions between *sys-4* and *sys-2/pop-1* implicate *sys-4* in the Wnt pathway. We have recently cloned *sys-4* and identified molecular lesions in 5/6 alleles. We find that *sys-4* does not encode a canonical member of the Wnt pathway, and will discuss ideas for how it regulates the Z1/Z4 division.

[1] Miskowski et al (2000) Developmental Biology 230: 61-73.

[2] Siegfried and Kimble (2002) Development 129: 443-453.

978630. The Wnt pathway and vulval lineage execution.

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The *C. elegans* Wnt signaling pathway has been shown to regulate cell-type decisions during embryonic and larval development. We have been examining whether this pathway regulates cell type decisions that occur within the primary and secondary lineages of the vulva. The transcription factor POP-1 is a component of the Wnt pathway and is expressed during vulval development in a pattern that correlates with the execution of the primary and secondary lineages. When each vulval precursor has divided to generate 4 descendant cells (PN.pxx), each of these descendants expresses a different level of POP-1 than its sister (Hill *et al*., I.W.M., 1999) and produces a different cell type(s) than its sister. We have used several genetic approaches to test if POP-1 directs sister PN.pxx cells to adopt different cell types.

Animals mutant for the gene *lin-17*, which encodes a putative Wnt receptor, are known to have defects in the secondary lineage of the vulval precursor P7.p. We observe that sister P7.pxx cells can inappropriately express equal levels of POP-1 in *lin-17(n671)* mutants. Thus, *lin-17* mutants provide a means of examining the possible consequence of misexpressing POP-1 during vulval lineage execution. We have used cell fusion patterns to look for the presence of cell-type transformations between P7.pxx sister cells. During normal vulval development, descendant cells of one cell type fuse exclusively with cells of the same cell type. We reasoned that if sister PN.pxx cells adopted the same cell type, then their descendants would undergo ectopic cell fusions. We have used the transgene *jcIs1*, that expresses a JAM-1::GFP fusion protein at adherens junctions, to detect ectopic fusions. We detect inappropriate fusions among the descendants of P7.p in 75% (n = 81) of *lin-17(n671)* animals. This result suggests that the misregulation of POP-1 expression that occurs in *lin-17* mutants is associated with cell fate transformations. To test whether misregulation of POP-1 could be responsible for these apparent cell fate transformations, we have used the transgene *huIs4* to express a dominant negative variant of the POP-1 protein during vulval development. We observe that the resulting animals also display ectopic cell fusions among the descendants of P7.p. These results suggest that POP-1 regulates the fate decisions made by the P7.pxx cells.

We have also examined whether ectopic expression of *C. elegans* Wnt signals can cause defects in vulval lineage execution. Transgenic animals that ectopically express the Wnt signal LIN-44 in response to heat shock display ectopic cell fusions among the descendants of vulval precursors that undergo the secondary lineage. Together, our results support the model that the Wnt pathway regulates cell type decisions that occur during the execution of the secondary vulval lineage.