

Program of the 2004 Midwest Worm Meeting

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Plenary Sessions I & II
Friday June 11, Alderson Auditorium, Kansas Union
Session I: 7:00 - 8:25 p.m.
Chair: Colette Witkowski

1. **Tim Walston**
The Dishevelleds and Casein Kinase I Regulate Spindle Orientation Through a Wnt Pathway Independent of Transcription
2. **Vandana Rajakumar**
Multiple Functions of PAX EGL-38 Coordinate the Development of the *Caenorhabditis elegans* Egg-Laying System
3. **Joseph D. Coolon**
Going back home: Development of a *C. elegans* Soil Culture System for Functional Genomic Studies
4. **Hongtao Qin**
The AHR-1 Transcriptional Complex Regulates Development of the Pseudocoelomic Neurons and Social Feeding Behavior
5. **Yieyie Yang**
Functional Study of Actin-Binding Protein UNC-115 and Its Binding Partner SWR-1 during Neuronal Morphogenesis in *C. elegans*

BREAK

Session II: 8:50 - 10:00 p.m.
Chair: Helen Chamberlin

6. **Stacie Hughes**
Characterization of Reproductive Senescence in *C. elegans* Hermaphrodites
7. **Kelly A. Flanagan**
UNC-82 is a Serine/Threonine Kinase Required for Thick Filament Organization in Body-Wall Muscle
8. **Jennifer L. Hueston**
Loss of the ELP-1 Microtubule-Binding Protein Exacerbates the Dystrophin Phenotype in *Caenorhabditis elegans*
9. **Vida Praitis**
SMA-1 Spectrin is Required for Epithelial Cell Sheet Morphogenesis in *C. elegans*

Plenary Sessions III & IV
Saturday June 12, Rm. 1005 Haworth Hall
Session III: 8:15 - 10:00 a.m.
Chair: Myeongwoo Lee

10. **Darin Blasiar**
Wormbase: Improvements to the Database

11. **Sinchita Roy Chowdhuri**
The *C. elegans* T-box gene *tbx-2* Is Required for Pharyngeal Development
12. **Courtney Wilkins**
C. elegans as a Model Host for Viral Infection
13. **Jana E. Harris**
MSP Signals Microtubule Reorganization in *C. elegans* Oocytes Prior to Fertilization
14. **Kenneth L. Jones**
Diversity Estimation to Determine the Changes in Nematode Community Composition in Response to Environmental Cues
15. **Kailiang Jia**
TOR and Insulin Pathways Converge at Raptor to Regulate *C. elegans* Larval Development, Metabolism, and Life Span

BREAK

Session IV: 10:30 a.m. - 12:15 p.m.
Chair: Shin Murakami

16. **April M. Orsborn**
To Degrade or Not to Degrade: Regulation of GLH Protein Levels
17. **Mary A. Allen**
Sex Determination Gene *laf-1* Is a Processed Non-Coding RNA
18. **Dana Byrd**
Regulation of Germline Stem Cells by the DTC Niche
19. **Rashmi Deshpande**
Mechanisms of Wnt Signaling During P12 Specification in *C. elegans*
20. **Maria Vidal**
spd-3 Is a Novel Gene Required for Spindle Alignment in *C. elegans*
21. **Ambrose R. Kidd III**
SYS-1, an Atypical β -Catenin, Acts with POP-1/TCF to Control Cell Fates in *C. elegans*

Plenary Sessions V & VI
Sunday June 13, Rm. 1005 Haworth Hall
Session V: 8:15 - 10:00 a.m.
Chair: Pamela Padilla

22. **Ahna Skop**
Conserved Cytokinesis Mechanisms Revealed through Dissection of the Mammalian Midbody Proteome
23. **Ge Gao**
Loss of *pan-1* Causes a Peter Pan-Like Phenotype in *C. elegans*
24. **Jo Anne Powell-Coffman**
C. elegans Hypoxia Response: Elucidating the Function and Regulation of the HIF-1 Transcription Factor
25. **Ellen L. Batchelder**
Investigations into the Role of Calmodulin in the Regulation of the Contractile Ring in the *Caenorhabditis elegans* Embryo

26. **Ryan W. Johnson**
Converging Genetic Pathways Restrict *vab-3* Transcription in the Male Tail to the B.a and Y.p Cell Lineages
27. **Elizabeth R. Leight**
Sumoylation of LIN-1 Promotes Recruitment of Chromatin Remodeling Enzymes and Inhibition of Vulval Cell Fates

BREAK
Session VI: 10:30 a.m. - 12:15 p.m.
Chair: Russell Hill

28. **Ginger R. Miley**
Analysis of LIN-1, an ETS protein Involved in Vulval Development
29. **Afaq Shakir**
Functional Redundancy of CED-10 and MIG-2 RACs with UNC-34 Enabled
30. **Xiangyan Tong**
Progress in Cloning *exc-9*
31. **Edward S. Davis**
Regulation of the Anaphase-Promoting Complex Function during Meiosis in *C. elegans*
32. **Rafal Ciosk**
ATX-2, the *C. elegans* Ortholog of Ataxin-2, Is Required for GLD-1 and MEX-3-Dependent Regulation of Translation in the Germline
33. **David Greenstein**
A Vesicle-Budding Model for the Release of MSP from *C. elegans* Sperm

Poster Session
Saturday, June 12, 1:30 - 5:00 p.m.
Kansas Union Ballroom

34. **Jessica Amrozowicz Kerins**
Genetic Studies of the Proliferation Versus Meiotic Development Decision
35. **Jacque Baca**
The Role of NXF-2 in Sexual Determination and *tra-2* mRNA Export
36. **Karen Bennett**
Cross-species RNAi: Several dsRNAs from *Ascaris* Sterilize *Caenorhabditis*
37. **Di Chen**
Positional Cloning and Functional Analysis of *daf-31*
38. **Nancy Cohen**
Electrophysiological Characterization of the PKD-2 Calcium Channel
39. **Frederick A. Danso**
Gut Expression Mechanism of Tropomyosin Isoforms III and IV in *Caenorhabditis elegans*
40. **Kim Evason**
Pharmacological Analysis of Aging in *C. elegans*
41. **Susan M. Fox**
Identification and Characterization of *C. elegans* Fos and Jun Homologs
42. **Ping Gong**
Mutational Analysis of the UNC-44 Axonal Guidance Ankyrin and PP2A B'-Subunit Interaction
43. **David I. Greenstein**
The Conserved DEAD-box Helicase CGH-1 Negatively Regulates MAP Kinase Activation in *C. elegans* Oocytes

44. **Wang Han**
Cloning and Analysis of *ne319* in *C. elegans*
 45. **Amy M. Hubert**
Investigating LAF-1's Involvement in *tra-2* Regulation
 46. **Andrew R. Jauregui**
Characterization of the Nephronophthisis Genes in *C. elegans*
 47. **Ke Jiang**
The Arp2/3 Activator Scar Acts in Parallel to MIG-2 Rac in Axon Pathfinding
 48. **Kenneth G. Johnson**
Identification of a Novel Gene Involved in Ras-Mediated Vulval Induction
 49. **Sushant Khandekar**
The Role of MUA-1 in Epidermal Tissue Integrity
 50. **Sarah M. LaMartina**
Characterization of a Suppressor of the *laf-1* Sex-Determination Gene
 51. **Tim Lindblom**
Nuclear Receptor Regulation of Drug Metabolizing Enzyme Gene Expression
 52. **Brendan C. Mattingly**
Searching for New Alleles in the GEF *exc-5*
 53. **Gary Moulder**
C. elegans Gene Knockout Project at OMRF
 54. **Shin Murakami**
Increasing Learning and Memory by Reducing Oxidative Stress in *C. elegans*
 55. **Garett M. Padilla**
Investigating the Role of a Putative STAR Domain Protein in *Caenorhabditis elegans*
 56. **Deanne S. Pruitt**
Postional Rescue Analysis of *mua-2*, *mua-5*, and *mua-10*
 57. **Vinita Prabhu**
Analysis of *C. elegans* Embryos Exposed to Anoxia
 58. **Vida Praitis**
The *unc-59(ru5)* Mutation Affects Morphogenesis of the Pharynx
 59. **Paramita Ray**
ceh-28 is Required for Feeding in *C. elegans*
 60. **Eric C. Struckhoff**
Interactions of Rho-Family GTPases in Axon Development
 61. **Prema Sundaram**
Cloning and Characterization of RNAi Defective Mutant *ne335* in *C. elegans*
 62. **Kristen Tews**
Determining the role of NUD-1 in hermaphrodite gonadogenesis by analysis of GFP reporters in *nud-1*(RNAi) Mutants
 63. **Koen Verbrugghe**
SPD-1 is Required for the Formation of the Spindle Midzone but Is Not Essential for the Completion of Cytokinesis in *C. elegans* Embryos
 64. **Colette M. Witkowski**
Distribution of AJM-1::GFP in Collagen IV Null Mutant *C. elegans* Embryos
 65. **Mingfu Wu**
lin(mh56) Controls the Asymmetric B Cell Division and Male Tail Development in *C. elegans*
 66. **Qun Zheng**
Analysis of the *rpc-1* Gene in *C. elegans*
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1. The Dishevelleds and Casein Kinase I Regulate Spindle Orientation Through a Wnt Pathway Independent of Transcription

Tim Walston^{1,2}, Christina Tuskey¹, Nancy Hawkins³, Gregory Ellis⁴, Bruce Bowerman⁴, Jeff Hardin¹

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Cell division in the early *Caenorhabditis elegans* embryo is a highly coordinated event involving several signaling pathways. Components of Wnt signaling play a role in positioning the mitotic spindle in the EMS and ABar blastomeres independent of β -catenin. Wnt signaling also regulates endoderm induction through β -catenin and gene transcription. Because Dishevelleds act as integrators of multiple Wnt pathways, determining their roles in cell division and differentiation is key to understanding early development. We identify novel components of a Wnt signaling pathway that controls spindle orientation in EMS and demonstrate that the same pathway orients the spindle in ABar. We show that all three *C. elegans* Dishevelleds act redundantly to position the spindles of both blastomeres. We identify KIN-19/CKI as a member of the Wnt spindle alignment pathway, which does not require WRM-1/ β -catenin or gene transcription. We also show that Src signaling affects spindle orientation in ABar. Finally, we show that a separate Wnt/ β -catenin signaling pathway controls the timing of spindle rotation in ABar. We conclude that Wnt signaling, through the Dishevelleds, KIN-19/CKI and GSK-3 regulate spindle orientation independent of β -catenin and transcription. Wnt signaling also regulates the timing of spindle rotation through a separate pathway requiring β -catenin and gene transcription.

2. MULTIPLE FUNCTIONS OF PAX EGL-38 COORDINATE THE DEVELOPMENT OF THE *Caenorhabditis elegans* EGG-LAYING SYSTEM

Vandana Rajakumar, Helen M. Chamberlin

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Communication between tissues in an organ system is necessary to co-ordinate their development and to ensure that they function as a unit. The egg-laying system in *C. elegans* offers a good model system for animal organ development. During the development of the egg-laying system, reciprocal interactions between the somatic gonad and the vulva co-ordinate development between these two tissues. The Anchor Cell (AC) of the somatic gonad initially induces 3 of 6 multipotential Vulval Precursor Cells (VPCs) to form the vulval cells by the LIN-3-LET-23 mediated signaling pathway. Subsequently, some cells of the 1° VPC lineage reciprocally signal to the uterine cells and induce uv1 cell fate using LIN-3-LET-23 again. Some genes important for the AC to vulva signal also mediate the vulva to uterus signal, whereas others function in one or the other developmental pathway. For example, only the vulval transcription of *lin-3* requires EGL-38, a PAX transcription factor, while the earlier AC expression is EGL-38 independent (1). EGL-38 is also required for the normal morphology of vulF cells (2). Thus it impacts both the somatic and the vulval cells to co-ordinate the development of the egg-laying system.

To investigate the different roles of *egl-38* in the egg-laying system development, we have studied the defects associated with different *egl-38* alleles. We have found that different *egl-38* alleles disrupt the different egg-laying features of egg-laying ability, *lin-3* expression, uv1 specification and vulF morphology to varying degrees. For example *egl-38(n578)* mutants are defective for all functions, *egl-38(gu22)* mutants are competent for all functions and *egl-38(sy294)* mutants are defective for *lin-3* expression but retain a high level of normal vulF morphogenesis.

To identify additional genes that function with *egl-38*, we performed a genetic screen for suppressors of the *egl-38(n578)* egg-laying defect (2, 3). We isolated four suppressor mutations from a screen of about 49,000 haploid genomes. Each suppressor is recessive and maps to LG IV. All four exhibit partial failure to complement in trans to each other. However they correspond to at least two genes since one (*gu68*) maps between *unc-24* and *sqv-1* while another (*gu51*) maps to the left of *unc-24* between *unc-5* and *lin-33*. Interestingly, these suppressors suppress the 1° vulval morphological defect and the uv1 specification defect though they still lack LIN-3 expression. We are currently doing further fine mapping and cosmid rescue experiments to identify the genes.

Identification of these suppressor genes along with the characterization of the egg-laying system in the different *egl-38* alleles will enable us to better understand at a molecular and cellular level, the 'crosstalk' occurring between tissues to co-ordinate organ development.

- (1) Chang, C., Newman, A.P and Sternberg, P.W. (1999) *Current Biology* 9: 237-246
- (2) Chamberlin, H. M., Palmer, R. E., Newman, A. P., et al (1997) *Development* 124:3919-3928
- (3) Trent, C., Tsung, N and Horvitz, H. R. (1983) *Genetics* 104: 619-647

3. Going back home: Development of a *C. elegans* soil culture system for functional genomic studies

Joseph D. Coolon¹, Timothy C. Todd², Ludek Zurek³, John M. Blair¹, Michael A. Herman¹

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³Department of Entomology, Kansas State University

Nematode communities at the Konza Tallgrass Prairie Biological Station near Manhattan, KS are known to respond to nitrogen addition and burning. Using a combination of morphometric identification and molecular methods, nematode "species" have been identified and preliminary results show differential responses to environmental perturbations at the genus level. (see abstract by Jones *et al.*) We are interested in linking the responses of organisms to environmental change at the genetic level. Little is known about how the environment affects organisms at the level of expression of individual genes. We are addressing this using laboratory soil cultures and the genetic model nematode *Caenorhabditis elegans*. We aim to use *C. elegans* to discover genes that are induced or repressed in response to changes in soil nitrogen and water by using laboratory soil cultures to model nematode environments on Konza. Homologs of the *C. elegans* genes exhibiting the greatest changes in expression will be identified in resident nematode "species" and their expression examined. As a first step we have developed a *C. elegans* soil culture system. We collected 13 soil bacterial isolates from native Kansas soils. N2 grew and developed normally on plates seeded with each of the isolates. Using the most abundant soil bacterial isolate, *Micrococcus luteus*, we have developed soil cultures that support soil bacterial and nematode growth. We have optimized growth conditions and found that water addition every six days maximizes nematode growth in soil culture. We also found that bacterial addition every three days increases nematode abundance in soil culture. We have been able to quickly extract nematodes from our soil cultures, which is essential for isolation of mRNAs for microarray studies. As a control for our future work, we are using cDNA microarrays to discover genes that are specifically expressed or repressed during soil growth. Microarray experiments comparing *E. coli* (OP50) fed N2 populations to *M. luteus* fed N2 populations grown on plates, as well as to those grown in soil culture, are currently under way.

4. The AHR-1 transcriptional complex regulates development of the pseudocoelomic neurons and social feeding behavior

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The mammalian aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor, and it mediates the toxic effects of dioxins and related compounds. Toxicological and genetic studies suggest that AHR regulates multiple developmental events, but the roles of AHR in neuronal development or function are not well understood. The *C. elegans* homologue of AHR is *ahr-1* (Powell-Coffman et al 1998, PNAS 95: 2844). To identify cells that express AHR-1, we constructed reporter genes in which the expression of GFP-tagged fusion proteins were directed by *ahr-1* regulatory sequences. During the first larval stage, *ahr-1*:GFP is expressed in twenty-eight neurons, several blast cells, and two phasmid socket cells. The neurons that express *ahr-1*:GFP represent multiple subtypes and include URXL, URXR, AQR and PQR, which directly contact pseudocoelomic fluid in the body cavity. Coates and de Bono (2002, Nature 419: 925) have demonstrated that these neurons modulate social feeding behavior. To further understand the role of the AHR-1 transcriptional complex in these and other neurons, we isolated and analyzed animals carrying a deletion in the *ahr-1* gene (Qin and Powell-Coffman 2004, Dev Biol. 270: 64). In collaboration with David Karow and Michael Marletta (U.C. Berkeley), we identified several genes that are expressed in URX, AQR, and PQR, including multiple soluble guanylate cyclases. We determined that expression of some cell-type-specific markers is dependent on *ahr-1* function, while expression of other markers appears to be *ahr-1*-independent. Interestingly, a strong loss-of-function mutation in *ahr-1* partially suppresses social feeding behavior in *npr-1*-deficient animals. These data suggest that *ahr-1* regulates a subset of the URX, AQR and PQR differentiation program. Further, specific *ahr-1*-dependent genes may modulate social feeding behavior. We are currently using a combination of biochemical and genetic assays to examine these hypotheses and to further understand the role of *ahr-1* in neuronal development. *C. elegans* AHR-1 and mammalian AHR share many molecular and biochemical properties, but invertebrate AHR homologs do not bind dioxin. Experiments are in progress to assay the ability of murine AHR to rescue *ahr-1* function in URX neurons in the presence or absence of an activating ligand.

This project is supported by NSF.

5. Functional Study of actin-binding protein UNC-115 and its binding partner SWR-1 during neuronal morphogenesis in *C. elegans*

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Rac GTPases have been shown to be key regulators of the dynamic movements and cell shape change of many different cell types, including axon outgrowth and guidance of neuron cells. Our previous studies showed that UNC-115, an actin-binding protein, acts downstream of RAC-2 during neuronal morphogenesis. Functional studies indicated that UNC-115 might be activated by translocation to the plasma membrane and by serine 617 dephosphorylation, possibly in response to RAC-2 signaling. Activated UNC-115 resulted in the ectopic formation of lamellipodia-like and filopodia-like structures in neurons that resemble those normally found on migrating growth cones. Also, these studies showed that the actin-binding villin headpiece domain (VHD) and the three UNC-115 LIM domains are required for UNC-115 activity in neuronal morphogenesis.

UNC-115-EGFP-transfected serum-starved NIH 3T3 fibroblasts showed striking morphological changes accompanied by actin cytoskeleton reorganization. The cells displayed a rounded morphology, a noted loss of actin stress fibers, and accumulation of peripheral actin bundles. TUNEL assays performed on *unc-115::egfp*-transfected cells precluded the possibility that UNC-115-EGFP induced apoptosis, which might have explained the rounded cell morphology. These results confirm the role of UNC-115 as a crucial actin cytoskeleton regulator. SWR-1, previously called AXM-1, is a 7 WD Repeat-Containing Protein and was identified by a yeast two-hybrid screen with the UNC-115 LIM domains. SWR-1 binds both to RAC-2 and UNC-115 in the yeast two-hybrid assay, suggesting SWR-1 as an adaptor for RAC-2 and UNC-115. Coimmunoprecipitation of UNC-115-EGFP and Myc-SWR-1 confirmed the two hybrid results. Coimmunoprecipitation of SWR-1 and RAC-2 is still under way. SWR-1 genetically acted as a repressor of RAC-2 in neuronal morphogenesis, indicating that SWR-1 might define a new class of Rac negative regulators. Study of these molecules in axon guidance pathways will provide a more comprehensive understanding of how these molecules work through the axon guidance signal pathways to modulate the seemingly diverse processes of neuronal development, plasticity and function, and thus contribute to the knowledge of human neuronal diseases.

6. 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 six of adulthood. This is caused by a depletion of sperm, since mated hermaphrodites that have an abundance of sperm produce progeny for several additional days. However, mated hermaphrodites display a sharp decrease in progeny production around day nine of adulthood, indicating that hermaphrodites undergo reproductive senescence despite ample sperm availability. We are using two approaches to analyze reproductive senescence. First, we have initiated F2 clonal screens to identify mutations that delay reproductive senescence. For one screening approach hermaphrodites were mated early in adulthood and then screened for greater than wild type progeny production on day nine and beyond. One candidate mutation was identified, *am117*, that caused hermaphrodites to produce ten times more progeny day nine and beyond and produce progeny for a greater length of time than wild type hermaphrodites when mated early in adulthood. *am117* mutants also produced significantly more progeny when mated on day nine of adulthood, appeared to be smaller in size and had a small increase in lifespan in comparison to wild type. Furthermore, we analyzed existing mutant strains for changes in reproductive senescence. Mutations that cause an increase in hermaphrodite lifespan had various effects on hermaphrodite fertility later in life. Pharmacological agents that have been found to increase longevity in our lab also increased progeny production late in life for N2 hermaphrodites when raised on media containing the drugs. Second, we are trying to identify morphological and molecular 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. The markers may suggest the cause of the observed functional decline. 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.

7. UNC-82 is a serine/threonine kinase required for thick filament organization in body-wall muscle.

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To elucidate the mechanisms that guide the assembly of thick filament proteins, we have undertaken an analysis of the *unc-82* gene. Mutations in *unc-82* cause abnormalities in thick filament morphology and organization (Waterston et al., 1980). A combination of genetic mapping, transformation rescue, and RNAi experiments demonstrate that *unc-82* encodes a ~1600 residue protein that contains a serine/threonine kinase active site domain at the N-terminus. No other regions of homology to known proteins have been detected. BLAST analysis identifies putative orthologs in vertebrates, ARK5 and SNARK, and in *Drosophila*. Sequencing of Yugi Kohara's cDNA clones revealed multiple splice variants that differ in the sequences C-terminal to the kinase domain. The most severe *unc-82* allele, *e1323*, contains a premature stop codon in the kinase domain, and is likely to represent a null mutation. The allele *e1220* contains a missense mutation (E205K) within a conserved residue of the catalytic loop. A rescuing UNC-82::GFP fusion is detected at or near the M-line in body-wall muscle. The *unc-82* phenotype indicates a role for the kinase in regulation of thick filament formation or function. Antibody staining of *e1323* embryos suggests that components of the contractile apparatus localize as wild type at the 1.5-fold stage, the time at which muscle contractions begin. However, defects in the localization of thick filament (myosin and paramyosin) and M-line (UNC-89) components are apparent by the 3-fold stage. The organization of the membrane adhesion structures (integrin) and thin filament system (vinculin and actin) appear normal at this stage, supporting the proposal that *unc-82* function is required specifically for thick filament organization. Our model is that *unc-82* activity is required for reorganization of thick filaments during growth and elongation of the muscle cell, perhaps by regulating filament attachment or mediating filament disassembly. The UNC-82::GFP fusion protein is expressed in non-muscle tissues, where it localizes to regions containing intermediate filaments. Therefore, UNC-82 may play a role in the organization of cytoskeletal structures in other tissue types.

8. Loss of the ELP-1 microtubule binding protein exacerbates the Dystrophin phenotype, in *Caenorhabditis elegans*

Jennifer L. Hueston, Kathy A. Suprenant

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The genome of *Caenorhabditis elegans* predicts a single gene encoding an EMAP-like protein, ELP-1. ELP-1 and analogs from several phyla describe the EMAP-like protein family, a group of microtubule-binding proteins with a prominent WD-repeat domain. In this study we have chosen *C. elegans*, a multicellular model organism, to investigate the function of the ELP family of proteins. To determine where and when ELP-1 is expressed, a GFP reporter construct was generated to examine the expression pattern of ELP-1 in the adult worm. In muscle, ELP-1::GFP is localized subcellularly in a periodic and punctate manner that corresponds to the dense bodies, regions where the myofilament lattice, resembling the human sarcomere, is anchored to the adjacent basement membrane. GFP labeled microtubule-like filaments were found to traverse the cytoplasm of body wall muscles in a crisscross pattern outside of the myofilament lattice. With clear expression of ELP-1 in the body wall muscles and possible sub-cellular localization to the dense bodies, we wanted to check for possible gene interactions, applying feeding based RNA interference, with potential mutant candidates that might confer defects in muscle integrity. A preliminary study was performed examining the development of each strain while growing on the RNAi feeding bacteria. A majority of the strains did not have any visible behavioral or morphological effects, however, a synthetic effect was seen with the Dystrophin-like gene, *dys-1*. In humans, mutations in the dystrophin gene may result in an inherited myogenic disorder such as Duchenne muscular dystrophy (DMD), where approximately 1 out of every 3500 males are affected. Loss of function of *dys-1* results in hyperactive worms that are slightly contracted and hypersensitive to the acetylcholinesterase inhibitor aldicarb. To more precisely define the genetic interaction between *elp-1* and *dys-1* we have performed preliminary morphological and physiological examinations and will also examine genes involved in the dystrophin-glycoprotein complex (DGC).

9. SMA-1 spectrin is required for epithelial cell sheet morphogenesis in *C. elegans*

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During *C. elegans* development, the embryo acquires its vermiform shape due to changes in the shape of epithelial cells, a process that requires an apically localized actin cytoskeleton. SMA-1, an ortholog of the actin binding protein beta_H-spectrin, is required for normal morphogenesis (McKeown, Praitis, & Austin, 1998). Using antisera to SMA-1, we have determined that the protein localizes to the apical membrane of epithelial cells, including the hypodermis, pharynx, gut, and excretory canal cell, during the period when these cells are rapidly elongating. We show that in the hypodermis, SMA-1 is required to maintain the association between actin and the apical membrane. Based on these results, we hypothesize that *sma-1(ru18)* null embryos fail to elongate because actin, which provide the driving force for cell shape change, dissociates from the apical membrane skeleton during morphogenesis. SMA-1 is a large, complex molecule with a series of distinct protein domains that include spectrin repeats, calponin homology, SH3, and pleckstrin homology domains. To elucidate the roles of these domains, we performed sequence and phenotype analysis of several classes of *sma-1* mutants and SMA-1 expression constructs. This analysis indicates that SMA-1 maintains the association between actin and the apical membrane via interactions at its N-terminus and this activity is independent of alpha-spectrin. Our analysis also shows that SMA-1, likely acting with SPC-1 alpha-spectrin (Norman & Moerman, 2002) has additional functions required for normal elongation. SMA-1 provides structural support for the apical membrane and it is required to preserve dynamic changes in the organization of the apical membrane skeleton. Taken together, our results demonstrate the SMA-1 spectrin-based membrane skeleton plays a dynamic role in converting changes in actin organization into changes in epithelial cell shape during *C. elegans* embryogenesis.

10. Wormbase: Improvements To The Database

Darin Blasiar

Washington University, Genome Sequencing Center, Campus Box 8501, 4444 Forest Park, St. Louis, MO, 63108

WormBase continues to grow and improve. In the last year, we have incorporated several new large-scale datasets, made improvements to the user interface, and provided new bioinformatics tools. Ongoing curation efforts have been focused on sequence data, literature, gene expression and antibodies, gene ontology, phenotype ontology, and anatomy ontology. New large-scale datasets include microarray, SAGE, interactome, 3D protein structure, ORFeome, and RNAi data. Improvements to the user interface include a streamlined homepage and efforts to make various web pages more uniform, informative, and stable. Textpresso is a new and useful feature that allows focused text searching of the *C.elegans* literature. New bioinformatics tools include batch downloads, periodic genome freezes, new mirror sites, and support for remote access to the ACeDB and MySQL databases that make up WormBase. Looking ahead, the WormBase data model and software architecture are being reorganized and optimized to accommodate the genomics and biology data from other nematode species.

11. The *C. elegans* T-box gene *tbx-2* is required for pharyngeal development

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T-domain transcription factors are crucial developmental regulators in all animals. In the vertebrate heart, T-domain factors interact genetically and physically with NK-2 homeodomain factors to promote cardiomyocyte differentiation. *C. elegans* contains 20 T-box genes, many of which have not been functionally characterized. We have focused on *tbx-2*, a member of the Tbx2 subfamily that includes factors involved in vertebrate heart development. Loss of *C. elegans* *tbx-2* function, either by RNAi or in deletion mutants, results in feeding defects, L1 arrest and abnormal pharyngeal morphology, although most markers of pharyngeal differentiation are expressed. These phenotypes are similar to those of animals defective in the pharyngeal muscle specific NK-2 homeobox gene *ceh-22*, and we hypothesize *tbx-2* functions with *ceh-22* in pharyngeal muscle development. A *ceh-22::gfp* reporter is expressed in *tbx-2* mutants, although the number of *ceh-22::gfp* expressing cells is reduced. We are currently examining the *tbx-2* expression pattern to understand its role in pharyngeal development and to determine if it is co-expressed with *ceh-22*. In addition we are currently examining possible genetic and physical interactions between *tbx-2* and *ceh-22*.

12. *C. elegans* as a model host for viral infection

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Susceptibility to infection and disease is highly dependent on the genetic background of the host. This is particularly true for viruses because they are obligate intracellular parasites and thus are highly dependent on the intracellular physiology of the host. The relative genetic simplicity of *Caenorhabditis elegans* makes it potentially a useful model host for identifying genes potentially responsible for determining susceptibility or resistance to viral infection. We will present data showing that *C. elegans* can be infected by the mammalian virus vesicular stomatitis virus (VSV). VSV infection of primary cell cultures from wild-type *C. elegans* leads to production of viral transcripts and proteins, demonstrating the ability of the virus to infect and replicate in these cells. Infection of *C. elegans* cells appears to be productive, resulting in increased viral titers in the culture. Consistent with its neurotropic phenotype in mammalian hosts, VSV infects primarily neuronal *C. elegans* cells. Using this infection model, preliminary data suggest that increased production of viral antigen and viral titers is observed during VSV infection of *rde-1* cells. This suggests that the RNA interference pathway may function as an antiviral defense mechanism in the worm. Thus we propose that VSV infection of *C. elegans* cells will provide a useful model for identifying and characterizing the genetic determinants of host susceptibility/resistance to viral infection.

13. MSP signals microtubule reorganization in *C. elegans* oocytes prior to fertilization

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The microtubule cytoskeleton of most animal oocytes differs from that of somatic cells in that the centrioles are lost during oogenesis. In most cases, the meiotic spindle develops without centrosomes serving as microtubule-organizing centers. In addition, microtubules play critical roles in controlling cell shape, protein trafficking, RNA localization, and cell polarity. Despite these essential functions, it is not well understood how extracellular signals regulate microtubule organization and function. It is essential to uncover the signaling mechanisms regulating microtubules in oocytes because of the striking age-related increase in non-disjunction during meiosis I in human females leading to congenital birth defects or miscarriage. In order to address this issue, we have been analyzing the behavior of microtubules during oocyte meiotic maturation in *C. elegans*.

In *C. elegans*, oocyte meiotic maturation is triggered by the major sperm proteins (MSPs) released from sperm. MSPs elicit several cellular responses in oocytes including MAPK activation, M-phase entry, nuclear envelope breakdown, and meiotic spindle assembly. MSP signaling transpires through two parallel pathways defined by VAB-1, an MSP/Eph receptor, and CEH-18, a POU-homeoprotein required for gonadal sheath cell differentiation and function. In the absence of MSP, both pathways negatively regulate meiotic maturation, whereas in its presence, the inhibition is relieved allowing ovulation and fertilization. To examine MSP's role in facilitating meiotic spindle assembly, we investigated the microtubule cytoskeleton in oocytes. We observed that the microtubule arrangement in oocytes differs in the presence and absence of sperm. In the presence of sperm, microtubules disperse evenly in a net-like fashion throughout the cytoplasm. By contrast, in the absence of sperm, microtubules are enriched 1.5-fold at the proximal-distal cortical edges of the oocyte. We also examined microtubules in oocytes of hermaphrodites over time, and observed that as sperm and MSP are depleted, cortical microtubule enrichment progressively increased from proximal to distal oocytes, correlating with the MSP distribution. To test whether MSP is sufficient to signal microtubule reorganization in oocytes, we injected female animals with purified MSP and analyzed microtubule distribution by confocal microscopy. Whereas, oocytes of buffer-injected females exhibited cortically enriched microtubules, oocytes of MSP-injected females did not. Based on these results, we propose that MSP signaling affects microtubule localization and/or dynamics in oocytes prior to fertilization. For future studies, our goal will be to trace the signal transduction pathway from the cell surface to the microtubule.

14. Diversity estimation to determine the changes in nematode community composition in response to environmental cues

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While much is known about the roles genes play in development and physiology, little is known about how the environment affects organisms at the level of expression of individual genes. Using resident nematode populations sampled from the Konza Tallgrass Prairie Biological Station near Manhattan, Kansas, we are attempting to link the responses of organisms to environmental change at the genetic level. We hypothesize that different species may have varying genetic capacities to respond to changes in the environment; either by differences in the genes they possess or in how those genes are regulated. We are currently testing these possibilities by looking at the responses of bacterial feeding nematodes to changes in soil chemistry. Although the Konza nematode community is known to respond strongly to nitrogen addition and burning at the family level, it is not known if there are differential responses to treatment effects at the genus level or below. As an initial step towards the identification of taxa that are sensitive to these treatment effects, the objective of this portion of the study was to define taxonomic diversity across treatment plots that differed in nitrogen addition and burning régime. In addition to morphometric identification to genus, we are using molecular methods to identify taxonomic diversity at the "species" level. We sequenced the 3' 500 bases of the 18S ribosomal RNA gene along with the entire ITS1 sequence. From 900bp of sequence, we have currently identified 17 "species" of nematodes across 4 different families. Preliminary results based on morphometric designations show differential effects of nitrogen and burning at the genus level. Using molecular information, further research will address treatment effects at lower taxonomic levels. Once treatment sensitivity has been identified at the lowest taxonomic scale possible, we will then attempt to identify the genes responsible for the observed responses.

15. TOR and Insulin Pathways Converge at Raptor to Regulate *C. elegans* Larval Development, Metabolism and Life Span

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In multicellular organisms, the control of growth depends on the integration of genetic and environmental cues. Cell growth in response to nutrients is controlled by the highly conserved TOR (target of rapamycin) protein kinases. TOR belongs to a family of phosphatidylinositol kinase-related kinases. Recently, TOR has been shown to interact with raptor (regulatory associated protein of mTOR) to relay nutrient signals to downstream translation machinery in mammals. We previously reported that *daf-15* mutants arrest development non-conditionally as dauer-like L3 larvae at the second molt. The *daf-15* gene encodes the *C. elegans* ortholog of raptor. Mutations in the genes encoding CeTOR also result in dauer-like larval arrest, implying that CeTOR regulates dauer diapause. Pre-dauer L2 larvae accumulate fat in preparation for a prolonged period of non-feeding. The *daf-15* (raptor) and *let-363* (CeTOR) mutants shift metabolism to accumulate fat. We also found that raptor mutations extend adult life span. *daf-15* transcription is negatively regulated by DAF-16, a FOXO transcription factor that is in turn regulated by *daf-2* signaling. This is a new mechanism regulating the TOR pathway. We propose that the CeTOR pathway responds to nutrient levels in *C. elegans*. It interacts with the DAF-2 insulin/IGF signaling pathway to control larval development, metabolism and life span.

16. To Degrade or Not to Degrade: Regulation of GLH Protein Levels

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By targeting proteins for degradation, the germline may maintain a delicate balance of essential proteins required for fertility. Both excess and absence of critical proteins have dire consequences in the germline of *Caenorhabditis elegans*. The COP9 signalosome (CSN) complex regulates protein stability in many organisms with phosphorylation of substrates crucial for targeting proteins for degradation. A component of the CSN complex, CSN-5, was identified as binding the *C. elegans* germline helicases (GLHs). The GLH family consists of four proteins, each of which contains conserved DEAD-box RNA helicase motifs and CCHC retroviral-like zinc fingers. The GLHs are constitutive components of P granules, non-membranous aggregates of protein and RNA that pattern the *C. elegans* embryo, segregating with the germline lineage throughout development. The proper regulation of GLH-1 seems critical for the mitotic/meiotic transition and for subsequent meiotic progression. Elimination of CSN-5 using RNA interference (RNAi) results in a phenotype like that seen after *glh-1/4* combinatorial RNAi. Loss of either CSN-5 or GLH-1/4 causes small, under-proliferated gonads and sterile worms. The GLHs also bind KGB-1, a novel MAP kinase. We find that levels of GLH-1 are greatly increased in the *kgb-1(um3)* deletion strain, which exhibits temperature sensitive sterility and endomitotic replication of oocytes (EMO) at 26°C. Pull-down experiments have shown that KGB-1 cannot bind to GLH-1 when GLH-1 lacks its C-terminus. This region of GLH-1 contains a MAP kinase docking site and a putative phosphodegron, both of which may be used by KGB-1 in its control of GLH levels. The CSN complex has associated kinase activity, with several kinases identified. We have shown that CSN-5 and KGB-1 physically interact through pull-down assays. Additionally, the *kgb-1(um3)* strain is able to rescue the sterility phenotype caused by *csn-5* RNAi. Based on evidence of CSN-5/KGB-1 interactions from RNAi and pull-down experiments, we predict CSN-5 may block KGB-1 mediated GLH-1 degradation. Recently, others have implicated KGB-1 in a MAP kinase pathway that responds to heavy metal stress (Mizuno et al, EMBO, 2004); we are exploring the potential role of this signaling pathway in GLH regulation and fertility.

17. Sex determination gene *laf-1* is a processed non-coding RNA

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In the sex determination pathway of *C. elegans*, regulation of the *tra-2* mRNA is essential for female cell fate. *laf-1* regulates translational repression of the *tra-2* mRNA through elements in the *tra-2* 3' UTR. The phenotypes of *laf-1* mutants include homozygous embryonic/larval lethality, feminization of some heterozygote, and additional germline defects. We are currently cloning *laf-1*. Based on genetic and SNP mapping *laf-1* has been located to a region of 400 kb between SNP Y71H2B.2 and *daf-2*. Injection of cosmids across this region identified a rescuing fragment of 5 kb. There are no complete predicted open reading frames in this fragment; however, there are at least two predicted Pol III genes. Sequence analysis of these genes suggest that *laf-1* may function as a non-coding RNA. A small fragment of 300 nt containing these two genes produces RNA transcripts in yeast Pol III extracts and *Xenopus* oocytes consistent with these genes being transcribed by Pol III. We believe one or both of these genes are *laf-1* for the following three reasons: *C. elegans laf-1* mutant strains are partially rescued by the injection of the 300 nt fragment, a mutation has been mapped for one of the *laf-1* alleles that is nine hundred nt upstream of this fragment, and three of the four *laf-1* alleles have been tested in an RNase protection assay and all three show an altered protection pattern compared to wildtype. Primer extension data and RNase protection experiments suggest that the transcript is processed to form the final product. Both the transcription and processing characteristics of this ncRNA indicate that *laf-1* may represent a new class of ncRNA distinct from miRNAs. Sequence based searches have identified a number of other similar predicted Pol III genes in *C. elegans*, *C. briggsae* and mouse. At least one of the *C. briggsae* genes and one of the mouse genes produce transcripts that may be processed in a similar manner to *laf-1*.

18. Regulation of germline stem cells by the DTC niche

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Stem cells are defined by their capacity for self-renewal and ability to produce at least one differentiated cell type. Their decision between self-renewal and differentiation is governed by both intrinsic signals and extrinsic stimuli from the surrounding microenvironment, or niche. The mitotic region in the *C. elegans* germline includes stem cells* since it is self-renewing and continuously generates gametes. In young wild-type adults, the mitotic region is composed of about 225 germ cells that extend 18-20 cell diameters along the distal-proximal axis. The somatic distal tip cell (DTC), which lies adjacent to the distal-most germ cells, promotes proliferation by GLP-1/Notch signaling and provides the stem cell niche.

Since stem cells defined in other systems divide slowly and/or asymmetrically, we are characterizing cell cycles and orientation of mitotic spindles within the germline mitotic region. Preliminary results using BrdU, Cy3-dUTP, and anti-PH3 reveal no differences along the distal-proximal axis with respect to frequency of mitosis or time from BrdU incorporation (S phase) to M phase. In addition, the orientation of mitotic spindles appears random throughout the region. We suggest that stem cells in the *C. elegans* germ line may be controlled at a population level rather than by asymmetric divisions. We are currently developing methods to track the lineage of individual cells within the mitotic region to examine this further.

The DTCs provide the LAG-2 Notch ligand required for maintenance of the germline stem cells. Whether the LAG-2 signal must reach all of the cells within the mitotic region is still unknown. To ask whether direct contact between the DTC and the germ cells defines the extent of the mitotic region, we examined the DTC and its processes using *lag-2::GFP* and *lag2::myrGFP* reporters. In animals of different ages and in mutants with longer and shorter mitotic regions, we found that DTC process length does not correlate with the length of the mitotic region. These findings extend the work of Hall et al. (1999) and confirm the idea that the length of DTC processes does not define the extent of the mitotic region. To identify additional genes that are important for DTC niche function, we are planning to use a DTC-specific RNAi screen.

Hall et al. (1999) *Developmental Biology* 212, 101-123.

* Note: While nuclei in the germline are syncytial, we call them "cells" for simplicity as they are partially enclosed by membranes and appear to function individually with respect to cell division regulation.

19. Mechanisms of Wnt signaling during P12 specification in *C. elegans*

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Wnt signal transduction regulates a wide variety of processes including cell type decisions in most animals. One such example is that of two neuroectodermal precursors, P11 and P12. These are born as a left-right pair and initially have equivalent developmental potential, but undergo different patterns of development in response to Wnt, EGF and Notch signaling.

We are investigating the nature of the Wnt pathway that specifies the P12 cell fate. Wnt pathways are often classified as canonical if they use beta-catenin as a transcriptional co-activator, or non-canonical if they do not. We have found that mutations in *lit-1(t1512/ts)* and *wrm-1(ne1982/ts)*, which disrupt a non-canonical pathway, have little effect on P11/P12 development. It was previously noted that null mutations in *bar-1* (beta-catenin) cause a P12 to P11 transformation. We found that expression of an activated form of BAR-1 (delta N BAR-1) causes the opposite (i.e. P11 to P12) transformation. These facts indicate that BAR-1 is necessary and sufficient to specify the P12 fate, and suggest that a canonical pathway functions in P12 specification. Time-course studies suggest that BAR-1 acts at the same time as EGF signaling to specify the P12 fate. Since BAR-1 can function as a co-activator of POP-1 (the sole TCF-1 in *C. elegans*), removing POP-1 activity should also cause a P12 to P11 fate transformation if this is a canonical pathway. We found that the *pop-1* hypomorphic mutations *hu9* and *q645* cause a low rate of P12 to P11 transformations. However disrupting POP-1 activity by RNAi caused the opposite effect. These results could indicate that POP-1 specifies the P12 cell fate when BAR-1 is present, but the P11 cell fate when BAR-1 is absent. Work in other animals has shown that TCFs can repress transcription in cells that do not respond to Wnt signals while activating transcription in cells that are responding to Wnts. Further analysis will be done to test whether POP-1 acts in this manner during P11/P12 development.

20. *spd-3* is a novel gene required for spindle alignment in *C. elegans*

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In order to develop a complex organism, cells must have the ability to divide asymmetrically to produce two daughter cells of different developmental potential. A cell accomplishes this by polarizing cytoplasmic components to opposite ends such that, upon cleavage, each daughter inherits distinct components. This segregation requires coordination of the axis of polarity and the cleavage plane. In animal cells, the cleavage plane is specified by the alignment of the mitotic spindle. To better understand the molecular mechanism of spindle alignment we are studying a temperature sensitive, maternal effect, lethal mutation in the *spd-3* gene of *C. elegans*. Cytological analysis reveals that the *spd-3(oj35)* mutant is defective in nuclear and spindle positioning. During the first mitosis the spindle fails to align along the anterior/posterior axis leading to abnormal cleavage configurations. *spd-3(oj35)* mutants also exhibit a failure to extrude polar bodies, which could be attributed to a misaligned meiotic spindle. Post-embryonic defects, including uncoordination and sterility, are consistent with cell division defects indicating that SPD-3 may be involved in spindle alignment in all tissues. The *spd-3(oj35)* strain contains a mutation converting Leucine130 to Phenylalanine in H34C03.1. The H34C03.1::GFP transgene rescues the *spd-3(oj35)* phenotype indicating that the novel gene H34C03.1 is *spd-3*. Surprisingly, SPD-3::GFP localizes to mitochondria. However, this may not be unprecedented because the kinesin Kip3 is required for microtubule instability and spindle alignment in *S. cerevisiae* (DeZwaan, 1997; Miller, 1998) and its *Drosophila* homolog localizes to mitochondria (Pereira, 1997). In studying genes such as *spd-3* we hope to reach a better understanding of factors involved in spindle alignment which is essential for defining the plane of cytokinesis and ensuring proper inheritance of segregated cytoplasmic components.

21. SYS-1, an atypical beta-catenin, acts with POP-1/TCF to control cell fates in *C. elegans*
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POP-1/TCF controls numerous asymmetric cell divisions during *C. elegans* development. Our focus has been on an asymmetric division that generates the proximal-distal axis of the gonad. Both POP-1 and other classical Wnt/MAPK signaling components promote the distal fate in daughters of somatic gonadal progenitor cells (1). Mutations in the *sys-1* (for symmetrical sisters) gene affect Z1/Z4 asymmetry in a similar way and interact genetically with *pop-1* (1,2). We now have five *sys-1* mutations. Two *sys-1* deletions are embryonic lethal, which appears to be the null phenotype. Three other *sys-1* mutations lead to sterility with defects in Z1/Z4 asymmetry; their molecular lesions include a missense mutation, an intronic change, and a silent mutation. The *sys-1* promoter drives GFP in many cells throughout development. Given its genetic interaction with *pop-1*, its effect on Z1/Z4 asymmetry, its broad expression pattern and embryonic lethal null phenotype, we suggest that SYS-1 may control many asymmetric divisions during *C. elegans* development. A major clue to the mechanism by which SYS-1 controls fates comes from its molecular identification as a divergent β -catenin. SYS-1 contains at least three ARM repeats, a motif typical of β -catenin/Armadillo, and rescues a *bar-1* null mutant when driven by a *bar-1*/ β -catenin promoter. Furthermore, by two-hybrid assay, SYS-1 binds POP-1 in a region that includes the β -catenin binding domain. Finally, a lack of SYS-1 does not affect POP-1 nuclear asymmetry (3). We suggest that SYS-1 works with POP-1 to control cell fates. Our favorite model is that SYS-1 acts with POP-1 as a transcriptional coactivator to control target genes. Predictions from this model are being tested and will be presented.

1. Siegfried and Kimble (2002) *Development* 129, 443-453.

2. Miskowski et al. (2001) *Developmental Biology* 230, 61-73.

3. Siegfried et al. (2004) *Genetics* 166, 171-186.

22. Conserved cytokinesis mechanisms revealed through dissection of the mammalian midbody proteome

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Cytokinesis is an essential process that partitions chromosomes, cytoplasm and organelles into daughter cells, yet few of the molecular factors involved are known. To rapidly identify and characterize essential cytokinesis proteins, we employed a functional proteomic and comparative genomic strategy. Midbodies were isolated from mammals, proteins were identified by multidimensional protein identification technology (MudPIT), and protein function was assessed in *C. elegans*. To validate our biochemical screen, we identified 57 known cytokinesis proteins (36%) and 45 known midbody proteins (28%). In addition, ten out of ten identified proteins tested localized at the midbody in HeLa cells. Of 172 homologs and paralogs disrupted by RNAi in *C. elegans*, 58% displayed defects in cleavage furrow formation, furrow completion or germline cytokinesis. Functional dissection of the midbody, a transient and previously mysterious organelle, highlights the importance of lipid rafts and vesicle trafficking pathways in cytokinesis and illuminates the role of over 100 proteins previously uncharacterized with respect to this process. The utilization of common components in diverse dynamic membrane events in the cytokinetic furrow, the germline, and neurons, indicates ancient mechanisms mediating cell division and complex morphogenetic cellular processes critical in human development and disease. Finally, we have begun to establish a midbody protein interaction map to further understand the complex biological functions of proteins that locate to this important structure during cytokinesis.

23. Loss of *pan-1* causes a Peter Pan-like phenotype in *C. elegans*

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P-granules are complexes of proteins and RNA found surrounding the nuclei of *C. elegans* germ cells and germ cell precursors. GLH (germline RNA helicase) proteins are components of the germline specific P-granules, which are necessary for fertility in *C. elegans*. PAN-1, a P-granule associated novel protein, was identified as a GLH-binding protein in yeast two hybrid assays. PAN-1 contains some conserved amino acids of N-terminal F-box motifs, as well as sixteen leucine-rich repeats and a weak FOG-2 homology (FTH) motif, each found in F-box proteins. F-box proteins, in the SCF (SKP-1, Cullin, F-box) complex, utilize ubiquitin-mediated substrate degradation. When *pan-1* is eliminated by RNA interference (RNAi), the larvae arrest between the L1 and L2 stages and can survive eight days at 20⁰C. A *pan-1(gk142)* deletion strain exhibits the same "forever-young" phenotype. mRNA analysis and protein expression show that PAN-1 is not germline specific but is germline enhanced. Experiments are ongoing to separate potential germline and somatic functions of PAN-1. If PAN-1 belongs to the family of F-box proteins, it may be implicated in regulating GLH protein levels, as are two other GLH binding proteins, CSN-5 and KGB-1.

24. C. elegans hypoxia response: Elucidating the function and regulation of the HIF-1 transcription factor

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The *C. elegans* *hif-1* gene is orthologous to the mammalian hypoxia inducible factor alpha units, which have been described as master regulators of transcriptional response to hypoxia (low oxygen). Mutants lacking *hif-1* function are viable in normoxic conditions, but they cannot adapt to hypoxia (Jiang et al. 2001, PNAS 98: 7916). The stability of the HIF-1 protein is regulated by the evolutionarily conserved EGL-9/ VHL-1 pathway. In normoxia, a proline in the oxygen-dependent degradation domain of HIF-1 is hydroxylated by EGL-9, enabling HIF-1 to bind VHL-1. VHL-1 targets HIF-1 for degradation (Epstein et al. 2001, Cell 107: 43). To further understand the role of HIF-1 in hypoxia response, we compared the mRNA expression patterns of wild-type, *hif-1*-deficient, and *vhl-1*-deficient animals in normoxic and in hypoxic conditions using microarray technology. We identified over 100 genes that exhibit a >2-fold difference in mRNA expression in hypoxic conditions ($p < 0.05$). The majority of these genes are regulated in a *hif-1*-dependent manner. We hypothesized that some of the transcriptional targets of HIF-1 might also be required for survival of hypoxia. To test this, we used mutation or RNAi to deplete gene function and assayed viability in normoxia and hypoxia. We report that several individual HIF-1 target genes have essential roles in adaptation to hypoxia. We aim to use genetic methods to identify VHL-independent regulators of HIF-1 and to isolate additional alleles of HIF-1. In pilot screens, we have identified several mutations that alter the expression of HIF-1 target genes. One of these mutations results in a constitutively expressed, truncated HIF-1 protein. The other mutations map to other regions of the genome. We are working towards identification and characterization of these potential HIF-1 regulators.

This project is supported by the American Heart Association.

25. Investigations into the Role of Calmodulin in the Regulation of the Contractile Ring in the *Caenorhabditis elegans* Embryo

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Cytokinesis in animal cells occurs through constriction of a cortical contractile ring that drives an ingressing cleavage furrow. This ring is composed of actin, myosin and other proteins in a bundled array. Mutant studies have shown that myosin is required for the constriction of this ring, and that phosphorylation of the regulatory myosin light chain subunit (rMLC) controls the force producing ability of myosin. Rho Kinase and Citron Kinase, both downstream of the small GTPase Rho, and Myosin Light Chain Kinase (MLCK), activated by Ca²⁺/calmodulin, have been shown to phosphorylate rMLC and to be present in the furrow in some cell types. *C. elegans* citron is missing the kinase domain. Rho Kinase does affect the level of rMLC phosphorylation in the contractile ring but does not appear to be essential, since Rho Kinase mutants have only low levels of cytokinesis failure and much of the rMLC is still phosphorylated (Piekny and Mains, 2002). To investigate the role of the MLCK pathway in activating myosin in the contractile ring in *C. elegans* we performed RNAi on the upstream regulator, *C. elegans* calmodulin (*cmd-1*). RNAi to this gene does not result in any early embryonic defects, but does result in a nearly 100% penetrant arrest at morphogenesis. A CMD-1::GFP fusion protein was introduced, where it localized to structures in the early embryo, but not the presumptive furrow. Additionally, exposing early embryos to pharmacological calmodulin inhibitors phenocopies the RNAi experiments in that they do not lead to early embryo defects, but do produce a later arrest. These observations suggest that the regulation of the cytokinetic furrowing in *C. elegans* is unusual in that it is not regulated by the kinases that have thus far been suggested to regulate myosin activation in cytokinesis in other systems.

26. Converging genetic pathways restrict *vab-3* transcription in the male tail to the B.a and Y.p cell lineages

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Members of the Pax family of transcription factors are well characterized as regulators with important roles in the specification of various tissues and organs. *Pax-6* is a key regulator of eye development in several species, and is important for the specification of other sensory tissues as well (Gehring, 2002). We study the role of the *C. elegans Pax-6* gene *vab-3* in sensory organ development.

Two sensory structures important for male mating are the copulatory spicules and the postcloacal sensillum. These structures develop from two of four post-embryonic blast cells in the male hindgut: F, U, B, and Y. The anterior daughter of B, B.a, gives rise to cells of the copulatory spicules, while the posterior daughter of Y, Y.p, produces the postcloacal sensillum (Sulston et al., 1980). *vab-3* is expressed in the B.a and Y.p lineages, and in *vab-3* mutant males, these blast cells fail to produce normal sensory structures (Zhang et al., 1998; Chamberlin and Sternberg, 1995).

To define the regulatory inputs important for *vab-3* expression, we have utilized a transcriptional GFP reporter transgene. We have found that *vab-3* expression within the hindgut is restricted to the male sensory structure lineages by converging genetic pathways.

One pathway corresponds to a transcriptional cascade involving the Pax-2,5,8 factor EGL-38 and the OVO-like zinc-finger protein LIN-48. In *lin-48* mutant males, the U cell lineage is transformed to a B.a-like lineage, and produces ectopic spicule cell types (Chamberlin et al., 1999; Jiang and Sternberg, 1999). EGL-38 is known to directly activate *lin-48* in the hindgut, and *egl-38* mutants also exhibit aberrant U lineage development (Johnson et al., 2001; Chamberlin et al., 1997). Consistently, we find *vab-3* is ectopically expressed in the U cell lineage of both *lin-48* and *egl-38* mutants. Thus, one function of these two genes is to negatively regulate *vab-3* in the U lineage. Additionally, the *egl-38* and *lin-48* genes display distinct developmental repression of *vab-3*, indicating that *egl-38*-dependent repression of *vab-3* is not mediated solely by *lin-48*.

A second mechanism restricting *vab-3* expression within the male tail involves a Wnt signal transduction pathway. Reception of the LIN-44/Wnt signal by LIN-17/Frizzled is essential for the asymmetry and orientation of the first division of B. In wild type males, B divides to produce the larger anterior daughter B.a, and a smaller posterior daughter, B.p. In *lin-44* mutant males, B often divides with a reversed asymmetry, and produces a corresponding reversal of cell fates between daughter cells (Herman and Horvitz, 1994). *lin-17* mutant males frequently exhibit a symmetric division of B, and both daughters develop with a presumptive B.a-like cell fate (Sternberg and Horvitz, 1988). We have found that *vab-3* transcription correlates with the B.a cell fate in each of these mutants. The transcription factor of canonical Wnt signaling is encoded by the *Tcf-1* ortholog *pop-1*, and *pop-1* mutants display independent defects in B cell behavior and *vab-3* expression. We conclude that Wnt signaling acts as a negative regulator of *vab-3* transcription in the B.p cell.

In order to identify cis regulatory elements important for *vab-3* expression, we have initiated a promoter analysis. We have uncovered elements in the proximal upstream region and the first intron necessary for early transcriptional repression in B.p, resembling the activities of the Wnt pathway genes. In addition, this analysis has revealed other cell-specific, timing-specific, and sex-specific regulatory features of *vab-3*.

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27. Sumoylation of LIN-1 promotes recruitment of chromatin remodeling enzymes and inhibition of vulval cell fates.

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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 MPK-1 MAPK and regulation of the LIN-1 ETS transcription factor, a critical inhibitor of the primary vulval cell fate. 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 small ubiquitin-related modifier (SUMO) to proteins. LIN-1 was shown to be sumoylated in yeast and cultured cells. Sumoylation of LIN-1 promoted its ability to repress transcription and inhibit vulval cell fates. Three proteins that bind sumoylated LIN-1 were identified. MAS-1 (*methyltransferase associated with sumoylated LIN-1*) is homologous to histone methyltransferases that cause transcriptional silencing. DAS-1 (*DNA-dependent ATPase associated with sumoylated LIN-1*) and MEP-1 are putative components of the *nucleosome remodeling and histone deacetylation complex* (NuRD) that also causes transcriptional silencing. These results suggest that sumoylation of LIN-1 mediates transcriptional repression of target genes by two distinct mechanisms: covalent modification of histones and nucleosome remodeling. SUMO was sufficient to mediate transcriptional repression and bind MAS-1, DAS-1 and MEP-1 in the absence of LIN-1, indicating that these are general mechanisms. We propose that SUMO forms an interface between several different DNA binding transcription factors and a common group of sequence-independent chromatin remodeling enzymes.

28. Analysis of LIN-1, an ETS protein, involved in vulval development.

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A highly conserved RTK/Ras/MAP kinase pathway promotes the primary vulval cell fate by 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. We used DNA sequencing to identify the molecular lesion in 27 loss-of-function alleles. The loss-of-function alleles include eleven missense mutations that affect nine different conserved residues in the ETS domain. Most of these mutations cause a severe loss-of-function, although the *ga56* mutation causes a partial loss-of-function. We examined the DNA-binding properties of the wild-type and mutant ETS domains using purified extracts in an electrophoretic mobility shift assay (EMSA). The wild-type LIN-1 ETS domain displays sequence specific binding to a consensus ETS binding site. These mutant proteins were severely defective in DNA binding. These findings indicate that the DNA binding activity of LIN-1 is essential for function *in vivo*.

Target genes that are directly regulated by LIN-1 not been characterized. We utilized our EMSA to test for LIN-1 binding sites in candidate genes involved in vulval development. One likely candidate is *lin-39*, which encodes a homeobox gene transcription factor. Javier Wagmaister and David Eisenmann have characterized the *lin-39* promoter and identified a 1.3Kb region that displays Ras responsive expression in transgenic animals. This region contains multiple GGA motifs that are potential ETS binding sites. EMSA experiments demonstrated that LIN-1 can bind some but not all fragments of the 1.3 Kb region. We are currently performing mutational analysis to identify the minimal regions that are necessary and sufficient for LIN-1 binding.

29. Functional Redundancy of CED-10 & MIG-2 RACs with UNC-34 Enabled

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Rac GTPases regulates cellular morphogenesis and axon pathfinding. Three *C. elegans* RAC proteins (CED-10, MIG-2 and RAC-2/3) are functionally redundant in cell migration and axon pathfinding. Previously, synthetic lethality has been reported in *ced-10; mig-2* and *ced-10; unc-34* doubles (1, 2), also *unc* phenotype was reported when *ced-10* mutation is heterozygous with *mig-2* mutant and vice versa. Still very little is known about how RACs interact with each other and what other molecules play role with RACs in cell migration and axon outgrowth and pathfinding in *C. elegans*. Interestingly, functional redundancy in single RAC protein(s) but synthetic lethality with double RAC mutation allowed us to screen for more partners for CED-10 RAC, using *ced-10* synthetic lethal phenotype.

To date, three new mutations, *lq13*, *lq17* and *lq20* have been isolated. *mig-2(lq13)* and *unc-34(lq17)* mutations are hits in the known CED-10 interacting molecules, whereas *ced-10(lq20)* is a new mutation in CED-10 itself. Interestingly all three are the Gain-of-Function mutations. *mig-2(lq13)* mutation (S75F) falls in the switch 2 region of RAC protein whereas *ced-10(lq20)* mutation (P29L) perturb the switch 1 region. *unc-34(lq17)* mutation is in 5' splice site which causes the use of cryptic splice site, hence causing premature stop codon, and resulting in truncated EVH2 domain of UNC-34 Enabled. Due to premature stop codon, degradation of *unc-34(lq17)* transcript by mRNA surveillance process might occurred which results in enhancement of *unc* and axon guidance phenotype, suggesting that *unc-34(lq17)* is a Gain-of-Function mutation, further *unc-34(lq17)* mutation probably blocking UNC-34 Enabled tetramerization in vivo.

Results from studies of cell migration, axonal guidance and axon outgrowth defects caused by *lq13*, *lq17* and *lq20* mutations, functional redundancy and Gain-of-Function in transgenic animals will be presented.

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30. Progress in Cloning *exc-9*

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The excretory canal cell of *C. elegans* is a single-cell tubular organ. It provides a simple model for understanding the formation and regulation of tubular shape and diameter. The *exc-9* gene regulates the apical diameter of the excretory canal. While the normal posterior canal is narrow and runs all the way to the tail, the posterior canals of *exc-9* mutants are wide, varicose, meandering, and generally end around the position of the vulva. In addition to these canal defects, *exc-9* worms also show impenetrant defects in the shape of the tail whip.

Using SNP mapping, we mapped *exc-9* (*n2669*) to a small region of 13 cosmids on LG IV (from C06A6 to B0218). Injection of cosmid F20D12 rescued both canal and tail defects of the *exc-9* mutants. RNAi of F20D12.5 showed a similar phenotype as that of *exc-9* mutants. Using a 3 kb fragment that includes the F20D12.5 coding sequence and 2 kb of upstream sequence rescued *exc-9*, too.

We are now sequencing the *n2669* allele to confirm the identity of F20D12.5 as *exc-9*. We are also integrating F20D12.5 into GFP vectors to see the expression pattern of this gene. F20D12.5 has a single LIM domain. While some LIM-domain proteins regulate transcription, proteins with a single LIM domain appear instead to mediate assembly of large protein aggregates. We intend to use biochemical and genetic methods to find out the binding partners of this protein.

31. Regulation of the Anaphase Promoting Complex function during meiosis in *C. elegans*. **Edward S. Davis**, Andy Golden

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Chromosome segregation defects during meiosis cause embryonic lethality and birth defects such as Down Syndrome. Our goal is to understand the mechanisms that control chromosome segregation during meiosis. The Anaphase Promoting Complex or Cyclosome (APC/C) is a multi-subunit E3 ubiquitin ligase that targets proteins for degradation during the metaphase-to-anaphase transition of the cell cycle. Most studies have focused on its role during mitosis. Previously, we and others identified ten APC/C subunits in *C. elegans* through sequence homology and RNAi studies. In addition, we described a large number of temperature-sensitive (ts) embryonic lethal mutations in genes encoding five of these subunits: *mat-1*, *mat-2*, *mat-3*, *emb-27*, and *emb-30*. *C. elegans* oocytes are arrested in prophase of meiosis I before fertilization by haploid sperm. Upon fertilization, the oocyte chromosomes go through the two meiotic divisions, discarding their extra chromosomes into polar bodies. Once meiosis is complete, oocyte and sperm pronuclei form, meet, and undergo their first mitotic division. In our mutants, the oocytes are fertilized but the oocyte chromosomes never progress past metaphase of meiosis I. No polar bodies are made and all further development ceases; these embryos arrest as 1-cell meiotic embryos.

We are interested in identifying proteins that could act as novel substrates and/or regulators of the APC/C during meiosis. To achieve this goal, we carried out a large-scale screen for suppressors of the *mat-3* (*or180ts*) mutation. We isolated 27 such suppressors. Among these suppressors of *mat-3* (*som*) mutations, all but one are extragenic, and 24 are dominant. We are working to determine the molecular identity of a subset of the *som* genes. Genetic mapping and complementation analysis indicates the presence of at least five separate *som* genes. Three of the suppressor alleles occur in two components of the spindle assembly checkpoint (SAC). The SAC inhibits the APC/C until all chromosomes are attached at their kinetochores to spindle microtubules. We are determining whether or not the genetic interaction that we have observed between the SAC and the APC/C is specific for the MAT-3 (CDC-23) subunit. Genetic mapping of several other *som* genes suggests that some of the remaining suppressors may not occur in known regulators of the APC/C.

Most of the suppressed embryos progress past the metaphase I arrest, though many display developmental abnormalities and fail to hatch. Of those embryos that do hatch and develop, we observe a high incidence of males (*him*) phenotype, due to non-disjunction of a single X chromosome. Thus, the *som* mutants allow *mat-3* (*or180ts*) chromosome segregation to occur, but not perfectly, thus leading to aneuploidy. This would account for the embryonic lethality and *him* phenotype in these suppressed strains. We hypothesize that the suppressor genes encode proteins that functionally interact with the APC/C during meiosis.

32. ATX-2, the *C. elegans* ortholog of ataxin-2, is required for GLD-1 and MEX-3-dependent regulation of translation in the germline.

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The human ataxin-2 protein is implicated in the neurodegenerative disorder spinocerebellar ataxia type 2, however its function is not known. We show that the *C. elegans* ortholog, ATX-2, is a widely-expressed cytoplasmic protein that forms a complex with PAB-1, a cytoplasmic poly(A)-binding protein that is essential for the development of the germline. We found that ATX-2 is required for the post-embryonic development of the germline. In the absence of ATX-2, proliferation of germline stem cells is reduced, and the hermaphrodite germline is abnormally masculinized. These and other *atx-2(RNAi)* defects suggest a loss, or de-regulation, of translational repression mediated by the conserved KH-domain protein GLD-1. We also found that another KH-domain protein, MEX-3, exhibits a novel, ATX-2-dependent role in preventing inappropriate translation in the germline stem cells. Together, our results support a model in which ATX-2 is involved in translational regulation, and suggest that ATX-2 may directly or indirectly link translational regulators like GLD-1 or MEX-3 with the core translation machinery through the activity of poly(A)-binding proteins.

33. A Vesicle-Budding Model for the Release of MSP from *C. elegans* Sperm

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Oocyte meiotic maturation is essential to prepare the oocyte for fertilization and embryonic development. *C. elegans* sperm stimulate oocyte meiotic maturation and gonadal sheath cell contraction using major sperm protein (MSP) as a signaling molecule. MSP promotes meiotic maturation and activates MAP kinase in oocytes in part by binding the VAB-1 Eph receptor protein-tyrosine kinase. The discovery of MSP's signaling role raised the question of how sperm release MSP to signal oocytes and sheath cells at a distance. MSP is a cytoskeletal protein that nematode sperm utilize for motility and it does not possess a hydrophobic leader sequence. In addition, *C. elegans* sperm lack many standard secretory components, such as ribosomes, ER, or Golgi. Thus, the release of MSP may depend on a novel mechanism.

Using specific antibodies, we detect MSP as far as 90 microns outside of spermatids and spermatozoa *in vivo*, consistent with its function as an extracellular signal. Labeling with vital dyes and sperm specific antibodies rules out sperm lysis as a potential mechanism. Wide-field and confocal microscopy shows extracellular MSP to be punctate with large (<0.5 microns) MSP-staining puncta near spermatids or spermatozoa. Confocal microscopy shows that MSP localizes to apparent membrane blebs at the surface of the sperm cell body, suggesting that the free puncta might originate from the membrane blebs. In the spermatheca, extracellular MSP appears to be more finely punctate suggesting that the large puncta may lose their integrity in this region to form a diffusible signal. Neither a pseudopod nor motility is required for MSP release because MSP puncta are located near wild-type or *spe-8* mutant spermatids. However, MSP puncta produced by spermatids appears to provide a longer acting more local signal. Using high-pressure freezing and freeze substitution to prepare samples for transmission electron microscopy, we have identified unusual free 150-300 nm double-layered vesicles located near spermatozoa in extracellular spaces of the spermatheca and uterus. We are currently testing if MSP is localized within these structures using immuno-EM.

Since, MSP release appears not to occur from spermatids within or dissected from males, we reasoned a cue from the hermaphrodite must initiate release. To test this hypothesis we devised an *in vitro* release assay. Spermatids treated *in vitro* with a female extract rapidly (20s to 2 min) form MSP-containing blebs at their cell surface and MSP appears to be lost from the cell upon further incubation. This activity is distinct from activation during spermiogenesis during which the sperm forms a pseudopod. Scanning electron microscopy also shows a difference in the cell morphology of spermatids treated with the extract. Activity from this extract is abundant, soluble, heat stable, yet abolished by boiling. MSP puncta are found within the uterus of mated germline deficient *glp-4(bn2ts)* animals, suggesting the proposed cue may originate from the soma. Based on these results we propose a model in which spermatids and spermatozoa receive a signal from the hermaphrodite soma and shed vesicles containing MSP in response to trigger meiotic maturation. In this model, MSP-containing vesicles released by spermatozoa in the spermatheca are unstable, forming a diffusible MSP signal that correlates with meiotic maturation rates and MAP kinase activation. Biochemical and cell biological tests of this model are underway.

34. 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 and functions to promote germ cell proliferation in *C. elegans*. Activation of the GLP-1 receptor, expressed in the germline, is spatially regulated by the transmembrane ligand, LAG-2, expressed in the distal tip cell. Binding of LAG-2 to GLP-1 is presumed to induce receptor cleavage generating GLP-1(INTRA), which then translocates to the nucleus, binds LAG-1, and alters the pattern of transcription. Disruption of this pathway causes all proliferating germ cells to prematurely enter meiosis. Conversely, constitutive activation by the *glp-1(oz112)* gain-of-function (gf) mutation results in the formation of a germline tumor where proliferative cells are found throughout the gonad. Two genes, *gld-1* and *gld-2*, function redundantly downstream of GLP-1 to inhibit proliferation and/or promote meiotic development. *glp-1* signaling, in effect, inhibits both genes to promote germ cell proliferation.

We have taken a forward genetic approach to identify genes that either function downstream of GLP-1 to promote entry of germ cells into meiosis, or that function to negatively regulate GLP-1 signaling. A number of screens have been conducted in sensitized backgrounds in order to identify mutations that confer a synthetic tumorous (Syt) phenotype. One of the first screens utilized a very weak *glp-1(oz112oz120 gf)* background and identified mutations in the genes *teg-1* and *teg-4*. *gld-2*; *teg-1* and *gld-2teg-4* are also tumorous. Interestingly, in contrast to the *gld-2gld-1*; *glp-1(null)* triple mutant, which is tumorous, *gld-2*; *glp-1teg-1* and *gld-2teg-4*; *glp-1* triple mutants display the Glp premature meiotic entry phenotype. Another screen using a *teg-1* mutant background identified two weak *glp-1(gf)* mutants, *oz264* and *oz274*; molecular lesions are located in the second and third LNG repeats, regions where other *glp-1/lin-12(gf)* mutations have been localized. Finally, another screen utilized the *glp-1(oz264 gf)* mutant background, which identified one Syt mutant, *oz273*. It appears to be associated with a Mog (masculinization of the germline) phenotype in the *glp-1(+)* background, similar to *teg-1*. Also similar to *teg-1*, the *gld-2oz273* mutant is tumorous, suggesting that *teg-1* and *oz273* could be functioning in the same pathway to regulate *glp-1*. The *oz273* mutation maps on LG1, and SNP mapping is currently underway identify the gene defined by the mutation.

35. The Role of NXF-2 in Sexual Determination and *tra-2* mRNA Export

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The sex determination pathway in *C. elegans* must be tightly controlled in order for proper sexual development to occur. *tra-2* encodes a transmembrane protein that is involved in promoting female sexual fate. Therefore, *tra-2* needs to be down-regulated in order for spermatogenesis to occur and up-regulated for oogenesis to occur. This switch in *tra-2* regulation is in part, governed by a link between nuclear export and translational control.

The control over nuclear export of *tra-2* is regulated by various factors, including NXF-2. Most mRNAs are exported by the NXF-1/TAP pathway. However, *tra-2* displays an unusual export mechanism because it appears to be CRM-1 dependent. Specific factors such as REF-1/REF-2 and NXF-2 are thought to associate with the *tra-2* 3'UTR to influence the choice of export pathway. When these factors are present, *tra-2* exits the nucleus in a CRM-1 dependent fashion. However, if one of these factors is missing, *tra-2* exits via the more typical NXF-1 route. Here, we focus on NXF-2 and its involvement in *tra-2* export. NXF-2 belongs to the same family of proteins as NXF-1, and it binds specifically to the *tra-2* 3'UTR consistent with NXF-2 controlling *tra-2* export. We have isolated a deletion of NXF-2 that displays pleiotropic phenotypes such as feminization, sterility and lethality, suggesting that NXF-2 has other mRNA targets. Currently, we are attempting to identify the precise NXF-2 binding site on *tra-2* so that these other mRNA targets may be identified.

36. Cross-species RNAi: several dsRNAs from *Ascaris* sterilize *Caenorhabditis*

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The parasitic nematode *Ascaris lumbricoides* is the most ubiquitous human parasite, infecting ~1/4th of the world's population, while a similar proportion of Missouri swine are infected with the closely-related species *A. suum*. *Ascaris*, worms lay 200,000 eggs/day! Because parasitic nematodes are rapidly developing multi-drug resistance and most anti-helminthic drugs eliminate the adults but have no effect on the eggs, we reasoned that RNA interference (RNAi) might be applicable as a novel anti-parasitic agent. Since ascarid worms cannot be maintained in the laboratory for extended periods, we have begun by testing *Ascaris* genes in *Caenorhabditis*. Several *Ascaris* cDNAs with stretches of identity to *C. elegans* of >21nts and with an overall match of >80% for ~200nts were amplified from *Ascaris* ovarian RNA. Thus far, after injection into *C. elegans*, two *A. suum* genes have been successful in sterilizing *C. elegans*. In our first tests ~90% of the offspring were either dead embryos or sterile F1 adult worms. Although both genes are highly conserved, neither has a mammalian counterpart with a match of 21nts. We have recently begun to test two other candidates that are nematode-specific and plan to expand our studies to the more-applicable methods of dsRNA delivery, including feeding and soaking. We plan to test isolated *Ascaris suum* adults in the laboratory to assure ourselves that RNAi works in this species of nematode before moving on to the daunting task of considering delivery in swine.

This work was supported by a pilot award from the MU PPAID (Program Project in Animal Infectious Diseases), funded by the USDA. SR was supported by a University of Missouri LS UROP (Life Science Undergraduate Research Opportunities Program) fellowship.

37. Positional cloning and functional analysis of daf-31

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Dauer-like larvae show some, but not all, of the normal features of dauer larvae. Fifty-four dauer-like mutants defining at least 14 genes were isolated from previous mutant screens. These dauer-like mutants fail to respond appropriately to environmental cues, and are incapable of executing either complete dauer or reproductive development. The development of daf-31 is arrested after the third molt. daf-31 shows some dauer characteristics, but it cannot complete dauer development either in the presence of dauer-inducing pheromone or under starved conditions. daf-31 is epistatic to daf-12 and daf-16, so it is judged to act downstream of insulin and TGF-beta pathways. Unlike some dauer-like mutants, daf-31 does not affect adult life span. daf-31 was identified using positional cloning methods; it encodes a putative acetyl transferase. Semi-quantitative RT-PCR revealed that DAF-12, a nuclear hormone receptor, promotes daf-31 transcription in vivo. Other biological functions of DAF-31 are being characterized.

38. Electrophysiological Characterization of the PKD-2 Calcium Channel

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The *C. elegans* *pkd-2* gene encodes a homologue of human Polycystin 2, a TRPC-related divalent cation channel. Homology between worm PKD-2 and human PKD2 is especially strong throughout the transmembrane region that forms the ion channel portion of the protein. Humans with defective *pkd2* develop polycystic kidney disease (PKD), whereas *C. elegans* males deficient for *pkd-2* exhibit impaired mating efficiency. The nematode gene regulates mating behaviors (Barr *et al.*, *Curr. Biol.* '01) and is expressed in the cilia and cell bodies of male-specific sensory neurons. We have found that worms deficient for PKD-2 also express a dispersal phenotype, i.e. they don't gather in clumps of males and hermaphrodites as do wild-type worms. This phenotype is probably effected via recognition defects and subsequent frequent loss of contact between males and hermaphrodites.

We have expressed *C. elegans* *pkd-2* in LLC-PK1 and MDCK cell lines. Calcium imaging and black lipid membrane analysis of single-channel electrophysiological properties of this channel in isolated membrane vesicles show that *C. elegans* PKD-2, like its mammalian counterparts, functions as a calcium channel.

We have prepared *pkd2* constructs containing either human or worm N- and C-termini (the human gene supplied by S. Somlo), to determine the ability of human and chimeric genes to substitute for the wild-type nematode gene and rescue the mutant phenotypes.

Finally, we are searching for modifier genes that will identify components of the polycystin signaling pathway. We have isolated several lines that exhibit partial rescue of wild-type dispersal behavior and mating efficiency. We are using genetic markers, including single nucleotide polymorphisms, to map the putative suppressor genes associated with phenotypic rescue.

39. Gut expression Mechanism of Tropomyosin isoforms III and IV in *Caenorhabditis elegans*

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The *Caenorhabditis elegans* single tropomyosin gene, *tmy-1/lev-11* encodes four isoforms- CeTMI, CeTMII, CeTMIII and CeTMIV. CeTMIII and CeTMIV are expressed in pharynx and gut via alternative splicing by the use of internal promoter of *tmy-1* while the other two isoforms are expressed in body wall, anal and sex muscles under the control of the external promoter. CeTMIII is further expressed in germ-line tissue. Mechanisms and transcription factors involved in production of these isoforms are unknown. Transcription factors such as *pha-4* and *ceh-22* regulate pharyngeal genes expression, and *end-1*, *elt-2*, *med-1* and *D2* associate with gut expression. PHA-4 and CEH-22 control pharyngeal expression of *myo-2* by targeting *B* and *C* subelements in its promoter. Similar to *myo-2*, internal promoter of *tmy-1* contains *B* and *C* subelements. It is of immense interest to know how PHA-4 involves in the transcription from the internal promoter of *tmy-1*. PHA-4 and other transcription factors cooperate to activate transcription of pharyngeal genes. Although these factors have been reported in other cells, none had been identified to associate with intestinal tropomyosin expression. In this study, screening *C. elegans* cDNA library by yeast one hybrid system using the internal promoter of *tmy-1* as a bait, *egl-4*, a cyclic-guanine monophosphate dependent protein kinase and *egl-18*, a GATA transcription factor were identified. Exons 5a, 5b and 5c of *tmy-1* has *ges-1*-like element. The *C. elegans ges-1* gene encodes a non-specific (with respect to substrate) carboxylesterase normally restricted to the gut. The presence of *ges-1* elements in CeTMIII and CeTMIV contributed to their further expression in the intestines. Reporter genes deficient in exons 5a or 5c may be expressed in pharynx only.

40. Pharmacological analysis of aging in *C. elegans*

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The identification of 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. We screened pharmacological compounds with known mechanisms of action for the ability to extend lifespan in *C. elegans*. Twenty compounds were tested, and two drugs were identified that reproducibly increase mean and maximum lifespan.

The ability of these compounds to delay the aging process suggests that their targets may regulate normal aging. We are using several approaches to investigate such targets. First, we are analyzing the lifespan of mutants with abnormal aging in the presence of the drugs to determine whether the drugs act by influencing known *C. elegans* aging pathways. Second, we are testing structurally or functionally similar compounds for an ability to extend lifespan in order to identify aspects of the drugs that are important for their potency. Third, we are quantifying non-aging phenotypes caused by the drug. Fourth, we are conducting a forward genetic screen to identify mutants that are resistant to the drugs.

41. Identification and characterization of *C. elegans* Fos and Jun homologs

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The basic region leucine zipper (bZIP) proteins constitute one of the largest families of transcription factors in mammals, *Drosophila*, *C. elegans* and plants. They bind to specific DNA sequences as heterodimers or homodimers, and regulate a variety of cellular processes. For example, the activator protein 1 (AP-1) group of bZIP proteins contains the Fos, Jun, ATF2 and Maf subfamilies, which bind to the AP-1 consensus sequences. AP-1 proteins activate a variety of target genes that regulate cellular proliferation, differentiation and death, as well as stress responses. Furthermore, genetic analyses using mouse and *Drosophila* have revealed that AP-1 proteins play pivotal roles in regulating specific developmental events.

P> P>

How do bZIP proteins form the correct heterodimers and homodimers at appropriate times during development, out of many other possible combinations? We are developing *C. elegans* as a system to answer this question. First, we searched the *C. elegans* genome sequence database and identified 21 unique bZIP-containing proteins. Sequence alignment and phylogenetic analysis of the bZIP domains along with those of *Drosophila* and human bZIP proteins showed that the proteins encoded by F29G9.4 and T24H10.2 are the closest homologs of *Drosophila* and human Fos and Jun, respectively. We tentatively name these genes *Ce-fos-1* and *Ce-jun-1*.

P> P>

To determine whether these genes regulate specific developmental events in *C. elegans*, we used RNA interference. Initial studies suggest that *Ce-fos-1* is required for normal vulva development, and *Ce-jun-1* is required for embryonic viability. To characterize their DNA-binding ability and dimerization selectivity, we carried out gel mobility shift assays, and found that they bind AP-1 consensus sequences as a heterodimer. As is the case with mammalian Fos and Jun proteins, Ce-JUN-1 can also form homodimers, but Ce-FOS-1 cannot. Furthermore, both Ce-FOS-1 and Ce-JUN-1 could form heterodimers with rat c-Fos to bind DNA, suggesting that they are functionally interchangeable in terms of DNA binding. Finally, these interactions have been confirmed in living cells and in living animals using a bimolecular fluorescence complementation (BiFC) assay. Taken together, our results strongly suggest that Ce-FOS-1 and Ce-JUN-1 are the *C. elegans* Fos and Jun proteins, and that these genes play important roles in *C. elegans* development. Our ability to assay dimerization *in vivo* should allow us to elucidate how their pattern of dimerization changes during development.

42. Mutational Analysis of the UNC-44 Axonal Guidance Ankyrin and PP2A B'-subunit Interaction

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In *Caenorhabditis elegans*, the neural-specific UNC-44 AO13 ankyrin is required for proper axon guidance and outgrowth (Otsuka *et al.*, *J. Cell. Biol.*, 129:1081-1092, 1995; Boontrakulpoontawee and Otsuka, *Molec. Genet. Genom.*, 267:291-302, 2002; Otsuka *et al.*, *J. Neurobiol.*, 50:333-349, 2002). Using a yeast two-hybrid approach, we found interactions between the carboxyl terminal domain (CTD) of AO13 ankyrin and the protein phosphatase 2A (PP2A) B' subunits encoded by the W08G11.4 and C13G3.3 model genes (Gong and Otsuka, *Molec. Biol. Cell* (suppl.), 14:124a, abst. #685, 2003). Using deletion subclones to define the binding domain, a 140 amino-acid (aa) region of the UNC-44 CTD (residues 6658-6798) was sufficient to bind W08G11.4 and C13G3.3a proteins. Threading of this sequence, which contains two 14-aa repeats, onto known protein structures by the 3D-PSSM computer program (<http://www.sbg.bio.ic.ac.uk>) revealed the best match to the two-EF-hand calcium-binding protein, calbindin D_{9k}. This observation may explain the axonal guidance defect resulting from the *unc-44(rh1042)* allele in which a Tc1 transposon is inserted between the two EF-hands. Blue staining of the recombinant UNC-44 protein with Stains-all is consistent with a metal-binding fold. Analysis of DNA primer-generated mutations showed that the 14-aa repeats [S(L/V)(T/S)SL(G/A)EFERLEKE] within this region are important for W08G11.4 binding, but have much less of an effect on C13G3.3a binding. Specifically, W08G11.4 binding is prevented by mutations that alter charge clusters in the putative EF-hand stem regions. Multiple mutations of serine to leucine in the EF-hand loop regions block W08G11.4 binding. As expected for a substrate of S/T phosphorylation and dephosphorylation, mimicking phosphoserine by single mutations of serines to aspartate or glutamate residues in the EF-hand loop regions did not prevent B'-subunit binding. We are currently exploring the possibility that UNC-44 is a calcium sensor modulating, or modulated by, UNC-44 phosphorylation-dephosphorylation.

43. The conserved DEAD-box helicase CGH-1 negatively regulates MAP kinase activation in *C. elegans* oocytes

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Oocyte meiotic cell cycle progression must be precisely coordinated with ovulation and fertilization in order to form a diploid zygote. In *C. elegans*, fully-grown oocytes arrest at diakinesis of meiotic prophase I, and this arrest is released by an extracellular signal provided by the sperm, the major sperm protein (MSP). MSP signaling activates the conserved MAP kinase (MAPK) pathway in oocytes and promotes diverse meiotic responses, including M-phase entry, cortical cytoskeletal rearrangement, and gonadal sheath cell contraction.

To clarify the mechanisms of MSP signal transduction and to identify downstream components in meiotic maturation regulatory pathways, we took a genetic approach. We isolated a dominant mutant *std-1(tn691d,ts)* (stuck in diakinesis) that affects MSP signaling responses, interferes with normal oocyte meiotic maturation processes, and disrupts meiotic chromosome segregation. Positional cloning of the gene revealed that *std-1* corresponds to *cgh-1* (1), which encodes a member of a highly conserved small subfamily of DEAD-box RNA helicases associated with germline development and meiotic progression.

According to phenotypic and molecular analyses, *cgh-1(tn691d,ts)* possesses dominant-negative character. Analysis of a protein null mutant, *cgh-1(ok492)*, indicates *cgh-1* is a negative regulator of MAPK activation in oocytes. MSP signaling activates MAPK in the most proximal one to three oocytes in the wild type. In contrast, in the *cgh-1* mutants, MAPK activation is observed in not only proximal but also distal oocytes. In females, in which the MSP signal is absent, MAPK activation is not observed; however, in feminized *cgh-1* mutants [*cgh-1(tn691d,ts);fog-2(q71)* and *cgh-1(ok492);fog-3(q443)*] signal independent MAPK activation is detected. Thus, *cgh-1* is required for: (i) the establishment of the response threshold in the presence of the MSP signal; and (ii) the inhibition of MAPK activation in the absence of sperm. OMA-1 and OMA-2 are two zinc finger proteins redundantly required for meiotic maturation (2). Without *oma-1/oma-2* function, MAPK activation in proximal oocytes is not observed. In either *cgh-1(RNAi);oma-1(te33);oma-2(te51)* or *oma-1(RNAi);oma-2(RNAi);cgh-1(ok492)*, MAPK activation is also not observed. Therefore, *cgh-1* functions upstream or in parallel to *oma-1* and *oma-2* for MAPK activation. In addition to germline phenotypes, *cgh-1* mutants exhibit elevated gonadal sheath cell contractions in both the presence and absence of sperm. Elevated sheath cell contractions are also observed in *cgh-1(RNAi);rrf-1(pk1417)* hermaphrodites and females, indicating that *cgh-1* functions in the germ line to modulate sheath cell response to MSP.

CGH-1 protein is specifically expressed in the germ line and localizes to the cytoplasm in proximal oocytes. In the wild type, CGH-1 is localized to a subcortical band that encircles the oocyte. In contrast, in the absence of sperm CGH-1 localizes subcortically, especially to large distinctive cytoplasmic foci. Similarly, in the dominant-negative mutant, *cgh-1(tn691d,ts)*, CGH-1 localizes to large subcortical cytoplasmic foci with and without sperm. Thus, CGH-1 could be in different kind of complexes or associated with different factors in the presence and absence of the MSP signal, and the dominant-negative mutation may alter such associations.

(1) Navarro et al. Development 128: 3221, 2001. (2) Detwiler et al. Dev. Cell 1: 87, 2001.

44. Cloning and analysis of *ne319* in *C. elegans*

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In *C. elegans*, dsRNA can induce systemic silencing of the corresponding gene in most tissues of the worm and in the progeny. *ne319* was identified in the rde screen aimed to isolate RNAi defective mutants. *ne319* has a method-of-delivery dependent RNAi phenotype since introduction of dsRNA by injection, but not feeding, elicits an RNAi response. The RNAi defect in *ne319* behaves as a temperature-sensitive recessive mutation. Preliminary study revealed that the mutation of *ne319* is located in a 0.2 map unit interval on IV chromosome. We will describe our phenotypic analyses and molecular data to reveal the nature of *ne319*.

45. Investigating *laf-1*'s involvement in *tra-2* regulation

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Sex determination in *C. elegans* is a complex process requiring precise temporal and spatial expression of many genes. *tra-2* encodes a large transmembrane protein and acts in the sex determination pathway to promote female development. *tra-2* is translationally repressed at specific stages in the hermaphrodite to allow spermatogenesis, and this repression is carried out by binding of factors (such as GLD-1) to elements called TGEs in the *tra-2* 3'UTR. Proper *tra-2* regulation requires *laf-1*, another gene involved in sex determination. Current data suggest that the *laf-1* gene product is a small non-coding RNA. *laf-1* mutations are homozygous lethal, and a proportion of *laf-1/+* animals are feminized (that is, they fail to produce sperm).

We are currently working to understand how *laf-1* interacts with *tra-2*. Reporter transgenes containing lacZ fused to the *tra-2* 3'UTR are misexpressed when placed in a *laf-1/+* mutant background, suggesting that *laf-1* is involved in the regulation of *tra-2* expression. Western blotting experiments indicate an increase in the amount of TRA-2 protein in *laf-1* mutant heterozygotes and that this increase is stage specific. This suggests that *laf-1* control may be developmentally regulated. Northern blots are currently in progress to ask if *laf-1* mutations affect the level of *tra-2* mRNA as well as the protein levels. We suspect that *laf-1* may be involved in the post-transcriptional regulation of *tra-2*, possibly acting in complex with GLD-1 on the *tra-2* 3'UTR. We plan to test this model using gel mobility shift and immunoprecipitation experiments and then go on to look for other possible targets of *laf-1* regulation.

46. Characterization of the Nephronophthisis Genes in *C. elegans*

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We are using *C. elegans* as a model system to study cystic diseases of the kidney. Nephronophthisis is an autosomal recessive renal cystic disease caused by mutations in at least four different loci (NPHP1, NPHP2, NPHP3, and NPHP4). The various disease lesions commonly show basement membrane disintegration, tubular atrophy with cyst development, and interstitial cell infiltration with fibrosis that leads to chronic renal failure. Differences exist between the NPHP genes in the age of onset of end-stage renal disease (ESRD)¹.

C. elegans has strong homologs of the NPHP1 and NPHP4 genes. To characterize the function of these genes, we are examining GFP expression patterns and knocking down gene function with RNAi and genetic mutations. We have found that at least one NPH gene is expressed in the male specific sensory neurons and localizes to the ends of male ray cilia. Interestingly, this expression pattern overlaps with that of *lov-1* and *pkd-2*, the *C. elegans* homologs of the human autosomal dominant polycystic kidney disease (ADPKD) genes PKD1 and PKD2². ADPKD is the most common monogenic cause of ESRD in humans. Clinical manifestations include cyst formation in the kidney, liver and pancreas³. Due to similarities in cyst formation and expression in *C. elegans*, we hypothesize that *lov-1*, *pkd-2* and the NPHP genes may share a common function. We are exploring links between autosomal dominant polycystic kidney disease and nephronophthisis.

1. Hildebrandt F. Journal of the American Society of Nephrology. 11(9):1753-61, 2000 Sep.

2. Barr MM. Sternberg PW. Nature. 401(6751):386-9, 1999 Sep 23.

3. Arnaout MA. Annual Review of Medicine. 52:93-123, 2001.

47. The Arp2/3 activator Scar Acts in Parallel to MIG-2 Rac in Axon Pathfinding.

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Axons are guided to their targets in the developing nervous system by the growth cone. In response to guidance receptor signals, the growth cone actin cytoskeleton is modulated to achieve growth cone movement. The actin cytoskeleton can mediate diverse functions in the growth cone, including guidance and the rate of outgrowth. The Arp2/3 complex is a major actin-nucleating complex and is involved in many morphogenetic processes including *Drosophila* axon pathfinding (Zallen et al., 2002, Pollard et al., 2000). Scar/WAVE is an activator of Arp2/3 complex that has extensive homology with WASP-Homology (WH) proteins in its C-terminal domain and a Scar-specific N-terminal Scar homology domain (SHD). Rac small GTPases are known to activate Arp2/3 actin nucleation via Scar, but the interactions of these molecules during axon development remain unclear. We have undertaken a study of the role of the *C. elegans* Scar gene *wve-1* in axon pathfinding.

A fusion of the *wve-1* promoter and the SHD coding region to *gfp* was expressed in most cells in early embryogenesis. At the two-fold stage of elongation, *wve-1::gfp* expression was prominent in the nerve ring, suggesting that *wve-1* is expressed in neurons. To determine if *wve-1* is required for axon development, we ablated *wve-1* gene function by RNAi. *wve-1*(RNAi) caused approximately 60% early embryonic lethality. The arrested embryos displayed morphogenesis defects as early as gastrulation, suggesting *wve-1* is important for proper embryonic morphogenesis. However, viable *wve-1*(RNAi) progeny displayed no axon development defects. Three *C. elegans* rac genes *ced-10*, *mig-2* and *rac-2/3* act redundantly in axon development. To determine if *wve-1* acts redundantly with *mig-2* and *ced-10* in axon development, we performed *wve-1* RNAi in *mig-2* and *ced-10* mutants. In both cases, embryonic arrest increased to ~80%, indicating that *wve-1* and the racs might act together during embryonic morphogenesis. Furthermore, *wve-1*(RNAi); *mig-2* viable animals displayed axon development defects, most notably ectopic axon branching, whereas *wve-1*(RNAi); *ced-10* viable animals did not, suggesting that *wve-1* acts in parallel to *mig-2* rac but not *ced-10* rac during axon development. Analysis of *wve-1*; *ced-10* and *wve-1*; *mig-2* double mutants showed similar results. Thus, *wve-1* might be required for embryonic morphogenesis and might function redundantly with *mig-2* rac in axon development, possibly in the *ced-10* rac pathway.

48. Identification of a novel gene involved in Ras-mediated vulval induction

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The development of the *Caenorhabditis elegans* vulva requires the activity of the Ras signaling pathway. To identify components of this pathway, we screened for mutations that suppress the multivulval phenotype caused by *let-60(n1046gf)*, a mutation that constitutively activates Ras. 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*). One of these alleles, *n2508*, defines a new complementation group. The *n2508* mutation strongly suppressed the *let-60(n1046)* multivulval phenotype, but did not suppress the *lin-1(n383)* multivulval phenotype, suggesting that the affected gene may act downstream of *let-60* but upstream of the transcription factor *lin-1*. In a wild-type genetic background, the *n2508* mutation did not dramatically affect vulval development, but *n2508* did cause a partially penetrant larval lethality. The *n2508* lethal phenotype was enhanced by loss-of-function mutations in *let-60 ras* and *sur-8*, indicating that the gene affected by *n2508* functions in other Ras-mediated processes such as excretory duct cell development. We used single nucleotide polymorphisms in the *C. elegans* strain, RC301, to map the *n2508* mutation to a 1.2kb region on chromosome II that encompasses regions of three predicted genes. Transgenic animals were constructed expressing wild-type fragments of genomic DNA from within this region. One gene was identified that was capable of restoring the multivulval phenotype in the *n2508;n1046* strain. The identified gene encodes a protein that is similar to a human tumor suppressor protein. Interestingly, RNA inhibition of the *n2508* gene increases the penetrance of the multivulval phenotype in both the *n1046* and the *n2508;n1046* strains, suggesting that this gene product exerts a negative regulatory influence upon Ras signaling in *C. elegans*.

49. The role of *mua-1* in epidermal tissue integrity.

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Mechanical attachments linking muscle to cuticle via basal lamina and hypodermis form in the embryo and are essential for musculo-skeletal system development and function. The *mua* genes are required for the proper development of these muscle-cuticle linkages. Mutations in *mua-1* disrupt not only attachments involved in the transmission of muscle contractions to the cuticle but also attachments between the uterus and the body wall, resulting in paralysis of movement and prolapse of the uterus through the vulval opening. Analysis of tissue separation in *mua-1* mutants shows that the hypodermis separates from cuticle. Previous rescue analysis suggested but did not definitely prove that *mua-1* is F54H5.4, a gene encoding a xKLF homolog. The protein encoded by this gene is more closely related to group II of KLF proteins than SP1. In vertebrates these group II proteins are involved in activating or repressing tissue specific gene. RNAi of F54H5.4 results into a prolapsed uterus that is similar to the *mua-1* (*rh160*) allele. However, no muscle detachment in RNAi treated worms has been observed under confocal microscope. In the two of four *mua-1* alleles that have been sequenced, no alterations in this gene have yet been identified. Complicating the issue, F54H5.4 may share an operon or a promoter region with F54H5.3, as only 267 bases separate the coding regions of the two genes. However, for the two sequenced alleles, no alterations were detected in F54H5.3 either. RNAi of F54H5.3 gave no observable phenotype. I am determining the DNA sequence alterations of the remaining mutant alleles of *mua-1* and defining a minimum-rescuing fragment of DNA to demonstrate that F54H5.4 is the *mua-1* gene. Also, I am doing GFP localization of both F54H5.4 and F54H5.3 genes to find out if both have a genuine promoter region. F54H5.4 promoter is expressed in early hypodermis, throughout development in anterior pharynx and at L4 stage in uterus. This expression is consistent with the tissue disruption in *mua-1* mutants. Although, there are no visible gross pharyngeal phenotype it was determined that these mutants have slow rate of pharyngeal pumping. We are also examining the regulatory elements of *mua-1* that are tissue specific.

50. Characterization of a suppressor of the *laf-1* sex-determination gene

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The sex-determination gene *tra-2* is essential for female development and its translation must be repressed for male cell fate decisions to occur. A factor called GLD-1 binds two elements in the *tra-2* 3'UTR to affect translational control. The function of another gene, *laf-1*, is also important for its regulation through these elements.

laf-1 heterozygotes exhibit a partially penetrant feminization phenotype in both hermaphrodites and males presumably due to its effect on *tra-2* translation. Interestingly, *laf-1* homozygotes arrest at about the 1.5- to 2- fold stage of embryogenesis or as L1 larvae. Since *tra-2* and other downstream sex-determination genes do not have a lethal phenotype, this suggests that *laf-1* may be involved in more processes than just sex-determination.

Our lab has isolated a mutation (*nw75*) that suppresses both the feminization and lethal phenotypes. When crossed away from *laf-1*, *nw75* has a sterile, uncoordinated phenotype, which often is indicative of defects in cell division. Consistent with this, *nw75* worms show evidence of cell division failures in the intestine and ventral nerve cord. Since the double mutant strain is not sterile, these mutations must be mutual suppressors. Taken together, these data are suggestive of a role for *laf-1* in cell division.

Using both genetic and SNP mapping techniques, I have mapped *nw75* to within a 100KB region on the right arm of chromosome III. One exciting candidate gene in this region is *tim-1*, which was recently found to be involved in chromosome cohesion, and exhibits some germline defects seen in *nw75*. Preliminary evidence suggests that RNAi of *tim-1* in a *laf-1* background can rescue *laf-1* lethality, which supports the idea that *nw75* is *tim-1*.

51. Nuclear Receptor Regulation of Drug Metabolizing Enzyme Gene Expression

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We are interested in understanding how environmental chemical signals impact the expression of drug metabolism enzymes (DMEs). DMEs of Phase I and II metabolism are actively involved in detoxification of xenobiotics and endogenous compounds and are a major source of drug inactivation and interference. Therefore, understanding the molecular events that begin with the ingestion of environmental compounds or drugs and result in DME upregulation is a key component of predicting drug-drug and drug-environment interactions. In the past several years, the nuclear receptor (NR) PXR has been implicated as a key mediator of xenobiotic induction of DME gene expression in several vertebrate species. Among the more than 260 nematode NRs are several that are closely related to PXR including NHR-8, which *C. elegans* requires for wild type resistance to xenobiotics. The role of NHR-8 in xenobiotic resistance suggests that like its vertebrate homolog, NHR-8 responds to the presence of xenobiotic toxic compounds by upregulating the expression of a detoxification network to remove the offending compounds. We are testing this prediction by 1) describing the members of the nematode detoxification network within the *C. elegans* genome, 2) defining the DME loci that are upregulated by NHR-8, and 3) investigating the regulation of NHR-8 expression. The description of the *C. elegans* detoxification network has allowed the production of a DME-specific DNA microarray that we are probing to discover the DME loci that may be regulated by NHR-8.

52. Searching for new alleles in the GEF *exc-5*

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The *C. elegans* excretory canals are formed from a single cell body located just behind the pharyngeal bulb. During the larval stages of the animal, this cell extends two tubular processes that eventually form two long canals, one on the left and one on the right, that stretch from the head of the animal to the anus. The result is a large H-shaped excretory organ.

A series of genes have been identified that when mutated, cause the canals to form fluid-filled cysts in various positions and sizes. These genes have been termed *exc* genes (short for excretory canal abnormal).

One of these genes, *exc-5*, when mutant, causes the animal to have a canal that stops approximately midway in length, and ends in one or several large cysts. EXC-5's closest human homologue is FGD1. FGD1 is involved with the genetic condition FacioGenital Dysplasia, which causes multiple defects in skeletal structure.

exc-5 encodes a putative guanine nucleotide exchange factor (GEF); these proteins replace GDP for GTP in order to activate small GTPase proteins such as Rho, Rac, and Cdc42. We have previously found that EXC-5::GFP is expressed primarily in the excretory canals, where we believe it regulates the ratio of apical:basal cytoskeletal construction.

Genetic evidence suggests that EXC-5 acts upstream of the Rho GTPase *mig-2*, a gene implicated in axonal guidance. The *mig-2* mutant phenotype, however, does not include any canal defects, which suggests that EXC-5 has several downstream targets, one of which is involved in cytoskeletal formation and maintenance of canal structure.

We are currently performing a non-complementation screen in order to isolate a new EMS-induced temperature-sensitive allele of *exc-5*. Such an animal would allow the temporal requirements of EXC-5 to be investigated as well as allowing control over the amount of downstream activation in the EXC-5 pathway.

To date there have been 15 alleles isolated from this screen, and more are continuing to be isolated. As expected, most so far have been found to be non-temperature sensitive. Once a temperature-sensitive allele has been isolated, all of the alleles will be sequenced and experiments involving the exact nature of the developmental requirements of EXC-5 utilizing the new temperature-sensitive allele will begin.

53. *C. elegans* Gene Knockout Project at OMRF

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The *C. elegans* Gene Knockout Project at the Oklahoma Medical Research Foundation represents the NHGRI funded node of the *C. elegans* Gene Knockout Consortium.

At the OMRF we produced over 450 deletion alleles in the last 12 months. During this period, *ok* alleles represented about 50% of the submissions to the *C. elegans* Genetics Center. At present, the unfilled request list stands at 1500 targets. At our present rate, we will address all present requests in two years.

Last year we launched a new Consortium website. You are encouraged to visit the site, register with the consortium, and confirm your contact information.

<http://www.celeganskoconsortium.omrf.org/>

Once registered, you are encouraged to update your gene request list.

Until recently, balancing mutations in essential genes was done exclusively by the University of British Columbia node of the Knockout Consortium. This year at the OMRF we launched a group to undertake a portion of this responsibility.

We will discuss our present and future efforts to increase throughput and reduce costs.

54. Increasing learning and memory by reducing oxidative stress in *C. elegans*

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Oxidative stress is associated with age-related declines of biological functions. However, the nervous structure is preserved during aging in *C. elegans* and, thus, it was not well explored whether aging and oxidative stress affect nervous functions. We investigated whether age-related decline can be observed in an associative learning behavior, called isothermal tracking. Isothermal tracking declined with increasing age, while motor activity was more severely declined. We also examined the effects of mutants with altered sensitivity to oxidative stress on the learning behavior and motor activity in young adults. The *isp-1* and *clk-1* mutants are members of Clk mutants and have deficits in the function of mitochondrial respiratory chain, leading to reduced levels of oxidative stress, increased longevity, delayed rhythmic behaviors and other phenotypes. Both the Clk mutants increased an ability to show isothermal tracking and modestly reduced motor activity. Similarly, pretreatment of a metabolic antioxidant, alpha-Lipoic acid, can cause increased learning behavior and reduced motor activity. In contrast, mutants with increased oxidative stress showed severely impaired learning behavior and modestly reduced motor activity. Therefore, physiological levels of oxidative stress may limit the performance of learning and memory, which are important functions of cognitive function. The results may also explain how *C. elegans* limits their cognitive function during evolution.

55. Investigating the Role of a Putative STAR Domain Protein in *Caenorhabditis elegans*

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TRA-2 protein is necessary to promote female sexual development. Male development, at least in part, requires translational repression of *tra-2* by two elements called TGEs (*tra-2* and GLI elements) located in the 3' UTR. *tra-2* gain of function mutants that disrupt the TGEs feminize hermaphrodites. These mutations also feminize male animals, resulting in yolk production in the intestines and oocyte development in the germline. Therefore, TGE control regulates *tra-2* activity in the germline and soma. GLD-1 is a germline specific protein that contains an RNA binding Maxi-KH/STAR domain that regulates *tra-2* translation; however, a *tra-2* somatic regulator has yet to be identified.

We have identified a STAR domain containing protein in *C. elegans*, T21G5.5. This gene has two putative transcripts which differ in a 3' exon and is predicted to bind the same mRNAs as GLD-1 (Ryder *et al*, 2004). T21G5.5 are 63% identical throughout the STAR domain and 74% identical in the KH RNA binding domain. Both transcripts have been cloned and we are currently investigating their biochemical functions. Western analysis of a deletion strain of T21G5.5 demonstrates increased levels of TRA-2 protein relative to wildtype worms. In addition, RNA interference against T21G5.5a results in some in embryonic lethality and a decrease in fecundity. However, unlike GLD-1 gross germline phenotypes are not observed. Together these results indicate that T21G5.5 may function in the soma as a translational repressor in a role equivalent to GLD-1. Given slight germline defects observed, T21G5.5 may also function in the germline as well.

Our future experiments include investigating the tissue specific and subcellular localization of T21G5.5, phenotypic analysis of the deletion strain, and determination of biochemical function.

Ryder SP, Frater LA, Abramovitz DL, Goodwin EB, and Williamson JR. RNA target specificity of the STAR/GSG domain post-transcriptional regulatory protein GLD-1. *Nature Structural and Molecular Biology* **11**, 20-28 (2004).

56. Positional rescue analysis of *mua-2*, *mua-5*, and *mua-10*

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Locomotion in *Caenorhabditis elegans* requires the maintenance of a complex series of cytoskeletal proteins, junctional complexes, and matrix ligands. Force is initially transmitted from contracting muscle cells via dense bodies to the underlying basal lamina, it then moves across the hypodermis to the cuticle via hemidesmosomes and associated cytoplasmic intermediate filaments. Mutations in the *mua-2*, *mua-5*, and *mua-10* genes of *C. elegans* cause postembryonic paralysis due to failure of these mechanical linkages. The molecular identity of *mua-2*, *mua-5*, and *mua-10* has yet to be determined.

To identify the DNA sequences that encode *mua-2*, *mua-5*, and *mua-10* a positional rescue strategy is being used. Three factor mapping has positioned *mua-5* between the markers *unc-44* and *lin-45* on chromosome IV. Initial mapping using SNPs (Single Nucleotide Polymorphisms) has positioned *mua-2* between snp *pkP3060* and snp *pkP3074* on chromosome III, and *mua-10* between snp *pkP6107* and snp *pkP6151* on the X chromosome. Further mapping by the three factor method has placed *mua-2* between the markers *dpy-18* and *spe-6* on chromosome III and *mua-10* between the markers *lon-2* and *unc-97* on the X chromosome. Cosmids and YACs containing genomic DNA from these intervals have been obtained. Microinjection of *mua-5* and *mua-2* heterozygous animals, and *mua-10* homozygous mutants with individual cosmids and YACs is being utilized to generate worms carrying a heritable extrachromosomal array containing the genomic DNA of interest. Transgenic worms will be self fertilized and their progeny screened to identify *mua-2*, *mua-5*, and *mua-10* homozygotes carrying the array and to determine the ability of the cosmid or YAC to rescue the phenotype. New alleles of *mua-2* and *mua-10* are also being generated using ethylmethanesulfonate (EMS). A poster at the Midwest meeting will be presented showing our most current results.

57. Analysis of *C. elegans* Embryos Exposed to Anoxia

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A variety of organisms have adapted mechanism to survive oxygen deprivation. *C. elegans*, at all stages of their life cycle, are capable of surviving at least one day of anoxia. Embryos exposed to anoxia enter into a state of suspended animation in which there is an arrest of developmental and cell cycle progression. These embryos are capable of surviving anoxia for at least three days. We are interested in understanding the molecular mechanisms *C. elegans* use to survive oxygen deprivation. Analysis of chromosomal, microtubule, and kinetochore structure in blastomeres of embryos exposed to anoxia was done to investigate the signaling pathway between low oxygen concentrations and cell cycle arrest. Previously, the spindle checkpoint genes, *san-1* and *mdf-2*, were identified to be required for embryonic anoxia survival. Further phenotype analysis of *san-1* (RNAi) and *mdf-2* (RNAi) was conducted to understand the signaling pathway from low oxygen concentrations to spindle checkpoint activation. A histone::GFP strain was used to analyze chromosome segregation in N2, *san-1* (RNAi) and *mdf-2* (RNAi) embryos exposed to anoxia. Additionally, SAN-1 localization in embryos exposed to anoxia was examined and results indicate that the localization is altered in embryos exposed to anoxia. An understanding of how *C. elegans* survive oxygen deprivation will lead to a greater understanding of the metazoan response to oxygen deprivation and how embryonic development and cell cycle progression is effected by environmental changes.

58. The *unc-59(ru5)* mutation affects morphogenesis of the pharynx

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In *C. elegans*, pharyngeal morphogenesis is divided into 3 discrete steps: (1) reorientation of the anterior pharyngeal cells, (2) formation of epithelial contacts between the pharyngeal cells, buccal cavity cells, and anterior hypodermis, and (3) contraction, which brings the pharynx, buccal cavity, and mouth together (Portereiko & Mango, 2001). We have identified a temperature-sensitive embryonic lethal mutation which causes defects in pharyngeal morphogenesis, resulting in embryos with the *pun* (pharynx unattached) phenotype. Genetic experiments show the *ru5* mutation is weakly semi-dominant but maternal or zygotic product is sufficient to rescue the temperature-sensitive lethal phenotype. *ru5* embryos maintained at the restrictive temperature undergo normal development until the bean stage when pharyngeal morphogenesis fails to occur properly. *ru5* embryos exhibit defects that include mis-oriented anterior pharynxes, excess basement membrane anterior to the pharynx, as assayed by anti-NID-1 antisera (Kang & Kramer, 2000), and, even when the orientation of the pharynx appears normal, failure to create a stable epithelium. We believe the defects we observe are due to a failure in morphogenesis rather than altered cell fate or number because the unattached pharynx is otherwise normal as development proceeds. Temperature shift experiments show the gene product is required for viability from ~4 to 6 hours after the two cell stage, prior to pharyngeal morphogenesis. Larva and adults, grown at the permissive temperature or at the restrictive temperature after the temperature sensitive period, have phenotypes that include uncoordination and vulva morphology defects.

Using conventional and snp-snp mapping strategies, we mapped the *ru5* mutation to a small interval on LG I. Recent cosmid rescue and sequencing experiments have revealed the *ru5* mutation is a new allele of the *unc-59* septin gene. Previously identified mutations in *unc-59* cause defects in distal tip cell and axon migration and post-embryonic cytokinesis (Nguyen, Sawa, Okano & White, 2000; Finger, Kopish & White, 2003). There is also a low penetrance embryonic lethality caused by failure of the pharynx to attach to the mouth (Finger, personal communication). We are currently examining the expression of UNC-59 protein in *ru5* mutants grown at the restrictive temperature to characterize the nature of the *unc-59(ru5)* allele. We believe the defects we observe in *unc-59(ru5)* mutants support the model that septins may be generally required for cell migration and tissue morphogenesis.

59. *ceh-28* is required for feeding in *C. elegans*

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NK-2 family homeobox genes are important developmental regulators in many organisms. The *C. elegans* genome contains 4 members of this gene family (*ceh-22*, *ceh-24*, *ceh-27* and *ceh-28*). *ceh-22* is expressed in the pharyngeal muscles where it regulates gene expression with the pan-pharyngeal factor PHA-4, and we are determining if additional NK-2 family members function similarly in other pharyngeal cell types. Brian Harfe and Andy Fire have previously shown a *ceh-28::gfp* transcriptional fusion is expressed exclusively in the M4 pharyngeal neuron. We have similarly found a *ceh-28::gfp* translational fusion containing 3.7 kb of 5' flanking DNA is expressed in M4. Expression initiates during embryogenesis in two cells, and we speculate these cells are M4 and its sister cell, which dies during normal development. We are currently verifying the expression pattern of the endogenous *ceh-28* gene using anti-CEH-28 antibodies. M4 is a motor neuron essential for pharyngeal isthmus peristalsis and feeding. To functionally characterize *ceh-28*, we have isolated a deletion mutant *ceh-28(cu11)*. This deletion removes much of the *ceh-28* homeobox and is likely a null allele. Homozygous *ceh-28(cu11)* strains can be maintained, although these mutants appear starved and exhibit slow growth and partially penetrant larval lethality. Similar phenotypes were observed in *ceh-28(RNAi)* animals. All *ceh-28* mutant worms have a stuffed pharynx phenotype similar to animals in which M4 has been killed by laser ablation (Avery and Horvitz, 1987), suggesting M4 is defective. We are currently examining M4 differentiation, morphology and function in *ceh-28(cu11)* mutants to understand the role of *ceh-28* in pharyngeal development.

60. Interactions of Rho-Family GTPases in Axon Development

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Rho-family GTPases (Rho, Rac, and Cdc42) regulate cell morphology by influencing the structure and dynamics of the actin cytoskeleton. Actin responses controlled by Rho GTPases include induction of focal adhesion complexes, assembly of actin stress fibers, and formation of filopodia and lamellipodia at the leading edge of axon growth cones in neurons. The three *C. elegans* Rac proteins CED-10, MIG-2 and RAC-2/3 function redundantly to control axon development: rac loss-of-function double mutants display premature axon termination, axon guidance errors and axon defasciculation as well as formation of ectopic axon branches. Furthermore, constitutively-active RAC(G12V) mutant molecules lead to extensive ectopic axon branch formation and induce the formation of ectopic lamellipodial and filopodial structures. RAC(G12V) molecules also cause weak defects resembling rac loss of function. Thus, Rac's are required for axon outgrowth, guidance, fasciculation and branch suppression and likely modulate growth cone lamellipodial and filopodial dynamics. Cdc-42 and Rho have also been implicated in controlling actin-based cell morphology, and *C. elegans* rho-1 has been implicated in regulation of P cell migration. To determine if rho-1 and cdc-42 act in axon development, we constructed transgenes containing wild-type and constitutively active forms (G12V mutation) of rho-1 and cdc-42 and examined PDE neuron morphology in transgenic animals. Both the wild-type and G12V forms of rho-1 and cdc-42 induced axon defects similar to constitutively-active Rac's, including: ectopic axon branching; ectopic lamellipodia and filopodia formation; defasciculation in the ventral nerve cord (VNC); and PDE axon ventral guidance errors.

That overactivity of the three Rac's, RHO-1 and CDC-42 causes similar defects in axon development suggests that they might act in the same processes during axon development. To test if rho-1, cdc-42 and the rac's act in the same pathway in axon development, we tested the ability of RHO-1 and CDC-42 transgenes to suppress rac loss of function. In preliminary studies, expression of wild-type cdc-42 via an extrachromosomal array conferred a partial but significant suppression of PDE guidance defects in mig-2(mu28); ced-10(n1993) double mutants whereas wild-type rho-1 did not. Both cdc-42 and rho-1 exacerbated the VNC defasciculation and ectopic axon formation of ced-10; mig-2 doubles, possibly in an additive manner. These results indicate that overexpression of cdc-42 but not rho-1 is able to compensate for loss of rac function, suggesting that cdc-42 and the rac genes are influencing the same process during axon development.

61. Cloning and characterization of RNAi defective mutant *ne335* in *C. elegans*

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RNA interference (RNAi) is a highly conserved gene silencing mechanism that plays major roles in anti-viral defense in plants, in silencing of transposon mobilization, and in development. The process involves dicer-directed cleavage of a dsRNA molecule to produce siRNAs, which in turn degrades mRNAs in a sequence specific manner. A mutant strain of *C. elegans*, *ne335*, has temperature-sensitive RNAi defects. We have mapped the mutation to chromosome I and have found a 34 bp deletion in the coding region of the *haf-6* gene. Future studies include structural and functional analyses of the gene to uncover its role in RNAi.

62. Determining the role of NUD-1 in hermaphrodite gonadogenesis by analysis of GFP reporters in *nud-1*(RNAi) mutants

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The *C. elegans* hermaphrodite gonad is a complex organ consisting of multiple cell-types that are organized into morphologically and functionally distinct tissues. We are interested in identifying proteins necessary for gonad formation and elucidating the mechanisms by which these proteins act. The *C. elegans* NUD-1 protein was identified based on its sequence conservation with the NudC protein of fungi, which has been shown to be involved in nuclear migration (1). However, the exact function of NudC in this process is unknown. NUD-1 plays a role in many aspects of *C. elegans* development, including gonadogenesis (1). Hermaphrodites where NUD-1 has been depleted by RNAi are sterile and have defects in oogenesis and somatic gonad formation. In particular, the proximal region of the gonad is filled with spermathecal tissue with little to no uterine tissue. Furthermore, a NUD-1::GFP reporter protein is expressed in uterine progenitor cells at the somatic gonadal primordium stage in hermaphrodites (SPh). To better characterize the gonadal defects in *nud-1*(RNAi) animals, cell-specific GFP reporter constructs have been employed. Analysis of GFP expression patterns in *nud-1*(RNAi) mutants will hopefully shed light on the particular gonadal cell(s) and/or tissue(s) affected by loss of the NUD-1 protein.

(1) Dawe et al., Dev. Genes Evol. 211, 434 (2001).

63. SPD-1 is required for the formation of the spindle midzone but is not essential for the completion of cytokinesis in *C. elegans* embryos.

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Cytokinesis, the physical process that partitions the duplicated genome and cytoplasm into two daughter cells, can be divided into two steps: the assembly and constriction of an actomyosin ring and the final breaking and resealing of the intercellular channel that this first process creates. Mutations in several genes of *Caenorhabditis elegans*, including *zen-4* and *cyk-4*, disrupt the spindle midzone (an array of anti-parallel microtubules and associated proteins that forms between the spindle poles), and give rise to failures in the terminal phase of cytokinesis suggesting that the midzone is required for this process.

We show that loss-of-function of *spd-1* causes midzone disruptions, although cytokinesis generally completes, failing only in a subset of cells. We identified SPD-1 as a conserved microtubule bundling protein that localizes predominantly to the spindle midzone but also to microtubule bundles in the cytoplasm. The spindle midzone localization is disrupted in embryos depleted of other spindle midzone components but the cytoplasmic bundles are not affected. We found that ZEN-4 and CYK-4 localize to the ingressing furrow, in addition to the spindle midzone in wild-type embryos; however, only furrow localization is preserved in embryos depleted of SPD-1.

We conclude that SPD-1 is required for an early step of spindle midzone formation. We also conclude that the spindle midzone is not essential for the completion of cytokinesis but that spindle midzone components that also localize to the ingressing furrow are generally capable of effecting the completion of cytokinesis in the absence of the midzone.

64. Distribution of AJM-1::GFP in Collagen IV Null Mutant *C. elegans* Embryos

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Collagen IV is an important structural molecule contributing to the basic three dimensional network of basement membranes. In *C. elegans*, the predominant form of collagen IV is a heterotrimer consisting of an $(\alpha 1)_2 \alpha 2$ composition initially expressed and secreted by the body wall muscle cells. The collagen IV network forms between the body wall muscle cells and the hypodermal cells of the young embryo such that the *C. elegans* epidermis shares a basal lamina with the body wall muscle cells. Adherens junction molecule (AJM-1) is an important structural adhesion molecule located in the apical junctions of adjacent epidermal cells and along with the muscle sarcomere function is important in embryonic elongation. Previous data demonstrated that the hypodermal cells in the collagen IV null animals show abnormal shape just prior to embryonic arrest.

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To identify if abnormalities in hypodermal cell shape occur earlier in collagen IV null embryos, previously generated transgenic strain, MZ1 expressing *ajm-1::gfp*, and time-lapse digital epifluorescence microscopy was used to identify the embryonic stage where abnormalities in hypodermal cell shape could be observed. Data show that abnormalities in hypodermal cell shape begin approximately at the 1.25 fold stage and followed through arrest with altered cell boundaries and distribution of adherens junction molecules.

65. *lin(mh56)* Controls the Asymmetric B Cell Division and Male Tail Development in *C. elegans*
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The B cell is a male-specific blast cell that divides to produce many of the cells that make up the male tail. The B cell divides asymmetrically during the L1 stage. The anterior daughter, B.a, is larger than the posterior daughter, B.p. The polarity of the asymmetric B cell division is controlled by LIN-44/Wnt and LIN-17/Fz, which also regulates the polarities of other cells in tail, including that of the TL and TR cells. However, mutation of some components that function to regulate T cell polarity, such as LIT-1, do not affect B cell polarity, suggesting that the pathways that regulate T and B cell polarities may differ. Recently, we demonstrated that a conserved Planar Cell Polarity-like pathway might regulate B cell polarity (M. Wu and M. Herman, unpublished). In order to identify additional components that affect B cell polarity, a screen for genes that affect B cell first asymmetric cell division was carried out. We screened over 7000 genomes and identified two *lin-44* alleles, one *mab-9* allele, and three alleles that may define new genes involved in the control of B cell polarity. We have begun characterization of one of these, *lin(mh56)*.

While *lin(mh56)* mutants do not display T cell polarity defects, the B cell division is defective. Specifically, 30% of *lin(mh56)* males have B.a and B.p cells of equal size and in five percent B.p was larger than B.a (n=80), which is reversed from what is observed in wild-type males. In addition, *lin(mh56)* hermaphrodites have a small brood size, are slightly Dumpy and have blunt tails. 90% (n=70) of *lin(mh56)* males have abnormal tail morphologies with missing, fused or swollen rays. Furthermore, the spicules are crumpled or missing in 53% (n=74) of *lin(mh56)* males.

We used Snip-SNP mapping to localize *lin(mh56)* to LG III and three factor crosses to further define its location to an interval of several cosmids on the physical map. Rescue experiments are currently underway.

66. Analysis of the *rpc-1* gene in *C. elegans*

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In a collection of EMS-induced larval arrest mutations, two were found to be in *rpc-1*, which encodes the largest subunit of RNA polymerase III. *rpc-1* mutants grow slowly and arrest development at the third larval (L3) stage. The two alleles carry missense mutations: m654 (G644E), which is in the channel domain, and s1139 (G1054E), which is in the cleft domain of the RNA polymerase large subunit. Both mutations are nulls. RNAi treatment of N2 resulted in developmental arrest of the progeny at the L2 stage; while identical treatment of an RNAi sensitive strain caused L1 arrest. Taken together, the data suggest that embryonic *rpc-1* mRNA is maternally inherited. With transgenic animals, we found that the *rpc-1* promoter / reporter construct was not equally expressed in all cells. It was expressed predominantly in head neurons, tail neurons and the intestine, and was weakly expressed during the dauer stage. Starvation silenced the promoter activity, whereas food triggered *rpc-1* promoter activity in dauer recovery and in starved animals. The expression pattern suggests that *rpc-1* transcription may be regulated by food availability.

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Last modified: Tue May 25 15:04:45 2004