# ECWM 2000 Abstracts

## Platform Sessions
### WHSCAB Auditorium
(10 min. presentation + 2 min. Q&A)

### Friday, June 9
### 7:00-8:48 p.m., Session 1
### Andy Singson, Chair

Two new cell-death genes and cell-corpse engulfment genes both contribute to cell killing
Peter Reddien, Scott Cameron, Michael Huang, Bob Horvitz

The cell-corpse engulfment protein CED-1 is a transmembrane receptor that recognizes cell corpses
Zheng Zhou, Bob Horvitz

Testing Functions of Phagocytosis Receptor Homologs in Cell Corpse Elimination and Gonadal Outgrowth
Sambath Chung, Monica Driscoll

Components of the cytoskeleton, involved in the biogenesis of membrane channels, interfere with mec-4(d)-induced cell death.
Nektarios Tavernarakis, Shiliang Wang, Monica Driscoll

Inhibition of touch cell fate in C. elegans
Ji Wu, Anne Duggan, Martin Chalfie

pag-3 and unc-3 interact to determine neural fates in the ventral cord, and their mammalian counterparts may similarly interact during blood cell development
Scott Cameron, Brinda Prasad, Joan B. McDermott, Eric Aamodt, Randall R. Reed, Bob Horvitz

Identification of sns-8, a serine/ threonine kinase, and additional genes required for the development of the AWB olfactory neurons.
Anne Lanjuin, Piali Sengupta

The bHLH transcription factors LIN-32 and HLH-2 specify multiple aspects of ray development in the C. elegans male tail
Douglas S. Portman, Scott W. Emmons

seu-1 Encodes Novel Nuclear Proteins Required for the Function of Ectopic UNC-5
D.C. Merz, H Zheng, A. Colavita, J.G. Culotti

### 8:48-9:10 p.m., BREAK
### 9:10-10:34 p.m., Session 2
### Bill Kelly, Chair

Molecular and genetic analyses of unc-69, a gene required for axon guidance
Suzanne Tharin, Bruce Wightman, Nancy Tsung, Erika Hartwieg, Gian Garriga, Bob Horvitz, Michael Hengartner

The C. elegans ORFeome project
Jerome Reboul, Philippe Vaglio, Cindy Jackson, Troy Moore, Jean Thierry-Mieg, Danielle Thierry-Mieg, Jim Hartley, Gary Temple, Mike Brasch, Nia Tzellas, Marc Vidal

Assigning function to ovary expressed genes using RNAi.
F. Piano, A. Schetter, D. Morton, K. Kemphues, V. Reinke, S. Kim, M. Mangone, L. Stein

A comprehensive reverse genetic screen of Chromosome I
Andrew Fraser, Ravi Kamath, Peder Zipperlen, Julie Ahringer

EGO-1: A link between development and RNAi
Eleanor Maine, Karin Schneider, Valarie Vought

Role of mut-7 and rde genes in the initiation of transgene silencing in the germline
Alla Grishok, Tae Ho Shin, Hiroaki Tabara, Craig Mello

Mechanisms of response to environmental dsRNA
Lisa Timmons, Hiroaki Tabara, Craig Mello, Andrew Fire

### 10:34 p.m.-midnight Workshop: Gene Knockouts
### Michael Koelle, organizer
### Saturday, June 10
### 8:30-10:06 a.m., Session 3
### Jill Schumacher, Chair

Parent of Origin Effects in C. elegans: Worm Imprinting or an Insight Into Hybrid Dysgenesis?
William G. Kelly
Towards the Molecular Genetics of Sex Drive in C. elegans
Jonathan Lipton, Scott W. Emmons
Haldane's Rule in Caenorhabditis is implemented by sexual transformation.
Scott Everet, Baird, Wei-Chih, Yen
Germ-Soma Distinction in C. elegans
Yingdee Unhavaithaya, Tae Ho Shin, Craig C. Mello.
The germline protein PIE-1 has two functions, each important for germ cell fate
T. Tenenhaus, K. Subramaniam, G. Seydoux
CGH-1, an essential and conserved germline RNA helicase
RE Navarro, EY Shim, Y Kohara, Y Shi, TK Blackwell
The Locations and Predicted Roles of MES-2, MES-3, MES-4, and MES-6 in Control of Gene Expression in the Germline
Lei Xu, Youyi Fong, and Susan Strome
Histone deacetylase HDA-1 is involved in gonadogenesis and germline maturation in Caenorhabditis elegans
P. Dufourcq, M. Victor, D. Calvo, J. Hodgkin, Y. Shi

## 10:06-10:40 a.m. BREAK

## 10:40 a.m.-12:16 p.m., Session 4
## Chris Rongo, Chair

glp-1 Proximal Proliferation (Pro) Mutants
Te-Wen Lo, E. Jane Albert Hubbard
Chromosome segregation is not required for polarized segregation of organelles during C. elegans sperm meiosis
Don Fox, Penny Sadler, Michelle Reed, Lori Blanchfield, Allen Dvarkas, Diane Shakes
Genes required for sperm-oocyte interactions at fertilization
Andrew Singson, Sonia Zannoni, Pavan Kadandale, Brian Geldziler, Steven L'Hernault
Several novel complementation groups regulate cell survival of vulva precursor cells in Pristionchus pacificus
Jagan Srinivasan, Ralf J. Sommer
Synthetic Vulvaless/Lethal mutations define new regulators of Ras signaling
Meera Sundaram, Ranjana Kishore, Robyn M. Howard, Alissa Goldman, Gaurang Patel
The Caenorhabditis elegans COG-4 protein mediates vulval cell morphogenesis
Wendy Hanna-Rose, Min Han
Expression of the small regulatory RNA let-7 across phylogeny
Brenda Reinhart, Amy Pasquinelli, Ashok Srinivasan, Mark Fishman, Frank Slack, Mitzi Kuroda, Gary Ruvkun
The C. elegans let-7 RNA controls temporal patterning by regulating the timing of expression of the lin-41RBCC regulatory gene.
Frank J. Slack, Betsy R. Maller

## 12:16-1:45 p.m., lunch, Dobbs University Center ("The DUC")
## 1:45-5:30 p.m., Poster Session
## Dobbs University Center (Winship Ballroom, Faculty & Trustees Dining Rooms)

## 5:30-6:54 p.m., Session 5
## Frank Slack, Chair

G protein regulators RGS-1 and RGS-2 allow C. elegans to rapidly alter egg-laying behavior when re-fed after starvation
Meng-Qiu Dong, Dan Chase, Georgia Patikoglou, Michael Koelle
daf-5 cloning identifies a Sno oncogene homolog as a TGF-beta signaling output
Li Sun, Garth Patterson
Cloning and Characterization of lon-1, a gene downstream of TGFb signaling involved in body size regulation
Lisa L. Maduzia, Pradnya Shetgiri, Srikant Krishna, Cathy Savage-Dunn, Huang Wang, Richard W.

Padgett
sma-9 and TGFb Signaling
Yuval Hiltzik, Rafal Tokarz, Robyn Lints, Scott Emmons, Cathy Savage-Dunn
Toward steroid endocrinology: cholesterol accumulation in C. elegans
John Lenard, William G. Wadsworth
rme-1, a new endocytosis gene implicated in recycling
B Grant, Y Zhang, M Paupard, DH Hall, D Hirsh
rme-8 is an essential gene required for endocytosis
Yinhua Zhang, David Hirsh

## 7:00-8:30 p.m., "wet bar" & banquet, Cox Hall Ballroom (3rd floor)
## 8:30-11:30 p.m., band, dancing etc.

## Sunday, June 11
## 8:30-9:42 a.m., Session 6
## Jane Hubbard, Chair

Postembryonic Muscle Patterning
Steve Kostas, Andy Fire
LAD-1, a homologue of vertebrate L1CAMs, is a general receptor for UNC-44 at sites of cell-cell contact throughout development, and participates in a novel ankyrin-independent signaling pathway
Lihsia Chen, Bryan Ong, Vann Bennett
MUP-4 is a novel matrix receptor with essential functions in epithelial cell adhesion, morphogenesis and maintenance of muscle position.
L. Hong, T. Elbl, C. Franzini-Armstrong, J. Ward, K. Rybicka, E. Bucher
The unc-78 gene encodes a homolog of actin-interacting protein 1 (AIP1) and is involved in actin filament assembly in body wall muscle
Shoichiro Ono, Henry F. Epstein, Guy M. Benian
PDZ containing Protein Kinases are Implicated in Contractile Functions.
M Land, C.S. Rubin
A calcium-binding protein of SR (sarcoplasmic reticulum), calsequestrin, is not essential for body-wall muscle function in C. elegans
Jeong Hoon Cho, Young Soo Oh, Tomoyo Hamada, Hiroaki Kagawa, Joohong Ahnn

## 9:42-10:10 a.m., BREAK

## 10:10-11:58 a.m., Session 7
## Scott Clark, Chair

The extracellular domain of the C. elegans amyloid precursor-related protein is essential for viability
Angela Hornsten, Chris Li
Analysis of C. elegans nlp genes reveals new neuropeptide families
Arif, Nathoo, Rachael Moeller, Anne C. Hart
Elongation factor-2 kinase affects Caenorhabditis elegans life span by regulating protein turnover
Nektarios Tavernarakis, Bradley S. Nefsky, Charmaine E. Mendola, Karen S. Pavur, Zeynep Altun-Gultekin, William G. Wadsworth, Monica Driscoll, Alexey G. Ryazanov
A wee-1 kinase homolog is required for meiosis during spermatogenesis in C. elegans
Todd Lamitina, Steve L'Hernault
Absence of CUL-4 leads to endoreplication
Weiwei Zhong, Edward Kipreos
Regulation of progression through G1 phase in C. elegans
Mike Boxem, Sander van den Heuvel
Regulation of microtubule dynamics during mitosis: identifying AIR kinase substrates in C. elegans.
John D. Bishop, Jill M. Schumacher
Cytoplasmic dynein light-intermediate chain is required for distinct aspects of cell division in C. elegans
John H. Yoder, Min Han
The survivin-like C. elegans protein BIR-1 acts with the aurora-like kinase AIR-2 to mediate chromosome behavior and spindle midzone formation
Liz Speliotes, Anthony Uren, David Vaux, Bob Horvitz
Negative regulation of MEC-3 by zinc finger protein SEM-4
Anne Toker, Martin Chalfie

## Posters

Genetic and molecular analysis of intron requirements for gene activity
Rosa Alcazar, Andrew Fire
CRB-1 may function in synaptic transmission and dauer formation
Mark Alkema, Bob Horvitz
In vivo Studies of Troponin T Functions.
T. Allen, J. Ward, L. Hong, A. Burkeen, E.A. Bucher
Genetic analysis of neuroendocrine controls of fat metabolism in C. elegans
Kaveh Ashrafi, Gary Ruvkun
Functional Characterization of Calcineurin, a serine/threonine protein phosphatase, in C. elegans
Jaya Bandyopadhyay, Jiyeon Lee, Byung-Jae Park, Joohong Ahnn
A Genetic Screen for Components of the Goalpha Signaling Pathway in C. elegans
I. Amy Bany, Michael Koelle
SQV-7, a protein involved in vulval invagination and embryonic development, transports UDP-glucuronic acid, UDP-N-acetylgalactosamine and UDP-galactose
Patricia Berninsone, Ho-Yon Hwang, Bob Horvitz, Carlos Hirschberg
The ClC family of Chloride Channels in C. elegans
Laura Bianchi, Carol L. Beck, David M. Miller III, Alfred L. George Jr
Protein interaction mapping of DNA repair and DNA damage checkpoint proteins in C. elegans
Simon Boulton, Nick Dyson, Marc Vidal
dig-1 encodes an adhesion molecule involved in sensory map formation.
Christopher Burket, Stacy Hubbard, Elizabeth F. Ryder
The C. elegans lissencephaly (LIS1)-like gene lis-1 is required for embryonic development
Ned Buttner, Bob Horvitz
The ER stress response in C. elegans
Marcella Calfon, Scott Clark, David Ron
POP-1, A C. elegans TCF/LEF1 family member, possesses activation and repression domains and is a target of acetylation by CBP-1
Dominica Calvo, Yang Shi
Analysis of male sex myoblast migration
Robert Cavallo, Michael Stern
Identification and characterization of genes that act with lin-35 Rb to negatively regulate vulval induction
Craig Ceol, Frank Stegmeier, Melissa Harrison, Bob Horvitz
G-protein beta-2 regulates the activity of two RGS proteins
Dan Chase, Georgia Patikoglou, Michael Koelle
A Mutation That Affects Early Germline Proliferation
Aisha Chaudhary, E. Jane Albert Hubbard
An Approach to Identifying the Lateral Signal in Vulval Development
Ning Chen, Iva Greenwald
Anteroposterior Axon Guidance Genes
Catherine Chiu, Ray Squires, Scott Clark
Studies on two isoforms of putative C. elegans SERCA (Sarco/Endoplasmic Reticulum Calcium ATPase)
Jeong Hoon Cho, Joohong Ahnn
Characterization of the class a synthetic multivulva genes lin-8 and lin-56
Ewa M. Davison, Bob Horvitz
Characterization of new genes required for the negative regulation of vulval induction, including the new class B synMuv gene lin-61
John Doll, Xiaowei Lu, Bob Horvitz
C. elegans era, homolog of an E. coli cell cycle gene?
Hilary M. Ellis, Bradford S. Powell, Andy Golden, Donald L. Court
Genetic and molecular analysis of polyglutamine toxicity in C. elegans
Peter W. Faber, Daphne King, Anne C. Hart
Investigation of cul-2 E3 complexes and Interaction between cul-2 and vhl</WBGTITLE>
Hui Feng, Edward T. Kipreos
Boy Is My Bursa Red: Automated Detection and Sorting of Fluorescent Stained C. elegans Males From a Mixed Population
A Ferrante, L Thibodeau, G O'Connor, WP Hansen
Characterizing lin-5 interactions identified in the two-hybrid system
Ridgely Fisk, Marian Walhout, Monique Lorson, Marc Vidal, Sander van den Heuvel
MIP-4 Affects Embryonic Polarity in C. elegans
Michael C. Fitzgerald, Nancy N. Huang, Craig P. Hunter

Two screens for new genes involved in programmed cell death
Brendan Galvin, Scott Cameron, and Bob Horvitz
Nuclear receptor genes in free-living and parasitic nematodes
Puneet Gandotra, Megan O'Donnell, Claude V. Maina, Kirsten Crossgrove
Semaphorin 1a and 1b mutants exhibit male tail morphological defects.
Val E. Ginzburg, Peter J. Roy, Joseph Culotti
Conserved nuclear receptors function during the C. elegans molting cycle
Chris R. Gisssendanner, Ann E. Sluder
Visualizing synapses in the motor neuron circuit
Christina Gleason, Dave Piston, David Miller
Characterization of the pvl-2/mig-14/mom-3 locus, which functions in multiple Wnt signaling processes in C. elegans
Julie Gleason, David Eisenmann
Isoform-specific functions of the EGL-15 FGFR
SJ Goodman, CS Branda, MJ Stern
Caenorhabditis elegans: A Model for Studying the Virulence of Burkholderia cepacia
Alex D. Green, Anne Morris Hooke
ced-12 is required for phagocytosis and cell migration
Tina L. Gumienny, Sambath Chung, Monica Driscoll, Michael Hengartner
Isolation and characterization of recessive suppressors of age-1
Josie Haduong, Catherine A. Wolkow, Gary Ruvkun
Regulation of cell-cell associations in C. elegans
Andrew Hahn, Scott W. Emmons
Improved Tissue Preservation Using Metal Mirror Fixation or High Pressure Freezing for TEM
David H. Hall, Frank Macaluso, Gloria Stephney, Marie Christine Paupard
A protocol for the use of electron microscopy in studies of axonal pathfinding
Erika Hartweig, Peter Reddien, Bob Horvitz
The C. elegans cysteine protease genes Ce-cpz-1 and Ce-cpl have a role in worm's Development
Sarwar Hashmi, Jing Liu, Sara Lustigman
n3194, identified in a screen for suppressors of ced-9(n1950), may be required for cell viability
Brad Hersh, Erika Hartwieg, Bob Horvitz
CED-9 and EGL-1 regulate the subcellular localization of CED-4
Brad Hersh, Fangli Chen, Barbara Conradt, Zheng Zhou, Bob Horvitz
Characterization of the 13 C. elegans RGS Proteins
Heather Hess, Michael Koelle
Phenotypic and Genetic Analysis of spe-16, a Gene Required for C. elegans Spermatogenesis
Katherine L. Hill, Carey A. Dobbins, Steven W. L'Hernault
egs-1 and egs-2 are required for HSN death
Dan Hoeppner, Mona S. Spector, Michael O. Hengartner
C. elegans at 10 times earth gravity
David Hoffman, Ruth Globus, Stuart Kim, Catharine Conley
Genetic analysis of DNA damage induced germline apoptosis
Edward R. Hofmann, Anton Gartner, Stuart Milstein, Bjorn Schumacher, Michael O. Hengartner
Characterization of eor-1 and eor-2 : two newly identified positive regulators of Ras signaling in C. elegans
Robyn M. Howard, Alissa Goldman, Meera Sundaram
Identifying lin-14 targets with the help of a DNA microarray
Marta Hristova, Victor Ambros
Identification of Novel Components of the daf-2 Signaling Pathway
Patrick Hu, Gary Ruvkun
CLR-1 and Fibroblast Growth Factor Receptor (FGFR) Signaling in C. elegans
Peng Huang, Matthew K. Robinson, Christina Z. Borland, Michael J. Stern
Histamine: a possible neuromodulator in C. elegans
Melissa Hunter-Ensor, Bob Horvitz
Defects in glycosaminoglycan biosynthesis cause the C. elegans Sqv phenotype and human Ehlers-Danlos syndrome
Ho-Yon Hwang, Tory Herman, Patricia Berninsone, Carlos Hirschberg, Bob Horvitz
The timeless-related protein TIM-1 is required for embryonic viability
Mili Jeon, Heather Gardner, Ann Rougvie
Ray neuron targeting in the male tail
Lingyun Jia, Scott W. Emmons
Screening for Precocious Sexual Maturation
Steven M. Johnson, Frank J. Slack

pvl-5 may prevent abnormal Pn.p cell death during vulval development
Pradeep M. Joshi, David M. Eisenmann
Fine mapping of lethal mutants on chromosome I of Caenorhabditiselegans
Sunki Jung, Jinsook Lee, Joohong Ahnn
What is the role of protein phosphatase 2A in vulval development?
Gautam Kao, Meera Sundaram
HLH-2 expression during the AC/VU decision
X Karp, M. Krause, I. Greenwald
A novel protein functions as a bridge between the GEX-2/GEX-3 complex and intermediate filaments in C. elegans.
Katsuhisa Kasuya, Makiko Inoue, Daisuke Tsuboi, Hiroshi Qadota, Kozo Kaibuchi
Pheromone Regulation of Neuroendocrine Outputs in C. elegans
Scott Kennedy, Gabriel Hayes, Gary Ruvkun
Using Ivermectin to Find Nervous System Mutants
Parul Khare, Shaun Segal, Joseph A. Dent
Function of the family of FMRFamide-related neuropeptides in C. elegans
Kyuhyung Kim, Chris Li
UNC-13 in the C. elegans Nervous System
R. Eustance Kohn, J.S. Duerr, J. McManus, A. Duke, G.L. Moulder, R. Barstead, J. B. Rand
Molecular cloning of the elusive unc-20
Holbrook Kohrt, Son Nguyen, Bruce Wightman
Toward an understanding of ASH circuit signaling pathways
Hidetoshi Komatsu, Shinya Matsumoto, Emily Bates, Anne C. Hart
nop-1 and the establishment of asymmetries in the early C. elegans embryo
Jean-Claude Labbé, Bob Goldstein
Structure and Expression of Calcineurin B, the regulatory subunit of the Ca2+/ CaM dependent protein phosphatase 2B: biological relevance in C. elegans
Jiyeon Lee, Jaya Bandyopadhyay, Sunki Jung, Joohong Ahnn
Identification and characterization of a Ubiquitin C-terminal hydrolase in C. elegans
Jungsoo Lee, Moon Hee Lee, Chin Ha Chung, Joohong Ahnn
Isolation of genetic suppressors of daf-16, a forkhead class transcription factor mediating insulin-like signaling
Raymond Lee, Siu Sylvia Lee, Gary Ruvkun
Chemosensory modulation of the daf-2/insulin signaling pathway
Siu Sylvia Lee, Gary Ruvkun
A deletion of C. elegans hcf-1 causes small brood size and a cold-sensitive hatching defect.
S. Lee, M. O. Hengartner, W. Herr
Isolation of Mutants Defective for the Initiation of Postembryonic Development in Caenorhabditis elegans
Shaolin Li, Chantal Pare, Victor Ambros, Richard Roy
Evidence for a daf-16/FH-independent daf-2/IR signal
Weiqing Li, Gary Ruvkun
Initial Characterization of aph-1 Suppressors
Shin-Yi Lin, Camila Libel, Katie Scangos, Caroline Goutte
The spe-10 and spe-21 genes encode sperm transmembrane proteins that contain a DHHC-CRD zinc finger motif
Wesley C. Lindsey, Steven W. L'Hernault
Patterning of neurotransmitter phenotype among male ray sensory neurons
Robyn Lints, Scott W. Emmons
Evolution of cyclin-dependent kinases (CDKs) and CDK-activating kinases (CAKs): Differential conservation of CAKs in yeast and metazoa
Ji Liu, Edward T. Kipreos
The cyclin B and B3 genes have distinct functions during cell division in C. elegans
Monique Lorson, Huihong Xu, Karen Bennett, Sander van den Heuvel
Candidate genes that govern motorneuron synaptic specificity
Charles Ma, Martin Chalfie
pat-4 Mediates Integrin Signaling Events During Assembly of the Myofilament Lattice
Craig Mackinnon, Ben Williams
The late acting genes let-7 and lin-41 interact to control temporal development in C. elegans.
Betsy R. Maller, Monica C. Vella, Frank J. Slack
The function and evolution of Latrophilins and Celsr, G-protein coupled receptors of the secretin family that are conserved in C.elegans, Drosophila and vertebrates
Surjeet S. Mastwal, Edward M. Hedgecock

LIN-13 Update
Alicia Melendez, I. Greenwald
sns-10(oy42) is involved in the development of the AWA chemosensory neuron<//WBGTITLE>
Tali Melkman, Piali Sengupta
Dissecting the Signaling Pathways Regulated by the PTEN Tumor Suppressor Homolog DAF-18 in C. elegans
Valia Mihaylova, Judy Pepper, Christina Borland, Michael Stern, Michael Koelle, Hong Sun
A screen for genes that control programmed cell death in the germ line
Stuart Milstein, Anton Gartner, Michael Hengartner
Expression and functional analysis of genes containing RING finger domains in Caenorhabditis elegans
Rhonda Moore, James R. Hudson, Jr., Lynn Boyd
Progress towards cloning egl-47, a putative component of a G protein-coupled signaling pathway
James J. Moresco, Michael R. Koelle
Regulation of the heterochronic gene lin-28 independently of the lin-4 RNA
Eric G. Moss, Lingjuan Tang, Kathy Seggerson
Functional studies on the FAX-1 nuclear receptor
Elissa Murphy, Nick Bianco, Jessica Tanis, Bruce Wightman
Structure-function characterization of BAR-1 and other b -catenins in C. elegans
Lakshmi Natarajan, David M. Eisenmann
Contributions of WRN-related Helicases to C. elegans Aging
K. M. Nycz, L.A. Herndon, M. Driscoll
Food-deprivation and modulation of locomotory behavior: mod-6 and a screen for new genes
Daniel Omura, Rajesh Ranganathan, Bob Horvitz
Arrays containing the cki-1 promoter region cause inappropriate dauer formation in C. elegans
Jimmy Ouellet, Yang Hong, Victor Ambros, Richard Roy
Screens for Transgenic Expression of Maternal Genes
Ka Ming Pang, Tae Ho Shin, Craig C. Mello
Characterization of Calreticulin (crt-1), a calcium-binding protein, in Caenorhabditis elegans
Byung-Jae Park, Duk Gyu Lee, Kyu Yeong Choi, Joohong Ahnn
Following the in vivo fate of the double-stranded trigger RNA during RNA-mediated interference (RNAi)
Susan Parrish, Andrew Fire
Two conserved domains of the EGL-10 RGS protein cooperate to inhibit G protein signaling in the C. elegans nervous system
Georgia A. Patikoglou, Dan Chase, Michael R. Koelle
An RNAi Screen for Genes Involved in PIE-1 Localization
Jason Pellettieri, Kimberly Reese, Melanie Dunn, Valerie Reinke, Stuart Kim, Geraldine Seydoux
Characterization of proximal proliferation (Pro) mutants
Anita Pepper, E. Jane Albert Hubbard
Characterization of sup-11 and sup-18, two regulators of the SUP-9/SUP10/UNC-93 two-pore potassium channel complex
Ignacio Perez de la Cruz, Bob Horvitz
Chemosensory Control of Surface Antigen Switching in C. elegans.
David Phu, Douglas P. Olsen, Laura L. Miceli, Samuel M. Politz
INS-1, one of many insulin-related genes in C. elegans, can regulate dauer formation
S. B. Pierce, M. Costa, S. Devadhar, S. Homburger, A. Buchman, K. Ferguson, J. Heller, D. Platt, S. Doberstein, R. Wisotsky, L. Liu, G. Ruvkun
Functional analysis of PKN-1, an effector of Rho GTPase, in C. elegans muscle
Hiroshi Qadota, Kozo Kaibuchi
mod-1 and mod-5 control serotonergic neurotransmission and experience-dependent modulation of locomotory behavior
Rajesh Ranganathan, Megan Higginbotham, Beth Sawin, Bob Horvitz
The CED-2 CrkII, CED-5 DOCK180, CED-10 Rac Pathway Controls Cell-corpse Engulfment and Cell Migration, and CED-10 functions redundantly with THE MIG-2 Rho-type GTPase to control Axon guidance
Peter Reddien, Erik Lundquist, Erika Hartweig, Cori Bargmann, Bob Horvitz
Regulation of FMRFamide-related expression by fax-1 and unc-42, and the Tailless subfamily of nuclear hormone receptors
Kristy Reinert, Sheila Mathieson, Bruce Wightman
Characterization of mutants defective in postembryonic morphogenesis of the male tail tip
Tania Del Rio, Can Q. Nguyen, Ying Yang, David H.A. Fitch
Analysis of Body Size Regulation in C. elegans by TGF-beta
Andrew Roberts, Steve Cohen, Lisa L. Maduzia, Cathy Savage-Dunn, Richard W. Padgett

sem-3 encodes a cis-prenyltransferase homolog, an enzyme required for production of an essential lipid
Matthew K. Robinson, Michael J. Stern
UNC-43 CaMKII regulates the density of central glutamatergic synapses in vivo
Christopher Rongo
Molecular Genetics of Li+ Sensitivity in C. elegans
Dewey Royal, Mary Anne Royal, Nicole Sirotin, Ellie Hsieh, Suk-Mei Kwok, Gautam Kao, Bruce Bowerman, John White, Monica Driscoll
elm-1, a Negative Cell Cycle Regulator
R. Mako Saito, Sander van den Heuvel
Genetic analysis of neural inputs to the dauer pathway
GM Sandoval, G Ruvkun
Specification of the AWA Chemosensory Neurons
Trina R. Sarafi-Reinach, Piali Sengupta
CDC27, an Anaphase Promoting Complex subunit, is required for the metaphase to anaphase transition during meiosis
J. Schumacher, M. Abdolrasulnia, L. Wille, P. Sadler, D. Shakes, A. Miller, A. Golden
A screen for mutants defective in the specification of the programmed cell deaths of the male-specific CEM neurons
Hillel Schwartz, Bob Horvitz
Searching for factors responsible for PAR-2 cortical localization
Peter Schweinsberg, Lynn Boyd
Post-transcriptional control of C. elegans lin-28 expression through its 3'UTR
Kathy Seggerson, Eric Moss
A C. elegans gene related to S. pombe pom1 is required for microtubule-based processes in the C. elegans embryo
Christopher A. Shelton, Caretha L. Creasy, George P. Livi
Specialized Roles of DRIP/TRAP/ARC/Mediator Transcription Complex Components in C. elegans.
Eun Yong Shim, Leonard P. Freedman, T. Keith Blackwell
Verification of gene prediction in the Caenorhabditis elegans genome
Nataliya Shmeleva, Mark Borodovsky
Kinesin motors moving chromosomal cargo
Shahid S. Siddiqui
WormBase; a Model Organism Database
John Spieth, WormBase Consortium
Biochemical purification of mitotic LIN-5 protein complexes
Dayalan Srinivasan, Sander van den Heuvel
Genetic characterization of osmosensation: structure-function analysis of OSM-10
Hana Sugimoto, Anne C. Hart
The whole enchilada (degenerin flavor)
Nektarios Tavernarakis, Beate Gerstbrein, Yun Ning, Ami Modi, Monica Driscoll
Protease-related features of the intracellular amino-termini ofDEG/ENaC Ion Channels
Nektarios Tavernarakis, John Everett, Monica Driscoll
Regulation of Hox Genes in Male Tail Ray Developmental Pathway
Yingqi Teng, Scott W. Emmons
Investigating the peroxisome in C. elegans:GFP analysis and functional knockout of 5 peroxin genes
Heather Thieringer, Britta Möllers, Gabriele Dodt, Wolf-H. Kunau, Monica Driscoll
Potential downstream targets of TGF-beta signaling in dauer formation
Guene L. Thio, Garth I. Patterson
UNC-89 is a putative muscle specific activator of rhoA
Tina Tinley, Anne Blangy, Rachel Kindt, Guy Benian
Further progress in understanding unc-98, a gene important for intermediate filament protein organization in nematode muscle
Tina Tinley, Denise Flaherty, Kristie Mercer, Xuexin Tang and Guy Benian
Characterizing the TGF-beta Small Mutants
Rafal Tokarz, Cathy Savage-Dunn
The aph-2 gene of C. briggsae
Makoto Tsunozaki, Janine Serebro, Caroline Goutte
CBP-1 in C.elegans development
Martin Victor, Craig Mello, Yang Shi
Regulation of lin-39 activity by Ras and Wnt signaling pathways in vulval development
Javier A. Wagmaister, Tao Wu, David M. Eisenmann
Protein interaction mapping in C. elegans
A.J.M. Walhout, N. Thierry-Mieg, L. Matthews, S. Boulton, J. Reboul, P. Vaglio, M. Vidal

A conserved transcription motif suggesting functional parallels betweenC. elegans SKN-1 and Cap 'n' Collar-related bZIP proteins
Amy K. Walker, Raymond See, Jae Hyung An, Yang Shi, T. Keith Blackwell
In vivo analysis of C. elegans TAFII homologs in gene expression and embryogenesis
Amy Walker, Morris Maduro, Joel Rothman, Yang Shi, T. Keith Blackwell
Biochemical Characterization of C. elegans RNA Helicase A and Malate Dehydrogenase
Katherine M. Walstrom, Ira P. Do, Yuliya Kislyak
CeMi-2, a Component of a Histone Deacetylase Complex, Is Essential for C. elegans Development
Shi-Liang Wang, Nektarios Tavernarakis, Yi Zhang, Monica Driscoll
Screening for new alleles of gon-11
Ian White, Marc Ginsburg, Eric Lambie
A Chemical Genetic Screen for Lethal Compounds in C. elegans
William M. Winston, Stuart L. Schreiber, Craig P. Hunter
The daf-2/insulin-like pathway functions from secretory cell types to control life span.
Catherine A. Wolkow, Kotaro Kimura, Mingsum Lee, Gary Ruvkun
A genetic screen for paternal effect mutations in C. elegans.
Amanda J. Wright, Craig P. Hunter
Screens for New Components in TGF-beta signaling Pathway from A Lon-2 Suppressor Screen<//WBGTITLE>
Shi-Lan Wu, Huang Wang, Stephen Cohen, Archana Sinha, Lisa L. Maduzia, Richard W. Padgett
tlp-1 encodes a protein similar to Drosophila NocA, a zinc finger protein
Ying Yang, Xiaojun Zhao, Michael Herman, David H. A. Fitch
Genetic Analysis of Coelomocyte Specification and Patterning
Judith Yanowitz, Andrew Fire
Idendification of new rde (RNAi deficient) loci in C. elegans
Erbay Yigit, Hiroaki Tabara, Craig C. Mello
TGF-beta Signaling and Egg Laying.
Ling Yu, Rafal Tokarz, Cathy Savage-Dunn
Phenotypic and Genetic Analysis Of Mutations Required for Fertilization
Sonia Zannoni, Pavan Kadandale, Brian Geldziler, Andrew Singson
sop-3, a novel component in the transcriptional regulation of Hox genes
Hong Zhang, Scott W. Emmons
A mec-3-Dependent Gene Isolated by Reverse Genetics
Yun Zhang, Marty Chalfie
A New Mutation Disrupting Membrane Morphogenesis during C. elegans Spermatogenesis
Guang-dan Zhu, Steven W. L'Hernault
Defining new components of TGF-beta signaling pathways in C. elegans
Cole M. Zimmerman, Lisa L. Maduzia, Richard W. Padgett

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# TWO NEW CELL-DEATH GENES AND CELL-CORPSE ENGULFMENT GENES BOTH CONTRIBUTE TO CELL KILLING

## Peter Reddien, Scott Cameron, Michael Huang, Bob Horvitz

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The gene *ced-3*, which is the most downstream known component in the cell-death execution pathway, encodes a cysteine protease or caspase. How the CED-3 protease kills cells is unknown. Mutations in genes controlling parallel cell-killing activities downstream of *ced-3* might not have been previously identified because of their redundant nature. Animals with strong loss-of-function mutations in *ced-3* lack most if not all programmed cell deaths. However, there are weaker *ced-3* mutants that lack only a small percentage of programmed cell deaths. We reasoned that mutations in genes controlling cell-killing activities downstream of CED-3 might enhance a cell-killing defect conferred by a partial loss-of-function mutation in *ced-3*.

We found that weak *ced-3* mutations are enhanced by mutations in genes involved in cell-corpse engulfment (*ced-1, -2, -5, -6, -7,* and *-10*). Because cell corpses are generated in engulfment-defective mutants, the proposed function of engulfment has long been solely the removal of unwanted apoptotic cell bodies. We have discovered, however, that in addition to functioning in cell-corpse removal, engulfment assists in the killing of dying cells. Specifically, mutations in engulfment genes result in a low-penetrance survival of some cells that normally die in the ventral cord. Lineage analysis shows that cells that fail to die initially show some morphological characteristics of programmed cell death but ultimately appear morphologically indistinguishable, using Nomarski optics, from living cells. Surviving Pn.aap cells in the ventral cord lineages express a VC cell-type specific reporter *lin-11::gfp*, suggesting they can differentiate. We conclude that a block in engulfment can result in the survival and differentiation of cells programmed to die.

We have performed a screen for enhancers of a partial *ced-3* loss-of-function allele and isolated 37 mutations. Of these, at least three are mutations in *ced-9*, two in *ced-4*, and six in *ced-3*. Nine mutations confer defects in cell-corpse engulfment. On the basis of complementation tests and map positions, we have defined at least two new cell-killing genes. We will discuss our characterization of these genes.

# THE CELL-CORPSE ENGULFMENT PROTEIN CED-1 IS A TRANSMEMBRANE RECEPTOR THAT RECOGNIZES CELL CORPSES

## Zheng Zhou, Bob Horvitz

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Engulfment of apoptotic cells by their neighbors is an evolutionarily conserved process that removes apoptotic cells before they release harmful contents. In *C. elegans*, mutations in seven genes that define two partially redundant pathways cause cell corpses to persist abnormally. *ced-2*, *-5*, *-10 ,* and *-12* are part of a CrkII/DOCK180/Rac signaling pathway proposed to mediate cytoskeletal reorganization. On the basis of genetic interactions, *ced-6* and *ced-7* appear to act together with *ced-1* to control cell-corpse recognition and to initiate phagocytosis. *ced-6* encodes an adaptor-like protein, and *ced-7* encodes an ABC transporter.

We have cloned *ced-1* and found that it encodes a transmembrane protein similar to human SREC (Scavenger Receptor from Endothelial Cells) in its extracellular domain and that contains candidate binding motifs for adaptor proteins in its intracellular domain. Scavenger receptors bind a variety of substrates, including lipoproteins and phospholipids, and some members such as SR-A and CD36 are thought to mediate the recognition of apoptotic cells by phagocytes. Our molecular studies indicate that *ced-1* is expressed and functions in engulfing, but not in dying, cells. More specifically, we found that CED-1 is localized to the surface of engulfing cells and clusters in the region of the plasma membrane adjacent to cell corpses. Our results suggest that CED-1 is a receptor that recognizes cell corpses and mediates their engulfment. We postulate that SREC, the *in vivo* function of which is unknown, and other CED-1-related proteins in mammals may be involved in the phagocytosis of apoptotic cells.

In addition to our study of *ced-1*, we have performed a large-scale genetic screen for new engulfment mutants. We isolated 68 potential engulfment mutants. These mutants define distinct phenotypic categories, some of which have not been described before. For example, six mutants display engulfment defects in embryos that fail to develop to hatching. We have cloned one gene identified from this screen. We hope the further characterization of these mutants will both help us understand the known engulfment genes as well as identify additional genes involved in this process.

# TESTING FUNCTIONS OF PHAGOCYTOSIS RECEPTOR HOMOLOGS IN CELL CORPSE ELIMINATION AND GONADAL OUTGROWTH

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Cell death can occur as a normal event in development or as a consequence of cell injury. Effective elimination of cell corpses is essential for maintaining tissue homeostasis, recycling cellular metabolites, and removing potentially harmful residual cellular contents. Both *C. elegans* programmed cell death corpses and necrotic-like corpses (such as those generated by *mec-4(d), deg-3(d)* and other stimuli) are removed via the action of seven engulfment *ced* genes--*ced-1, ced-2, ced-5, ced-6, ced-7, ced-10,* and *ced-12* (see Gumienny *et al*.). We have been interested in identifying genes that might specifically be involved in the recognition/elimination of necrotic cell corpses. Because the receptors that initially mediate recognition of the necrotic cells might be different from those recognizing the morphologically distinct programmed cell death corpses, we considered the hypothesis that a subset of nematode genes related to phagocytosis receptor genes in other organisms might be required for recognition of necrotic cell corpses.

Mammalian CD36 and *Drosophila* Croquemort are related scavenger receptors that function in cell corpse removal. We searched the *C. elegans* genomic database and identified six homologs of the CD36/Croquemort family. We generated a deletion mutation affecting the gene most closely related to CD36/Croquemort. This allele harbors a deletion of approximately 1kb, starting about 200 bp upstream of the receptor open reading frame. We named this locus *scr-1*, for scavenger receptor-like. The *scr-1* deletion mutant does not exhibit necrotic or programmed cell death corpse persistence, nor does this mutation enhance corpse persistence when present in combination with any of the seven engulfment *ced* mutations. Interestingly, however, a significant percentage of *scr-1* mutants arrest at the L1 larval stage and appear to have programmed cell death corpses throughout their bodies. *scr-1* mutants do exhibit distinctive defects in distal tip cell migration, similar to that observed in *ced-2, ced-5, ced-10,* and *ced-12* engulfment mutants. This observation suggests that SCR-1 might function as a receptor important in gonadal outgrowth in the process involving CED-2, -5, -10, -12.

We have also tested for effects of the other five *scr* homologs using RNAi.

# COMPONENTS OF THE CYTOSKELETON, INVOLVED IN THE BIOGENESIS OF MEMBRANE CHANNELS, INTERFERE WITH *MEC-4(D)*-INDUCED CELL DEATH. 

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In our analysis of mutations that block *mec-4(d)*-induced degeneration of the six touch cells, we identified lesions in a gene that encodes a novel protein, with a low level of similarity to microtubule-associated proteins. This gene spans cosmids F08B6 and C37A2 and was originally predicted by the Genome Sequencing Consortium as being two separate genes (F08B6.5 and C37A2.5, on the two cosmids). Deletions of the gene cause animals to arrest growth early in development. We tested for complementation of lethal mutations in the region and determined that this gene corresponds to *let-398*. It also appears to be allelic to *vms-1*, an essential gene that genetically interacts with the kinesin-like gene *vab-8*. Animals with lethal mutations in *vms-1* can be rescued by introduction of multiple copies of *vab-8* (vms: vab-8 multicopy suppressible). *let-398/vms-1* is expressed broadly in the *C. elegans* nervous system including sensory neurons, interneurons and motor neurons of the ventral nerve cord. Additionally, strong expression is observed in the pharynx, the canal cell and some muscles. Interestingly, expression of the kinesin-like gene *vab-8* extensively overlaps with *let-398* expression. We have thus investigated the possibility that *vab-8* mutations might interfere with degenerative cell death. Examination of *mec-4(d);vab-8* double mutants shows a marked decrease in degeneration of the six touch cells expressing the toxic *mec-4(d)* allele. Our findings implicate a novel cytoskeletal component in biogenesis, function and/or stability of degenerin channels. Our working hypothesis is that *let-398* is involved in the intracellular trafficking of proteins across long neuron axons to their destined sub-cellular localization. We are currently in the process of evaluating this model by directly examining the sub-cellular localization and stability of MEC-4 and UNC-8 in the absence of LET-398/VMS-1 and VAB-8.

# INHIBITION OF TOUCH CELL FATE IN *C. ELEGANS*

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In wild-type *C. elegans* only six cells develop as receptors for gentle touch. In *egl-44* and *egl-46* mutants, two other neurons, the FLP cells, express touch receptor-like features. *egl-44* and *egl-46* were first identified by egg-laying defective mutants with defects in the development of the HSN neurons . Thus, *egl-44* and *egl-46* mutations affect the differentiation of several different neurons.

*egl-44* encodes a member of the TEF (transcription enhancer factor) family. TEF proteins, found from yeast to human, are involved in a variety of developmental processes. Like other family members, EGL-44 protein contains a specific DNA-binding domain (the TEA/ATTS domain) and a transcriptional regulation domain. *egl-44::gfp* reporter constructs are expressed in different cell types, including neurons, hypodermal cells and some intestinal cells The product of the *egl-46* gene and similar proteins in *Drosophila*, mouse and human, define a new family of Zn-finger proteins, which are expressed in neural precursor cells. *egl-46::gfp* fusions are expressed in the Q lineages, which undergo extra division in *egl-46* mutants. Both *egl-44* and *egl-46* are expressed in FLP neurons and HSN neurons (as well as other cells), but only in the former cells is *egl-44* needed for full expression of *egl-46*. Both the EGL-46 protein and the 3' end of its mRNA contain sequences that may target the gene products to rapid degradation. Consistent with these sequence elements, expression of *egl-46::gfp* fusions is transient in HSN, touch cells and ventral nerve cord neurons. Since expression of both genes or *egl-44* in wild-type touch cells causes touch insensitivity and loss of touch cell-specific gene expression, but *egl-46* in wild-type and *egl-44* in a *egl-46* mutant do not significantly affect the touch cells, both genes are required to repress touch cell fate. The sequences of these genes and their nuclear location suggest that these genes repress transcription of touch cell characteristics in the FLP cells.

# *PAG-3* AND *UNC-3* INTERACT TO DETERMINE NEURAL FATES IN THE VENTRAL CORD, AND THEIR MAMMALIAN COUNTERPARTS MAY SIMILARLY INTERACT DURING BLOOD CELL DEVELOPMENT

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To understand how programmed cell death is regulated during development, we are studying mechanisms that determine the pattern of programmed cell deaths in the ventral cord. In the midbody the six Pn.aap cells survive and differentiate to form VC motor neurons, while lineally-equivalent cells in the anterior and posterior die. Through two genetic screens we have found that *unc-3* and *pag-3* mutants have both extra cell corpses and extra VC motor neurons in the ventral cord. In *pag-3* mutants these phenotypes are a consequence of a P cell lineage defect wherein the Pn.aaa neuroblast reiterates the fate of its mother, Pn.aa, to generate extra Pn.aap cells, some of which live and some of which die. Analysis of the number of Pn.aap cells in *unc-3 pag-3* double mutants suggests that *unc-3* and *pag-3* may function together to determine the number of Pn.aap cells. This functional interaction is not obligatory in all cells as *unc-3 pag-3* mutants, but neither single mutant, are Mec, suggesting redundant functions, and *unc-3* functions without *pag-3* to prevent dauer-formation at 27°C. UNC-3 and PAG-3 are coexpressed in ventral cord motor neurons.

Our finding of similar phenotypes in the ventral cord of *pag-3* and *unc-3* mutants prompted us to ask whether mammalian homologues of *pag-3* and *unc-3*, the *Gfi-1* and *O/E* genes respectively, might also function together to promote particular cell fates. *Gfi-1* and *O/E-1* are known to be coexpressed in hematopoietic cells, and *O/E-1* is required for development of the B cell lineage in mice. *Gfi-1* may directly regulate apoptosis. The O/E-1 protein is known to physically interact with a $C_2H_2$ Zn-finger protein ROAZ, suggesting the possibility that PAG-3, which encodes a $C_2H_2$ Zn-finger protein, might interact directly with UNC-3 in the ventral cord to specify Pn.aaa neuroblast fate and/or to regulate terminal neuronal fates, including programmed cell death, and that a Gfi-1 protein might interact with an O/E protein in hematopoietic cells. We are currently testing these ideas.

# IDENTIFICATION OF *SNS-8*, A SERINE/ THREONINE KINASE, AND ADDITIONAL GENES REQUIRED FOR THE DEVELOPMENT OF THE AWB OLFACTORY NEURONS. 

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Our lab is interested in understanding how chemosensory neurons acquire their unique identities during development. The AWB olfactory neuron pair must be properly specified in order to mediate the worm's avoidance from volatile repellents such as 2-nonanone. Recent work has shown that the *lim-4* encoded transcription factor is required for the development of the AWB neurons (Sagasti *et al*, 1999, Genes Dev. 13:1794-1806). To identify additional genes required for AWB development and specification, we performed screens to isolate mutants that fail to correctly express the odorant receptor, *str-1* specifically in the AWB neurons (Troemel *et al*, 1995, Cell 83: 207-18). These screens have resulted in the isolation of mutants in components of a pathway required for AWB development. These include: *sns-9*, a gene required to restrict AWB fate to the single neuron pair, and *sns-11*, a gene required for the regulation of *lim-4* expression within the AWB neurons. In addition, our screens have identified *sns-8*, a gene that is required downstream of *lim-4* to promote high levels of *str-1* odorant receptor expression.

*sns-8* encodes a putative serine/threonine kinase that is broadly expressed. It is localized to the nuclei of most, if not all, neurons in the head. In addition to promoting high levels of *str-1* receptor expression in the AWB neurons, *sns-8* is also required to promote expression of another candidate receptor, *sra-6,* in the ASH neurons. *sns-8* appears to have a fairly specific role in the regulation of subsets of receptors within these neurons, since *sns-8* mutants exhibit largely wild-type ASH and AWB mediated behaviors. We are currently investigating where SNS-8 acts to promote high levels of expression of these receptors. Other phenotypes exhibited by *sns-8* mutants include growth and brood size defects, indicating a role for this kinase in other aspects of development as well. We are also further characterizing and attempting to clone *sns-9* and *sns-11*.

# THE BHLH TRANSCRIPTION FACTORS LIN-32 AND HLH-2 SPECIFY MULTIPLE ASPECTS OF RAY DEVELOPMENT IN THE *C. ELEGANS* MALE TAIL

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During the male L3 larval stage, nine pairs of posterior hypodermal cells begin to execute the ray sublineage, generating the two neurons and one structural cell that comprise each ray. We are interested in understanding how the fates of ray neuroblasts and differentiated ray cells are determined, and how the asymmetric cell divisions of the ray sublineage allocate these fates.

We have found that two genes encoding bHLH transcription factors, *lin-32* (belonging to the *atonal* family of proneural genes) and *hlh-2* (the worm ortholog of the E/*daughterless* family of heterodimerization partners), are required for, and expressed during, ray development. In both *lin-32* and *hlh-2; lin-32* mutants, a spectrum of ray development defects can be observed. In strong loss-of-function backgrounds, the ray precursor cell usually gives rise only to hypodermal cells, suggesting an early role for these genes in neuronal commitment. In weaker alleles, a variety of partially-successful sublineages occurs. Execution of the anterior and posterior branches of the sublineage can become uncoupled from each other; that is, A-type ray neurons can be generated in the absence of their cognate ray structural cells, and vice versa. This implies that the two branches of the sublineage independently require *lin-32* and *hlh-2* function. Finally, when ray cells are generated, they often have abnormal or absent processes. We conclude that *lin-32* and *hlh-2* are required for several cell-fate determination steps, from early commitment to ray fate, to intermediate fates in the ray sublineage and the establishment of differentiated characteristics.

To understand these functions at a biochemical level, we are studying the DNA-binding and heterodimerization properties of these proteins, and have found that LIN-32 and HLH-2 can heterodimerize and bind to E-box-containing sequences *in vitro*. We are currently determining the effects of mutations on these activities and investigating the sequence-specificity of DNA binding. In collaboration with Stuart Kim, we have also begun to use microarrays to identify potential LIN-32:HLH-2 target genes expressed during the ray sublineage and in mature rays.

# *SEU-1* ENCODES NOVEL NUCLEAR PROTEINS REQUIRED FOR THE FUNCTION OF ECTOPIC UNC-5

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The UNC-6/netrin guidance cue and its receptors UNC-5 and UNC-40/DCC form a highly conserved system for circumferential guidance of migrating cells and growth cones. In *C. elegans*, ectopic expression of the UNC-5 receptor causes repulsion of touch neuron growth cones away from sources of UNC-6. A genetic suppressor screen identified 8 genes required for this repulsion: *unc-6*, *unc-34*, *unc-40*, *unc-44*, *unc-129*, *seu-1*, *seu-2*, and *seu-3*. We have cloned *seu-1* and report that it encodes 2 novel proteins (SEU-1A and SEU-1B), which differ through alternative splicing at their C-termini. Both SEU-1 isoforms contain acidic and proline-rich regions and nuclear localization sequences, but no recognizable DNA- or RNA-binding domains. The *seu-1(ev520)* allele contains an early nonsense mutation and is likely to be a null allele.

*seu-1::GFP* is nuclear localized and dynamically expressed throughout development in neural and non-neural tissues. Similar to *unc-5* reporter constructs, expression in the hermaphrodite distal tip cells was observed only at the time of the circumferential second migration phase. Transgenic expression of the combination of SEU-1A and SEU-1B in the touch neurons (using the *mec-7* promoter) was sufficient to restore the function of UNC-5 in the touch neurons in a *seu-1* mutant background, suggesting a cell autonomous function. Neither the SEU-1A nor the SEU-1B isoform alone had rescuing ability. Although *seu-1* mutations alone have subtle defects in axonal morphology, over-expression in neurons caused more severe cell migration and axon guidance defects. We propose that SEU-1 in neurons regulates growth cone responses to guidance cues. We are currently examining genetic interactions between *seu-1* and *unc-5,6,40*.

# MOLECULAR AND GENETIC ANALYSES OF *UNC-69*, A GENE REQUIRED FOR AXON GUIDANCE

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The *unc-69* gene is required for axon guidance, outgrowth and fasciculation in *C. elegans*. In *unc-69* mutants many axons, including those of the HSN and DD/VD neurons, exhibit guidance defects. Furthermore, the axons of HSN, ALM and AVM terminate prematurely, and the dorsal and ventral nerve cords are defasciculated in these mutants. *unc-69* encodes a novel 108 amino-acid protein with a predicted coiled-coil motif near its C-terminus. Rescuing *unc-69::gfp* fusion constructs are expressed throughout development in the nervous system, predominantly in axons. This expression pattern, combined with the lack of a signal sequence, suggests that *unc-69* acts cell-autonomously in axon guidance. UNC-69 shows significant similarity to the predicted products of previously uncharacterized human, mouse and *Drosophila* genes (*hunc-69*, *munc-69* and *drunc-69*, respectively), identified by expressed sequence tags. The predicted vertebrate proteins also have coiled-coil structures near their C-termini and are 77% identical to UNC-69 over the coiled-coil regions. *hunc-69* mRNA is enriched in human fetal brain, although its expression is not restricted to this tissue. A *hunc-69* transgene rescues the uncoordinated phenotype of *unc-69* mutants, suggesting that the two gene products are functionally homologous. The high degree of sequence conservation over the coiled-coil domain strongly suggests that protein-protein interactions mediated by this domain are required for UNC-69 function. We are currently carrying out a yeast two-hybrid screen to identify candidate UNC-69 interaction partners. We speculate that UNC-69 participates in a conserved signaling pathway that transduces extracellular guidance cues to the axonal cytoskeleton.

# THE *C. ELEGANS* ORFEOME PROJECT

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In addition to gene-based functional genomics approaches such as large-scale gene knock-outs and microarray or chip analysis, it is also important to develop protein-based approaches, e.g. protein interaction mapping, protein localization mapping, and biochemical and structural genomics. Most of protein-based approaches rely upon the availability of near complete set of open reading frames ("ORFeomes") cloned into various expression vectors (i.e., for each ORF: the sequence between the start and the stop codons, in the absence of 5' and 3' untranslated sequences and introns).

To clone the *C. elegans* ORFeome into various expression vectors, we use a Recombination Cloning technique (RC) referred to as Gateway™ (Walhout et al., 2000, *Science*, 287, 166-122). RC allows both the initial cloning of ORFs and their subsequent transfer into different expression vectors by site-specific recombination *in vitro*. In addition, RC is amenable to automation in 96-well (or 384-) plate settings, which is crucial for large-scale ORFeome cloning. So far we have cloned 2,000 *C. elegans* ORFs. At the current throughput (~400 ORFs/week), ~70% of the *C. elegans* ORFeome should be cloned by the end of the year. We will present: i) the details of the method used, ii) illustrations of our current throughput, iii) a description of the cloning quality, iv) how this resource will be made available to the community, and v) how the ORFeome project will help the protein interaction mapping project (see abstract by Walhout et al).

# ASSIGNING FUNCTION TO OVARY EXPRESSED GENES USING RNAI.

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We have been performing RNAi analysis of genes expressed in the ovary. In a pilot screen, we tested 216 cDNA clones randomly picked from an ovary library and found that 76 were required for embryogenesis. Sequencing 69 of these revealed 52 different essential genes. To decrease redundant RNAi analyses, we next used filter hybridization to identify 142 (out of 184 tested) new ovary clones not previously analyzed. From this new set we found 31 clones required for embryogenesis. This subtractive approach appears efficient in decreasing redundancy as none of the 30 clones we sequenced were duplicates and only three had been identified in our previous set.

More recently, we have been testing a set of 766 genes identified as ovary enriched by microarray experiments (Worm Breeder's Gazette 16(2): 29). We have completed the RNAi analysis of over 200 genes from this set and find that over 25% are required for embryogenesis. Data from the two approaches lead us to speculate that as much as one fifth of ovary-expressed genes are required for embryogenesis.

Of the more than 140 genes found to be required for embryogenesis so far, less than 10% encode genes previously studied either molecularly or genetically in C. elegans. However, we can assign a putative biochemical function to ~70% of these genes using similarity based searches while ~10% do not seem to share any sequence similarity with any other gene present in the Genbank database. The remaining ~20% show weak or partial similarity to other proteins.

For every gene identified as strong RNAi-embryonic lethal we are collecting time lapse digital movies of the first two cleavages (at least one embryo from each of three injected mothers). Using these data we are categorizing the genes according to the first defect observed in the early embryo. Thus far, over 70% of the embryonically-required genes we identified exhibit defects during early cleavages and/or oogenesis.

We will make the results from these tests (positives, negatives, phenotypic descriptions and movies) available through the Web using a database and interface being designed for this purpose.

# A COMPREHENSIVE REVERSE GENETIC SCREEN OF CHROMOSOME I

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*C. elegans* is an ideal model system for the dissection of diverse biological processes using reverse genetics. Firstly, the entire sequence of the genome is known and is predicted to contain ~19,000 genes; secondly, RNA-mediated inhibition allows the directed and specific inhibition of individual genes. RNAi is thus an ideal tool for a genome-wide analysis of gene function in *C. elegans*. However, the most widely used methods for RNAi in *C. elegans* require the sythesis of RNA *in vitro*; these methods are labour intensive, high cost and ultimately result in limited amounts of dsRNA, thus precluding multiple subsequent screens. It has previously been shown that RNAi can be carried out by feeding bacteria that express dsRNA to adult worms. We have determined conditions for which feeding dsRNA-expressing bacteria to worms results in potent RNAi effects; once constructed, such bacterial strains can be used for unlimited experiments at minimal cost.

We present here the construction and screening of a bacterially-expressed dsRNA library spanning the entire of Chromosome I, which contains ~17% of all *C. elegans* genes. We will describe our results for the screen so far. This is the first attempt at a comprehensive reverse-genetic analysis in any eukaryote and marks a significant advance in functional genomics.

# EGO-1: A LINK BETWEEN DEVELOPMENT AND RNAI

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*ego-1* gene function is required for normal germline development and, consequently, for fertility (1, 2). In addition to its pleiotropic germline phenotype, *ego-1* is also required for a robust response to RNAi by many germline-expressed genes (2). EGO-1 protein is a member of the RNA-directed RNA polymerase (RdRP) family, whose other members include *N. crassa* QDE-1, *A. thaliana* SGS-2, and tomato RdRP. These proteins have been implicated in RNA silencing phenomena, as well. Consistent with these results, *ego-1* appears to be expressed mainly, if not entirely, in the germ line (2).

We are studying *ego-1* to understand better its role in development and if/how its developmental role is related to its role in RNAi. We are carrying out a variety of studies aimed at describing the EGO-1 expression pattern in the gonad and identifying potential targets of EGO-1 regulation. For comparison with other RNAi-defective mutants (Rde and Mut genes), we are examining whether transgenes are desilenced in the *ego-1* mutant germ line, and whether Rde mutations enhance the Ego-1 developmental phenotype. The results of these ongoing studies will be presented.

(1) Qiao, L., Lissemore, J.L., Shu, P., Smardon, A., Gelber, M.B., and Maine, E.M. (1995) Genetics 141:551-569. (2) Smardon, A., Spoerke, J.M., Stacey, S.C., Klein, M.E., Mackin, N., and Maine, E.M. (2000) Current Biology 10:169-178.

# ROLE OF *MUT-7* AND *RDE* GENES IN THE INITIATION OF TRANSGENE SILENCING IN THE GERMLINE

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Transgene silencing in *C.elegans* has been shown to require both *mes* genes implicated in chromatin-based mechanisms (Kelly and Fire, 1998) and genes required for post-transcriptional gene silencing or RNAi -- *mut-7, rde-2, rde-3* - (Tabara et al, 1999). Desilencing role of the genes involved in RNAi was apparent only at 25 C, while there is no temperature dependence for their RNAi-resistance. We reasoned that the silencing process might require initiation and maintenance steps. RNAi-pathway genes might be required only for initiation of transgene silencing or re-initiation at 25 C, while maintenance of silencing might be independent of RNAi genes but sensitive to temperature. As the role of RNAi genes in silencing was previously studied only with transgenic lines where the transgenes where introduced in the wild type background and then crossed into mutants, their role in the initiation step was not determined. When we introduced repetitive arrays of *let-858::GFP* transgene directly into *mut-7* and *rde-6* backgrounds, the transgene did not show signs of progressive silencing in the germline even under normal culture conditions (20° C). When "naïve" *let-858::GFP* transgene array in the *mut-7* background was crossed into wild type it became silent immediately and remained silent even when crossed back to *mut-7* background. To determine directly if transgene silencing can be induced by dsRNA we injected *gfp* dsRNA into the transgenic strain expressing *pie-1::GFP* from recombinant YAC and observed that transgene became immediately silent and remained silent in generations. Induction of silencing in this case required all RNAi pathway genes acting in response to dsRNA, but maintenance in generations occurred by different mechanism. We conclude that silencing of germline transgenes can be triggered either by copy number or dsRNA and this triggering is dependent on *mut-7* and *rde-6* in both cases.

# MECHANISMS OF RESPONSE TO ENVIRONMENTAL DSRNA

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C. elegans responds to environmental presentation of double-stranded RNA which results in interference with gene function in a sequence-specific manner (RNAi) in C. elegans. RNAi has proven to be a useful tool in C. elegans for the analysis of gene function, and this technique has also been applied successfully in other organisms. Injection of exogenous dsRNA sequences results in the disappearance of the corresponding mRNA. In addition a consequence the transcriptional level cannot be ruled out. The mechanism underlying RNAi may prove to have similarities to co-suppression in C. elegans and other organisms and viral suppression in plants.

We have also demonstrated that *C. elegans* can respond in a gene-specific manner to dsRNA encountered in the environment. This was done by producing bacteria that express dsRNAs corresponding to *C. elegans* genes, then allowing the worms to feed on these bacteria. This produced marked and gene-specific effects on activity of several genes tested. Recent work analyzing the interactions between bacteria and their nematode predators will be described. The ability to respond to dsRNA from the environment suggests that worms have a natural defense against dsRNAs, or more specifically viruses that produce dsRNAs during a part of their life cycle.

We are taking a genetic approach toward understanding how C. elegans can acquire information from the environment via dsRNA. We are especially interested in learning how dsRNA is dispensed to all cells throughout the worm. Mutants that respond to dsRNA when introduced by injection but fail to respond to dsRNA when introduced by "feeding" are candidates for further analysis. These mutants may aid in the identification of factors involved in uptake and dispersal. Several such mutations are being analyzed.

# PARENT OF ORIGIN EFFECTS IN *C. ELEGANS*: WORM IMPRINTING OR AN INSIGHT INTO HYBRID DYSGENESIS?

## William G. Kelly

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Parent of origin effects in gene expression describe how the sex of a parent transmitting a particular allele affects the expression of that gene in its offspring. A gene that exhibits a differential expression in the offspring that depends on whether it was inherited from the mother or the father is considered to be *imprinted*. Imprinting has been shown in mammals to closely correlate with cytosine methylation, yet parent of origin effects have been frequently reported in *Drosophila*, an organism in which methylated cytosine is not observed (*e.g.* Lloyd et al, Genetics **151**:1503). Imprinting in *C. elegans* has previously been investigated by looking at cross-progeny that were homozygous for a single chromosome from either parent, using *him-6* to generate disomic or nullisomic gametes in each cross (Haack and Hodgkin, Mol.&Gen. Genet **228:**482). No effects on viability were observed, leading to the conclusion that either chromosomal imprinting effects do not occur in the worm, or that their effects are minor. *C. elegans* also has no detectable methylcytosine.

During crosses to build transgenic strains for testing germline desilencing, a high frequency of transient activation of silenced transgenes was observed in the germ cells of the offspring. Upon further investigation it was shown that this activation only occurs when the silenced transgene is inherited from the father, and the effect is temperature-sensitive. The activation is generally restricted to the F1 progeny, although a low frequency of heritable activation can be observed. Interestingly, the presence of a transgene in the recipient oocyte that bears partial homology to the incoming reporter suppresses the activation of the reporter. This suppression is still seen in animals arising from oocytes that have lost the suppressing DNA during meiosis. The effect of different mutations on this phenomenon, its applicability for germline transgene expression protocols, and its natural role in worm biology will be discussed.

# TOWARDS THE MOLECULAR GENETICS OF SEX DRIVE IN *C. ELEGANS* 

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We are analyzing a sexually dimorphic appetitive behavior in *C. elegans.* Hermaphrodites placed on a food source will forage and lay eggs, rarely wandering away from the food. In contrast, individual males cultured in the absence of hermaphrodites will wander away from food - a behavior we refer to as "leaving." We have developed a quantitative leaving assay with the intention of dissecting the molecular mechanisms governing both sex drive and the interactions between the sexes.

Animals are placed singly on a small bacterial lawn in the center of a 9cm plate. A worm that has wandered as far as 3.5 cm from the plate's center, or off the plate entirely, is scored as a leaver. We have found that leaving can be modeled as a stochastic process characterized by probability of leaving per hour ($P_L$). For wild-type males, $P_L$ is 0.1 whereas for hermaphrodites, $P_L$ is 0. $P_L$(male) is independent of time separated from hermaphrodites, indicating that males do not become motivated to leave with mating deprivation. $P_L$ is also independent of prior mating experience. Plating males with hermaphrodites completely abrogates leaving whereas plating males with males, does not. Males plated with hermaphrodites for several hours leave instantaneously upon hermaphrodite removal and with $P_L$ commensurate to males plated alone. This result suggests both that sexual experience does not affect leaving behavior and that a putative hermaphrodite-derived signal is short-lived. *lin-39*, *let-23*, and *lin-4* hermaphrodites were also capable of attenuating leaving suggesting that neither the vulva nor successful copulation are required to "keep" males. $P_L$ for *mab-3(e1240)* males plated with hermaphrodites resembled that of males plated alone, suggesting that *mab-3* is required for males to respond to hermaphrodites. We have found that neither L4 males nor L4 hermaphrodites leave ($P_L$ is 0); male leaving commences with the onset of sexual maturity. Neither dopamine- nor serotonin-deficient mutants showed significant alterations in leaving behavior.

We screened for mutants with decreased $P_L$(male). Several *lad* (**l**eaving **a**ssay **d**efective) mutants have been isolated. $P_L$ of *lad-1* males is approximately 0. Although *lad-1* males do not appreciably leave food, they move well, mate, and are morphologically wild-type.

# HALDANE'S RULE IN *CAENORHABDITIS* IS IMPLEMENTED BY SEXUAL TRANSFORMATION.

## Scott Everet Baird[1,2], Wei-Chih Yen[1]

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Crosses between *C. briggsae* strain AF16 males and *C. remanei* strain EM464 females resulted in gender-biased broods in which all F1 adults were sterile females. This was consistent with Haldane's Rule: 'When in the F1 offspring of two different animals races one sex is absent, rare, or sterile, that sex is the heterozygous [heterogametic] sex' (Haldane, 1922). The observed gender bias in AF16::EM464 adults resulted from sexual transformation, not male-specific lethality. This was determined using a single-worm PCR assay to detect the *C. briggsae* homolog of the X-linked *unc18* gene (*Cb_unc18*) in F1 adults. *Cb_unc18* was detected in approximately half of F1 adult hybrids consistent with a karyotypic XX:XO ratio of 1:1. *C. briggsae* and *C. remanei* variants have been identified that suppress this hybrid sexual transformation phenotype. In crosses between *C. briggsae* strain HK104 males and *C. remanei* strain EM464 females adult male and female hybrids were obtained. In these hybrids, a strong correlation was observed between karyotype and gender. Similar results were obtained for crosses between *C. briggsae* strain AF16 males and *C. remanei* strain PB228 females and for crosses between *C. briggsae* strain HK104 males and *C. remanei* strain PB228 females. The effects of the HK104 and PB228 variants on the suppression of sexual transformation of XO hybrids was cumulative. In hybrid males obtained from crosses employing HK104 or PB228, the copulatory bursa and sensory rays were absent or greatly attenuated and the spicules were short and/or crumpled. In hybrid males obtained from crosses employing HK104 and PB228, the fan and rays were slightly attenuated and the spicules usually were well formed. These results implicate defects in sex determination as a reproductive isolating mechanism in *Caenorhabditis*. Genetic studies of the HK104 and PB228 variants will be used to identify the genes that implement this reproductive barrier.

Haldane, J. B. S. (1922) Sex Ratio and Unisexual Sterility in Hybrid Animals. J. Genetics 12: 101-109.

# GERM-SOMA DISTINCTION IN *C. ELEGANS*

## Yingdee Unhavaithaya, Tae Ho Shin, Craig C. Mello.

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During development, cells that constitute somatic tissues must differentiate from the germ cells while the latter are required to remain undifferentiated. How is the distinction between the germ and somatic cells maintained? Early *C. elegans* embryos keep the germ-soma difference through differential transcription; the germ cell-specific protein PIE-1 suppresses transcription in the germ cells while tissue-specific transcription programs are activated by maternal transcription factors in the soma. We think that transcriptional mechanisms can also account for the germ-soma distinction in later stages and that *eps-1* (ectopic P-granules in the soma) is essential for this process. *eps-1* encodes a ubiquitous nuclear protein related to *Drosophila* Suppressor of Hairy Wing (Su(HW)). A loss of both maternal and zygotic *eps-1(+)* activity (via RNAi or germline mosaicism of the rescued *eps-1* mutant strain [A. Puoti and J. Kimble]) causes numerous somatic cells to adopt germ-like characteristics. For example, both intestinal and hypodermal cells lose their proper cell-cell adhesions and adopt nuclear morphology resembling that of the germ cells. The transformed somatic cells ectopically express germ cell-specific proteins including PGL-1, GLH-2 and GLH-3, which appears to reflect *de novo* synthesis of the P-granule components. *eps-1(+)* is not required for asymmetric segregation of maternal P-granules during embryogenesis, and furthermore, *in situ* hybridization reveals ectopic accumulation of mRNAs for the P-granule proteins, but not for somatic genes, in the *eps-1* animals. The latter observation is consistent with the hypothesis that EPS-1 functions at the level of transcription. Interestingly, in addition to its suppressive role in the soma, *eps-1* is zygotically required for germ cell development, indicating its dual function in the germ-soma distinction. Su(HW) binds to chromatin insulators and is thought to repress transcription by blocking the enhancer-promoter interaction. Subnuclear distribution of EPS-1 is consistent with its potential involvement in the insulator function. Furthermore, *eps-1* genetically interacts with *C. elegans* genes proposed to affect higher order chromatin structures. EPS-1 may thus regulate transcription by reorganizing chromatin structure into distinct domains and may function differentially between germ and somatic cells.

# THE GERMLINE PROTEIN PIE-1 HAS TWO FUNCTIONS, EACH IMPORTANT FOR GERM CELL FATE

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PIE-1 is a maternal protein which segregates with the early germ lineage where it is required to maintain germ cell fate. We have shown previously that one function of PIE-1 is to inhibit mRNA transcription in germline blastomeres. Consistent with this PIE-1 can inhibit transcription when introduced in Hela cells (Batchelder et al., 1999). Recently we have found that PIE-1 is required for another aspect of germline blastomere identity: NOS-2 expression.

*nos-2* is a *nanos*-related gene which codes for a maternal RNA preferentially maintained in germline blastomeres. NOS-2 protein is expressed specifically in P4 and its daughters Z2 and Z3. In *pie-1(zu154)* embryos *nos-2* RNA is lost prematurely from the germ lineage and NOS-2 protein is not expressed. Two other maternal RNAs are also lost prematurely in *pie-1(zu154)* embryos, suggesting that PIE-1 maybe generally required to maintain RNAs in germline blastomeres.

To test whether PIE-1's effect on NOS-2 expression is dependent on PIE-1's ability to repress transcription, we examined *pie-1(zu154) smg-1(cc545ts)* embryos which express a truncated form of PIE-1 missing the domain required in Hela cells for transcriptional repression. These embryos fail to repress transcription, but still produce NOS-2, indicating that NOS-2 expression is not dependent on transcriptional repression. This result raises the possibility that PIE-1 has two independent functions: one to repress transcription and one to maintain maternal mRNAs.

To explore this hypothesis further we have tested mutant *pie-1* transgenes for rescue of each function *in vivo*. Among these we have identified two new mutants which affect one function without affecting the other. Embryos expressing a *pie-1* transgene with a mutation in a sequence required for efficient nuclear entry can still produce NOS-2, but fail to repress transcription. Embryos expressing a *pie-1* transgene with a different mutation have the opposite phenotype: they can still repress transcription but fail to express NOS-2 as efficiently as wild-type. Interestingly this latter mutant is sterile, suggesting that PIE-1's 2$^{nd}$ function is also important for germ cell fate. Our results support a model in which PIE-1 performs two functions, both of which are important for germ cell development.

# CGH-1, AN ESSENTIAL AND CONSERVED GERMLINE RNA HELICASE

## RE Navarro[1], EY Shim[1], Y Kohara[2], Y Shi[3], TK Blackwell[1]

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RCK is a conserved DEAD-box type RNA helicase that is present in the germline. In *Drosophila* and *Xenopus*, RCK is expressed specifically during oogenesis and early embryonic stages, and in *Xenopus* it is associated with maternal mRNA. *S. pombe* RCK (*ste13*) is required for entry into either $G_0$ or meiosis, however, and in mice RCK is expressed in oocytes and also in some adult tissues, suggesting the possibility of a conserved role in self-renewing cells.

In a screen for proteins that bind the germline protein PIE-1, we identified the *C. elegans* RCK homolog, which we refer to as conserved germline helicase, *cgh-1*. In adults, *cgh-1* mRNA is present predominately in the germline. CGH-1 protein is detected within germline P granules beginning at the L1 stage. It is present in mitotic germline cells but its levels increase dramatically in both males and hermaphrodites during meiotic entry, then it disappears during sperm development but persists at high levels in oocytes and the early embryo. CGH-1 is present in P granules until the 1-200 cell embryonic stage, but is also found in other cytoplasmic granules that are present in somatic cells. Somatic CGH-1 staining declines after the 4 cell stage until it is undetectable after 50 cells. This embryonic pattern approximates the previously described distribution of maternal mRNA. It also suggests that CGH-1 is unlikely to be involved directly in germline silencing by PIE-1, but might contribute to other aspects of PIE-1 function.

RNAi experiments indicate that *cgh-1* is essential for fertility in both males and hermaphrodites. In *cgh-1 RNAi* F1 males, the gonad appears superficially normal. *Cgh-1 RNA*i F1 hermaphrodites also appear normal through the early L4 stage and generate sperm, but then produce grossly abnormal oocytes. Multiple P granule components are localized appropriately in *cgh-1 RNAi* animals, and their germline cells express appropriate markers of mitotic, pachytene, and developing oocyte stages. Apparently, CGH-1 is not essential for P granule structure or germline fate. The data suggest, however, that regulatory mechanisms required for fission yeast to become quiescent and self-renewing are linked to P granules, and required for germ cell function in a metazoan.

# THE LOCATIONS AND PREDICTED ROLES OF MES-2, MES-3, MES-4, AND MES-6 IN CONTROL OF GENE EXPRESSION IN THE GERMLINE

## Lei Xu, Youyi Fong, and Susan Strome

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*mes-2, mes-3, mes-4 and mes-6* mutants produce sterile but otherwise healthy hermaphrodite progeny. The MES proteins are predicted to function in complexes to repress gene expression in the germline: 1) MES-2 and MES-6 are homologs of Polycomb Group (PcG) proteins, which in *Drosophila* function in complexes to repress gene expression. 2) MES-2 and MES-6 depend on each other and on MES-3 for correct nuclear localization. 3) Transgenes on extrachromosomal arrays are silenced in wild-type *C. elegans* germlines, but can be desilenced in *mes* mutant germlines (Kelly & Fire), suggesting that MES proteins normally participate in repressing germline gene expression.

We have investigated the localization of MES-3 and MES-4. MES-3 is a novel protein, and MES-4 is a SET-domain protein with PHD fingers. Both proteins resemble MES-2 and MES-6 in being abundant in all nuclei of early embryos and remaining detectable only in the primordial germ cells in later stage embryos. However, MES-4 shows much more dramatic localization to chromosomes than any of the other MES proteins, and intriguingly is associated with the autosomes but not the X chromosomes. In the germline, MES-3 levels are high in the distal mitotic region, low in pachytene nuclei, and increasing in maturing oocytes, while MES-4 is only detectable in the distal mitotic region.

Coimmunoprecipitation experiments have demonstrated that MES-2, MES-3, and MES-6 are in a complex in embryo extracts. The size of this complex is predicted to be ~300kD, based on sucrose gradient and gel filtration analyses. *In vitro* binding assays have shown that MES-2 and MES-6 interact directly with each other, but not with MES-3, suggesting that an additional component(s) mediates the association of MES-3 with MES-2 and MES-6. MES-4 protein did not coprecipitate with any of the other three MES proteins. Our finding that MES-4 protein spreads to the X chromosomes in *mes-2*, *mes-3*, or *mes-6* mutants suggests that the MES-2,3,6 complex somehow regulates MES-4's binding pattern.

These new results, along with our previous findings that the Mes mutant phenotype is highly sensitive to X-chromosome dosage, suggest that in wild-type worms, MES-2, MES-3, and MES-6 act in a complex to exclude MES-4 from the X chromosomes and that this leads to repression of X gene expression.

# HISTONE DEACETYLASE HDA-1 IS INVOLVED IN GONADOGENESIS AND GERMLINE MATURATION IN *CAENORHABDITIS ELEGANS*

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Our lab is interested in chromatin remodeling activities in *C. elegans* development with a particular emphasis on histone acetylases and histone deacetylases. We showed previously that a histone acetylase, CBP-1, is essential for all non-neuronal cell differentiation in *C. elegans* embryogenesis. In endodermal cells, CBP-1 appears to promote their differentiation by antagonizing the repressive activity of a histone deacetylase gene, *hda-1* (1). Inhibition of *hda-1* expression by RNAi results in embryonic arrest at the comma stage, and the cause of this embryonic lethality is currently unclear. To understand the dynamic genetic interaction between *cbp-1* and *hda-1* in *C. elegans* development, we analyzed the zygotic function of *hda-1*.

To inhibit *hda-1* zygotic expression, we took advantage of the *rde-1* strain generated by the Mello lab. We find by RNAi experiment in the *rde-1* genetic background that loss of zygotic *hda-1* expression results in sterile animals that have abnormal somatic gonad tissues and a defect in germline development. The *hda-1* gene is located on chromosome V in a region where a previously isolated genetic mutant termed *gon-10* is mapped to. We find that *gon-10* mutants share many of the phenotypes displayed by the animals that lack zygotic *hda-1*. We have sequenced the complete *hda-1* gene isolated from the homozygotic *gon-10* mutant (including its promoter and 3' untranslated regions) and have identified a single nucleotide change that alters a conserved amino acid near the catalytic domain of HDA-1. Using affinity-purified HDA-1 antibodies that we developed, we found *hda-1* expression to be virtually undetectable in these mutants. Thus, the finding of a point mutation in the *hda-1* gene coupled with a lack of *hda-1* expression in the *gon-10* mutant are highly suggestive that *gon-10* may be an *hda-1* mutant. To determine whether this is indeed the case, experiments are in progress to rescue the *gon-10* defects. Analysis and comparison of the phenotypes of the *gon-10* mutant and the *rde-1; hda-1/RNAi* animals will be presented.

1. Shi, Y., and Mello, C.C. (1998). Gen.& Dev. 12, 943-955.

# *GLP-1* PROXIMAL PROLIFERATION (PRO) MUTANTS  

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The adult *C. elegans* hermaphrodite gonad is organized along a distal to proximal axis. Mitosis occurs distally and is followed proximally by meiosis and gametogenesis. Mutations that cause a proximal proliferation (Pro) phenotype alter this pattern without affecting the ability of the cells to execute their normal fates (Schedl, 1997). Specifically, in addition to the normal distal-to-proximal pattern of "mitosis, meiosis, gametogenesis", a second cluster of mitotic germ cells is observed proximal to mature gametes. Identification and analysis of genes that, when mutant, cause this phenotype will reveal mechanisms by which the germline pattern is normally generated and maintained.

As part of a genetic screen to identify loci involved in germline development, we identified three temperature sensitive Pro mutants that proved to be new *glp-1* alleles. Activity of the well-characterized GLP-1 receptor, a member of the LIN-12/Notch family, is associated with maintenance of mitosis and/or inhibition of meiosis in the germ line (Austin and Kimble, 1987; Schedl, 1997). The distal germ line remains mitotic due to GLP-1-mediated signaling from the distal tip cell (DTC) to the distal germ line (Austin and Kimble, 1987).

The current understanding for spatial control of meiotic entry in the L3 is that as the germ line grows, cells in the proximal germ line enter meiosis by default as their distance from the DTC increases. Our *glp-1(Pro)* alleles offer additional insight into mechanisms regulating the spatial control of L3 meiotic entry. To distinguish between several possible models for the cellular origin of the *glp-1(Pro)* phenotype, we performed a detailed time-course analysis. In the wild-type L3, the proximal-most germ cells are the first cells to enter meiosis. In *glp-1(Pro)* mutants, however, the proximal-most germ cells do not enter meiosis whereas more distally-located cells adopt the appropriate distal-to-proximal pattern. Therefore, our data suggest that perhaps two mechanisms exist in the wild type, one proximal and one distal, that act in concert to restrict meiotic entry to the proximal-most region of the L3 germ line. On-going genetic and phenotypic analysis is aimed at determining the precise nature of these alleles and their dependence on ligands and effectors in the LIN-12/Notch signaling pathway.

# CHROMOSOME SEGREGATION IS NOT REQUIRED FOR POLARIZED SEGREGATION OF ORGANELLES DURING C. ELEGANS SPERM MEIOSIS

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C.*elegans* sperm meiosis consists of two uniquely different divisions. Meiosis I involves an actin/myosin based contractile ring which symmetrically splits a primary spermatocyte into two secondary spermatocytes. In contrast, meiosis II, involves a actin independent mechanism which asymmetrically cleaves the secondary spermatocytes into two spermatid buds and a large, central cytoplast known as the residual body. These resulting spermatids contain only those cellular components which are essential for sperm function, such as mitochondria and sperm motility proteins. Other cellular components, including protein synthesis machinery and many cytoskeletal proteins, segregate to the residual body. Recently, we have examined several temperature sensitive mutants which fail to segregate chromosomes due to metaphase block during sperm meiosis I. Although their chromosomes remain located in a metaphase I meiotic spindle, these mutant primary spermatocytes continue to differentiate; they subsequently polarize and divide forming two anucleate spermatids and a DNA-containing residual body. To investigate whether proper chromosome segregation is required for the segregation of other sperm specific components during spermatid budding, we analyzed the localization of mitochondria and the sperm specific FB-MO complex in these DNA segregation-defective mutants. Also, to study the effect of these meiosis defects on possible components of the segregation system, we analyzed both actin and tubulin structures. We have found that missegregation of chromosomes disrupts cytoskeletal structures during meiosis I but does not affect the segregation of either organelles nor cytoskeletal proteins during spermatid budding. Our findings suggest that (a) spermatid budding does not require normal progression through meiosis I and (b) separate mechanisms control segregation of DNA and organelles to daughter cells during *C. elegans* spermatogenesis.

# GENES REQUIRED FOR SPERM-OOCYTE INTERACTIONS AT FERTILIZATION

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The reproductive biology of the worm facilitates the identification of mutations that affect sperm and no other cells. This provides a unique opportunity to define sperm components required for sperm-egg interactions. Worms with mutations in the *spe-9* gene produce spermatozoa with wild type morphology and motility that cannot fertilize oocytes even after contact between gametes. From this phenotype we infer that these mutants disrupt either sperm-egg signaling, adhesion or fusion. The *spe-9* gene encodes a sperm transmembrane protein with an extracellular domain that contains ten epidermal growth factor (EGF)-like repeats. A common feature of proteins that include EGF-like motifs is their involvement in extracellular functions such as adhesive and ligand-receptor interactions. In order to gain a better understanding of the role of SPE-9 during fertilization, we are following several lines of investigation. First, we will report on our structural analysis of the SPE-9 protein. Second, we are expanding our studies to include other genes that phenocopy *spe-9* mutants. Finally, we will report our progress with both forward and reverse genetic approaches to identify additional genes required for fertility in the worm.

# SEVERAL NOVEL COMPLEMENTATION GROUPS REGULATE CELL SURVIVAL OF VULVA PRECURSOR CELLS IN *PRISTIONCHUS PACIFICUS*

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We are studying the evolution of cell fate specification, using vulva development as a model system and compare *Pristionchus pacificus* with *C. elegans*. In *P. pacificus*, seven ventral epidermal cells P(1-4,9-11).p undergo programmed cell death during late embryogenesis, whereas P(5-8).p survive and participate in vulva formation. It has previously been shown that the *lin-39* homologue of *P. pacificus* prevents apoptosis of P(5-8).p and that mutations in *Ppa-lin-39* result in a generation vulvaless phenotype. Double mutants of *lin-39* with the cell death gene *ced-3* indicated that *Ppa lin-39* has an early role in vulva development in *P. pacificus*. It prevents cell death but is dispensable for vulva induction, which is in contrast to *Cel lin-39*.

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

*gev-1* animals are also egg-laying defective, but have a lower penetrance than *ped-12*. Additional pleiotropic defects like uncoordinated movement,distended body, malformed tails are also observed. Characterisation of the third complementation group *gev-2* is in progress.

Another interesting mutant involved in vulval development of *P.pacificus* is *ped-6*. *ped-6* animals are egg-laying defective, with P(3,4).p surviving and ectopically invaginating resulting in a traditional Multivulva phenotype. To check whether *ped-6* and *ped-12* lie in the same pathway, we constructed the double mutant between *ped-12* and *ped-6*. The double mutant was Egg-laying defective with no surviving Pn.p cells indicating that *ped-6* and *ped-12* lie in the same pathway, with *ped-12* being upstream of *ped-6*.

# SYNTHETIC VULVALESS/LETHAL MUTATIONS DEFINE NEW REGULATORS OF RAS SIGNALING

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Vulval fate induction is controlled by several different signaling pathways, including an RTK/Ras/ERK pathway. Some genes that influence vulval development have wild-type or nearly wild-type null phenotypes, and their roles are only revealed in appropriate sensitized genetic backgrounds. For example, mutations in *ksr-1* and *sur-8* do not cause strong phenotypes singly, but do cause highly penetrant Vulvaless and Lethal phenotypes in combination with each other, or in combination with weak alleles of other Ras pathway genes such as *lin-45 raf*. To identify additional genes acting with *ksr-1* or *sur-8* (or in other parallel pathways), we have used both forward and reverse genetic approaches to identify additional synthetic Vul/Let mutations.

In the forward genetic approach, we screened for enhancers of two weak, "phenotypeless" *lin-45 raf* alleles. Because most Ras pathway mutations will cause complete lethality in these *lin-45* backgrounds, this screen has allowed us to avoid most of the usual suspects and instead isolate mutations in more peripheral regulatory genes like *ksr-1, sur-6, eor-1* and *eor-2* (see abstracts by Howard et al. and by Kao and Sundaram). As a reverse genetic approach, we also directly tested candidate genes for synthetic effects using existing mutations or RNAi, and we observed synthetic effects for *mig-2(rh17gf)* and for R01H10.8 (*cnk-1*) RNAi. By testing for synthetic effects of these different mutation/RNAi pairs, we have tentatively placed the various genes into three functional groups that may define different parallel pathways that positively regulate Ras signaling.

The SynVul/Let effects of *mig-2(rh17gf)* are particularly intriguing since *mig-2* encodes a Rac-like GTPase, and Rac has been proposed to be an important target of Ras signaling in mammalian cells. The molecular lesion in *rh17* suggests this is an activating mutation; however, several of the *rh17* mutant phenotypes are also seen in *ced-10/rac-1(lf); mig-2(lf)* double mutants, suggesting that *ced-10* and *mig-2* have overlapping functions and that *rh17* somehow interferes with the functions of both genes.

# THE *CAENORHABDITIS ELEGANS* COG-4 PROTEIN MEDIATES VULVAL CELL MORPHOGENESIS

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In screens for *Caenorhabditis elegans* mutants defective in vulval morphogenesis, we isolated multiple mutants in which the uterus and the vulva fail to make a functional connection, resulting in an egg-laying defective phenotype. Two of these connection of gonad defective (cog) alleles define the gene *cog-4* which is allelic to *egl-26*. In *cog-4/egl-26* mutants, an abnormally thick layer of vulval tissue forms at the apex of the vulva, blocking the exit to the vulva from the uterus. We have determined that the vulval lineages in *cog-4* mutant animals are normal. However, by examining the shape of the vulval cells using a *jam-1::gfp* transgene, we determined that one vulval cell, the most dorsal cell called vul F, adopts an abnormal morphology at its apex.

*cog-4* is predicted to encode a novel protein that has a very interesting expression pattern. A functional, translational fusion protein of COG-4 and GFP becomes localized to the apical edges of cells, and the fusion protein is observed lining the uterine lumen and portions of the vulval lumen. Mosaic analysis indicates that *cog-4* activity is required in the vulva and not in the uterus for proper vul F morphogenesis. And specifically, mosaic analysis indicates that *cog-4* is required in the primary vulval lineage for proper function. vul E and vul F are the only two cells that arise from the primary lineage. Closer analysis of the expression pattern indicates that vul E is the only cell in the primary lineage that expresses *cog-4*. Our working model suggests that vul E acts to instruct morphological changes in vul F in an interaction mediated by COG-4. This model is being tested by cell ablation experiments. Furthermore, we are seeking clues to the function of this novel protein by searching for protein interactors using a yeast two-hybrid screen.

# EXPRESSION OF THE SMALL REGULATORY RNA *LET-7* ACROSS PHYLOGENY

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The heterochronic gene pathway consists of a set of temporally controlled regulatory genes that specify the timing of developmental events in *C. elegans*. Two small regulatory RNAs, *lin-4* and *let-7*, have been proposed to act as negative regulators in this pathway via binding to complementary sites in the 3' UTRs of their regulatory targets. *lin-4* is expressed in late L1 and negatively regulates LIN-14 to allow progression from early to late larval fates, while *let-7* expression begins late in the third larval stage and negatively regulates LIN-41 to allow the progression through later larval fates.

We have found that *let-7* is conserved across a wide range of metazoans. By Northern analysis, we have detected 21 or 22nt RNAs in species from *Drosophila melanogaster* to *Homo sapiens*. We have not detected a *let-7* like message in more distant species such as *S. cerevisiae*, *E. coli*, and *Arabidopsis thaliana*. In contrast, *lin-4* is either not conserved or is poorly conserved in other species, for we have not detected *lin-4* like transcripts.

Profiles of *let-7* expression in *Drosophila* and the zebrafish *Danio rerio* have suggested that developmental regulation of *let-7* is also conserved. For example, expression of *Drosophila let-7* expression begins in the late third instar larva and continues through pupation and adulthood, suggesting that *let-7* may play a similar role in developmental progression in worms and flies. Furthermore, the *let-7* regulatory target LIN-41 is conserved in *Drosophila*, where *lin-41* has as least one *let-7* complementary site in its 3' UTR. Zebrafish *let-7* expression is also temporally regulated: expression was first detected at 48 hours post-fertilization (late embryogenesis, completed rapid morphogenesis of primary organ systems) and is maintained in adults. Our observations suggest that the *let-7* small RNA-based regulation of gene expression is widely conserved in metazoans. We are currently exploring the function of *let-7* in these other species.

# THE *C. ELEGANS LET-7* RNA CONTROLS TEMPORAL PATTERNING BY REGULATING THE TIMING OF EXPRESSION OF THE *LIN-41*RBCC REGULATORY GENE.

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Heterochronic genes regulate the timing of cell fate decisions. In *C. elegans, lin-41* mutant animals precociously express adult characteristics at earlier larval stages, while overexpression of *lin-41* causes some cells to reiterate late larval fates at the adult stage. *lin-41* negatively regulates the expression of *lin-29*, a transcription factor required for adult fates, that appears to be translated at the L4 and adult stages from an mRNA expressed from the L2 stage. In a *lin-41* mutant, LIN-29 protein appears as early as the L2 stage. In the epidermis *lin-41* expression is down-regulated during the L4 stage, which is when expression of *lin-29* protein is observed. Thus LIN-41 may regulate the timing of *lin-29* mRNA translation or protein stability.

*lin-41* is a divergent members of the RBCC (RING finger, B-Box, Coiled coil) family of regulatory proteins, that includes the RNA binding protein SSA/Ro, and the tumor suppressor genes PML, TIF1 and rpt. A fusion of GFP to a functional LIN-41 protein reveals that the protein is predominantly cytoplasmic. Based on precedents from the RBCC gene family, LIN-41 may regulate *lin-29* expression by binding to its mRNA to control its translation, or by binding to the LIN-29 protein to regulate its stability.

We isolated *lin-41* mutations as suppressors of *let-7* mutations. *let-7* encodes a small untranslated RNA that is expressed just prior to the L4 stage and is complementary to two sites in the 3'UTR of *lin-41*. This complementarity and the temporal down-regulation of *lin-41* expression at the time when *let-7* RNA is up-regulated suggests that *let-7* inhibits *lin-41* expression by binding to its 3' UTR. Thus, activation of the *let-7* RNA during the L4 stage may down-regulate LIN-41 expression to then relieve the inhibition of *lin-29*.

The heterochronic genes *let-7, lin-29* and *lin-41* are conserved (and expressed) in *Drosophila* and mammals (also see abstract by Reinhart et al). Moreover, the *Drosophila lin-41* homologue has a *let-7* complementary sequence in its 3'UTR, suggesting that the mechanism for regulating gene expression by small RNAs may be conserved across animal phylogeny.

# G PROTEIN REGULATORS RGS-1 AND RGS-2 ALLOW *C. ELEGANS* TO RAPIDLY ALTER EGG-LAYING BEHAVIOR WHEN RE-FED AFTER STARVATION

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Regulators of G protein signaling (RGS proteins) act as G protein GTPase activators *in vitro*, but little is known about why these G protein inhibitors exist. *C. elegans* egg laying is controlled by antagonistic signaling of the G proteins GOA-1 (Go) and EGL-30 (Gq), which are negatively regulated by the RGS proteins EGL-10 and EAT-16 respectively[1, 2]. In a survey of the functions of all 13 *C. elegans* RGS genes, we found that overexpression of *egl-10*, *rgs-1*, or *rgs-2* mimics the loss-of-function phenotype of *goa-1*. Thus, these three RGS proteins might all regulate the G protein GOA-1. We generated deletion alleles for both *rgs-1* and *rgs-2*; neither single nor double mutants of *rgs-1* and *rgs-2* strongly affect egg laying under constant food conditions. However, *rgs-1; rgs-2* double mutants fail to rapidly induce egg laying when re-fed after egg laying has been halted by starvation. This defect is not seen in either *egl-10* or *eat-16* RGS mutants. Although *egl-10* and *eat-16* mutants exhibit abnormal egg-laying behavior, they still rapidly adjust this behavior according to food availability. This suggests that EGL-10 and EAT-16 may set baseline levels of G protein signaling, while RGS-1 and RGS-2 redundantly alter signaling after re-feeding to cause appropriate behavioral changes.

Several lines of evidence suggest that RGS-1 and RGS-2 induce behavioral change by negatively regulating GOA-1. First, *goa-1* null mutants completely fail to alter egg-laying behavior when food conditions are changed, indicating that GOA-1 is a key component in directing behavioral changes. Second, overexpression of *rgs-1* or *rgs-2* mimics, to different degrees, the *goa-1* loss-of-function phenotype. Third, some GOA-1 expressing neurons also express RGS-1 and RGS-2. Lastly, purified RGS-1 and RGS-2 proteins both stimulate the GTPase activity of purified GOA-1. We propose that *C. elegans* utilizes three RGS proteins to regulate different aspects of GOA-1 signaling: EGL-10 sets the baseline signaling level, while RGS-1 and RGS-2 redundantly inhibit GOA-1 to rapidly induce egg-laying behavior in response to feeding after food deprivation.

1. Koelle, M.R. and Horvitz, H.R. (1996) *Cell* 84, 115-125.
2. Hajdu-Cronin YM, Chen WJ, Patikoglou G, Koelle MR, Sternberg PW. (1999) *Genes Dev.* 13, 1780-93.

# *DAF-5* CLONING IDENTIFIES A SNO ONCOGENE HOMOLOG AS A TGF-BETA SIGNALING OUTPUT

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Rutgers U

The reproductive/dauer developmental decision is controlled by environmental cues, such as food, pheromone and temperature. A TGF-beta-related pathway promotes reproductive over dauer development. Mutations in *daf-7* (a TGF-beta-like ligand), *daf-1*and *daf-4* (type I and type II receptor kinases), and *daf-8* and *daf-14* (Smad transcription factors) induce dauer at restrictive temperature regardless of environmental cues. The Daf-c phenotype of these mutants is suppressed by mutations in *daf-3* (a Smad) or *daf-5*. Epistasis analysis suggests that *daf-5* and *daf-3* are either antagonized by this TGF-beta-related pathway or acting in a parallel pathway. The similarity of the dauer pathway to other TGF-beta pathways suggested that DAF-5 may represent a new molecule functioning in a variety of TGF-beta pathways. We have cloned *daf-5* so as to study it at a molecular level.

We show that *daf-5* encodes a transcription factor homologous to the human oncogene Sno. Mapping and cosmid rescue placed *daf-5* in a 8 kb interval of chromosome II. We PCRed a 3.9 kb region of W01G7 from fifteen *daf-5* alleles and digested the products with BstUI; we detected a polymorphism in *daf-5* (*sa211*). This region of W01G7 encodes a Sno homologue. Sequencing of *daf-5* alleles is in progess. Recently, vertebrate Sno has been shown to be capable of acting as an inhibitor of TGF-beta pathways that functions by interacting with Smad proteins. The genetic function of *daf-5* suggests that the function of Sno homologues in TGF-beta pathways is broader than suggested by the vertebrate tissue culture studies, in that *daf-5* is not simply acting to block signaling by the receptors.

In addition to function in the TGF-beta-related pathway, *daf-5* has been shown by various groups to genetically interact with other dauer genes: *daf-11* (encoding guanyl cyclase), *daf-21* (encoding Hsp90), *daf-2* (encoding an insulin-like ligand), and *daf-28*. Moreover, some *daf-5* alleles have a longer life span than N2, implying *daf-5* regulates life span as well as dauer formation. One allele of *daf-5* does not affect life span independently; however, it further extends the life span of long-lived *daf-2* mutants. These results indicate that *daf-5* may control aging in parallel to the *daf-2* pathway. We hope to identify the role of *daf-5* in integrating information from different pathways that control dauer and other developmental events.

# CLONING AND CHARACTERIZATION OF *LON-1*, A GENE DOWNSTREAM OF TGFB SIGNALING INVOLVED IN BODY SIZE REGULATION

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*C. elegans* TGFb-like signaling ligands affect body size morphogenesis and male tail patterning (*dbl-1*), dauer formation (*daf-7*) and neuronal migration (*unc-129*). Interestingly, two of these ligands, *dbl-1* and *daf-7*, share a common type II serine/threonine kinase receptor, *daf-4*, that interacts with either, *sma-6* or *daf-1*, type I serine/threonine kinase receptors, to transmit the body size and male tail (Sma/Mab) or dauer signals respectively. Each pathway signals to specific sets of downstream mediators, identified as Smads, which then regulate transcription of target genes. In *C. elegans*, however, very few target genes have been identified. Our focus is on the Sma/Mab pathway with an interest in isolating and characterizing target genes regulated by this signaling process. Therefore, we examined existing mutants displaying body size phenotypes that might be associated with the Sma/Mab pathway.

One of the mutants analyzed, *lon-1*, is 1.5 times greater in length than wild type animals and displays a phenotype opposite to that seen in any of the existing Sma/Mab pathway mutants. We have cloned *lon-1* and find that it encodes a novel secreted protein containing a GPI-linkage site. Our results suggest that *lon-1* is epistatic to all existing pathway components. To test whether *lon-1* is in fact regulated by the Sma/Mab pathway, we are conducting two primary experiments. First, we are analyzing *lon-1::gfp* expression patterns in *sma* mutant backgrounds. We find that *lon-1::gfp* expression is observed in overlapping regions where *sma-2* and *sma-4* are expressed as well, therefore, localizing *lon-1* in tissues where Sma/Mab pathway components are present. Secondly, we are determining whether any of the Smads, *sma-2*, *-3* and/or *--4*, are capable of binding regions with in the *lon-1* promoter. In addition to implicating *lon-1* as a Sma/Mab regulated gene, we are also interested in determining how *lon-1* functions in regulating body size morphogenesis. To further aid in identifying the genes that might function with *lon-1*, we are carrying out a genetic screen to isolate mutations that suppress the *lon-1* phenotype.

# *SMA-9* AND TGFB SIGNALING

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A TGFb -related signaling pathway regulates body size and male tail morphogenesis in *C. elegans*. Mutations in *dbl-1* ligand, *sma-6* type I receptor, *daf-4* type II receptor, and *sma-2, sma-3*, and *sma-4* Smads result in similar defects. Mutants hatch at about the same size as wild type, but grow more slowly and are half the normal size at adulthood. In the male tail, defects in morphogenesis and patterning result in crumpled spicules and sensory ray fusions. In addition, mutants show less frequent expression of dopamine in sensory neurons in rays 5, 7, and 9, the same rays that are involved in ray fusions and changes in morphology. In a genetic screen for additional Small mutants (C.S.D. and R.W.Padgett, unpublished), four alleles of a novel gene *sma-9* were isolated. *sma-9* mutants have defects in all of the tissues described above, but in each case, the *sma-9* phenotype differs slightly from that of the other mutants. In body size, L1 and L2 *sma-9* animals have the same size and growth rate as TGFb Sma mutants, but after this time, their growth rate increases to a wild-type rate. In the male tail, *sma-9* animals have crumpled spicules, but at a lower penetrance. Sensory ray fusions are seen between rays 8 and 9, but never between rays 4 and 5 or rays 6 and 7. Four *sma-9* alleles from a noncomplementation screen show the same ray and spicule phenotypes. Finally, the frequency of dopamine expression in neurons of rays 5, 7, and 9 is reduced, but unlike in the TGFb Sma mutants, the levels of expression are also reduced in neurons that still express the neurotransmitter. Based on the phenotypic analysis, we suggest that *sma-9* is a co-factor or modulator of the TGFb pathway. To understand better how *sma-9* interacts with the signaling pathway, we are analyzing double mutants between *sma-9* and TGFb Sma mutants. Surprisingly, the phenotype of *sma-3;sma-9* doubles is less severe than that of *sma-3* alone. We have also initiated a molecular analysis of *sma-9*. After mapping *sma-9* X, we obtained transformation rescue with the cosmid C44C10. Progress in identifying the *sma-9* open reading frame will be presented.

# TOWARD STEROID ENDOCRINOLOGY: CHOLESTEROL ACCUMULATION IN C. ELEGANS

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*C. elegans* requires cholesterol for growth. In the absence of cholesterol, growth slows by day 3 after hatching and the number of eggs laid is decreased. In the second generation of growth in the absence of cholesterol, growth is severely retarded from the start and ceases by day 3 after hatching, with the animals remaining in an intermediate larval stage. Cholesterol undergoes extensive enzymatic modification in *C. elegans* to form other sterols of unknown function[1]. We postulate that these or related sterols function as classical steroid hormones, which activate nuclear hormone receptors. Using cholesterol-specific staining with filipin, we observed that cholesterol accumulates only in five cells in both larval and adult hermaphrodites. These include two amphid socket cells, two phasmid socket cells and the excretory gland cell. The latter has connections with both the excretory canal (which extends nearly the entire length of the animal) and with the pharyngeal nerve ring. Male animals showed a different pattern of accumulation in the tail region. Two laterally symmetric posterior rays of the male tail were specifically stained, arising from the same cell lineages that form the phasmid socket cells in the hermaphrodite. Staining was also observed in the pharynx and intestine, decreasing continuously from the intestinal valve distally to the anus. Intestinal staining disappeared after a 4h chase on cholesterol deficient medium, with no change or an increase in the staining intensity of the five cholesterol accumulating cells, suggesting that cholesterol uptake occurs from the intestine. Evidence will be presented to show that the sterol requirement for growth is stereospecific, requiring a free 3b -OH group, and that the functional sterol pathway is sensitive to some drugs that disrupt steroid hormone pathways.

[1]Chitwood, D.J. 1999. Crit. Rev. Biochem. Mol. Biol. 34: 273-284.

# *RME-1*, A NEW ENDOCYTOSIS GENE IMPLICATED IN RECYCLING

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Endocytosis is essential to many aspects of metazoan life, including nutrient uptake, synaptic vesicle recycling, and growth factor receptor down-regulation. Much remains to be learned about this important cellular pathway.

We have isolated a collection of endocytosis defective mutants, the *rme* genes. Recently, we focused on the analysis of *rme-1,* a gene that may regulate endocytic recycling. *rme-1* mutants are defective in endocytosis in multiple cell types including oocytes and coelomocytes. *rme-1* mutants also develop large vacuoles in the intestine and hypodermis. The intestinal vacuoles accumulate endocytosis markers, but only from their basolateral (pseudocoelomic) surfaces. Specifically, we found that fluorescent dextran microinjected into the pseudocoelom rapidly accumulates within *rme-1* mutant vacuoles. Another endocytosis marker microinjected into the pseudocoelom, the membrane dye FM4-64, is excluded from *rme-1* mutant vacuoles and instead accumulates in autofluorescent gut granules (lysosomes). Because fluid-phase markers like dextran can enter the recycling pathway, and FM4-64 does not, *rme-1* mutant vacuoles may represent aberrant recycling endosomes.

We found that *rme-1* encodes a member of a new family of proteins bearing a C-terminal EH, or eps15 homology, domain. The EH domain has been found at the N-terminus of a small number of proteins, each of which has been implicated in endocytosis. RME-1 also contains a predicted P-loop sequence associated with ATP or GTP binding in many proteins, as well as a predicted coiled-coil domain.

Homologs of RME-1 are found in the human and fly genome databases, but have not been studied functionally. No homologs of RME-1 are found in the complete yeast genome sequence. Sequencing of *rme-1* cDNAs and 5' RACE analysis identified four different *rme-1* mRNAs differing at their 5' ends, encoding four predicted RME-1 isoforms. Sequencing of our *rme-1* mutant alleles revealed mutations in highly conserved residues of the RME-1 region shared by all isoforms. Immunofluorescence with anti-RME-1 Abs shows that RME-1 is a cytoplasmic protein expressed in all cells, and that it is associated with the periphery of basolateral endosomes in the wild-type intestine.

# *RME-8* IS AN ESSENTIAL GENE REQUIRED FOR ENDOCYTOSIS

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Endocytosis is required in cells for diverse functions such as nutrient uptake, synaptic vesicle recycling, plasma membrane recycling and receptor internalization. We have isolated a number of *C. elegans* endocytic mutants using a vitellogenin::GFP assay (Grant and Hirsh, 1999). Here we describe the molecular analysis and phenotypic characterization of *rme-8* (*r*eceptor-*m*ediated *e*ndocytosis mutant 8).

*rme-8* encodes a predicted protein containing 2279 amino acids with a central DnaJ domain. DnaJ domains are known to stimulate the ATPase activity of chaperone proteins of the hsc70 family. Together they function to modulate the folding and unfolding of proteins. *rme- 8* homologues are found in humans, Drosophila and Arabidopsis, but not in yeast. This sequence conservation suggests RME-8 may define a conserved step in endocytosis in multicellular organisms.

*rme-8* is required for endocytosis in most worm cells. *rme-8(b1023*) is defective in both receptor-mediated endocytosis in oocytes and fluid-phase endocytosis in coelomocytes. This allele is also temperature sensitive for lethality, reflecting a broad requirement for *rme-8* activity in other cells. Temperature-shift experiments indicate a requirement for *rme-8* activity during all developmental stages.

Consistent with its role in endocytosis, RME-8 is localized to intracellular endocytic structures. Using a reporter consisting of an RME-8::GFP chimera that rescues the *rme-8* mutant, we found that *rme-8* is expressed in many cells. RME-8::GFP localizes to small intracellular punctate structures, except in coelomocytes, where it localizes to the periphery of large vesicles beneath the plasma membrane. These large RME-8::GFP vesicles are pre-lysosomal endosomes. By antibody staining, we found that RME-8 localizes to cortical punctate structures in the oocyte which are not early endosomes.

In summary, we have identified a new conserved endocytic gene *rme-8*. We showed that *rme-8* is essential and is required for endocytosis in many cells. We propose that *rme-8* functions in an intermediate intracellular trafficking step prior to lysosome formation.

# POSTEMBRYONIC MUSCLE PATTERNING

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We are interested in understanding the genetic and molecular mechanisms that execute muscle patterning programs. The worm has a variety of muscle types that arise through a combination of lineage-specific programs and cell-cell interactions. While most of the muscle cells are born embryonically, a subset of muscles (14 striated muscles, eight uterine muscles and eight vulval muscles) arise postembryonically. All of these are derived from the lone postembryonic mesodermal blast cell, M. Analysis of mutations affecting some of the known developmental control genes has provided an intriguing but still fragmentary picture of pattern regulation in this lineage.[1,2,3]

Using *green fluorescent protein* (*gfp*) reporters that are active in cells derived from M we have screened for new mutants with altered muscle lineages. Our initial analysis has focused on a distinct and easily-scored cell fate decision: the distinction between uterine and vulval muscle. Three mutations that transform presumptive uterine to vulval muscles were isolated from the screen. The mutations are allelic and define a gene (designated *mls-1*) required for this decision. We found that this gene encodes a member of the T-Box family of transcription factors. Currently efforts are directed toward testing interactions between *mls-1* and other regulatory pathways thought to be active within the lineage. Surprisingly, we found that mutations in several of the *mog* genes result in a similar sex muscle transformation. We are interested in determining if the *mog* genes interact with a tissue-specific regulatory site downstream of the *mls-1* coding region.

[1] Corsi, A. et al., Development, in press.

[2] Harfe, B. et al., Development 125: 421-429 1998.

[3] Greenwald, I. et al., Cell 34: 435-444 1983.

# LAD-1, A HOMOLOGUE OF VERTEBRATE L1CAMS, IS A GENERAL RECEPTOR FOR UNC-44 AT SITES OF CELL-CELL CONTACT THROUGHOUT DEVELOPMENT, AND PARTICIPATES IN A NOVEL ANKYRIN-INDEPENDENT SIGNALING PATHWAY

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The L1 family of neural cell adhesion molecules (L1, NrCAM, NgCAM, neurofascin, and CHL1 in vertebrates, and neuroglian in *Drosophila*) are thought to function in axon outgrowth, guidance, and fasciculation[1]. Consistent with this hypothesis, mutations in human L1 result in mental retardation[1].

We have identified the *C. elegans* L1 family homologue, LAD-1 (**L**1-like **AD**hesion). As with the L1 family members[1], LAD-1 has the conserved structure of six extracellular Ig domains and five fibronectin III domains, a transmembrane domain, and a cytoplasmic tail containing a conserved motif required for binding to ankyrin, a cytoplasmic protein that links several transmembrane proteins to the cytoskeleton. Interestingly, the *C. elegans* ankyrin homologue is UNC-44, a protein shown to function in axon guidance[2].

We show that LAD-1 does indeed bind and recruit ankyrin to the plasma membrane. Moreover, immuofluorescence studies indicate that LAD-1 and UNC-44 (UNC-44 antisera is a gift from A. Otsuka) have similar expression patterns; *ie* both are expressed in the plasma membrane at sites of cell-cell contact in almost all cells. Thus LAD-1, unlike its vertebrate homologues, has nonneuronal functions in addition to neuronal roles. Moreover, the colocalization of ankyrin and LAD-1, together with the ankyrin-LAD-1 interaction, identify LAD-1 as the general receptor for UNC-44.

The ankyrin-binding motif in LAD-1 contains a conserved tyrosine residue, which when phosphorylated, abolishes ankyrin binding[3]. To understand the significance of this phosphorylation, we determined the expression pattern of phosphorylated LAD-1 (LAD-1P) via immunofluorescence studies. We show that LAD-1P is expressed in polarized epithelial tissues, with its localization complementary to that of non-phosphorylated LAD-1. LAD-1P is also localized in regularly-spaced puncta along axons. This expression pattern indicates that LAD-1 participates in a novel signaling pathway in addition to its structural role.

[1] Brummendorf, T. et al. 1998. *Curr. Op in Neurobiol* 8:87-97

[2] Otsuka, A. et al. 1995. *J. Cell Biol*. 129:1081-1092

[3] Garver, T.D. et al. 1997. *J. Cell Biol*. 138:703-714

# MUP-4 IS A NOVEL MATRIX RECEPTOR WITH ESSENTIAL FUNCTIONS IN EPITHELIAL CELL ADHESION, MORPHOGENESIS AND MAINTENANCE OF MUSCLE POSITION.

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*mup-4* is essential for maintenance of *mu*scle *p*osition and for epithelial (hypodermal) cell organization during *C. elegans* embryonic development (Gatewood, B.K. and Bucher, E.A. *Genetics*. 146:165-183). We now report that MUP-4 is a large transmembrane protein comprised of an extracellular domain with twenty-eight EGF-like repeats, a single von Willebrand Factor A module and two SEA modules. The intracellular domain has homology to a class of intermediate filament (IF) associated proteins, the filaggrins, which are known to regulate IF compaction in vertebrate epithelium, but which have not previously been identified as a component of junctional complexes. MUP-4 colocalizes with IF structures, consistent with a possible role in IF regulation and/or association in response to extracellular signals. Ultrastructural studies demonstrate a lesion in *mup-4* mutants at the apical, cuticular surface of the hypodermal epithelium. We propose that MUP-4 functions as an ECM receptor that regulates and maintains hemidesmosome-like structures, some of which function to transduce muscle force to the exoskeletal cuticle. Additional expression and phenotypic data support other roles for this novel matrix receptor in postembryonic muscle development and in epithelial morphogenesis.

# THE *UNC-78* GENE ENCODES A HOMOLOG OF ACTIN-INTERACTING PROTEIN 1 (AIP1) AND IS INVOLVED IN ACTIN FILAMENT ASSEMBLY IN BODY WALL MUSCLE

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Mutations in the *unc-78* gene result in slow-moving worms that have large aggregates of thin filaments at the ends of spindle-shaped body wall muscle cells. Therefore, the *unc-78* gene has been implicated in proper organization of actin filaments into myofibrils. In addition, *unc-78* mutations affect total integrity of the myofilament lattice (Welborne et al., 1997 Int. Worm Meeting). We characterized phenotypes of homozygous *unc-78* mutants. By observing their motility, *unc-78(su152)* was very slow, whereas *unc-78(e1217)* and *unc-78(su135)* were only slightly slower than wild-type and *unc-78(e1221)* and *unc-78(su187)* moved comparable to wild-type. Phalloidin-staining of whole worms revealed that large aggregates of actin filaments were formed in almost all body wall muscle cells of *unc-78(e1217)*. In contrast, *unc-78(e1221)* had well-organized actin filaments as well as relatively smaller actin aggregates only in a subset of body wall muscle cells. In addition, we noted that actin filaments in L1 to L3 larvae appeared normal even in severe *unc-78* mutants, suggesting that *unc-78* is involved in growth and/or maintenance of the myofibrils. We identified sequence alterations in a gene, C04F6.4, from four *unc-78* alleles. C04F6.4 has been predicted to encode a protein of 65 kDa that contains 9 WD-repeats. All four alleles are missense mutations at conserved residues within WD-repeats. The mutation sites are located in the forth repeat for *unc-78(su135)*, the fifth repeat for *unc-78(e1217)* and the eighth repeat for *unc-78(e1221)* and *unc-78(su187)* (same mutations). The predicted C04F6.4 protein is homologous to actin-interacting protein 1 (AIP1). AIP1 has been identified in yeast, slime molds and vertebrates and shown to be involved in dynamic reorganization of actin cytoskeleton. Moreover, AIP1 has been demonstrated to interact with ADF/cofilin and potentiate its actin-depolymerizing activity. Likewise, preliminary results showed that UNC-60B, a worm ADF/cofilin isoform, was mislocalized to the actin aggregates in *unc-78* mutants. Thus, UNC-78 is a likely regulator of actin filament dynamics in body wall muscle.

# PDZ CONTAINING PROTEIN KINASES ARE IMPLICATED IN CONTRACTILE FUNCTIONS.

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PKL1 and PKL2 are large S/T protein kinases (PKs) encoded by *kin-4*. PKLs and mouse MAST205 have 70% identical catalytic domains and share a conserved PDZ domain. However, sequences of N and C terminal regions of the *C. elegans* and mouse kinases diverge. Immunostaining of *C. elegans* revealed that PKL1 is aligned along filaments of body-wall muscle, adjacent to actin. PKL2 is enriched in pharyngeal muscle (adjacent to actin) and the actin rich "midbody" in embryos. Both PKLs associate with punctuate structures in many other cell types. *kin-4* promoter activity is evident in cells identified by immunostaining.

Several approaches were used to assess PKL functions. PDZ-containing *C. elegans* syntrophins were considered as PKL binding targets. However, syntrophins were poor ligands for PKL *in vitro*. $^{32}$P-labeled PDZ domains from MAST205 and PKL avidly bound mouse skeletal muscle b -tropomyosin (TM) and retrieved b -TM cDNAs from expression libraries. Thus, the relationship between PKL and *C. elegans* TMs (TMI and TMIII) was probed. PKL binds and phosphorylates CeTMI and CeTMIII. Mouse TMs are phosphorylated by an unknown kinase *in vivo* and this modification is thought to regulate myofibril assembly and function. PKLs may be targeted to actin filaments in body wall muscle via interaction with TM. Anchored PKL may phosphorylate TM and/or associated troponin subunits, thereby modulating contractile physiology.

Transgenic worms that express anti-sense PKL RNA (under control of the myo-3 promoter) were generated to probe PKL function in body wall muscle. Late larval transgenic worms move slowly and L1 larvae are paralyzed. Western analysis demonstrated that PKL1 protein content declined by >90% in transgenic L3 larvae. Thus a PKL deficit is linked to contractile dysfunction. Expression of anti-sense PKL RNA in pharynx elicits delayed larval development. This may be due to a defect in pumping of the pharynx that prevents ingestion of food. Injection of PKL antisense oligonucleotides resulted in embryos containing binucleate cells, suggesting a failure in cytokinesis. Embryonic lethality ensued. These studies suggest that PKL is involved in regulating aspects of the assembly and/or contractile functions of a key physiological structure- the actomyosin filament.

# A CALCIUM-BINDING PROTEIN OF SR (SARCOPLASMIC RETICULUM), CALSEQUESTRIN, IS NOT ESSENTIAL FOR BODY-WALL MUSCLE FUNCTION IN *C. ELEGANS*

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Calsequestrin is the major calcium-binding protein of cardiac and skeletal muscles whose function is to sequester $Ca^{2+}$ in the lumen of the sarcoplasmic reticulum (SR). Here we describe the identification and functional characterization of a *C. elegans* calsequestrin gene (*csq-1*). CSQ-1 shows high similarity (50% similarity, 30% identity) to rabbit skeletal calsequestrin. Unlike mammals, which have two isoforms of calsequestrin, the cardiac and fast-twitch skeletal muscle forms encoded by two different genes, CSQ-1 is the only calsequestrin in the *C. elegans* genome. We show that *csq-1* is highly expressed in body-wall, pharyngeal and vulval muscle cells beginning during mid-embryogenesis and maintained through the adult stage. In body-wall muscle cells, CSQ-1 is localized to sarcoplasmic membranes surrounding sarcomeric structures, in the regions where ryanodine receptors (UNC-68) are located. Mutation in UNC-68 affects CSQ-1 localization, suggesting that the two interact in vivo. Genetic analyses of chromosomal deficiency mutants deleting *csq-1* show that, CSQ-1 is not essential for initiation of embryonic muscle formation and contraction. Furthermore, double-stranded RNA injection resulted in animals completely lacking CSQ-1 in body-wall muscles with no observable defects in locomotion. These data indicate that although CSQ-1 is the major calcium-binding protein interacting with UNC-68 in *C. elegans*, it is not required for body-wall muscle formation and contraction.

# THE EXTRACELLULAR DOMAIN OF THE *C. ELEGANS* AMYLOID PRECURSOR-RELATED PROTEIN IS ESSENTIAL FOR VIABILITY

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Alzheimer's disease is a neurodegenerative disorder characterized by the accumulation of dense extracellular plaques in the brain. The major component of these plaques is the b-amyloid peptide, which is a cleavage product of the Amyloid Protein Precursor (APP). The early-onset form of Alzheimer's Disease has been linked to mutations in several genes, including *APP*. The function of APP remains unclear. There is a large family of APP-related proteins, suggesting that their function is conserved throughout evolution. These proteins are putative single pass transmembrane proteins that contain two highly conserved regions, E1 and E2, in the extracellular domain. We are studying the function(s) of the *APP*-related gene, *apl-1*, in the nematode *Caenorhabditis elegans*. APL-1 shares many similarities with the APP family of proteins, although it does not contain the b-amyloid peptide. *apl-1* is expressed in over 50 cells throughout the animal.

To analyze the function of APL-1, we have isolated three *apl-1* alleles, *yn10*, *yn23*, and *yn5*. Western blot analysis indicates that *yn10* and *yn23* animals do not produce an APL-1 protein, suggesting that these alleles represent complete loss-of-function alleles. Homozygous *yn10* and *yn23* animals have a larval lethal phenotype. The lethality is rescued by expression of APL-1 in neurons. The extracellular domain of APL-1 appears to be essential for viability since expression of only the extracellular domain of APL-1 is sufficient to rescue the lethality. We are analyzing which regions of the extracellular domain are essential for APL-1 function. The E2 region of the extracellular domain does not appear to be important since microinjection of a construct encoding the APL-1 protein with a deletion of the E2 region rescues the lethality.

By western blot analysis, *yn5* animals produce a truncated APL-1 protein that presumably corresponds to only the extracellular domain of APL-1. *yn5* animals show several phenotypes that appear to be due to an increased production of the extracellular domain of APL-1. These results suggest that the extracellular domain of APL-1 is involved in multiple behaviors.

# ANALYSIS OF *C. ELEGANS NLP* GENES REVEALS NEW NEUROPEPTIDE FAMILIES

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Neuropeptide neurotransmitters play critical roles in simple and complex behaviors of many organisms. Neuropeptides are translated as preproproteins with a signal sequence and with endopeptidase sites flanking the neuropeptides. Multiple copies of related neuropeptides are found within the previously characterized *C. elegans FMRFamide-like protein-1* (*flp-1*) gene and most other invertebrate neuropeptide preproproteins.

To identify additional *C. elegans* neuropeptides we searched for predicted genes encoding preproproteins. We identified 20 of the 22 *C. elegans flp* genes and found 32 new *C. elegans neuropeptide-like protein* (*nlp*) genes. Many *nlp* genes contain novel putative neuropeptides with no homology to previously characterized neuropeptides; none contain the C-terminal R-F-G motif of the *flp* family. We have tentatively grouped 24 of the 32 *nlp* genes into 7 new families of neuropeptides based on varied similarities at the amino acid level. Each of these families includes at least one homolog or EST from another species. The remaining 8 *nlp* genes encode novel putative neuropeptides with no homologs in *C. elegans* nor in other species.

To determine cellular expression patterns of *nlp* genes, we have characterized 21 *nlp* GFP reporter constructs to date. Twenty of the 21 *nlp* gene reporter constructs are expressed primarily or exclusively in neurons. These predominantly neuronal expression patterns further suggest that *nlp* genes encode neuropeptides. Some *nlp* reporter constructs are expressed in neurons that play specific roles in well-characterized *C. elegans* behaviors: 5 *nlp* genes are expressed in ventral nerve cord neurons, 7 in the pharyngeal nervous system and 2 in the ASH sensory neurons. (The latter are of particular interest to our laboratory.)

Analysis of *C. elegans nlp* genes provides the opportunity to address neuropeptide function using genetic and molecular tools and will allow us to test the role of these neuropeptides in the behavior and development of a simple nervous system.

# ELONGATION FACTOR-2 KINASE AFFECTS *CAENORHABDITIS ELEGANS* LIFE SPAN BY REGULATING PROTEIN TURNOVER

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**Accumulation of damaged proteins is postulated to be a major contributor to senescent decline. Decreases in both protein synthesis and degradation rates may result in the persistence of defective or modified proteins and thus the overall rate of protein turnover could affect the rate of aging. We characterized a *Caenorhabditis elegans* mutation disrupting the *efk-1* gene which encodes elongation factor-2 (eEF-2) kinase, an enzyme that specifically phosphorylates eEF-2, inhibiting the elongation step of protein synthesis. We find that loss of eEF-2 kinase activity increases protein synthesis and degradation rates and extends lifespan. Conversely, over-expression of eEF-2 kinase in transgenic nematodes shortens lifespan. *efk-1* mutants appear nearly normal in morphology, co-ordination and fertility but exhibit slowed development and altered rates of several rhythmic behaviors, reminiscent of the *clk* lifespan extension mutants. Interestingly, double mutants of *efk-1* and *age-1* or *clk-1* mutants live no longer than either single mutant. This suggests both *age-1* and *clk-1* mutants may affect lifespan, at least in part, by modulating protein turnover. Strikingly, eEF-2 kinase activity is also decreased in starved nematodes, suggesting that up-regulation of protein synthesis via decreased eEF-2 kinase activity could contribute to the extension of lifespan by caloric restriction. Our data provide the first direct evidence that an increase in protein turnover can extend lifespan.**

# A WEE-1 KINASE HOMOLOG IS REQUIRED FOR MEIOSIS DURING SPERMATOGENESIS *IN C. ELEGANS*

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The switch from mitosis to meiosis is a critical decision in the life of all metazoan germ cells. Once this commitment to meiosis is made, the sperm and oocyte must coordinate differentiation with the meiotic cell cycle. While examining spermatogenesis defective (*spe*) mutants in the nematode *C. elegans*, we discovered two dominant mutants that show major defects in sperm proliferation. Light and electron microscopic examination reveal that both mutants show similar aspects of cellular differentiation in the absence of either cell division or successful meiosis. Both dominant mutants affect sperm, but do not affect oocytes or any somatic cells.

Using these dominant alleles, one can select for a second mutation within *spe-37* (*gf*) that allows production of functional sperm by *spe-37* (*gf*) / wild type heterozygotes. Several of these "second site" *spe-37* mutants have been selected and all have recessive phenotypes. Genetic analysis of these recessive mutants uncovers a role for this gene in early embryogenesis and germline proliferation, in addition to its role in sperm as revealed by the dominant mutants). These recessive mutants allow utilization of positional cloning techniques, which reveal that *spe-37* encodes the metazoan specific wee-1 homolog Myt1. Many higher eukaryotes, including humans, have a soluble Wee-1p as well as Myt1, which is a single pass integral membrane protein that localizes to the endoplasmic reticulum and Golgi. The kinase region of Myt1 is presumed to face the cytoplasm while the C terminal tail lies within these organelles. The C terminal region is known to be essential for proper Myt1 function, but prior work has not revealed its role. Two independently derived dominant *spe-37*/Myt1 mutants have an identical missense mutation in this C-terminal region, so they should provide crucial insight into the function of this domain. We have initiate a large scale mutant hunt to recover new dominant and recessive *spe-37*/Myt1 mutants and to identify other genes in the pathway in which Myt1 functions. This is the first time that it has been possible to combine cell biological and genetic techniques to study this important metazoan kinase. It should be possible to determine the genetic pathway that controls exit from mitosis and entry into meiosis through study of *C. elegans* Myt1.

# ABSENCE OF CUL-4 LEADS TO ENDOREPLICATION

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CUL-4 is a member of the cullin family. Cullins are components of Cullin-Ring finger ubiquitin ligases (E3s), which determine target specificity for ubiquitin-mediated degradation. The functions of several cullins have been characterized to be essential cell cycle regulators. Previously, our lab reported that in *C. elegans, cul-1* is required for cell cycle exit, while *cul-2* is required for the G1-to-S phase transition and chromosome condensation. However, the function of one cullin, CUL-4, is completely unknown. Its human ortholog, CUL-4A, is amplified and overexpressed in breast cancer cells (Chen et al 1998), suggesting a possible role of CUL-4A in cancer progression. Here we report that, like *cul-1* and *cul-2*, *C. elegans cul-4* also functions as a cell cycle regulator.

In order to study the function of *cul-4*, we performed RNAi. We observed 10-50% embryonic lethality in the F1 progeny, with the remainder arresting development as L2 larvae. Huge seam nuclei were observed in these arrested larvae. DNA quantitaion revealed that the DNA content in these nuclei varied from 8N to 42N. Cell lineage studies revealed that no mitoses occurred during the seam cell lineages, although nuclear volume increased. This indicates that these cells were undergoing endoreplication (in which cells bypass mitosis and rereplicate their DNA). A similar phenotype of cell cycle arrest with increased DNA content was observed, at a lower percentage, in somatic gonadal cells and P cells. None of these cells normally endoreplicate. Our results suggest that *cul-4* is required to keep somatic cells from undergoing endoreplication. Finally, in a percentage of the L2 arrested animals, germ cells differentiated into sperm. The mechanism of this developmentally precocious differentiation is unclear.

# REGULATION OF PROGRESSION THROUGH G1 PHASE IN *C. ELEGANS*

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Cell division must be coordinated with growth and differentiation during the development of multicellular organisms. This coordination likely involves transduction of extracellular signals to the cell-cycle machinery. Although several signal transduction pathways have been implicated in cell-cycle regulation, the molecular mechanisms involved remain largely unknown.

We performed a screen to identify regulators of cell-cycle entry in *C. elegans*. To select mutants that arrest cell division in G1 phase, we used a transgene that expresses GFP under the control of the ribonucleotide reductase promoter (*rnr::GFP,* gift from R. Roy and V. Ambros) as a marker for S-phase.

Six strains were identified that showed arrest of cell division and absence of *rnr::GFP* expression. DNA staining of mutants of each strain confirmed that cells failed to undergo DNA replication. Two non-complementing mutations mapped close to *cyd-1,* the *C. elegans* homolog of cyclin D. A truncating mutation in the *cyd-1* gene was found in one of these mutant strains. The remaining four mutations all mapped to chromosome X. The phenotype caused by three of these mutations was rescued by expression of wild-type *cdk-4* (construct a gift from M. Park and M. Krause), and mutations were found in the *cdk-4* gene in these three strains. We are currently examining whether or not the fourth mutation is also an allele of *cdk-4*.

The isolation of a *cyd-1* mutant allele allowed us to address the importance of cyclin D in regulating cell division through inactivation of retinoblastoma (Rb) protein family members *in vivo*. If the main function of cyclin D *in vivo* is to inactivate pRb family members, the cell-cycle arrest caused by a *cyd-1* mutation should be rescued by loss-of-function of these proteins. To test this hypothesis, we created a double mutant strain carrying mutant alleles of *cyd-1* and *lin-35*, the only identified *C. elegans* gene that encodes a pRb related protein.

Our preliminary results showed that cells are able to go through S-phase in *lin-35; cyd-1* double mutants. This indicates that *lin-35* is an important negative regulator of the G1/S transition and a major target of *cyd-1*. We will present a detailed analysis on the roles of *cyd-1* and *lin-35* in G1 progression.

# REGULATION OF MICROTUBULE DYNAMICS DURING MITOSIS: IDENTIFYING AIR KINASE SUBSTRATES IN *C. ELEGANS.*

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Eukaryotic cells have evolved an elaborate cellular machinery, the mitotic spindle, which ensures the orderly placement of one copy of each chromosome into each daughter cell during cell division. Malfunctioning of the mitotic spindle can cause defects in both chromosome segregation and development of the cleavage furrow during cytokinesis. Both of these defects can produce cells with abnormal chromosomal complements, a condition often seen in cancerous cells. We are therefore interested in elucidating the regulatory pathways that control the multiple functions of the mitotic spindle. Hopefully, this analysis will aid in understanding how errors in mitosis lead to disease. We have previously identified two novel *C. elegans* members of the Aurora/Ipl1 kinase family, AIR-1 and AIR-2. Each of these proteins is dynamically associated with the microtubules that affect chromosome movement and cytokinesis during the cell cycle. *C. elegans* embryos in which either AIR-1 or AIR-2 expression is abolished by RNA interference (RNAi) display defects in mitotic spindle formation and cytokinesis respectively. Our current goals are to determine how these two highly related kinases regulate proper microtubule functioning during cell division, and to identify additional components in these regulatory pathways.

We have characterized the cellular localization of a candidate substrate of the AIR-2 kinase, the kinesin-like protein *C. elegans* Eg5, In wild-type cells, CeEg5 is associated with centrosomes and spindle microtubules. Like AIR-2, CeEg5 appears to be present at the spindle midzone during meiotic and mitotic anaphase. However, CeEg5 localization is completely disrupted in *air-2*(RNAi) embryos. CeEg5 is no longer found on meiotic spindles in the absence of AIR-2, and is not present on the multiple centrosomes found in *air-2*(RNAi) embryos. The AIR-2 and CeEg5 proteins physically interact with one another in the yeast two-hybrid assay and both proteins can be coimmunoprecipitated from *in vitro* translation reactions. Further experiments addressing whether CeEg5 is an *in vitro* and/or *in vivo* substrate of the AIR-2 kinase are underway.

# CYTOPLASMIC DYNEIN LIGHT-INTERMEDIATE CHAIN IS REQUIRED FOR DISTINCT ASPECTS OF CELL DIVISION IN *C. ELEGANS*

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We have cloned the *C. elegans* homologue of cytoplasmic dynein light intermediate chain (*dli-2)* and shown through RNAi analysis that the gene product is required for several aspects of cell divisions in *C. elegans.*

Cytoplasmic dynein is a multisubunit complex consisting of two heavy chain motor proteins associated with subunits categorized as light, light-intermediate or intermediate chains. Cytoplasmic dynein has been shown to be required for distinct aspects of mitosis and this activity requires association with another multisubunit complex, dynactin. Previous RNAi analysis of dynein heavy chain (*dhc-1*) and two dynactin components (*dnc-1* and *dnc-2*) revealed a variety of mitotic defects (Gönczy et al., 1999). Particularly, RNAi against each of these three genes shows early embryonic defects including failed pronuclear migration and failed centrosome separation in the one cell stage embryo. Interestingly, RNAi against *dli-2* reveals similar but distinct phenotypes. Pronuclear migrations are slowed but not prevented while centrosome separation occurs but proper spindle alignment fails (a phenotype also observed when dynein heavy chain function is diminished only partially with RNAi).

Cytoplasmic dynein light intermediate chain (LIC) was only recently identified as a component of the dynein complex. Rat LIC-2 was found to directly interact with pericentrin, a conserved component of the centrosome but a proposed function has yet to be ascribed. It is possible, based on the observed differences in RNAi phenotypes, that dynein light intermediate chain may function in a regulatory or targeting capacity for the dynein complex and therefore be required only for a subset of dynein function. Sequence analysis of LIC-2 and DLI-2 reveal a region of extended sequence similarity with the nucleotide binding P-loop domain with strongest similarity to this region in members of the ABC transporter family (ATPases). To investigate the requirement of nucleotide binding for DLI-2 function we are currently generating point mutations in conserved residues within its P-loop domain and testing these constructs for their ability to rescue the phenotype in a null background.

# THE SURVIVIN-LIKE *C. ELEGANS* PROTEIN BIR-1 ACTS WITH THE AURORA-LIKE KINASE AIR-2 TO MEDIATE CHROMOSOME BEHAVIOR AND SPINDLE MIDZONE FORMATION

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Baculoviral IAP repeat proteins (BIRPs) have been proposed to affect cell death, cell division, and signal transduction. How BIRPs function in these processes remains unclear. To learn more about BIRP function we characterized the *C. elegans* BIRP BIR-1.

BIR-1 localizes to chromosomes and to the spindle midzone, a tubulin structure that forms between segregating homologues and sister chromatids and that may play a role in cytokinesis. *bir-1*(RNAi) embryos have defects in chromosome condensation, alignment, and segregation as well as in spindle midzone formation and cytokinesis. The localization of BIR-1 is identical to that of the Aurora-like kinase AIR-2 [1]. *air-2*(RNAi) and *bir-1*(RNAi) embryos are phenotypically indistinguishable. Both BIR-1 and AIR-2 are required for histone H3 phosphorylation, which is necessary for chromosome condensation and for localization of the kinetochore component HCP-1, which may be necessary for chromosome alignment and segregation. BIR-1 is required to localize AIR-2 to chromosomes, but AIR-2 is not required to localize BIR-1.

We propose that BIR-1 localizes AIR-2 to chromosomes and to the spindle midzone, where AIR-2 phosphorylates proteins that control chromosome behavior and spindle midzone organization. Other known *C. elegans* proteins that function in cell division have localization patterns and loss-of-function phenotypes distinct from those of BIR-1 and AIR-2. Therefore, *bir-1* and *air-2* define a new class of *C. elegans* cell division genes.

BIR-1 is most similar in size and structure to the human BIRP survivin. Survivin, which is upregulated in tumors and can prevent programmed cell death when overexpressed, partially rescued the cytokinesis defect of *bir-1*(RNAi) embryos. This defect was not rescued by blocking programmed cell death, suggesting that BIR-1 and survivin share an evolutionarily conserved function separate from preventing cell death. Human Aurora homologs localize to the same structures as does survivin. We propose that survivin may contribute to tumorigenesis by promoting aneuploidy via an evolutionarily conserved mechanism that includes interaction with an Aurora-like kinase.

1. Schumacher *et al*., (1998). J. Cell. Biol. *143*, 1635-46.

# NEGATIVE REGULATION OF MEC-3 BY ZINC FINGER PROTEIN SEM-4

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Although the LIM homeodomain protein MEC-3 is the master regulator of touch cell fate, the mechanism by which MEC-3 itself is regulated is not completely understood. MEC-3 is expressed in the six touch receptors and in the FLP and PVD neurons. Mitani *et al*. found that *sem-4* animals express MEC-3 ectopically in extra cells in the tail, in addition to the PLM touch neurons (Mitani *et al*., *Development* 119: 773-783 (1993); Basson and Horvitz, *Genes & Dev*.10: 1953-1965 (1996)). This result suggests the possibility that SEM-4 negatively regulates MEC-3 expression, at least in these additional tail cells. We have demonstrated the ability of SEM-4 to repress MEC-3 expression and touch cell function in the touch cells. In addition, we have discovered that negative regulation of MEC-3 by SEM-4 is important for proper touch cell development.

When SEM-4 was overexpressed in the touch cells of wild-type worms, touch sensitivity was significantly decreased and the fluorescence of a *mec-3::GFP* reporter construct was substantially diminished. Furthermore, we found that *sem-4* mutants are partially touch insensitive, that their AVM and PVM neurons express MEC-3 earlier in development than wild type worms, and that the AVM neuron does not migrate properly in some *sem-4* animals. These results are consistent with the finding that SEM-4 is expressed in at least two of the six touch cells, AVM and PVM (Michael Basson, pers. comm.), and point to a regulatory role for SEM-4 in normal touch cell development.

To determine which regions of the SEM-4 molecule are important for its function in touch cells, we have studied four different alleles of *sem-4*. The varying effects of these mutations on touch sensitivity and on the fluorescence of the *mec-3::GFP* reporter demonstrate that the N terminal pair of zinc fingers, 2 and 3, is particularly critical for the function of SEM-4 in the touch cells.

Mutations in a small region of the *mec-3* promoter in a *mec-3::lacZ* reporter construct produce transient, ectopic staining in two tail cells (Ding Xue & M. Chalfie, unpublished). We are investigating the binding of SEM-4 to this promoter sequence. We have purified full length SEM-4 protein and are carrying out gel shift assays with the putative SEM-4 binding site.

# GENETIC AND MOLECULAR ANALYSIS OF INTRON REQUIREMENTS FOR GENE ACTIVITY

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We are interested in elucidating the molecular and genetic basis of an observed requirement for introns in the normal processing of mRNA. Previous work shows that messages that are intronless are retained in the nucleus. Insertion of one intron allows some expression, while additional introns are required for optimal expression.

In other systems, cis-acting sequences have been identified which allow expression of intronless mRNAs. We are screening the worm genome for endogenous sequences that have similar properties in *C.elegans*.

In an effort to understand and identify the genes involved in this process, we are also conducting a screen for trans-acting factors . Using intronless GFP as a reporter gene ( very low expression) we have been screening for mutants with enhanced expression. Further analysis of these mutants will involve a characterization of transport effects on intronless mRNAs and (if warrented) cloning and characterization of the corresponding genes.

# CRB-1 MAY FUNCTION IN SYNAPTIC TRANSMISSION AND DAUER FORMATION

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The cyclic AMP-response element binding protein CREB plays a central role in long-term memory in *Aplysia*, *Drosophila* and mice. We characterized the *C. elegans* and *C. briggsae* CREB genes (*crb-1*) and found that the encoded proteins are 85% identical. The DNA-binding bZIP domain and cAMP-dependent kinase site, as defined in the mammalian and *Drosophila* CREB family members, are highly conserved in the nematode proteins. The *C. elegans crb-1* gene has two alternatively-spliced isoforms. Our immunohistochemical studies indicate that CRB-1 is ubiquitously expressed throughout development and in adults. CRB-1 can bind to cyclic AMP-response element (CRE) sites and can be phosophorylated by cAMP-dependent protein kinase (PKA) *in vitro*.

To determine the function of *crb-1* in the worm, we isolated three deletion alleles of *crb-1* from a chemical deletion library. Two are predicted to cause early truncations, and the third deletes part of the bZIP domain. No CRB-1 protein is detected by western blot analysis of these three mutant strains, suggesting that all three are null alleles.

*crb-1* mutants are viable and show no obvious abnormalities in brood size, locomotion, mechanosensation, chemotaxis or thermotaxis. However,*crb-1* mutants tend to accumulate at the edge of the bacterial lawn (bordering) and form clumps of animals. In addition, mutations in *crb-1* confer a dauer-constitutive phenotype (Daf-c) at 27C but not at 25C. The Daf-c phenotype of *crb-1* animals is suppressed by mutations in *daf-16* and *daf-12* but not by mutations in *daf-5*. This finding suggests that *crb-1* acts in the DAF-2 insulin receptor-like signaling pathway. A similar role has been suggested for *unc-31* and *unc-64*,[1] which are implicated in synaptic vesicle release. Double mutants between *crb-1* and either *unc-31* or *unc-64* show a strongly enhanced Daf-c phenotype at 25C as has been shown for *unc-31; unc-64* double mutants.[1] This synergy suggests that mutations in *crb-1*, like mutations in *unc-31* and *unc-64*, impair synaptic function. Unlike *unc-31* or *unc-64* mutants, *crb-1* mutants do not have an increased life span. We are further investigating the role of *crb-1* in synaptic transmission, dauer formation, and behavior.

1. Ailion, M., Inoue, T., Weaver, C.I., Holdcraft, R.W. and J.H. Thomas, (1999) PNAS **96**: 7394-7397.

# *IN VIVO* STUDIES OF TROPONIN T FUNCTIONS.

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The *in vivo* functions and interactions of TnT in muscle contraction are poorly understood. Thus, we are studying the four *C. elegans* TnT genes, which give rise to at least eight distinct transcripts, by comparing their encoded proteins, their expression patterns, their individual and combined RNAi phenotypes, and the effects of site-directed mutagenesis.

The *mup-2/TnT-1* gene encodes a single isoform expressed in embryonic and larval body wall muscles, and in the hermaphrodite oviduct. RNAi of TnT-1 phenocopies the *mup-2* null embryonic Mup phenotype. The *TnT-2* gene encodes the major larval and adult body wall isoform. RNAi of TnT-2 causes an uncoordinated phenotype and larval arrest. The *TnT-3* gene, which is expressed in early embryonic body wall muscle and in pharyngeal muscle and other minor muscle groups, generates at least four distinct transcripts. No phenotypes have yet been associated with RNAi of TnT-3. The TnT-4 gene, which is expressed in pharyngeal muscle, gives rise to two isoforms differing in the N-terminal region. RNAi of TnT-4 causes L1 arrest, consistent with a likely defect in pharyngeal function (starvation). We are examining the TnT-2 and TnT-4 phenotypes further, and undertaking RNAi of TnT loci in combination.

Comparisons of *C. elegans* and vertebrate TnTs reveal a highly conserved cluster of charged residues located in the N-terminal region of TnT believed to interact in a calcium-independent manner with tropomyosin. Mutations in human cardiac TnT near this cluster are linked with familial hypertrophic cardiomyopathy. There is also a highly conserved heptad repeat of leucines in the C-terminal region, which is thought to allow interaction with troponin I and to be essential for TnT's role in regulating muscle contraction. One of the TnT-3 isoforms replaces, the heptad repeat with a sequence of about 70 amino acids having some similarity to filaggrin, which binds intermediate filaments, as well as to the Wolf-Hirschhorn Syndrome Critical Region 1 protein (WHSC1), which is expressed in rapidly growing embryonic tissues. The effects *in vivo* of site-directed mutations of these domains appear to cause dominant hypercontracted phenotypes suggesting essential roles in acto-myosin inhibition.

# GENETIC ANALYSIS OF NEUROENDOCRINE CONTROLS OF FAT METABOLISM IN *C. ELEGANS*

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Body fat regulation is a complex process that affects behavior, physiology, and metabolism of an organism in order to balance energy intake with energy expenditure*. C. elegans* is an attractive choice for the systematic identification of components of fat homeostasis and cell biology. As in mammals, an insulin signaling pathway in *C. elegans* couples nutritional status to the tempo and mode of metabolism, while neuronal outputs, e.g. serotonin, translate food sensation to various motor and endocrine outputs.

We have used the phenoxazine dye Nile Red to visualize fat droplets in the intestinal and hypodermal cells of living *C. elegans*. The Nile Red staining pattern closely matches the pattern observed with the traditional methods of fat staining in *C. elegans* (e.g Sudan Black B). Moreover, the known and predicted patterns of fat accumulation arising from mutations in insulin-like (*daf-2* / *daf-16*) and TGF-b like (*daf-7* / *daf-3*) signaling pathways are well recapitulated by Nile Red staining. Thus, Nile Red staining of living animals provides a means for genetic screens based on fat metabolism and homeostasis. Two such screens are presented:

(i) To identify genes involved in the biogenesis and cell biology of fat storage, we screened EMS-mutagenized N2 animals for mutant worms with abnormalities in lipid droplet size or number. In a screen of 15,000 mutagenized F2, we identified four mutants that display abnormally large droplet sizes.

(ii) The *C. elegans* insulin signaling pathway determines whether larvae grow to a fast metabolizing adult stage or enter a slow metabolizing dauer stage. Reduced signaling through the DAF-2 insulin-like receptor promotes a shift in metabolism to fat storage and dauer arrest. The forkhead transcription factor DAF-16, is the key target of DAF-2 signaling pathway and is required for the shift in fat metabolism and dauer arrest. Mutant adult *daf-2* animals have a diffuse pattern of Nile Red staining. In contrast, Nile Red appears in very discrete droplet spots in *daf-16* animals. To identify downstream metabolic targets of DAF-16, we have begun screening EMS-mutagenized *daf-16* worms to find mutants that recapitulate the *daf-2* pattern of staining.

# FUNCTIONAL CHARACTERIZATION OF CALCINEURIN, A SERINE/THREONINE PROTEIN PHOSPHATASE, IN *C. ELEGANS*

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Calcineurin is a cytoplasmic, calcium and calmodulin dependent serine - threonine protein phosphatase that plays a vital role in the development and regulation of several cellular processes including gene transcription. It is a heterodimer of a 58~64 kDa catalytic sububit, Calcineurin A (CnA), and a calcium binding 19 kDa regulatory subunit, Calcineurin B (CnB). Although the overall sequence of CnA is divergent in different organisms, the regions that play an important role in the phosphatase activity are well conserved. Until date, very little has been known about calcineurin in *C. elegans*, and, hence, we attempted to characterize this protein and elucidate its function using molecular biology and genetical approaches in this model. *C. elegans* calcineurin A (CeCnA) is mapped to the LG IV on the cosmid C02F4 and calcineurin B (CeCnB) is located on the cosmid F55C10 (LGV). We have cloned the full-length CeCnA cDNA by screening a mixed stage worm cDNA library. CeCnA shows approximately 77% sequence identity with mammalian CnA. It contains a large catalytic domain at the N-terminus, and a C-terminus regulatory domain for CnB and CaM binding. CeCnA expresses strongly in neuronal cells, body-wall and vulvul muscles. Northern blot analysis reveals a single predominant transcript of 3.2 kb. Furthermore, the developmental profile of CeCnA mRNA suggests that it is detectable at all stages of development. Disruption of calcineurin function by RNA-mediated interference (RNAi) resulted in male progeny suggesting that calcineurin probably has an important role in sex-chromosome disjunction. Additionally, the cosmid C02F4 is genetically mapped closely to the *him-8* locus which is also tempting to speculate that *him-8* encodes CeCnA. However, preliminary examination by northern analysis has revealed no change of CeCnA transcript size in *him* mutants when compared with wild type indicating the possibility of a point mutation rather than deletions. Currently, we are sequencing calcineurin A in the *him* alleles and performing rescue experiments using cDNAs to further characterize the function(s) of calcineurin in *C. elegans*.

# A GENETIC SCREEN FOR COMPONENTS OF THE $G_O$ ALPHA SIGNALING PATHWAY IN *C. ELEGANS*

## I. Amy Bany, Michael Koelle

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Neurotransmitters can mediate neural communication by activating heterotrimeric G proteins that signal through poorly understood pathways. GOA-1, a G protein expressed in the *C. elegans* nervous system, is 80% identical to human $G_O$a, the major G protein of the brain. Neural signaling defects in *goa-1* mutants result in hyperactivity of several behaviors, including feeding, locomotion, and egg laying.

To identify components of the GOA-1 signaling pathway, we screened 39,000 mutagenized haploid genomes for mutations that confer a phenotype similar to that of *goa-1* loss-of-function mutants. Like *goa-1*, these hyperactive egg-laying mutants lay their eggs soon after fertilization, presumably due to the hyperstimulation of the egg-laying machinery. This results in worms that accumulate very few eggs within their uterus and eggs that are laid at an early stage of development. In the past, it has been difficult to identify mutants with this phenotype (thin and empty of eggs) because they resemble both young adults and the partially sterile animals that appear at a high frequency following mutagenesis. To overcome this difficulty we developed an efficient selection for the early-stage eggs laid by hyperactive egg-laying mutants that is followed by a visual rescreening for animals resembling *goa-1* mutants. This procedure allows for the identification of mutants with neurotransmitter signaling defects due to the altered function of proteins both upstream and downstream of GOA-1.

This screen has isolated mutants with the desired hyperactive egg-laying phenotype. We are currently characterizing 17 isolates that lay greater than 70% of their eggs at the 8-cell stage or earlier. The strength of this phenotype distinguishes these mutants from previously described "Egl-C" mutants which, in a limited survey, typically lay a much lower percentage of early-stage eggs. Only loss-of-function mutations in *goa-1* and *eat-16*, components of G-protein signaling pathways that control egg-laying behavior, have comparably strong phenotypes. Interestingly, all of the mutations analyzed from this screen show some degree of semi-dominance, as do *goa-1 (n363)* and *eat-16(ad702)*. We plan to clone and characterize the genes identified in this screen to understand GOA-1 signaling at a molecular level.

# SQV-7, A PROTEIN INVOLVED IN VULVAL INVAGINATION AND EMBRYONIC DEVELOPMENT, TRANSPORTS UDP-GLUCURONIC ACID, UDP-N-ACETYLGALACTOSAMINE AND UDP-GALACTOSE

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Mutations in eight *sqv* (squashed vulva) genes (*sqv-1* to *8*) cause defects in vulval invagination and early embryonic development (1). SQV-1 and SQV-4 are similar to enzymes involved in nucleotide sugar metabolism (2), while SQV-3 and SQV-8 are similar to glycosyltransferases (3). SQV-7 is a multi-transmembrane protein resembling Golgi membrane nucleotide sugar transporters (4); these proteins specifically translocate nucleotide sugars from the cytosol into the lumen of the endoplasmic reticulum and Golgi apparatus where they are used as sugar donors by glycosyltransferases (4). Translocation is essential for glycosylation: yeast, protozoa and mammalian cell mutants defective in this function have a severe deficiency of the corresponding sugar in their glycoconjugates (4). Because GDP-mannose and UDP-glucose are the only nucleotide sugars transported into *S.cerevisiae* Golgi and ER vesicles *in vivo* and *in vitro* (5), this organism was used for expression of SQV-7 to determine its substrate specificity.

We found that SQV-7 transports UDP-glucuronic acid, UDP-N-acetylgalactosamine and UDP-galactose *in vitro*, the first protein known to transport the former two nucleotide sugars. All three nucleotide derivatives are competitive, alternate, non-cooperative substrates, which suggests there is a single active site for all three substrates on SQV-7. Expression of the two mutant *sqv-7* missense alleles results in significant reduction of the three transport activities. We hypothetize that in these mutants the biosynthesis of chondroitin, the major glycosaminoglycan in *C. elegans* (6), is more likely to be impaired than that of heparan sulfate since chondroitin polymerization requires both UDP-glucuronic acid and UDP-N-acetylgalactosamine while heparan sulfate polymerization does not require UDP-N-acetylgalactosamine.

(1)Herman T. et al (1999) PNAS 96:968

(2)Hwang H. et al.(2000) East Coast *C.elegans* Meeting

(3)Herman T. and Horvitz H. R. (1999) PNAS 96: 974

(4)Hirschberg C. et al (1998) Annu. Rev. Biochem.67: 49

(5)Berninsone P. et al (1997) J. Biol. Chem. 272: 12616

(6)Toyoda H. et al (2000) J. Biol. Chem. 275: 2269

# THE CLC FAMILY OF CHLORIDE CHANNELS IN *C. ELEGANS*

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Voltage-gated ClC $Cl^-$ channels participate in various physiological processes including sarcolemmal excitation, cell volume regulation, and renal epithelial $Cl^-$ transport. Nine mammalian ClC isoforms have been identified to date. Three human ClC genes were found responsible for inherited diseases (*CLCN1*, myotonia congenita; *CLCN5*, Dent's disease; and *CLCNKB*, Bartter's syndrome) revealing the clear importance of ClC channels. Unfortunately most isoforms have no known physiological function in mammalian tissues due to the difficulty in studying these channels in complex organisms. In the *C. elegans* genome, six ClC predicted coding sequences have been identified with significant amino acid sequence homology with the mammalian counterparts. These six nematode ClC channels offer unique opportunities to study the functional biology of ClC channels in a well-defined model organism. To verify the existence of each putative ClC channel in *C. elegans*, we isolated and sequenced cDNAs encoding all six isoforms (*Clh-1* through *Clh-6*). RT-PCR experiments revealed that mRNAs corresponding to 5 of 6 ClC channels are present in both adult and embryonic worms. Only *Clh-4* is uniquely expressed in adult worms. Green fluorescent protein fusion constructs revealed expression of *Clh-5* in the excretory system, intestinal cells, neurons and muscle and expression of *Clh-6* in RMEL and RMER neurons. Experiments employing knock-out techniques are in progress to determine the function of each *C. elegans* ClC channel.

# PROTEIN INTERACTION MAPPING OF DNA REPAIR AND DNA DAMAGE CHECKPOINT PROTEINS IN *C. ELEGANS*

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One of the outstanding questions that remains within the DNA repair field is how proteins involved in the checkpoint response to damage communicate and coordinate with those proteins implicated in the repair of DNA lessions. Also, it remains to be seen how repair and DNA damage responses differ in higher Eukaryotes.

In an attempt to begin to address these questions we are generating a protein-protein interaction map between DNA repair and DNA damage checkpoint proteins in *C.elegans*. The *C.elegans* model system has the advantage of a complete genome sequence and is amenable to RNA mediated interference (RNAi), a standard genetic tool that enables the elimination of gene-function in a rapid and reproducible manner.

Using database analysis we have identified 75 proteins present in the worm that are homologues of proteins previously in DNA repair or in the DNA damage response in other model organisms. We have cloned the majority of these genes using rapid recombination cloning (RC) technology and have performed a yeast 2-hybrid matrix experiment (Gal4-DB proteins verse Gal4-AD proteins) to confirm previously described interactions and to identify novel protein-protein interactions between all of these proteins. In order to identify novel proteins potentially involved in the DNA damage response we are performing high throughput yeast 2-hybrid analysis with each Gal4-DB fusion against the entire *C. elegans* genome.

These analyses may identify possible cross talk between DNA repair and DNA damage checkpoint proteins. Novel proteins identified in this study will then be validated as relevant to the DNA damage response by analyzing the consequences of DNA damage in the absence of gene function following RNAi.

# *DIG-1* ENCODES AN ADHESION MOLECULE INVOLVED IN SENSORY MAP FORMATION.

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We are interested in the development of topographic maps. Topographic maps coordinately map spatial information from the environment to the brain. We are studying the maps formed by two sets of head sensory neurons in *C. elegans*, the IL1's (visualized by an *unc-5*::GFP construct kindly provided by J. Culotti) and the IL2's (visualized by DiO staining; C. Bargmann, pers. comm.). Currently, we are studying mutations in the gene *dig-1* that affect the formation of these maps. In *dig-1* mutant animals, IL1 and IL2 sensory processes are aberrant, and sometimes fail to reach their normal destinations in the nose. Mutant animals exhibit additional characteristics consistent with a defect in cell adhesion.

*dig-1* has been mapped to a region on chromosome III near *sma-3*. Two *dig-1* alleles show changes by PCR and Southern analysis in a very large cell adhesion molecule in this region (K07E12.1; predicted cDNA about 40 kb). We have sequenced the affected regions of this gene in both alleles. Allele *nu336* is a point mutation towards the 5' end of the gene resulting in a serine being changed to a phenylalanine. Allele *n1480* is a rearrangement involving about 900 base pairs towards the 3' end of the gene. It is not yet clear whether this rearrangement would result in truncation of the predicted gene product, although this seems a likely possibility. Interestingly, the phenotype of the *nu336* allele is more severe than that of the *n1480* allele. We are conducting northern and RT-PCR experiments, as well as constructing a promoter::GFP fusion, to determine the cDNA structure and expression patterns of the *dig-1* gene. CB was supported by a GAANN fellowship from the US DOEd (P200A50010). EFR is supported by a NSF CAREER Award (IBN-9984662).

# THE *C. ELEGANS* LISSENCEPHALY (LIS1)-LIKE GENE *LIS-1* IS REQUIRED FOR EMBRYONIC DEVELOPMENT

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In the human disease lissencephaly, abnormal neuronal migration during brain development leads to reduced cerebral convolutions. Afflicted patients suffer epilepsy and mental retardation. The most common cause of lissencephaly is haploinsufficiency of the gene LIS1. The predicted *C. elegans* LIS-1 protein is 58% identical to human LIS1. We have isolated two deletion alleles of *lis-1*, one that removes 1465 bp, including predicted exons 4-5, and one that removes 2019 bp, including predicted exons 4-6. These 2 alleles fail to complement.

Deletion homozygotes are Unc, Egl, and Mel. Progeny of deletion homozygotes appear to arrest at the 50-100 cell stage and contain enlarged, heterogeneous, asymmetrically distributed nuclei. We have analyzed the cell biological basis of the defects in embryonic development and in egg-laying. A translational *lis-1::gfp* fusion gene driven by the worm *lis-1* promoter is expressed in multiple tissue types in transgenic worms; expression in the nervous system is restricted to a subset of neurons. We are now attempting to confirm this expression pattern using antibody staining. To test whether the human and worm genes are orthologous, we are attempting to rescue the phenotype of the worm deletion mutants with the human LIS1 cDNA driven by either the heat-shock or the endogenous *lis-1* promoters.

We are seeking *lis-1* interactors both by screening for suppressors of the *lis-1* Mel phenotype and by testing candidate genes. An F1 screen of ~10,000 haploid genomes failed to yield suppressors of the *lis-1* Mel phenotype. We now plan to extend this screen to the F2 generation.

# THE ER STRESS RESPONSE IN *C. ELEGANS*

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Protein malfolding in the endoplasmic reticulum (ER) causes at least two distinct cellular responses. The accumulation of malfolded proteins induces transcription of ER chaperones like Hsp70/GRP78 to promote proper folding and causes an inhibition of protein translation. Protein synthesis is inhibited via PERK phosphorylation of eIF-2a . We have identified *C. elegans* homologues of several proteins involved in the *S. cerevisiae* and mammalian ER stress response pathways including GRP78, PERK, ATF6, and IRE1. The presence of these genes suggests that the ER stress response pathways are conserved and that *C. elegans* provides an ideal model system to study ER stress. We are currently using transgenic and reverse genetic strategies to analyze the expression and function of these genes. In addition, we are conducting genetic screens to identify novel components of these pathways.

# POP-1, A *C. ELEGANS* TCF/LEF1 FAMILY MEMBER, POSSESSES ACTIVATION AND REPRESSION DOMAINS AND IS A TARGET OF ACETYLATION BY CBP-1

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CBP-1 is the *C. elegans* homolog of mammalian histone acetyltransferase p300/CBP. Our lab has shown recently that CBP-1 is essential for the differentiation of endoderm, mesoderm and hypodermal tissues. In the endoderm differentiation CBP appears to antagonize the repressive activity of histone deacetylases, suggesting that the acetyltransferase activity of CBP plays an important role in endodermal gene activation. Studies in Drosophila and mammals have shown that, besides histones, p300/CBP also regulate the activities of a number of transcription factors through acetylation. These targets of p300/CBP include a Drosophila HMG-containing protein TCF that is related to the *C. elegans* POP-1protein.

During *C. elegans* early embryogenesis, POP-1 plays an essential role in anterior-posterior cell fate decisions in *C. elegans* embryogenesis. Lack of POP-1 results in a mesodermal founder cell to adopt endodermal cell fate, suggesting that POP-1 functions to suppress genes important for endodermal fate in the mesodermal founder cell. To investigate the functional relationship between POP-1 and p300/CBP, we wish to determine whether p300/CBP can acetylate POP-1 in vitro and to analyze the consequence of POP-1 acetylation in *C. elegans* development. We have been able to show that p300 acetylates POP-1 efficiently in vitro and have identified the main lysine residue in POP-1 that is acetylated by p300. We have generated polyclonal antisera specific for this acetylation site and the results of these studies will be discussed.

To investigate the molecular mechanisms that underlie the developmental function of POP-1, we also analyzed the transcriptional activity of POP-1. Consistent with its biological activity, we show that POP-1 can repress transcription. We identify a strong transcriptional repression domain that is necessary and sufficient for POP-1 to mediate repression in a GAL-4 fusion protein-based assay. We find multiple sequence motifs within the repression domain that each on its own can mediate repression. Further dissection of one such motif reveals amino acids that are important for the repression activity. POP-1 also possesses an activation domain, similar to its vertebrate homologs that have been shown to both activate and repress transcription.

# ANALYSIS OF MALE SEX MYOBLAST MIGRATION

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Cell migration is a complex process requiring the coordinated production of external guidance information to direct the migrating cell as well as factors within the migratory cell that are responsible for reading the guidance information, choosing a polarity for response, and generating cellular movement. To understand the molecular mechanisms underlying these processes, we have been studying the migrations of the sex myoblasts (SMs). While both male and hermaphrodite SMs are born in the same place, they subsequently migrate in opposite directions to localize the sex muscles to sexually distinct positions; hermaphrodite SMs migrate anteriorly and male SMs migrate posteriorly. While much is known about the mechanisms that guide hermaphrodite SM migration, male SM migration guidance is not well understood. Interestingly, studies have shown that *tra-1* and *mab-5* are required cell autonomously for SMs to follow their sexually-appropriate trajectories. Since the SMs' external environment remains normal in these circumstances, these results indicate that some of the cues that guide SM migration are present in both males and hermaphrodites but are interpreted differently by sexually distinct SMs. Thus, mutations that alter the general direction of SM migration could help elucidate the mechanisms underlying the correct polarized response to these cues.

I have initiated a genetic screen looking for mutations that cause male SMs to migrate anteriorly. To facilitate this screen, I am using the sex muscle-specific GFP marker *ayIs2[egl-15::GFP]* to screen for anteriorly-misplaced male sex muscles. In addition to screening for new mutations which confer this defect, I have begun an analysis of the *cod-11(sy35)* mutant,donated to us by the Sternberg Lab, which has a similar defect. As with *mab-5* mutant males, *cod-11* males have anteriorly-misplaced sex muscles. However, the SM migration defects in *sy35* males are less penetrant than that of *mab-5* null mutants; only a random subset of the SMs migrate in the wrong direction, whereas all SMs migrate improperly in *mab-5* mutant males. It is not yet clear whether this is because of gene-specific differences, or if *sy35* represents a weak allele of *cod-11*. Further analysis of both *cod-11* and *mab-5* will be conducted to determine the nature of their functions in male SM migration.

# IDENTIFICATION AND CHARACTERIZATION OF GENES THAT ACT WITH *LIN-35* RB TO NEGATIVELY REGULATE VULVAL INDUCTION

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The synthetic multivulva (synMuv) class A and class B genes define two functionally redundant pathways that antagonize Ras signaling during vulval induction. Molecular analyses of the class B synMuv genes have defined a *C. elegans* pathway that includes a counterpart of the mammalian retinoblastoma, or Rb, tumor suppressor gene. Members of this pathway include *lin-35* Rb, genes encoding the presumed heterodimeric transcription factor DPL-1 DP / EFL-1 E2F, and genes, such as *lin-53* RbAp48 and *hda-1* HDAC, predicted to modulate chromatin structure.

The class B synMuv gene *lin-54* was originally identified in screens conducted by Jeff Thomas, a former graduate student in our laboratory. We cloned *lin-54* and found that it encodes a novel protein with cysteine-rich motifs. These motifs have cysteine signatures unlike those found in proteins with known functions; however, these motifs are conserved in proteins predicted from mammalian, plant and fly ESTs. A rescuing *lin-54::gfp* transgene is expressed broadly and is localized to nuclei, an expression pattern like that of all synMuv genes thus far assayed. We obtained two deletion alleles of *lin-54* and found that, in addition to a Muv phenotype in a *synmuvA* background, they cause sterility in a wild-type background. We are currently characterizing this sterile phenotype.

We are also identifying new class B genes. Using a *lin-15A* background, we screened 6,500 mutagenized haploid genomes and obtained 95 Muv mutant strains. A partial analysis of these mutants has identified at least three previously unknown class B synMuv genes. We will describe our further characterization of these mutants.

# G-PROTEIN BETA-2 REGULATES THE ACTIVITY OF TWO RGS PROTEINS

## Dan Chase, Georgia Patikoglou, Michael Koelle

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Heterotrimeric G proteins are signaling molecules that permit cells to adapt to changing extracellular conditions. Termination of the G protein signal is stimulated by a class of GTPase activating proteins known as regulators of G protein signaling or RGS proteins. We want to understand how RGS proteins select their proper G protein target *in vivo*. The Ga proteins GOA-1 and EGL-30, while normally expressed in the same cells of the *C. elegans* nervous system, have opposite effects on several behaviors including egg laying. Genetic analysis has identified two RGS proteins, EGL-10 and EAT-16, that negatively regulate GOA-1 and EGL-30, respectively. Such specificity of RGS proteins for Ga is not generally observed *in vitro* and encouraged further study of these signaling pathways. EGL-10 and EAT-16 share a region of similarity to Gg proteins known as the G-gamma-like or GGL domain. Mammalian GGL-containing RGS proteins have been shown to bind to the unique Gb protein, Gb5. To determine if the *C. elegans* ortholog of Gb5, GPB-2, plays a role in G protein signaling, or if GPB-2 contributes to signaling specificity, we isolated a deletion allele of *gpb-2*. This allele, missing the first two exons and 500 bases of promoter sequence (and thus a presumptive null allele), has little affect on egg-laying behavior in a wild-type background apparently because it has equal and opposing effects through EGL-10 and EAT-16. In mutants of either *egl-10* or *eat-16* in which we can isolate one RGS protein in the absence of the other, the *gpb-2* deletion allele has dramatic effects. These effects are consistent with GPB-2 being required for full activity of these RGS proteins.

If EGL-10 and EAT-16 physically interact with GPB-2 there may exist novel types of heterotrimeric complexes consisting of Ga, GPB-2 and RGS protein. Activation of these complexes by a receptor would then liberate GTP-bound Ga from the Gb-RGS complex. One might imagine that the proximity of the active Ga and Gb-RGS molecules could lead to a dramatically increased rate of GTP hydrolysis and hence termination of the Ga signal. We are currently using *in vitro* biochemical analyses of purified proteins to independently establish the interaction of GPB-2 with both EGL-10 and EAT-16. If these complexes form we will also determine the effect of GPB-2 binding on RGS activity and Ga subunit selection.

# A MUTATION THAT AFFECTS EARLY GERMLINE PROLIFERATION  

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We would like to understand the molecular basis for early germline development. The somatic and germ components of the gonad are descendents of four cells that constitute the gonad primordium, Z1, Z2, Z3 and Z4. Cell-cell communication is necessary for the proper development of the germ line. In particular, ablation of Z1 and Z4, cells that give rise to the somatic gonad, prevents the germline cells Z2 and Z3 from dividing or entering meiosis (Kimble and White, 1981). Therefore, cell-cell communication is necessary for the proliferation and meiotic competence of Z2 and Z3. The effect of Z1/Z4 ablation differs from the *glp-1* null mutant phenotype in which Z2 and Z3 divide several times and enter meiosis (Austin and Kimble, 1987). This effect also differs from the phenotype of certain germ line proliferation mutants in which Z2 and Z3 divide several times but do not enter meiosis (e.g., *glp-4*, Beanan and Strome, 1992 and *glp-3*, Kadyk et al., 1997). The molecular components of the Z1/Z4-to-Z2/Z3 signaling event are not known.

We have identified mutants in which the adult hermaphrodite somatic gonad is normal but apparently contains no germ line (Nog phenotype). Several different developmental mishaps could cause a Nog phenotype. For example, (1) Z2 and Z3 or their descendents may die, (2) Z2 and Z3 may not be generated, (3) Z2 and Z3 may be generated but may not reach the gonad primordium, or (4) they may reach the primordium but fail to proliferate. Interference with cell-cell communication between the somatic and germ lineages suggested by the Z1/Z4 ablation experiment could account for the last scenario.

One of the Nog mutants,*ar228*, develops a normal L1 gonad primordium containing Z1, Z2, Z3 and Z4. During the L1, however, Z2 and Z3 take on a slightly abnormal morphology and fail to produce a germ line. The worms display no other obvious behavioral or morphological defects. Therefore, the gene defined by the *ar228* allele could provide molecular means to explore events necessary to initiate the proliferation of Z2 and Z3. *ar228* is recessive, fully penetrant and maps to the left arm of LGV, under sDf50. *ar228/Df* displays the same phenotype as an *ar228* homozygote, suggesting that *ar228* is a strong loss-of-function allele. At the meeting, we will present further genetic and phenotypic analyses of this mutant.

# AN APPROACH TO IDENTIFYING THE LATERAL SIGNAL IN VULVAL DEVELOPMENT

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In C. elegans, six vulval precursor cells (VPCs), P3.p through P8.p, are capable of generating vulval cells. In wild-type hermaphrodites, several discrete signaling events (i.e., inducing, lateral and inhibitory signaling) specify the VPC cell fate pattern 3° -3° -2° -1° -2° -3° . P5.p, P6.p and P7.p adopt vulval fates (1° and 2° ), while the other three cells adopt a non-vulval fate (3° ).

Previous studies showed that the lateral signal is sufficient to specify the 2° cell fate and ensures that only one VPC has the 1° cell fate. The receptor for the lateral signal is encoded by a Notch family member *lin-12*. However, the lateral signal itself is not known yet. Well-studied ligands of LIN-12/Notch all belong to the DSL protein family and share common structural motifs. The three C. elegans DSL proteins that have been examined, Lag-2, Apx-1 and Arg-1, do not appear to be the lateral signal. We therefore searched the worm genome database for other DSL homologous sequences and identified 7 candidates. Currently, we are using both GFP and LacZ constructs to determine the expression patterns of the candidate genes in vivo.

# ANTEROPOSTERIOR AXON GUIDANCE GENES

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Cell and growth cone migrations along the dorsoventral and anteroposterior body axes establish the pattern of the *C. elegans* nervous system. We are performing several screens for axon guidance mutants to identify genes needed specifically for longitudinal axon growth as well as genes required generally for all axon extension and pathfinding.

The PVQ neurons, which can be visualized directly in a *sra-6::gfp* strain, are located in the tail and each extends a single axon that enters the ventral nerve cord and continues to the nerve ring in the head. We mutagenized *sra-6::gfp* animals and isolated over sixty mutants with PVQ axon outgrowth and pathfinding defects. About half caused the PVQ axons to terminate their growth prior to reaching the nerve ring, while the rest caused both axon outgrowth and pathfinding defects, such as the inappropriate extension along the dorsal nerve cord and lateral midline or across the ventral midline. We also found twenty mutants with defects in either cell fate, cell migration or programmed cell death. For example, *pag-3* mutants lacked or had a reduced expression of GFP in PVQ, suggesting a defect in PVQ cell fate, whereas *ced-3, ced-4* and *vab-7* mutants had additional GFP-expressing cells in the tail.

A second screen to identify genes needed for the growth of the AVA, AVB and AVD axons is also underway. AVA, AVB and AVD are located in the head and each extends a single axon that runs in the ventral nerve cord to the tail. The mutations found in these two screens define at least 37 genes, including the known genes *cam-1, ced-3, ced-4, epi-1, mig-2, mig-11, pag-3, unc-6, unc-14, unc-22, unc-33, unc-40, unc-42, unc-44, unc-51, unc-53, unc-71, unc-73, unc-76, unc-119, vab-1, vab-7* and *vab-8*. We are continuing our screens for new mutants as well as further characterizing our existing mutants. We are pursuing the molecular characterization of several new guidance genes and investigating the roles of candidate guidance genes identified in the *C. elegans* genomic sequence.

# STUDIES ON TWO ISOFORMS OF PUTATIVE *C. ELEGANS* SERCA (SARCO/ENDOPLASMIC RETICULUM CALCIUM ATPASE)

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SERCA is a calcium ATPase pump located in sarcoplasmic and endoplasmic reticulum. A putative *C. elegans* SERCA (ce-SERCA) shows 70% identity in amino acid sequence with that of *Drosophila*, rat, rabbit, and other species. *C. elegans* SERCA::GFP reporter construct is shown to express in body-wall, vulval, pharyngeal muscles, and some neuronal cells. Sequenced cDNA clones have revealed two alternatively spliced isoforms of ce-SERCA. In order to address possible functions of the two isoforms of SERCA, dsRNAs targeted for specific regions of each isoform and as well as common regions were injected. Interestingly, high frequency of dead embryos was observed for one isoform and mostly larval lethal phenotype was observed for the other. Monoclonal anti-SERCA-1 antibody staining showed signals in pharyngeal, vulval muscles, and some neuronal cells but not in body-wall muscles. We are currently constructing reporter genes (CFP and YFP) to locate tissue specific expression of each isoform and determine the temporal expression pattern of SERCA in *C. elegans*.

# CHARACTERIZATION OF THE CLASS A SYNTHETIC MULTIVULVA GENES *LIN-8* AND *LIN-56*

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The receptor tyrosine kinase/Ras pathway essential for vulval induction in *C. elegans* is negatively regulated by two redundant pathways. Hermaphrodites mutant in only one of these two pathways (A or B) appear wild-type for vulval induction. Hermaphrodites mutant in both pathways (A and B) exhibit the synthetic Multivulva (synMuv) phenotype. Various screens for multivulva animals have defined four genes in the synMuv class A pathway: *lin-8*, *lin-15A*, *lin-38*, and *lin-56*. Of these genes, only the *lin-15A* locus was cloned previously; *lin-15A* encodes a novel protein with no recognizable functional or structural motifs (1).

The class B synMuv genes inhibit Ras-mediated vulval development via an Rb/E2F/DP-mediated pathway, which suggests that the transcriptional repression of genes required for vulval development is the molecular mechanism of this inhibition (2). The class A synMuv genes function in parallel to this Rb pathway, but the molecular mechanism by which they inhibit vulval development is not known. The recent identification of *egr-1* and *egl-27* as possible class A synMuv genes suggests that the synMuv class A pathway may also act via transcriptional repression, as *egr-1* and *egl-27* are homologous to MTA1, a component of the mammalian NURD chromatin remodeling complex (3). In addition, evidence from two-hybrid experiments suggests that the products of class A and class B synMuv genes may be associated in a complex *in vivo* (4).

To further our understanding of the mechanism by which the class A synMuv genes inhibit the Ras pathway, we have cloned *lin-56* and *lin-8.* Both appear to encode novel, highly-charged proteins. We have identified molecular lesions associated with both *lin-56* alleles and with all but one of the nine *lin-8* alleles. We are working to characterize the expression patterns of *lin-56* and *lin-8* and plan to determine their sites of action using mosaic analysis.

1. Clark, S.G., Lu, X., and H.R. Horvitz. (1994). Genetics **137**: 987.

   Huang, L.S., Tzou, P., and P.W. Sternberg. (1994). MBC **5**: 395.

2. Lu, X. and H.R. Horvitz. (1998). Cell **95**: 981.

   C. Ceol and H.R. Horvitz, unpublished observations.

3. Solari, F. and J. Ahringer. (2000). Current Biology **10**: 223.

   Solari, F., Bateman, A., and J. Ahringer. (1999) Development **126**: 2483.

4. Walhout, A.J.M., et al. (2000). Science **287**: 116.

# CHARACTERIZATION OF NEW GENES REQUIRED FOR THE NEGATIVE REGULATION OF VULVAL INDUCTION, INCLUDING THE NEW CLASS B SYNMUV GENE *LIN-61*

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The regulation of vulval induction in *C. elegans* provides a useful model system for the study of genetic and molecular mechanisms involved in signal transduction, transcriptional repression and cell-fate determination. Previous work has defined two classes of genes (A and B) that act redundantly to negatively regulate the adoption of vulval cell fates by vulval precursor cells. The elimination of a member of both gene classes results in a synthetic multivulva (synMuv) phenotype. The class B synMuv genes include genes similar to members of the mammalian Rb signaling pathway. By contrast, determination of the molecular nature of the known class A synMuv genes has thus far yielded relatively little insight into the mechanism of their function (see abstract by Davison and Horvitz).

The mutation *sy223* (a generous gift from Paul Sternberg) defines a new class B synMuv gene, *lin-61*. We have found that this gene, located on LG I, encodes a protein weakly similar to *Drosophila* Polycomb group members, and more strongly similar to predicted proteins of unknown function from *C. elegans* and human. Three alleles of *lin-61* are currently under study, each of which contains a point mutation in the C-terminus of the putative protein product. We are currently seeking a null allele of this gene. RNAi of *lin-61* produces embryonic lethality both in a synMuv A (*lin-15A(n767*)*)* and in a wild-type background, suggesting that the gene has an essential function in addition to its role in the inhibition of vulval induction.

In addition to the analysis of *lin*-61, we are also attempting to identify new class A synMuv genes. Previous screens to isolate class A genes would not have recovered synMuv mutations that also cause sterility or maternal-effect lethality. For this reason, we are now performing a clonal screen to seek alleles of new class A synMuv genes as well as new alleles of known genes. Beginning with a strain containing a strong *lin-15B* allele, *n744,* we have screened more than 10,000 genomes and isolated more than 20 mutants that display a Muv phenotype. Complementation testing and mapping of these mutants is currently underway.

# *C. ELEGANS ERA*, HOMOLOG OF AN *E. COLI* CELL CYCLE GENE?

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Members of the GTPase superfamily are found in organisms ranging from bacteria to humans. These proteins function as molecular switches, regulating a variety of cellular processes including signal transduction, cell cycle regulation and protein translocation. The *E. coli* GTPase, ERA is an essential protein required for normal progression through the cell cycle1. Reduction of *era* function results in cell cycle arrest after chromosome partitioning, but prior to cytokinesis. Sequence comparisons suggest that ERA and its homologs in other bacteria, *C.elegans*, mouse and human form a new subfamily of GTPases. These proteins show extensive homology both in the GTPase domain and in a C-terminal RNA binding domain.

In searches of the *C.elegans* database with the conserved carboxy-terminal region of *E. coli* ERA we identified a single homolog, E02H1.2, which we will refer to as Ce-ERA-1. Ce-ERA-1, is 31% identical (49% similar) to *E.coli* ERA in the GTPase domain and 19% identical (43% similar) to *E.coli* ERA in the C-terminal region. RT-PCR experiments generated an SL2 spliced cDNA with an exon structure matching that predicted by the genome project. The first gene in this operon E02H1.1 is homologous to the bacterial rRNA methyltransferase ksgA, which interacts genetically with *E. coli era*. Thus there might be a functional relationship between the two genes in the *C. elegans* operon. RNA-mediated interference of E02H1.1 results in a severe developmental delay and larval lethality. The function of *Ce-era-1* is unknown. Attempts to disrupt *Ce-era-1* function by RNA-mediated interference did not generate any obvious phenotype. A null allele of *Ce-era-1* was recently identified in a PCR screen (R. Ranganathan and H.R. Horvitz, personal communication). Characterization of this allele will be presented.

1. Britton, R.A., Powell, B.S., Dasgupta, S., Sun, Q., Margolin, W., Lupski, J.R., and Court, D.L.(1998) Cell cycle arrest in Era GTPase mutants: a potential growth regulated checkpoint in *Escherichia coli. Molecular Microbiology* 27(4), 739-750.

# GENETIC AND MOLECULAR ANALYSIS OF POLYGLUTAMINE TOXICITY IN *C. ELEGANS*

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Huntington's Disease (HD) is one of 8 dominant human neurodegenerative disorders caused by expansion of a polyglutamine (polyQ) tract. Generally, more than 40 consecutive glutamine residues leads to disease. Age of onset is dependent on the length of the polyQ tract. The mechanisms underlying polyQ toxicity are unclear. To gain insight in this toxicity, we are using a *C. elegans* model for polyQ-mediated cellular dysfunction. N-terminal fragments of the HD protein huntingtin (Htn) containing polyQ tracts of 2, 23, 95 or 150 residues are expressed in the ASH sensory neurons (PNAS 96, 179-184, 1999). Expression of Htn-Q150, but not Htn-Q2, Htn-Q23 or Htn-Q95, caused age dependent ASH degeneration without ASH cell death in 8-day-old animals (as measured in dye-filling assays and immunohistochemical experiments). This degeneration was partially dependent on *ced-3* caspase function suggesting the involvement of the apoptotic cell death pathway.

We undertook an F2 EMS screen for mutations that lead to enhanced polyQ toxicity in the ASH neurons. The corresponding genes may be involved in turn-over and stability of Htn-Q150 or form part of the unknown cellular pathways perturbed by expanded polyQ segments. Screening 30,000 F2 animals yielded 7 independent mutations:*rt13*, *rt58*, *rt61*, *rt62*, *rt64*, *rt65* and *rt67*. In *rt13* mutant animals expressing Htn-Q150 about 80% of the ASH neurons are absent in 3 day old animals. But, *rt13* does not generally impair ASH survival nor function. In the absence of Htn-Q150, no ASH cell death was observed in *rt13* in 8 days old animals. *rt13* animals respond normally to two stimuli detected by ASH: nose-touch and high osmolarity. Importantly, neither expression of Htn-Q2 nor a toxic control protein devoid of polyglutamines induced ASH cell death in 3 or 8 day old *rt13* animals. The Htn-Q150 mediated cell death in *rt13* animals is again partially dependent on *ced-3* caspase function.

Phenotypic and complementation analysis of the 6 remaining mutations is under way. Identification of the molecular nature of *rt13* and other mutations might provide important insights in the molecular nature of polyQ pathogenesis and lead to novel therapeutic approaches for these neurodegenerative disorders.

# INVESTIGATION OF *CUL-2* E3 COMPLEXES AND INTERACTION BETWEEN *CUL-2* AND *VHL*

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The ubiquitin-proteasome proteolytic pathway plays a critical role in the regulation of cell cycle progression. Ubiquitin ligases (E3s) function in this pathway to target substrates for degradation. There are two major types of E3 complexes, the APC and Cullin/Ring finger complexes. The cullin/Ring finger complexes contain a cullin, a RBX1 ring finger protein, a SKP1-like molecule, and a substrate-binding adaptor. The first described cullin member, CUL-1, functions to allow the cell cycle exit. Our recent study (Feng et al, *Nature cell Biology*, Vol.1(8), 1999) described the function of another member of the cullin family, CUL-*2*. CUL-2 is a positive cell cycle regulator that is required for the G1 to S phase transition, functioning in part by negatively regulating CKI-1 (a cyclin-dependent kinase inhibitor). Surprisingly, CUL-2 is also required for mitotic chromosome condensation. In humans, CUL-2 was found in an E3 complex with the von Hippel-Lindau (VHL) tumor suppressor, Elongin C (a SKP1 like protein), Elongin B (a ubiquitin like protein), and RBX1. This CUL-2/VCB complex functions to degrade hypoxia inducible transcription factor 1a. In order to gain insights into the composition of the CUL-2 E3 complexes that regulate CKI-1 degradation and chromosome condensation, we took advantage of the RNAi technique to look for genes which phenocopy *cul-2* after RNAi. RNAi of Elongin C gave phenotypes similar to *cul-2.* We think that it is likely that Elongin C functions with CUL-2 in a complex to regulate the cell cycle. However, VHL does not appear to function with CUL-2 in cell cycle regulation since the inactivation of this gene either by RNAi or deletion mutant (obtained by Gary Moulder in Robert Barstead's lab) doesn't phenocopy *cul-2*. In addition, in humans, a removal of VHL decreases the level of the CKI p27, while in contrast overexpression of VHL increases p27. We propose that there are adaptor proteins other than VHL in CUL-2 complexes that are required for cell cycle regulation. VHL may sequester CUL-2 from other CUL-2 complexes containing different adaptor molecules. The absence of VHL may lead to an increase of these CUL-2 complexes, causing rapid degradation of CKIs, thereby contributing to more cell division and tumor formation.

# BOY IS MY BURSA RED: AUTOMATED DETECTION AND SORTING OF FLUORESCENT STAINED *C. ELEGANS* MALES FROM A MIXED POPULATION

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We have developed a male-specific, fluorescent stain that can be used in conjunction with the LPD $1000_{NF}$ (Union Biometrica, Inc.) to purify male worms from a mixed population. The LPD $1000_{NF}$ is a high throughput system that analyzes and sorts *C. elegans* using parameters of size and fluorescence. The stain is comprised of a specific ligand to the male copulatory bursa. The ligand is coupled to a dye that is excited by a standard 633nm HeNe laser and emits in the far red spectra. A *him-8* strain, CB1489, was used in order to provide sufficient males for development of the reagents used in this study. Mixed populations treated with the stain were analyzed and sorted on a LPD $1000_{NF}$ system. A sort region was set on a size versus fluorescence two-parameter display and the brightest worms were dispensed. The sorted males were successfully used in mating studies and the stain in no way inhibited copulation.

The sorted male worms were mated with a strain which has high-level GFP expression in the pharynx, in order to demonstrate that the stain did not inhibit copulation. The progeny were examined using the LPD $1000_{NF}$ system. Three populations were observed on size versus fluorescence, two-parameter plots. One population had a fluorescence level equal to that of the GFP expressing strain; a second population showed no GFP expression, indicating that mating had indeed occurred; and the third population showed an intermediate level of fluorescence. The intermediate population consisted of heterozygous worms that had only one chromosomal copy of GFP.

# CHARACTERIZING *LIN-5* INTERACTIONS IDENTIFIED IN THE TWO-HYBRID SYSTEM

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To better understand the process of mitosis, we have focused on the *lin-5* gene and its roles in chromosome segregation and spindle orientation. We have shown that *lin-5* encodes a novel coiled-coil component of the spindle apparatus and hypothesize that LIN-5 is required for spindle force generation. However, the mechanisms by which *lin-5* functions and the proteins with which it interacts remain unknown. To address these questions, we are characterizing LIN-5 interactors identified in a two-hybrid screen using *in vivo* selected mutations.

In the two-hybrid screen, LIN-5 was shown to interact with itself and other coiled-coil proteins, including ZC8.4. The ZC8.4 clone encodes a homolog of PUMA1, which localizes to the *P. univalens* mitotic apparatus. We mapped the interaction domains of LIN-5 using deletion constructs. We found that the coiled-coil domain is sufficient to promote homodimerization, but the C terminus is required for most of the other interactions. Since *lin-5* mutations may prevent key interactions, we introduced two mutations previously identified *in vivo*, *ev571ts* and *e1457*, to determine whether they disrupted any two-hybrid interactions. The *e1457* allele was of interest because it behaves as a genetic null even though it only contains a single missense mutation in the 5' end of the ORF. The *e1457* product, though expressed in yeast, failed to bind any of the interactors. The *ev571ts* product contains a 3 amino acid insertion in the coiled-coil domain and localizes properly at the nonpermissive temperature in *C. elegans*. In the two-hybrid system, the *ev571ts* construct showed decreases in four of the binding interactions, including ZC8.4.

In a previous screen, we isolated 68 intragenic suppressors of the *ev571ts* mutation. In 8 of the 10 suppressors examined, we identified a compensatory mutation upstream of the *ev571ts* mutation. We introduced one suppressor mutation into the two-hybrid system and found that it restored binding particularly to the ZC8.4 clone, suggesting that ZC8.4 binding may be important for *lin-5* function. We are further characterizing the interactors by examining their localization and RNAi phenotypes.

# MIP-4 AFFECTS EMBRYONIC POLARITY IN *C. ELEGANS*

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We are interested in understanding how polarity and patterning is carried out in the early *C. elegans* embryo and have begun studies aimed at identifying proteins that interact with MEX-3. MEX-3 is a cytoplasmic KH domain protein that is necessary to repress *pal-1* translation in oocytes and anterior blastomeres, thereby restricting PAL-1 expression to the posterior blastomeres. By genetic analysis, we have learned that although MEX-3 is required to repress *pal-1* translation, it is not sufficient. To identify additional proteins that pattern PAL-1 expression, we have identified MEX-3 interacting proteins (MIPs) in yeast two-hybrid screens[1]. A MEX-3 interacting protein, called MIP-4, was identified multiple times in independent screens using different MEX-3 domains as bait. MIP-4 contains an RNA recognition motif and, by RNAi analysis, is required to repress PAL-1 expression in anterior blastomeres. MIP-4 (RNAi) embryos also show a variety of other phenotypes consistent with disruption of embryonic polarity. These polarity defects indicate that the requirement for MIP-4 to repress PAL-1 expression is likely a consequence of an earlier requirement for MIP-4 to establish or maintain embryonic polarity.

[1] NN Huang, et al. Identification of proteins that may interact with MEX-3 to regulate *pal-1* translation. 1999 International Worm Meeting, abstract 418.

# TWO SCREENS FOR NEW GENES INVOLVED IN PROGRAMMED CELL DEATH

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Many genes involved in programmed cell death in *C. elegans* have been identified by screening for mutations that allow survival of cells that are normally destined to die. The opposite approach, screening for mutations that result in the deaths of cells, has been comparatively unexplored, in part because of a lack of efficient methods to identify such mutations. A *lin-11::gfp* reporter (see abstract by Cameron and Horvitz) expresses in Pn.aap cells. In wild-type animals, only the six Pn.aap cells of the P3-P8 lineages survive and express GFP, while in mutants defective for cell killing all 12 Pn.aap cells survive and express GFP. A strain carrying this reporter allows one to monitor the survival of cells using a dissecting microscope rather than Nomarski optics, greatly facilitating screening. In this way, I am performing a *ced-4* suppression screen by looking for a reduction in the number GFP-positive cells and thus an increase in cell death in the ventral cords of partial loss-of-function *ced-4* mutants.

To identify substrates of the caspase CED-3, which is required for programmed cell death, I am using a modified two-hybrid screen. An active caspase is composed of two subunits proteolytically derived from an inactive procaspase. The active site of the caspase contains a cysteine, which when mutated prevents the cleavage activity of the enzyme. In this screen the two active subunits of the CED-3 caspase are expressed separately, with the active-site cysteine changed to a serine. This amino acid substitution should permit an interaction with substrates but prevent their cleavage, allowing for the identification of interacting proteins. Other groups have identified substrates of mammalian caspases using this approach (1, 2). Candidates from the screen will be analyzed by testing *in vitro* cleavage by CED-3 and assayed by RNAi to examine their roles in programmed cell death.

1. Van Criekinge, W., van Gurp, M., Decoster, E., Schotte, P., Van de Craen, M., Fiers, W., Vandenabeele, P., and Beyaert, R. *Analytical Biochemistry*. 263, 62-66 (1998).

2. Kamada, S, Kusano, H., Fujita, H., Ohtsu, M., Koya, R. C., Kuzumaki, N., and Tsujimoto, Y. *Proc. Natl. Acad. Sci. USA*. 95, 8532-8537 (1998).

# NUCLEAR RECEPTOR GENES IN FREE-LIVING AND PARASITIC NEMATODES

## Puneet Gandotra[1], Megan O'Donnell[1], Claude V. Maina[2], Kirsten Crossgrove[1]

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The *C. elegans* genome project facilitated the discovery of the extensive representation of genes encoding members of the nuclear receptor (NR) superfamily of transcription factor proteins. The specific role of the vast majority of these genes in *C. elegans* remains unknown. NRs are required for developmental processes in a wide variety of multicellular organisms. One extensively studied example of the developmental role of NRs is in the control of molting and metamorphosis in *Drosophila*. Nematodes also undergo a series of molts during development and loss of function of at least one *C. elegans* NR (nhr-23) has been associated with molting defects. Nhr-23 is one of a number of NRs identified in *C. elegans* that have putative homologs in *Drosophila*. Another gene, *nhr-85*, was identified by sequence homology (Ann Sluder, personal communication) as a putative homolog of the *Drosophila E75* gene. In *Drosophila*, *E75* is induced by the molting hormone 20-OH ecdysone. 20-OH ecdysone can induce premature molting from the third to the fourth larval stage of the parasitic nematode *Dirofilaria immitis* (the causative agent of dog heartworm disease), suggesting that molting in this parasitic nematode may be under hormonal control similar to *Drosophila*. We have identified *dinhr-6*, a putative *D. immitis* homolog of the *Drosophila E75* gene. The protein encoded by *dinhr*-6, is 83% identical to E75A in the DNA binding domain. Northern blot analysis suggests that *dinhr-6* encodes multiple isoforms and is female-specific in adults. We are currently cloning the *dinhr6* homolog from the human parasite *Brugia malayi* to expand our understanding of *dinhr6* structure and function. However, working in parasite systems has certain limitations, particularly with the feasibility of genetic manipulation. Therefore, we are also attempting to characterize the structure and expression of the *C. elegans nhr-85* gene with the eventual goal of using transgenic analysis and dsRNAi to understand the function of the *nhr-85* gene. We discuss the potential of using *C. elegans* to help understand parasitic nematode development.

# SEMAPHORIN 1A AND 1B MUTANTS EXHIBIT MALE TAIL MORPHOLOGICAL DEFECTS.

## Val E. Ginzburg, Peter J. Roy, Joseph Culotti

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The semaphorin protein family has been implicated in axon guidance and morphogenesis in vertebrates and invertebrates, as described by Kolodkin et al., Cell 75, 1389-1399, 1993. Analysis of the *C.elegans* genome has revealed three genes that encode semaphorins designated Sema-1a, Sema-1b and Sema-2a according to their predicted protein structure. Recently, P. Roy has isolated and characterized mutants of the secreted semaphorin Sema-2a and found it has mild axon guidance defects and epidermal cell migration defects (P. Roy et., al 2000). To understand what role transmembrane semaphorins play in the development of *C.elegans* we decided to utilize reverse genetics to isolate Sema-1a and Sema-1b mutants.

With mutants of all three semaphorin genes, we can study their functions using classical genetics, and moreover we can look for downstream components by designing various enhancer and suppressor screens. First, we have constructed a deletion library of approximately 1.7 million haploid genome, using a standard mutagenesis protocol. We have designed several sets of nested PCR primers for Sema-1a and Sema-1b genes. Screening of the deletion library found several candidates for deletions in the genes and we isolated two alleles of Sema-1a (ev715, ev708) and one allele of Sema-1b (ev709). The alleles were sequenced and revealed that ev715 is a deletion of ~300bp that puts the next three exons out-of-frame, ev708 is a first intron deletion of ~800bp and ev709 is a deletion of two exons. We found that backcrossed versions of the mutants exhibit several abnormalities, which we are further characterizing. Ectopic male tail ray1 (R1) and swollen head are two common phenotypes for both genes. The penetrance of an ectopic R1 in ev715 is 41% (n=201), and in ev709 is 17% (n=170). The penetrance of this unique phenotype is enhanced in a Sema-1a, Sema-1b double mutant (ev715 ev709) to 91% (n=202).

Currently, we are attempting to rescue these phenotypes with the cloned genes. We are also further characterizing single, double and triple semaphorin mutants.

P>P>

# CONSERVED NUCLEAR RECEPTORS FUNCTION DURING THE *C. ELEGANS* MOLTING CYCLE

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We are investigating the role of NR genes in the regulation of the *C. elegans* molting cycle. The *C. elegans* genome encodes fifteen nuclear receptor (NR) genes that are conserved in sequence in both arthropods and vertebrates. Among these are four NR genes that are orthologs of *Drosophila* NRs that function in the ecdysone cascade. *nhr-25, nhr-41,* and *nhr-85* are orthologs of *Drosophila Ftz-F1, DHR78,* and *E75*, respectively. Initial RNAi and/or GFP expression data implicate these genes in the regulation of nematode molting. Another gene, *nhr-23,* (ortholog of *Drosophila DHR3*), has been shown by others to be required for molting as well (Kostrouchova et al, *Development* 125: pg. 1617-1626 (1998)).

*nhr-25(RNAi)* and *nhr-41(RNAi)* larvae have defects in shedding molted cuticle. *nhr-25* also functions during embryogenesis and development of the male tail and somatic gonad while the function of *nhr-41* appears to be specific to the molting cycle. *nhr-41(RNAi)* larvae display a prolonged lethargus and the molting process can sometimes take many hours to complete. Once animals complete the molt they usually have similar difficulties at the next molt. L1 lethality is also observed in *nhr-41* RNAi experiments and appears to result from a complete block in the molting process. GFP expression data indicates that *nhr-41* is expressed in a variety of cell types while *nhr-25* and *nhr-85* are expressed exclusively in the epidermis and specialized epithelia.

We are currently performing a detailed RNAi and gene expression analysis to further dissect the roles of these NRs during the molting cycle.

# VISUALIZING SYNAPSES IN THE MOTOR NEURON CIRCUIT

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A major emphasis of this laboratory is to unravel the mechanism of synaptic specificity. The UNC-4 homeodomain protein regulates the pattern of synaptic input to one class of motor neurons in the *C. elegans* ventral nerve cord. *unc-4* mutant animals are unable to execute backward locomotion because the usual inputs to VA motor neurons are replaced with connections normally reserved for VB motor neurons. The *unc-4* wiring defect was initially detected by John White and colleagues by EM reconstruction of serial sections. We are now developing an alternative approach to detect neuron-specific synapses in living animals in the confocal microscope. The idea is to label presynaptic and postsynaptic proteins with different colored GFPs and then to use specific promoters to drive expression of these GFP-tagged markers in neurons that synapse with each other. The Zeiss LSM 510 confocal microscope provides argon laser lines that are ideal for discrete excitation of CFP (458 nm) and YFP (514 nm). An authentic synapse in the *C. elegans* motor neuron circuit should appear as superimposed CFP and YFP "spots" in the ventral nerve cord. We have constructed YFP-tagged synaptobrevin, a membrane component of neurotransmitter vesicles, and used the *nmr-1* promoter to drive expression in AVA and AVD command interneurons. These "A-type" interneurons extend axons into the ventral nerve cord to synapse with VA motor neurons. Animals expressing the *nmr-1*-VAMP-YFP transgene show punctate YFP spots (pseudocolored "Red") which correspond to presumptive presynaptic zones in the ventral nerve cord. We are now using the *unc-4* promoter to test candidate proteins for CFP-labeling ("Green") the postsynaptic density in the VA motor neurons. Authentic synapses between A-type command interneurons and VA motor neurons should be detected as superimposed Red and Green spots. Our goal is to exploit the known wiring defect in *unc-4* mutants to confirm the reliability of this approach and then to utilize this strategy to define wiring defects in other mutants that presumptively alter synaptic specificity in the *C. elegans* motor neuron circuit.

# CHARACTERIZATION OF THE *PVL-2/MIG-14/MOM-3* LOCUS, WHICH FUNCTIONS IN MULTIPLE WNT SIGNALING PROCESSES IN *C. ELEGANS*

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During early larval life, P3.p -- P8.p express the Hox gene *lin-39* and become the vulval precursor cells (VPCs). Later, the VPCs adopt one of three cell fates (1°, 2°, 3°) in response to activation of Ras and Notch signaling pathways. This is true in 50% of animals; in the remaining 50% P3.p loses its VPC identity and fuses with the hypodermis without dividing (the Fused or F fate). Mutations in *bar-1* were found in a screen for mutants with a Protruding Vulva (Pvl) phenotype. In *bar-1* mutants P4.p -- P8.p can also adopt the F fate and lose their VPC identity, and VPCs that maintain their identity can adopt incorrect cell fates (3° instead of 1° or 2°). *bar-1* encodes a b-catenin/Armadillo-related protein, one of three in *C. elegans*. These proteins function in Wnt signaling pathways as transcription factors whose stability is regulated by Wnt signals. In *bar-1* mutants, VPCs that adopt the F fate have lost *lin-39* expression, indicating *bar-1* is required for *lin-39* regulation. This phenotype is enhanced by a mutation in *let-23*, indicating that the Ras and Wnt pathways coordinately regulate *lin-39*. *bar-1* mutants also have defects in the migration of the progeny of the neuroblast $Q_L$, and in fate determination by P12. In both of these cases *bar-1* appears to function in a Wnt pathway regulating Hox gene expression.

Several other loci identified in the Pvl screen cause vulval defects like *bar-1*. One of these is *pvl-2*, which causes defects in VPC fate specification, $Q_L$ migration and P12 fate specification, suggesting *pvl-2* may function in Wnt signaling with *bar-1*. Consistent with this, we found that the *pvl-2* F fate phenotype is also enhanced by loss of Ras signaling. *pvl-2* maps to LG IIR, and fails to complement *mig-14*, identified by the Kenyon lab in a screen for $Q_L$ migration mutants, and fails to complement *mom-3*, identified by Bruce Bowerman in a screen for mutants affecting mesoderm/endoderm specification. Both of these processes are known to function via Wnt signaling. Efforts to map and clone *pvl-2/mig-14/mom-3* are underway. There are no know Wnt pathway components or regulators in the interval where this locus lies, suggesting it may encode a novel Wnt pathway component in *C. elegans*.

# ISOFORM-SPECIFIC FUNCTIONS OF THE EGL-15 FGFR

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The *C. elegans* FGFR, EGL-15, is involved in both an essential function and a cell migration event. A larval arrest (Let) phenotype results from the complete loss of EGL-15, while certain hypomorphic alleles confer a scrawny body morphology which may represent a weaker manifestation of the Let phenotype. EGL-15 is also required for the sex myoblasts (SMs) to migrate in response to the FGF chemoattractant EGL-17. Failure of this migration to occur properly results in an egg-laying-defective (Egl) phenotype. Most *egl-15* alleles are pleiotropic; four alleles, however, exclusively compromise SM migration and lead solely to an Egl defect. Recent work has identified the molecular basis for the specificity of these alleles.

Alternative splicing is responsible for generating multiple forms of mammalian FGFRs as well as EGL-15. EGL-15 and the large isoforms of the mammalian FGFRs contain three IG domains, alignment of which reveals an EGL-15-specific insert between IG domains I and II. Two isoforms of EGL-15 that differ in this insert region appear to be generated by selecting one of two alternative exons: EGL-15(5a) is required for SM migration in response to EGL-17, but is not required for viability; EGL-15(5b) is sufficient for viability but not for responsiveness to the EGL-17 chemoattractant. Two of the four EGL-15 alleles specifically affecting SM migration (*n484*, *n1458*) contain nonsense mutations within exon 5a, and a third allele (*ay1*) alters the splice acceptor site at the beginning of exon 5a. These mutations represent putative null alleles of the EGL-15(5a) isoform.

*egl-15(n1457)* is also specifically defective for SM migration. This allele contains a premature stop mutation predicted to delete the carboxy-terminal domain (CTD) of EGL-15, just downstream of the kinase domain. The phenotype of this mutant strongly suggests an essential role of the CTD in EGL-15-mediated chemotaxis. We are investigating the structural element(s) within this domain that are required for chemotaxis.

EGL-15 antibodies will be used to determine the *in vivo* expression pattern of the 5a and 5b isoforms. Also, to determine where *egl-15* function is required for viability and SM migration, as well as the effect of complete loss of *egl-15* activity on SM migration, we are analyzing mosaic animals obtained from an *egl-15* null strain bearing an *egl-15*(+)-containing array.

# *CAENORHABDITIS ELEGANS*: A MODEL FOR STUDYING THE VIRULENCE OF *BURKHOLDERIA CEPACIA*

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*Burkholderia cepacia* and *Pseudomonas aeruginosa* are opportunistic pathogens that infect the lungs of cystic fibrosis patients. Animal models developed for studying the pathogenesis and virulence factors of these organisms are less than optimal and usually require large numbers of mice compromised in some way. A recently described model for testing the virulence of *P. aeruginosa* in the nematode *Caenorhabditis elegans* prompted us to test its suitability for studies with *B. cepacia*. *B. cepacia* strain Pc224c, a clinical isolate that produces phospholipase C, was grown in tryptic soy broth to early-, mid-, or late-log growth phase. We spread $1x10^8$ cfu from each phase on nematode growth agar and allowed the plate to dry; 20 nematodes (both L4 stages and adults) were placed on each of three plates; controls were placed on nematode growth agar with *Escherichia coli* as a food source. Dead and live nematodes were recorded every 6 h for approximately 84 h or until all the nematodes on the experimental plates had died. The time required to kill 50% of the nematodes ($LT_{50}$) was calculated (Systat 9.0). The $LT_{50}$ for the nematodes placed on late-log phase *B. cepacia* was 60 h; the $LT_{50}$ tended to increase when similar numbers of mid-log and early-log phase organisms were used. Death of the nematodes was usually due to matricide - the worms were unable to lay their eggs, which hatched inside them and literally consumed the parents from within. This model should prove useful for the study of potential virulence factors of *B. cepacia*.

# *CED-12* IS REQUIRED FOR PHAGOCYTOSIS AND CELL MIGRATION

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The CrkII/DOCK180/Rac pathway is required for cell process extension and cell migration. The evidence for this involvement has recently been strengthened by recent papers showing that *ced-2*, *ced-5*, and *ced-10*, genes required for the efficient removal of cell corpses and for correct DTC migration, encode CrkII, DOCK180 and Rac, respectively[1,2]. We have identified a fourth player in this group of engulfment genes, *ced-12*.

The two known alleles, *oz167* and *bz187*, are both recessive loss-of-function mutations. Like mutations in all other engulfment genes except *ced-1* alleles, *ced-12(lf)* persistent cell deaths are maternally rescued in the embryo but not in the adult germ line. *ced-12(lf)* mutants exhibit both a strong corpse persistence phenotype (for both programmed cell death an necrotic cell death), as well as the aberrant gonadal outgrowth displayed when other genes in this class are defective. The engulfment defect is suppressed by *ced-3* and *ced-4*, but the DTC migration defect is not, suggesting *ced-12*'s involvement in multiple activities involving cytoplasmic reorganization. c*ed-12* lies on chromosome I between *mec-8* and *lin-11*. Cloning information will be presented at the meeting.

[1]Wu and Horvitz (1998). Nature *392*: 501-504.

[2]Reddien and Horvitz (2000). Nature Cell Biology *2*: 131-136.

# ISOLATION AND CHARACTERIZATION OF RECESSIVE SUPPRESSORS OF *AGE-1*

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An insulin-like signaling pathway mediated by DAF-2, an insulin receptor homolog, regulates dauer arrest and longevity in *C.elegans*. *age-1* encodes a phosphatidylinositol 3-kinase homolog, which has been implicated as a direct target of *daf-2* signaling. Mutations in *age-1* are maternal effect and cause animals to constitutively arrest as dauer larvae. However, maternally rescued *age-1* mutants that lack zygotic *age-1* activity exhibit a two-fold increase in adult lifespan. *daf-2/age-1* signaling negatively regulates *daf-16*, a Forkhead-related transcription factor. *daf-16* mutants are dauer defective and completely suppress the dauer constitutive and long-lived phenotypes of *age-1* and *daf-2* null mutants.

To identify other components of the *daf-2/*insulin-like signaling pathway, specifically downstream components activated by *age-1* PI-3 kinase signaling, we screened for recessive suppressors of *age-1(mg109)* dauer arrest. *mg109* has the same phenotype as a nonsense allele; however, the point mutation it carries results in a complete, albeit mutated, protein. We screened 23,000 genomes and isolated 14 recessive suppressors of *age-1(mg109)*. Of these 14 *age-1(mg109)* suppressors, 8 have been roughly mapped. Four of these mutations lie on LGV, while the remaining four mutations map to LGIII. Interestingly, one of the mutations on LGV suppresses both the dauer constitutive and the long-lived phenotypes of *age-1(mg109).* The remaining three mutations rescue only the dauer arrest phenotype of *age-1(mg109)*. All four of the LGIII-linked mutations are allelic and fail to suppress the long-lived phenotype of *age-1.* Of these mutations, one has been further characterized and exhibits no obvious phenotype on its own. Experiments underway include complementation studies, finer mapping, and epistasis analysis.

# REGULATION OF CELL-CELL ASSOCIATIONS IN C. ELEGANS

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Morphogenesis of multicellular structures involves endowment of cells with individual identities that lead to correct cell-cell associations. We are studying this process in the cells that give rise to male sensory rays. During ray formation the three cells that will form a single ray become closely associated and at the same time separate from cells of neighboring rays. This separation does not occur in certain mutants, leading to formation of a fused ray. Two such mutants are *mab-26* and *mab-20*. *mab-26* encodes a GPI-linked ephrin, and *mab-20* encodes a predicted secreted Semaphorin.

We have found that a *mab-26* reporter is expressed by cells of alternating rays: cells of rays 1 and 3 seldom express the reporter, whereas cells of rays 2, 4 and 6 express at a high frequency. This pattern is similar to the expression of certain vertebrate ephrins in even-numbered rhombomeres only. Odd-numbered rhombomeres express the EphA4 receptor and therefore do not mix with ephrin-expressing cells. By analogy, *mab-26* may be expressed in a complementary pattern with an unidentified receptor and thus prevent associations between cells of neighboring rays. In contrast, *mab-20* reporters are expressed in cells of all rays, as also shown by Roy et al (*Dev.* 127, 755-767). Given the repulsive effect of Semaphorins on axon growth cones, *mab-20* may act constitutively to inhibit growth of processes from cells of all rays, thereby preventing formation of ectopic cell contacts.

We have found that the expression patterns of *mab-26* and *mab-20* reporters are unchanged in the ray fusion mutants *mab-18*, *mab-21*, and *sma-6*, suggesting that these mutations affect other components of *mab-20* or *mab-26* pathways or an additional, independent pathway. *egl-5* mutants have a normal *mab-20* expression pattern but appear to ectopically express the *mab-26* reporter in cells of ray 3. We are studying whether this alteration is the cause of fusion of rays 2, 3 and 4 in *egl-5* mutants.

We are using Nomarski and a reporter for the MH27 antigen to characterize ray cell positions and cell-cell contacts. In *mab-26* and *mab-20* mutants, ray cells that are normally separate form and maintain adherens junctions beginning at the Rn.aa stage. *egl-5* and *sma-6* mutants have similar defects, as well as significant changes in overall ray cell positions.

# IMPROVED TISSUE PRESERVATION USING METAL MIRROR FIXATION OR HIGH PRESSURE FREEZING FOR TEM

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Recent reports (see below) showed that high pressure freezing (HPF) followed by freeze substitution is superior to chemical immersion fixation for *C. elegans.* HPF captures a more "life-like" view of the worm's ultrastructure. We compared HPF and a related technique, rapid freezing onto a metal mirror (MMF). For MMF, live animals on a small piece of filter paper are plunged against a metal mirror in liquid nitrogen. Freezing damage is often a problem, but some animals seem to be well frozen throughout. For HPF, we have tried two methods to concentrate live animals into a small metal planchette (see Lavin and McDonald ref's below). Further processing is the same for both methods. While holding at very low temperatures, the samples are freeze substituted into 1% osmium tetroxide in acetone, then embedded into plastic resin and cured for thin sectioning.

By TEM fast-frozen worms reveal excellent views of membrane events and organelles. For instance, we see active endocytosis events that are not captured by chemical fixation. The microtubule network is better preserved and the basal laminae look strikingly different. Sample images are shown at www.aecom.yu.edu/wormem/new.html.

HPF and MMF also hold promise for high resolution immunoEM. By reducing the osmium content and adding a dilute aldehyde fixative to the freeze substitution medium, we can better preserve structure than by our microwave technique (Paupard et al., submitted). We have successfully localized epitopes in thin sections from HPF samples. We are conducting HPF trials with Stan Erlandson and Ya Chen at the U. of Minnesota. MMF equipment is available here at Einstein and elsewhere. HPF machines are available to outside users in Madison, Berkeley, Minneapolis, and Albany. As our skills improve, we will offer such services to the *C. elegans* community.

For further information on HPF, we recommend the following sources:

Colleen Lavin's website at www.geology.wisc.edu/~uwmr/coating.html

Martin Muller's website at www.em.biol.ethz.ch/

Kent McDonald, Methods in Molecular Biology, vol 117, pp. 77-97 (Humana Press) 1999.

# A PROTOCOL FOR THE USE OF ELECTRON MICROSCOPY IN STUDIES OF AXONAL PATHFINDING

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We have developed a protocol using electron microscopy for the analysis of axonal pathfinding in the ventral and dorsal nerve cords of *C. elegans*. We standardized our preparation technique, as described below, to compare animals of different genotypes to the wild type. For each genotype we embedded 30-40 animals, of which 10 animals were sectioned. We photographed the ribbons of sections and the individual worms at low magnification both to establish the orientation of the nerve cords (right, left, dorsal, ventral) and to determine the location of the section within the worm (e.g., at the gonadal reflex). We cut and examined two different points along the anterior-posterior axis of each worm at a distance separated by about 30 microns within a region between the gonadal reflex and the vulva, to compare defects within an animal and to check that our data were consistent within a single worm. From each worm and at each point, both dorsal and ventral cords were photographed at high resolution. Defects in axonal elongation were determined by counting the total number of axons, and defects in fasciculation were quantified by counting the number of axons separated from the main fascicles. Using this method we discovered synthetic nerve cord defects in *ced-10*; *mig-2* double mutants (see abstract by Reddien et al., for further information about these genes). We will present details of our techniques and representative pictures to demonstrate our methodology and results.

# THE *C. ELEGANS* CYSTEINE PROTEASE GENES *CE-CPZ-1* AND *CE-CPL* HAVE A ROLE IN WORM&RSQUO;S DEVELOPMENT

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We are currently characterizing two *C. elegans* cysteine proteases, *Ce-cpz-1* (cathepsin Z) and *Ce-cpl* (cathepsin L) family of enzymes. Previously we have reported that *Ce-cpz-1* dsRNA blocked embryogenesis and was lethal for the injected worm. While *Ce-cpl* dsRNA blocked egg production. To understand the potential distinct function of both enzyme, we initiated to study the temporal and spatial pattern of gene expression by creating several stable lines of transgenic *C. elegans* carrying *Ce-cpz-1: gfp* and *Ce-cpl:gfp* genes. All lines of both genes displayed similar expression patterns in all developmental stages of the worm. The *Ce-cpz-1* expression was observed along the length of the worm throughout worm's development and was particularly strong in the hypodermal region. The expression of *cpz-1* in the hypodermal region suggest that this gene possibly responsible in regulating activities such as molting and cuticle remodeling in *C. elegans*. In addition, to hypodermal region, the expression of *cpz-1* was also observed in the posterior bulb of pharynx. Few clusters of cells also expressed *cpz-1* in the vulval region. Intense *cpl:gfp* expression was observed in the pharyngeal bulb, pharyngeal muscles and hypodermal region throughout the nematode's body in all developmental stages. However, the hypodermal expression was more intense in the head and the tail. The expression of *cpl* in the hypodermal region suggests that the gene may also be involved in regulating activities such as molting, and cuticle remodeling in *C. elegans*. Furthermore, robust expression of *cpl* was also observed in the vulva, eggshell, uterus and spermatheca. Thus, *cpl* may also be involved in the control of egg production, egg laying and the maintenance of tissues that concentrates sperms and mediates fertilization of oocytes. Results of RNAi experiments with *Ce-cpl* have shown that injection of dsRNA prepared from *Ce-cpl* cDNA interfered with normal egg production. It is possible that CPL enzyme is also required for normal hermaphrodite fertility.

# *N3194*, IDENTIFIED IN A SCREEN FOR SUPPRESSORS OF *CED-9(N1950)*, MAY BE REQUIRED FOR CELL VIABILITY

## Brad Hersh, Erika Hartwieg, Bob Horvitz

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To identify genes that interact with the cell-death protector gene *ced-9*, we screened for suppressors of the Ced phenotype of *ced-9*(*n1950*gf) animals by looking for the reappearance of corpses in *ced-1*(*e1735*); *ced-9*(*n1950*) embryos. We identified one candidate, *n3194*, with a large number of refractile bodies in embryos. *n3194* maps to LG*III*, complements *ced-9*(*lf*), and causes a recessive maternal-effect lethal phenotype. Embryos arrest at approximately the two-fold stage of development. Two additional alleles, *n3264* and *zu223*,were isolated by others in unrelated screens. Neither the accumulation of refractile bodies nor the lethality of *n3194* is suppressed by loss-of-function mutations in *ced-3* or *ced-4*, suggesting that this gene acts downstream of or parallel to *ced-3*. We examined the morphology of the refractile bodies in *n3194* mutants using electron microscopy and determined that the bodies more closely resemble degenerative deaths than they do caspase-dependent apoptotic deaths.

*n3194* disrupts the gene R13A5.1, which encodes a predicted protein highly similar to the products of *Drosophila*, human and mouse ESTs of unknown function and with weak similarity to $K^+$ and $Ca^{2+}$ channels. *n3194* and *zu223* are nonsense mutations that likely represent molecular null alleles, and *n3264* is a missense mutation that alters a conserved glycine. An R13A5.1::GFP fusion capable of rescuing *n3194* is localized to the plasma membrane of all cells in early embryos. Later in development the GFP marker becomes restricted to the excretory canal cell and several neurons in the head. We have isolated a full-length R13A5.1 cDNA, which we will use for rescue experiments. In addition, we will test the effect of R13A5.1 overexpression on cell death.

R13A5.1 appears to be required for cell viability during embryogenesis. Its localization in the excretory canal and its similarity to ion channels suggest a possible role in maintaining ionic homeostasis. Loss of R13A5.1 function may activate a degenerative cell-death pathway or may aberrantly activate some but not all of the apoptotic pathway, leading to abnormal corpses. As redistribution of key ions such as $Ca^{2+}$ may play an important role in regulating apoptosis, R13A5.1 may represent a link between ionic balance and programmed cell death.

# CED-9 AND EGL-1 REGULATE THE SUBCELLULAR LOCALIZATION OF CED-4

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EGL-1, CED-9, CED-4, and CED-3, the core components of the *C. elegans* pathway for programmed cell death, have conserved mammalian counterparts with roles in apoptosis. EGL-1 is a cell-death activating BH3-domain protein that binds the cell-death inhibitor CED-9 and negatively regulates CED-9 activity. CED-9 is a member of the Bcl-2 family of cell-death regulators and negatively regulates CED-4 and CED-3. CED-4 is similar to mammalian Apaf-1, which activates caspases, cysteine proteases that effect cell killing. CED-3 is a caspase. Physical interactions among these components have been demonstrated *in vitro*, in yeast and in mammalian cells.

We generated antibodies against CED-9 and CED-4 and used them to determine the expression patterns and subcellular localizations of these two key cell-death regulators. Endogenous CED-9 and CED-4 are localized to mitochondria in wild-type embryos, in which the majority of cells survive. However, in embryos in which most cells have been induced to die, such as embryos homozygous for loss-of-function mutations in the cell-death inhibitor gene *ced-9* or overexpressing EGL-1, CED-4 is no longer seen at mitochondria but instead localizes to nuclear membranes. CED-4 redistribution induced by EGL-1 overexpression can be blocked by a gain-of-function mutation in *ced-9* but not by a loss-of-function mutation in *ced-3*, suggesting that this redistribution precedes the caspase activation step of programmed cell death. A missense mutation (P23L) within the CED-4 protein disrupts cell killing and CED-4 localization, causing CED-4 to be cytoplasmic both in the presence and absence of CED-9. We are now testing whether targeting this missense form of CED-4 to nuclear membranes is sufficient to rescue the Ced phenotype of this mutant. In addition, we are exploring whether CED-4-interacting proteins identified via two-hybrid screening are important for the localization of CED-4 to nuclear membranes.

Our findings suggest that the subcellular localization of CED-4 correlates with the life-or-death decision of a cell. Cells that survive maintain CED-4 localization at mitochondria, apparently through interaction with CED-9. Cells in which programmed cell death has been induced release CED-4 from mitochondria and relocalize CED-4 to nuclear membranes.

# CHARACTERIZATION OF THE 13 *C. ELEGANS* RGS PROTEINS

## Heather Hess, Michael Koelle

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Heterotrimeric G proteins mediate signaling through G protein-coupled receptors and are composed of three subunits (alpha, beta, gamma). The active GTP-bound Ga subunit has a slow intrinsic GTPase activity. A recently-identified class of Regulators of G protein Signaling (RGS) proteins interact with the Ga subunit to stimulate its GTPase activity, thus driving the heterotrimeric G protein to the inactive GDP-bound form.

There are exactly 13 RGS proteins in *C. elegans* and at least 24 in humans. Many of the mammalian RGS proteins act on the same G proteins *in vitro*, thus raising the question of why there are so many RGS proteins if their biochemical properties are so similar. One possibility is that their functions may be more specific *in vivo*.

RGS proteins may also play a role in the poorly understood mechanism of desensitization of G protein signaling, *i.e*. the ability of cells to stop responding to signals after prolonged stimulation. Most pharmaceuticals act through G proteins and desensitization thus limits their usefulness. One idea is that prolonged G protein signaling induces RGS proteins, which in turn shut down signaling.

To address the problems of *in vivo* specificity and desensitization, we are investigating the functions of the 13 *C. elegans* RGS genes. Mutations have already been generated in five RGS genes and the remaining knockout mutations are in process. We have also generated strains overexpressing each RGS gene by introducing multiple copies of the wild-type genes as transgenes. Overexpression of four of the RGS proteins resulted in egg-laying behavior defects, while the remaining eight showed no obvious abnormalities. We determined the expression patterns for 12 of the RGS genes by GFP fusion. Ten RGS proteins are expressed in many neurons, one is expressed only embryonically, one is only in the chemosensory neurons, and the last is being analyzed.

To analyze the functions of the RGS genes we will employ behavioral assays using stimuli that are known to act through G proteins. The knockout mutants and overexpressors will be tested for chemotaxis to various water-soluble and volatile chemoattractants and repellents and for response to neurotransmitters such as dopamine and serotonin. Specifically, we will test for the inability of mutant animals to respond normally to these chemicals and also for their inability to undergo desensitization.

# PHENOTYPIC AND GENETIC ANALYSIS OF *SPE-16*, A GENE REQUIRED FOR *C. ELEGANS* SPERMATOGENESIS

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*C. elegans* spermatogenesis has proven to be a useful model for studying the more general developmental processes of cellular differentiation and cell-cell interactions. Mutants in the *spe-16* gene show sperm-based sterility and pleiotropic defects in spermatogenesis, including abnormal meiotic spindle positioning and orientation, defects in nuclear and chromosomal segregation and inappropriate cytoplasmic polarization and organelle maturation. These defects may be more severe in hermaphrodite-derived sperm than in male-derived sperm. *spe-16* lies on LGIIIR, between the cloned genes *tra-1* and *dpy-18* (Hill, Harfe, Dobbins and L'Hernault, Genetics, in press). We have used polymorphism mapping to narrow the physical interval that must contain the *spe-16(hc54ts)* mutation. To date, *spe-16(hc54ts)* has been mapped to a ~ 27 kb region containing two primary candidate genes, an oxysterol binding protein and a ribosomal protein s6 kinase. Mapping continues with three additional polymorphisms located within the 27 kb interval, and will be followed by sequencing to identify the *spe-16(hc54ts)* mutation.

# *EGS-1* AND *EGS-2* ARE REQUIRED FOR HSN DEATH

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The process of programmed cell death eliminates diverse types of cells during development in *C. elegans*. All of these deaths are regulated by the core apoptotic machinery encoded by the *egl-1*, *ced-9, ced-4* and *ced-3* genes. However very little is known about the genes that regulate the life vs. death decision of specific cells. A subset of cells that are fated to die (*e.g.,* the sisters of the NSM and I2 neurons) requires the function of the cell type-specific genes *ces-1* and *ces-2.* In addition, there are sex-specific cell deaths: in males, the hermaphrodite-specific neurons (HSNs) die by programmed cell death.

In hermaphrodites, dominant gain-of-function mutations in *egl-1(egl-1(n1084))* cause the HSNs to die by programmed cell death resulting in adults that are defective in egg-laying. We took advantage of this strain background as a powerful tool with which to screen for novel mutants that are compromised in their ability to specify death of the HSN. We identified several classes of suppressors and have focused our efforts on two complementation groups, *egs-1 and egs-2*.

Loss-of-function mutations in either *egs-1* or *egs-2* prevent HSN death and restore egg-laying capacity to *egl-1(n1084)* hermaphrodites. They also prevent the death of the HSNs in males. These gene products appear to function in a cell-type specific manner as other cell types that normally undergo programmed cell death, such as the pharyngeal cells and the ventral nerve cord precursors maintain the ability to die in *egs-1* and *egs-2* mutants. To better understand how *egs-1* and *egs-2* mediate HSN apoptosis, we are cloning these two genes using SNP mapping.

In addition to their effect on HSN death, *egs-1* and *egs-2* mutants also display similar pleiotropic phenotypes such as uncoordinated movement and incompletely penetrant rod-larval lethality. Based on the pleiotropic phenotypes exhibited by these mutants, we speculate that *egs-1* and *egs-2* may play a broader role in development and function in the differentiation of multiple neuronal cell types.

# *C. ELEGANS* AT 10 TIMES EARTH GRAVITY

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All organisms on earth evolved at unit gravity (1xG), and thus are probably adapted to function optimally at 1xG. Organisms exposed to increased G levels show changes in physiological processes that compensate for novel stresses, such as increased weight and density-driven sedimentation. These physiological adaptations illustrate the plasticity of organisms exposed to environmental conditions in which they could not possibly have evolved. Investigating the mechanism(s) behind these adaptations may uncover biological pathways that have not previously been identified. During laboratory procedures C. elegans are routinely subjected to transient accelerations of >1000xG, with no obvious ill effects. To establish whether C. elegans display altered physiological processes after exposure to low levels of increased G (2-10xG), we developed a centrifugation protocol using axenic liquid cultures of C. elegans and the short-arm centrifuge at NASA-Ames. After exposure to 5 or 10x G for 4 days, more than 90% of the C. elegans were immobile and did not display a 'tap-response'. Mobility and the tap-response were regained by two hours after the return to unit gravity. To determine whether maintenance at 10xG for 4 days produced alterations in steady-state mRNA levels, we performed whole-genome microarray analysis on mRNA from centrifuged C. elegans compared to parallel cultures of stationary controls. Only 40 highly-expressed genes out of 19,600 were upregulated or downregulated more than 3-fold. Two HSP70 genes were upregulated by 3-fold, indicating that the centrifuged cultures were under mild stress. Most of the genes are of unknown function, but a number show similarity to proteins possibly involved in signal transduction, including G-protein receptor subunits and transcription factors. One member of the ODR10 family of chemosensory receptors was upregulated 8-fold, and others were down-regulated more than 3-fold. These data indicate that C. elegans do respond to increases in G level, but further work will be required to establish how the genes that we have identified may be involved in the responses of C. elegans to increased G.

# GENETIC ANALYSIS OF DNA DAMAGE INDUCED GERMLINE APOPTOSIS

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DNA damage induces cell cycle arrest that allows for repair of the lesion. However, in metazoans, when damage to the genome is excessive, cells undergo apoptosis. The importance of DNA damage in tumorigenesis has directed extensive research on DNA repair and cell cycle arrest in yeast and bacteria, yet not much is known about the molecular and genetic pathways of checkpoint mediated apoptosis. In *C. elegans,* gamma irradiation induces apoptotic cell death of meiotic germ cells as well as proliferation arrest of mitotic germ cells. The DNA damage-mediated apoptosis is dependent on *ced-3*, *ced-4* and is negatively regulated by *ced-9*. The positive death regulator, *egl-1,* participates in, but is not essential for radiation-induced apoptosis (1). Recently, three mutants - *dam-1(op241), rad-5(mn159)*, and *mrt-2(e2663)* - have been identified in *C. elegans* that block DNA damage induced apoptosis and cell cycle arrest (1). The MRT-2 protein is homologous to *S. pombe* RAD1, demonstrating for the first time that RAD1 homologs participate in apoptosis. We believe that many other genes are important for this DNA damage response. *C. elegans* provides an excellent genetic model to study DNA damage-induced apoptosis. In order to understand the mechanisms of DNA damage-induced germ cell cycle arrest and apoptosis, we plan to use *C. elegans* for a genetic and molecular analysis of these pathways. We will isolate and characterize three candidate checkpoint deletion mutants using reverse genetics. These deletion mutants will be characterized genetically for their involvement in DNA damage induced apoptosis and cell cycle arrest. We will use forward genetics to identify new, possibly metazoan specific, *dam* mutants that are defective in their apoptotic response to DNA damage. We expect that these studies will lead to a greater understanding of the genetic and molecular pathway(s) that regulate DNA damage-induced cell cycle arrest and apoptosis

1) Gartner, A., Milstein, S., Ahmed, S., Hodgkin, J., Hengartner, M.O. 2000. *Mol. Cell* 5:435-443.

# CHARACTERIZATION OF *EOR-1* AND *EOR-2* : TWO NEWLY IDENTIFIED POSITIVE REGULATORS OF RAS SIGNALING IN *C. ELEGANS*

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The RTK-Ras-Raf-MEK-ERK signaling pathway is involved in many biological processes such as cellular proliferation and differentiation. Our lab studies how Ras signaling controls vulval fate specification. In a genetic screen for enhancers of *lin-45 raf(ku112)*, we have isolated loss-of-function mutations in two genes, *eor-1* and *eor-2* (enhancer of raf). *eor-1(cs28)* and *eor-2(cs30)* mutations cause larval lethal and egg-laying defective (Egl) phenotypes at low penetrance and are also slightly uncoordinated. The *eor1; eor-2* double mutant shows the same low penetrant phenotypes as each of the single mutants alone. However, in combination with *ksr-1(n2526)* or *lin-45(ku112)*, these phenotypes are greatly enhanced. *eor-1* and *eor-2* also suppress the Multivulva phenotype of *let-60 ras(n1046).* Together, these results suggest that *eor-1* and *eor-2* both act as positive regulators of Ras signaling and act downstream of or in parallel to Ras. We also hypothesize that *eor-1* and *eor-2* function together to control the same aspect of Ras signaling in the same step of the pathway since their phenotypes are so similar and since the double mutant phenotype is no more severe than either of the single mutants alone. The molecular identity of EOR-1 suggests that EOR-1 and EOR-2 could function at the level of transcriptional regulation.

# IDENTIFYING *LIN-14* TARGETS WITH THE HELP OF A DNA MICROARRAY

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The heterochronic regulator *lin-14* affects certain somatic cell-fate choices during post-embryonic development in *C. elegans*. In *lin-14* mutants, cells express stage-specific developmental programs, such as cell divisions or dauer entry, at inappropriate times in development.1,2 Loss-of-function mutations in *lin-14* result in the precocious execution of L2-type programs during the first larval stage and the omission of L1-type programs, while gain-of-function mutations retard the execution of L2-specific programs during the second larval stage and instead cause the re-iteration of L1-specific programs. While the biochemical activity of *lin-14* remains unknown, we hypothesize that *lin-14* functions to either repress the expression/execution of L2-type fates during the first larval stage, and/or to promote the expression of L1-type fates during that stage. Since LIN-14 is a nuclear protein, it is likely that it controls state-specific fates by regulating gene expression at the level of mRNA abundance.

We chose to look for downstream targets of *lin-14* using a microarray of 12,000 ORFs prepared by the Kim lab, at Stanford University. The two synchronized mid-L1 populations compared were N2 and *lin-14* (*n179ts*) grown at the non-premissive temperature. We chose 4 genes for follow-up by Northern and GFP-fusion analysis of stage-specific and cell-type specific expression. Three of these four genes show mRNA misexpression in the L1 in the absense of *lin-14* function. The expression of promoter fusions to GFP suggests that at least one of the genes, *ins-33*, appears to be developmentally regulated by *lin-14* at the transcriptional level.

1. Ambros, V., and H.R. Horvitz, Genes and Development (1987) 1:398

2. Liu, Z., and V. Ambros, Genes and Development (1989) 3:2039

# IDENTIFICATION OF NOVEL COMPONENTS OF THE *DAF-2* SIGNALING PATHWAY

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The *daf-2* insulin-like signaling pathway plays a critical role in the regulation of dauer formation, metabolism, and longevity in *C. elegans*. Genetic analysis has identified multiple components of this regulatory cascade, including the DAF-2 insulin receptor, AGE-1 PI 3-kinase, PDK-1 and AKT-1 protein kinases, and the DAF-16 Forkhead transcription factor. Epistasis studies suggest the presence of an AGE-1-independent signal that emanates from DAF-2 to regulate DAF-16 activity. In an effort to identify novel components of the *daf-2* signaling pathway, we have performed a 25°C $F_2$ Daf-c screen in a *daf-2(e1370); daf-18(mg198)* double mutant. A pilot screen of 5,000 haploid genomes has yielded 3 distinct mutants (*mg290*, *mg291*, and *mg292*).

*mg292* is Daf-c on its own at 25°C and maps near *daf-11* on LGV. *mg291* has moderate penetrance of the Daf-c phenotype on its own at 25°C and is 100% 25°C Daf-c in a *daf-2; daf-18* background. It is also 100% 27°C Daf-c. The Daf-c phenotype of *mg291* is fully suppressed by *daf-16(mgDf47)*. *mg291* maps to the left arm of LGX near *pdk-1*, and its pleiotropies resemble the *pdk-1* loss-of-function phenotype. Sequencing of *pdk-1* exons and splice junctions in *mg291* revealed a point mutation at the splice donor site of exon 9. This is predicted to yield a PDK-1 protein that is prematurely truncated in its pleckstrin homology domain.

*mg290* is not 25°C Daf-c on its own but is 25°C Daf-c in a *daf-2; daf-18* background. It is also partially 27°C Daf-c. *mg290* maps to the left arm of LGX but appears to complement *mg291*, and it does not exhibit the same pleiotropies as *mg291* and other *pdk-1* mutants. Dye-filling defective (Dyf) mutants and other ciliary mutants are also 27°C Daf-c; amphid neuron dye-filling assays revealed that *mg290* is not Dyf. We are currently using visible markers and SNPs to establish finer mapping of *mg290*. Since this preliminary screen has identified known components of the *daf-2* signaling pathway, we are currently scaling up the screen.

# CLR-1 AND FIBROBLAST GROWTH FACTOR RECEPTOR (FGFR) SIGNALING IN *C. ELEGANS*

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Mutations in *clr-1*, which encodes a receptor tyrosine phosphatase (RTP) in *C. elegans*, result in a dramatic Clr (**Cl**ea**r**) phenotype characterized by extremely short, immobile, and sterile animals that accumulate clear fluid within their pseudocoelomic cavities. Genetic analysis suggests that CLR-1 negatively regulates an EGL-15 FGFR signaling pathway in *C. elegans* and that the Clr phenotype is the manifestation of hyperactivation of this pathway. To understand FGFR signaling in *C. elegans* better, we are taking advantage of two different components whose alteration can result in a Clr phenotype.

First, we utilize an allele of *let-60 ras* to identify additional genes involved in EGL-15 signaling. The involvement of LET-60 in EGL-15 signaling is analogous to the involvement of RAS in mammalian FGFR signaling. Two gain-of-function alleles of *let-60*, *ga89* and *ay75*, confer a late-onset Clr phenotype. By contrast, a third allele, *let-60(n1046gf)*, does not confer a Clr phenotype, but can synergize with weak alleles of *clr-1* to enhance their Clr phenotype. We have carried out an enhancer screen designed to identify mutations that lead to a Clr phenotype in a *let-60(n1046gf)* background. We have identified two mutations whose Clr phenotype is enhanced in a *let-60(n1046gf)* background. The first mutation is an allele of *clr-1*; the second mutation, *ay123*, confers a late-onset Clr phenotype and was mapped to LG I. The identity of *ay123* and its role in EGL-15 signaling are currently being investigated.

Second, to address how CLR-1 functions *in vivo*, we are determining the expression pattern of CLR-1 by immunofluorescence and its cellular site of action by mosaic analysis. We are also beginning to use "substrate-trapping" mutants of CLR-1 to identify its physiological substrates. "Substrate-trapping" mutants of CLR-1 presumably have no catalytic activity, but retain affinity for their substrates. Using a modified yeast two-hybrid system expressing a c-Src kinase, we are attempting to identify tyrosine phosphorylation-dependent interactions in yeast. The cytoplasmic domains of CLR-1 "substrate-trapping" mutants will be used as baits to screen a mix-staged *C. elegans* cDNA library for positive interactors.

# HISTAMINE: A POSSIBLE NEUROMODULATOR IN *C. ELEGANS*

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Many invertebrates use neuromodulators such as histamine to achieve behavioral flexibility. Histidine decarboxylase is a biosynthetic enzyme that synthesizes histamine from the amino acid histidine. We have identified a gene that encodes a putative L-aromatic amino acid decarboxylase (*adc-1*) similar to human and *Drosophila melanogaster* histidine decarboxylases. To identify *adc-1* expressing cells we generated several $P_{adc-1}$::*gfp* transcriptional fusions. We observed GFP expression in the AIA interneurons and the HSN neurons, as well as in a neuron within the ventral ganglion. Using antibodies against histamine, we identified histamine-like immunoreactivity in adult *C. elegans*. This histamine-staining appears to colocalize with the anti-GFP staining of $P_{adc-1}$::*gfp* expressing worms. We will generate antibodies against bacterially-expressed ADC-1 fusion proteins to aid in our identification of *adc-1* expressing cells.

Because ADC-1 is similar to dopa decarboxylase, a biosynthetic enzyme required for serotonin and dopamine synthesis, we tested whether *adc-1* mutants are defective in dopamine or serotonin biosynthesis. Immunocytochemistry with an anti-serotonin antibody demonstrated that *adc-1* worms synthesize serotonin. Dopamine levels, which we assayed using the method of formaldehyde-induced fluorescence, also appeared normal in *adc-1* animals.

To analyze the function of *adc-1* we obtained three independent deletion mutations in the *C. elegans adc-1* gene. These deletions, which were generously provided by M. Dong and M.R. Koelle, perturb coordination between the head and body during backward movement. Specifically, while wild-type worms move backwards in a smooth sinusoidal wave, *adc-1* mutants wag their noses back and forth during reversals. We plan to define the neural circuit involved in this behavior and to determine if histamine is synthesized by ADC-1.

# DEFECTS IN GLYCOSAMINOGLYCAN BIOSYNTHESIS CAUSE THE *C. ELEGANS* SQV PHENOTYPE AND HUMAN EHLERS-DANLOS SYNDROME

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Mutations in eight *sqv* (squashed vulva) genes result in several developmental abnormalities, including defective vulval invagination and maternal-effect lethality. The molecular identities of the six *sqv* genes cloned to date now suggest that the molecular basis for these defects lies in the disruption of the biosynthesis of glycosaminoglycans (GAG) of the structure (serine residue in the protein core)-xylose-galactose-galactose-glucuronic acid-(X-glucuronic acid)$_n$, where X is either N-acetylgalactosamine or N-acetylglucosamine.

Biosynthesis of GAGs requires the synthesis of nucleotide sugars in the cytoplasm and translocation of nucleotide sugars into the endoplasmic reticulum (ER) and/or Golgi, where polymerization of sugars is catalyzed by glycosyltransferases. SQV-4 is a UDP-glucose dehydrogenase, a key enzyme in UDP-glucuronic acid synthesis. SQV-7 is a multi-pass transmembrane protein that transports UDP-glucuronic acid, UDP-N-acetylgalactosamine and UDP-galactose from the cytoplasm into the ER and/or Golgi (see abstract by Berninsone *et al*.). Recently cloned mammalian homologs of *sqv-3* and *sqv-8* encode glycosyltransferases necessary for the biosynthesis of the GAG-protein linkage region of proteoglycans. SQV-3 is similar to galactosyltransferase I, and SQV-8 is similar to glucuronyltransferase I. SQV-1 is a cytoplasmic protein with weak similarities to nucleotide-sugar modifying enzymes, and SQV-5 is a novel protein with a single predicted transmembrane domain. We postulate that *sqv-1* and *sqv-5* are components of the same GAG biosynthesis pathway and that the GAGs are important for cell-cell or cell-matrix interactions in embryonic and vulval development.

Mutations in the human homolog of *sqv-3* are implicated as the cause of a progeroid variant of the connective-tissue disorder Ehlers-Danlos syndrome. The other five *sqv* genes also have close human counterparts, which suggest that a common pathway for modifying important cell surface and/or extracellular GAGs is present in humans and in *C. elegans*. Defects in the human counterparts of other *sqv* genes therefore may be responsible for aging disorders and connective tissue diseases such as Ehlers-Danlos syndrome.

# THE *TIMELESS*-RELATED PROTEIN TIM-1 IS REQUIRED FOR EMBRYONIC VIABILITY

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Our studies of the heterochronic gene *lin-42* revealed it encodes a protein with similarity to the *Drosophila* circadian rhythm protein PERIOD (PER). PER interacts with a second circadian rhythm protein TIMELESS (TIM) to control circadian rhythms. PER-TIM heterodimers participate in a negative autoregulatory feedback-loop resulting in rhythmic oscillation of their mRNA and protein levels with a 24-hour period. We want to determine if the similarity between LIN-42 and PER extends beyond the amino acid sequence level.

RT-PCR experiments show that *lin-42* message levels also oscillate, in this case with a 6-hour period synchronized to the molting cycle of postembryonic development. The mRNA levels peak during the inter-molt and are lowest during the molt. A rescuing LIN-42::GFP construct appears more intense during the molt, suggesting that protein levels may also oscillate. A *timeless*-related gene *tim-1* is present in the *C. elegans* genome. In contrast to *lin-42*, *tim-1* mRNA levels do not oscillate, but increase as development proceeds. Preliminary evidence suggests that TIM-1 does not bind LIN-42. Thus, although *timeless*- and *per*-related proteins are conserved in *C. elegans*, our results suggest they function separately.

We have begun to investigate the role of TIM-1 by silencing the expression of *tim-1* using RNAi. Experiments where two different regions of the gene were targeted separately, both resulted in dead embryos, indicating that TIM-1 is required for embryogenesis. Animals that underwent postembryonic development did not exhibit a *lin-42*-like phenotype. *lin-42* null mutants and *lin-42* (RNAi) animals synthesize adult cuticle one stage early, at the L3m. In contrast, 6/6 *tim-1* (RNAi) animals correctly synthesized larval-type cuticle at the L3m. Observation by DIC microscopy revealed that embryos failed to undergo morphogenesis. Preliminary experiments with the antisera raised against a TIM-1::GST fusion detect a nuclear antigen in 2 to 300-cell embryos. Embryos where TIM-1 was inactivated using RNAi lacked the antigen recognized by this antisera. We are currently investigating the role of TIM-1 during early embryogenesis.

# RAY NEURON TARGETING IN THE MALE TAIL

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We are using *C. elegans* male sensory rays as a model to study how neurons form precise and selective connections with their targets during neuronal development. Each ray consists of a structural cell and two neurons (type A and B) with distinct position, and possibly neurotransmitter. For example, we have found that rays 1, 3 and 9B neurons express serotonin, while rays 5, 7 and 9A neurons synthesize dopamine (see Lints and Emmons). These results raise the possibility that A and B neurons in different rays may synapse on different targets. Though previous studies by Sulston *et al* (1980) indicated that the ray axons terminate in the preanal ganglion, the precise synaptic connectivity of A and B ray neurons is still unknown. We are using both genetic and molecular approaches to address these issues.

By looking at the expression patterns of a *pkd-2::gfp* reporter, which is strongly expressed by one neuron in each ray (except ray 6) (Barr *et al*, 1999), we find that axons of the ray neurons exhibit a stereotyped pattern. The axons of anterior rays first extend anteriorly (ray 1) or ventrally (rays 2, 3, 4 and 5). Ray 1 then progresses to the ventral side before turning posteriorly, while rays 2-5 turn anteriorly and all run into the preanal ganglion. Axons of rays 7, 8 and 9 migrate anteriorly and ventrally to the same region. However, it is still unclear whether the target is a single cell or several cells specific to different neurons. We intend to ablate the putative target(s) in the preanal ganglion (the EF interneurons) to address whether the development of stereotyped connection is target-dependent.

Many transcription factors have been shown to regulate ray development. We found that axonal trajectories are dramatically changed in *egl-5* (a HOX gene) and *unc-37* (a groucho-like corepressor) mutants, indicating these genes specify neuronal connectivity. We are also investigating the LIM homeodomain proteins.

To look for molecules involved in target recognition, we have begun a screen using *pkd-2::gfp* transgenic lines, and obtained 5 mutants. Most of these mutants have abnormal axon patterns with anterior axons (always ray 1, also rays 2, 3 and/or 4 in some cases) extending anteriorly and apparently failing to reach their targets.

# SCREENING FOR PRECOCIOUS SEXUAL MATURATION

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Heterochronic genes regulate the temporal aspect of development in *C. elegans*. Mutations have been found that identify heterochronic genes that affect timing of the soma, but to date none have been identified that regulate the timing of the gonad. We are interested in the heterochronic genes that control the timing of gonadal development. In order to identify these genes we have conducted a genetic screen for precocious sexual maturation by selecting for mutants that produce precocious eggs. The screen was carried out by mutagenizing wild type (N2) worms and then selecting for precocious egg development in the fourth larval stage of the F2 generation using bleach.

We will carry out a detailed phenotypic analysis of the mutations we recover, including mapping all the mutations and cloning selected individual genes. We anticipate finding mutations in which the gonad matures precociously resulting in precocious egg development. In addition, due to our technique of synchronizing animals by hatching in the absence of food, we could possibly recover mutations which allow development to proceed in the absence of food.

# *PVL-5* MAY PREVENT ABNORMAL PN.P CELL DEATH DURING VULVAL DEVELOPMENT

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Our lab is studying the development of the vulva in *C. elegans.*The first stage in vulval development is the generation of the P blast cells that divide to form the vulva. The 12 P cells migrate from the sides into the ventral midline during the L1 larval stage where they divide to give a Pn.a neuroblast and a Pn.p hypodermal cell. Of the 12 Pn.p cells, six (P3.p - P8.p) become the vulval precursor cells. On induction by the anchor cell, P5.p, P6.p and P7.p adopt induced cell fates and divide to generate 22 vulval cells. This process involves Ras, Notch and Wnt signaling pathways. Although much is known about VPC fate specification, less is known about the generation of the VPCs.

*pvl-5(ga87*) was isolated in a screen for mutations causing a Protruding vulva phenotype. *pvl-5* affects the generation of Pn.p cells. In *pvl-5* mutants there are an average of 7.0 large Pn.p nuclei [n=172, range=4-10] in the ventral midline compared to 11 in N2 animals. Consequently, there are fewer than 6 VPCs and usually fewer than 22 cells available to form the vulva. The reduced number of Pn.p cells could be attributed to a loss of P or Pn.p cells by cell death, a failure of P cells to migrate into the ventral midline, or a Pn.p to a Pn.a cell fate transformation. We have used *jam-1::GFP* and lineage analysis to distinguish between these possibilities. *jam-1::GFP* defines the boundaries of hypodermal cells. Analysis of *jam-1::GFP* expression in *pvl-5* animals shows that the 12 P cells migrate correctly in *pvl-5* mutants. Lineage analysis shows that the P cells also divide correctly to generate 12 Pn.p cells. Further, the number of neurons in the ventral cord in *pvl-5* mutants and N2 are the same. These observations rule out a defect in cell migration, a Pn.p to a Pn.a cell fate transformation and P cell death as causes for the reduced number of Pn.p cells in *pvl-5* mutants. It is possible that the Pn.p cells may be undergoing abnormal cell death in *pvl-5* mutants, since we observe that Pn.p nuclei in *pvl-5* mutants lose their hypodermal morphology at the L1 molt and disappear. To test this we are building double mutants with *pvl-5* and genes required for apoptosis to look for suppression of the *pvl-5* phenotype. *pvl-5* has been localized to a two-cosmid region close to *dpy-10* on LG II and cloning results will be reported

# FINE MAPPING OF LETHAL MUTANTS ON CHROMOSOME I OF *CAENORHABDITISELEGANS*

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We have isolated six independent mutants (*jh1* to *jh6*) out of approximately 5,200 ethyl methanesulfonate (EMS) treated haploids. Four of the six mutants demonstrated embryonic lethal phenotypes, while the other two showed embryonic and larval lethal phenotypes. Terminal phenotypes observed in two mutants (*jh1* and *jh2*) indicated developmental defects restricted to the posterior part of the embryo, which appeared similar to the phenotypes observed in *nob* (no back end) mutants. Another mutant (*jh4*) resulted in an interesting phenotype of body-wall muscle degeneration at larval stage. These mutants were mapped by using three-factor crosses and deficiency mutants in this region. We are characterizing two mutants, *jh2* and *jh4*, in order to have more precise genetic map of these mutations by three factor crosses. Currently, we are attempting the cosmid rescue experiments.

# WHAT IS THE ROLE OF PROTEIN PHOSPHATASE 2A IN VULVAL DEVELOPMENT?

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*sur-6* positively regulates the ras pathway in vulval development and acts between Ras and Raf. *sur-6* encodes a protein belonging to the PR55 family of regulatory subunits of protein phosphatase 2A, thus implicating this phosphatase vulval development. PP2A is a heterotrimer in which various regulatory B subunits can associate with the core enzyme, which consists of a catalytic subunit (C) and a scaffolding subunit (A). The AC core enzyme dephosphorylates a wide variety of substrates and the B subunits regulate the enzyme activity by changing its sub-cellular location and by increasing or decreasing its affinity for different substrates.

We want to understand whether SUR-6 acts by activating or inhibiting PP2A activity during vulval development. To test these models we need to reduce the activity of the catalytic subunit of PP2A in vulva precursors. However our efforts have been hampered by the essential role of PP2A in embryonic and early larval development. Work from the Baillie lab and by Ogura and Kohara has shown that *let-92* encodes the catalytic subunit of PP2A. We have shown that *let-92(s504)* is a dominant (haplo-insufficient) lethal in a *sur-6* background indicating that SUR-6 is an activator of PP2A in early development. However the substrates acted upon by PP2A are probably different in vulval development. We are now performing similar genetic tests using weak mutations in the Ras-Raf-MAPK pathway genes to ask whether heterozygosity for a strong *let-92* mutation will make these animals vulvaless.

We will also attempt to reduce LET-92 levels during the L3 stage using conditional RNAi and by conditionally expressing dominant-negative alleles of *let-92* that we have constructed *in vitro.* Finally, predicated on the assumption that PP2A is required positively we have made a site-directed mutation in the first 14-3-3 binding site of LIN-45. This is a well-characterized site for an inhibitory phosphorylation on Raf. We will test the possibility that this site is acted upon by PP2A by asking whether the transgene can bypass the requirement for *sur-6.*

# HLH-2 EXPRESSION DURING THE AC/VU DECISION

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The AC/VU decision provides a simple model system to study lateral specification during development (reviewed in 1). Two cells of the somatic gonad, Z1.ppp and Z4.aaa, have equivalent developmental potential in that either one may become the anchor cell (AC) or a ventral uterine precursor cell (VU). LAG-2, a ligand of the DSL family, and LIN-12, a receptor of the LIN-12/Notch family, mediate interactions between Z1.ppp and Z4.aaa so that only one AC is formed. Genetic mosaic analysis and expression studies have suggested that at least two feedback loops operate during the AC/VU decision (2,3). Specifically, before the decision both Z1.ppp and Z4.aaa express *lin-12* and *lag-2*. As the decision progresses *lin-12* transcription becomes restricted to the presumptive VU whereas *lag-2* transcription becomes restricted to the presumptive AC (3).

We are interested in studying other genes which may be involved in these feedback loops. One such gene is *hlh-2*, which codes for a bHLH transcription factor homologous to *daughterless* in *Drosophila* and to mammalian E proteins (4). We find HLH-2 expressed in the presumptive anchor cell beginning at a time prior to a change in either *lin-12::lacZ* or *lag-2::lacZ* expression, suggesting that the AC/VU decision has not yet occurred. We are further analyzing the role of *hlh-2* via RNAi as well as various transgenic approaches.

1. I. Greenwald, Genes & Development 12, 1751-1762 (1998).

2. G. Seydoux, and I. Greenwald, Cell 57, 1237-1245 (1989).

3. H. A. Wilkinson, K. Fitzgerald, and I. Greenwald, Cell 79, 1187-1198 (1994).

4. M. Krause, M. Park, J-M Zhang, J. Yuan, B. Harfe, S-Q Xu, I Greenwald, M. Cole, B. Paterson and A. Fire. Development 124, 2179-2189 (1997).

# A NOVEL PROTEIN FUNCTIONS AS A BRIDGE BETWEEN THE GEX-2/GEX-3 COMPLEX AND INTERMEDIATE FILAMENTS IN *C. ELEGANS*.

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Tissue morphogenesis is indispensable for living functions of multicellular organisms. We have previously reported that loss-of-function of *gex-2* (Ce-Sra-1) or *gex-3* (Ce-HEM-2) leads to 100% embryonic lethality with abnormal tissue morphogenesis and that these molecules are localized at the cell-cell contact sites of all tissue cells throughout embryogenesis. (K. Kasuya *et al.,* M. Soto *et al.,* 12$^{th}$ international *C. elegans* meeting) Based on binding in a two-hybrid system and co-localization in immunostaining, GEX-2 and GEX-3 probably form a novel protein complex.

GEX-2 and GEX-3 proteins have no significant functional domain. To clarify the molecular mechanisms of GEX-2/GEX-3 functions in tissue morphogenesis, we screened physically and functionally interacting molecules. At first, by a two-hybrid screening, we isolated 29 positive clones that included 6 genes. Next, we performed RNAi of 6 genes. Among them, disruption of W07B3.2 resulted in 100% lethal embryos, some of which showed exploder phenotype of hypodermis and others arrested at the comma-stage. Although both phenotypes were weaker than Gex phenotype, the disorganization of molecular markers of muscle and intestine were also observed in W07B3.2(RNAi) embryos. The GEX-2/GEX-3-like localization pattern of W07B3.2 and the binding of W07B3.2 to GEX-2 and GEX-3 in the immunoprecipitation assay suggested that W07B3.2 functionally related to GEX-2 and GEX-3.

W07B3.2 gene product has a repeated structure which is weakly homologous to trichohyalin and raises the possibility to interact with intermediate filaments (IFs). To examine whether W07B3.2 interacts with *C. elegans* IFs, we performed two-hybrid binding assay. As a result, some IFs interacted with W07B3.2. Co-localization of GEX-2, GEX-3, and W07B3.2 with some IFs suggested that these molecules form a large complex. RNAi of *gex-2, gex-3,* and W07B3.2 eventually caused disorganization of the IF structure. Taken together, we proposed a model that W07B3.2 serves as a bridge between GEX-2/GEX-3 complex and intermediate filaments, and that the large complex essentially functions in tissue morphogenesis in *C. elegans*.

# PHEROMONE REGULATION OF NEUROENDOCRINE OUTPUTS IN *C. ELEGANS*

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A major regulator of the decision to enter the dauer stage is the environmental concentration of a constitutively secreted dauer promoting pheromone. One mechanism by which dauer pheromone controls dauer formation is via the regulation of the expression levels of the TGF-b like ligand DAF-7. To determine the mechanism(s) by which pheromone regulates the expression of DAF-7 we undertook a genetic screen to identify genes that when mutated would lead to both a loss of DAF-7 expression and a Daf-c (dauer constitutive) phenotype. In order to monitor DAF-7 expression we have utilized a *daf-7p::gfp* reporter construct, which is expressed predominantly in the ASI amphid neuron. From an initial screen of 20,000 haploid genomes we identified one mutation, *mg295*. Mapping data indicates that *mg295* maps to LGV, very near the *daf-11* locus. In addition, *mg295* fails to complement daf-11 (m47). Consequently we conclude that *mg295* is very likely to be a mutation in *daf-11*. *Daf-11* encodes a guanylyl cyclase, an enzyme that converts GTP to the intracellular second messenger cGMP. This suggests the possibility that pheromone signaling *in C. elegans* involves the second messenger cGMP, a molecule previously identified as essential for mammalian photoreceptor signal transduction.

To further our understanding of the mechanism(s) by which pheromone and *daf-11* control *daf-7* expression we have begun two additional genetic screens. The first screen seeks to identify mutants that lead to both a loss of daf-7p::GFP expression and a Daf-c phenotype under more stringent environmental conditions (27° ). We anticipate that this screen will identify additional genes involved in the regulation of dauer formation and *daf-7* expression. Progress on this screen will be reported.

We have also begun a genetic screen to identify mutations that both suppress the daf-11 Daf-c phenotype and lead to a reversion in daf-7p::GFP expression. From an initial screen of 250,000 haploid genomes we have identified two mutations; tentatively named *PollyAnna-1 (mg296)*, and *PollyAnna-2 (mg297*), that satisfy these criteria. Progress of this screen and on the characterization and mapping of *mg296* and *mg297* will be reported.

# USING IVERMECTIN TO FIND NERVOUS SYSTEM MUTANTS

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Ivermectin is an antiparasitic drug of great medical and commercial interest. Ivermectin activates glutamate-gated chloride channels (GluCls) found in worms and in other invertebrates. We have previously characterized the complex genetic interactions among two classes of genes that when mutated confer ivermectin resistance on C. elegans (Dent et al., PNAS 97:2674, 2000). One class, as might be expected, consists of genes encoding GluCls. But genes encoding innexins, subunits of invertebrate gap junctional channels, can also alter the structure of the nervous system to make worms less susceptible to ivermectin. Because selection for ivermectin resistance is so efficient, we are conducting further screens to identify new genes important for nervous system function in worms. We are looking for super-resistance, i.e. above the >1000 fold resistance that occurs when three GluCl genes are mutated. We have also ectopically expressed an avr-15 cDNA in the body muscle of a triple GluCl mutant using the unc-54 promoter. We hope to find a new spectrum of mutations conferring resistance to this ectopically expressed channel subunit. We have several mutants in hand and are in the process of characterizing them.

# FUNCTION OF THE FAMILY OF FMRFAMIDE-RELATED NEUROPEPTIDES IN *C. ELEGANS*

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FMRFamide-related peptides (FaRPs) are short peptide neurotransmitters that have been found throughout the animal kingdom. FaRPs have been shown to have many general functions, including cardioregulation, muscle control, pain modulation and learning. Our lab uses *Caenorhabditis elegans* as a model system to study FaRPs. In *C. elegans*, at least 50% of the neurons express FaRPs, which are encoded by at least 22 genes (*flp-1* through *flp-22*).

To determine the cell-specific expression of the *flp* genes in *C. elegans*, transgenic animals carrying a *gfp* (green fluorescent protein) reporter construct under the control of each *flp* promoter are being generated. To date, the cell-specific expression patterns of 13 *flp* genes have been determined. Each *flp* gene is expressed in distinct, but sometimes overlapping, sets of cells. The cell-specific expression patterns of the remaining *flp* genes are being determined.

To determine the function of the *flp* genes in *C. elegans*, several *flp* deletion mutants, including *flp-3, flp-4, flp-6, flp-8, flp-10,* and *flp-12*, were isolated by screening libraries of chemically-mutagenized *C. elegans*. Both *flp-3* and *flp-8* deletion mutants have no obvious behavioral phenotypes thus far, suggesting that these genes have overlapping functions with other *flp* genes. *flp-4, flp-6, flp-10,* and *flp-12* deletion mutants are being characterized.

To identify genes that may regulate expression of the *flp* genes, we are using transgenic animals expressing a *gfp* reporter construct under the control of a *flp-1* or *flp-12* promoter in genetic screens. These strains were mutagenized, and mutants showing altered expression of these markers were isolated. Several mutants which show no, faint, or strong GFP expression have been isolated. These mutants, called *mof* (modifier of *flp* expression), are being mapped, characterized, and cloned.

# UNC-13 IN THE *C. ELEGANS* NERVOUS SYSTEM

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*C. elegans unc-13* and its homologues in vertebrates and *Drosophila* are involved in neurotransmitter release. UNC-13 has several regions homologous to PKC regulatory domains; these domains confer it with calcium, phorbol ester and phospholipid binding properties (Maruyama and Brenner, *PNAS* **88**, 1991). A 5.9kb transcript coding for a 200kDa protein was initially identified (now designated L-R for left and right regions). We have identified two additional types of transcripts. One transcript includes a 1kb novel exon (L-M-R, M for middle region) and another transcript lacks the 5' region included in the other two transcripts (M-R). All three transcripts are identical at the 3' end (R). *C. elegans* with mutations in the 5' end of the gene (L) alter two types of transcripts (L-R and L-M-R) resulting in an uncoordinated coily phenotype and resistance to the anti-cholinesterease, aldicarb. A 2.7kb deletion near the 3' end (R) (identified by Bob Barstead using PCR analysis) affects all *unc-13* transcripts and results in a lethal phenotype. Antibodies recognizing the N-terminal region of UNC-13 (L) label synapses, but not synaptic vesicles, of most or all neurons; many mutations in L and R remove staining with this antibody. Supported by grants from the NIH and OCAST.

# MOLECULAR CLONING OF THE ELUSIVE *UNC-20*

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Our lab is studying the molecular mechanisms of nervous system development. Mutants bearing the canonical *unc-20* allele, *e112*, display a temperature-sensitive coiling phenotype. Phil Anderson's laboratory has identified six alleles of *unc-20* that cause a phenotype similar to *unc-20 (e112)* when heterozygous in a *smg-1* mutant background. These alleles cause a homozygous L1 lethal phenotype, suggesting that *unc-20* provides an essential function.

*unc-20 (e112)* and *unc-20 (r990)/+; smg-1 (r861)* strains display defects in HSN axon pathfinding and extension and AVKR axon pathfinding. *unc-20 (e112)* animals also display defects in VD/DD axon pathfinding (Y. Jin, personal communication), VC axon anatomy, and serotonin sensitivity (T. Rakow and B. Schafer, personal communication). Therefore, we propose that *unc-20* functions in nervous system development and an unknown essential process.

In order to understand the role of *unc-20*, we embarked on a positional cloning strategy to isolate the gene. Genetic mapping has demonstrated that *unc-20* lies to the right of *imo-1, bwP1,* and *gpd-2,3*; and to the left of *fax-1*. This defines a relatively small region that is covered by cosmids M01F3, C15C7, and W05A4. M01F3 and a subclone containing the left-hand 10kb of C15C7 fail to rescue *unc-20 (e112)*, suggesting that *unc-20* may lie in the C15C7 or W05A4 clones. We have not been successful at recovering stable transgene arrays with the entire C15C7 clone. The *gm27* deletion removes *fax-1* and flanking DNA, including most of cosmid W05A4 and the right-hand portion of C15C7. The *gm27* mutation complements *unc-20(e112)* suggesting that *unc-20* lies in the central portion of the C15C7 clone. This region contains four predicted genes. We have attempted RNAi with cDNA clones from two these predicted clones, and have not obtained phenotypes consistent with known *unc-20* phenotypes. We are currently amplifying DNA from *unc-20 (e112)* and *unc-20 (r997)* animals to determine if any of the four predicted genes in this region contain mutations in these strains.

# TOWARD AN UNDERSTANDING OF ASH CIRCUIT SIGNALING PATHWAYS

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The ASH sensory neurons play a critical role in the detection of three different noxious stimuli: nose touch, high osmolarity and 1-octanol. The direct synaptic targets of the ASH neurons include the interneurons which control locomotion and each stimulus evokes backward locomotion. Previous analysis of two cloned genes, *glr-1* and *osm-10*, suggests that distinct signalling pathways may mediate response to stimuli detected by ASH. We hope to elucidate the molecular and cellular mechanisms underlying stimulus detection and synaptic response in the ASH circuit through the analysis of additional genes which are required for response to just one or two ASH stimuli.

Two mutations are currently under analysis which severely perturb response to 1-octanol: *rt6* and *rt68*. *rt68* animals are severely defective in their ability to respond to high osmolarity, but *rt6* animals are virtually wild type For osmotic avoidance. Both mutations map to the right arm of the X chromosome and are normal in their ability to respond to nose touch, benzaldehyde, diacetyl and NaCl mediated by ASH, AWA, AWC and ASE, respectively. But preliminary noncomplementation and phenotypic analysis suggest that they may not be alleles of the same gene. *rt6/rt68* animals respond to 1-octanol.

Interestingly, the defective response of *rt6* to 1-octanol can be modulated by feeding status and exogenous serotonin. *rt6* animals respond as well as wild type animals to 1-octanol on the bacterial lawn. After 10 minutes off the bacterial lawn, *rt6* animals are severely defective in their response to 1-octanol (versus wild type animals). Consistent with the previously demonstrated connection between feeding status and serotonin for *C. elegans*, we found that exogenous serotonin rescues the *rt6* mutant phenotype. This data suggests that serotonin and feeding status modulate the response of the ASH circuit to volatile repellent chemicals. Additional cellular, phenotypic and molecular analysis should reveal the mechanisms underlying stimulus detection, synaptic encoding and serotonin modulation of sensory signalling in the ASH circuit.

# *NOP-1* AND THE ESTABLISHMENT OF ASYMMETRIES IN THE EARLY *C. ELEGANS* EMBRYO

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The generation of asymmetries before the first cell division in the early *C. elegans* embryo appears to be a complex mechanism that involves the activity of several genes. Cortical and cytoplasmic flows are among some of the first observed aspects of asymmetry. These flows appear to play important role(s) in the generation of early asymmetries, as many cellular components become localized concomitantly with them, and disruption of the actin cytoskeleton by treatment with cytochalasin D at the time of flows also affects embryonic asymmetries (Hill and Strome, 1990). We are interested in understanding how asymmetries are established in the early *C. elegans* embryo. One of the genes that might participate in some of these early events is *nop-1*. *nop-1(it142)* was shown by Rose *et al.* (1995) to be a viable mutant that lacks a pseudocleavage furrow, and has reduced flows. Furthermore, 20% of embryos fail to hatch. We were interested to study in more detail the phenotype of *nop-1(it142)*, in order to find out whether the embryos that don't hatch also have asymmetry problems. We found that a proportion of the *nop-1* mutant embryos had no cytoplasmic flows; these embryos failed to hatch. Interestingly, most of these defective embryos either had a Par phenotype or failed to complete first cytokinesis. Some of the *nop-1* mutant embryos that did have flows also failed to hatch, although their lethality did not appear to result from a defect in early asymmetries, but might be explained by the observation that *nop-1* mutant animals have a weak Him phenotype, suggesting a defect in chromosomal segregation. We are currently examining the possibility that *nop-1* might genetically interact with other genes involved in early embryogenesis. We set out to clone *nop-1* as a step toward further defining its role in early embryogenesis. This will help us to address the null phenotype of *nop-1*, as *it142* might be a weak loss-of-function allele. *nop-1(it142)* was previously mapped by Lesilee Rose between dpy-17 and unc-32, at approximately -1.5 cM on the left arm of chromosome III. We refined the mapping using several deficiencies in that area and obtained partial rescue with one YAC in this region. We are currently assessing rescue with cosmids in the region and will present further details at the meeting.

# STRUCTURE AND EXPRESSION OF CALCINEURIN B, THE REGULATORY SUBUNIT OF THE $CA^{2+}$ / CAM DEPENDENT PROTEIN PHOSPHATASE 2B: BIOLOGICAL RELEVANCE IN *C. ELEGANS*

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Calcineurin, a major calmodulin (CaM)-binding protein in the brain, is a $Ca^{2+}$/CaM-dependent serine/threonine protein phosphatase classified as protein phosphatase 2B (PP2B). PP2B is a heterodimer of a catalytic and CaM-binding subunit, Calcineurin A (CnA), tightly bound to a $Ca^{2+}$-binding regulatory subunit, Calcineurin B (CnB). Apart from the brain, calcineurin is also abundantly found in non-neural tissues. Although CnB and CaM share a relevant degree of similarity (35% identity), they cannot substitute for each other and both of them are needed for the activation of the phosphatase. Phylogenetic studies reveal that the protein structure of CnB is highly conserved from yeast to man. The *C. elegans* genome database revealed the presence of a predicted CnB (we termed CeCnB) on the cosmid F55C10 (LGV) and shows about 80% sequence identity with other organisms. CeCnB is also shown to contain four "EF-hand" motifs for $Ca^{2+}$ binding. We attempted to clone the full-length cDNA from a mixed stage worm cDNA library, using the partial cDNA clone from Kohara's data bank as a probe. Furthermore, northern blot experiments and cDNA sequencing confirm that the earlier gene prediction for CeCnB is incorrect. CeCnB expresses strongly in neuronal cells but faintly in muscles. Expression is also observed around spermatheca indicating that the gene is probably expressed in the male germline. To check the phosphatase activity *in vitro*, full-length proteins are being overexpressed and purified. To better understand the biological role of calcineurin in *C. elegans*, RNA mediated interference (RNAi) with CeCnB alone or/and with CeCnA had been undertaken. Resulting male progeny from RNAi in the F1 generation suggests that calcineurin is probably involved in sex determination. Presently, we have obtained a candidate CeCnB mutant by TMP/UV mutagenesis and have observed the appearance of male progeny. Hence, it seems likely that calcineurin has a key role in sex determination amongst other functions. Detailed characterization is still underway to solve this problem.

# IDENTIFICATION AND CHARACTERIZATION OF A UBIQUITIN C-TERMINAL HYDROLASE IN *C. ELEGANS*

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Ubiquitin (ub), a protein of 76 amino acid residues, is extremely well conserved in all eukaryotic cells. It plays a key role in a variety of cellular functions, including the regulation of intracellular protein breakdown, cell cycle regulation, and stress response. Ubiquitins, either by themselves or conjugated to protein, can also be ligated to additional ub molecules to form branched poly-ub molecules. Ubiquitin C-terminal hydrolase (UCH) is a thiol protease that recognizes and hydrolyzes the peptide bond at the C-terminal glycine of ubiquitin. Since we are interested in identifying and characterizing UCH in *C. elegans*, we searched the worm genome database and located a putative homologue of UCH in the cosmid, ZK328 (LGIII). *C. elegans* UCH (ZK328.1) consists of 12 exons encoding 1041 amino acids and contains two functional domains that are well conserved in other organisms. As a first step to characterize the worm UCH, expression pattern was investigated using the GFP reporter system. ZK328.1 is shown to express in neuronal cells, muscle cells, and seam cells throughout the body. Northern blot experiments with total RNAs from wild type mixed-staged animals reveal two mRNA transcripts, the dominant one being 3.6 kb in size. To better understand the function of UCH in *C. elegans*, RNA-mediated interference (RNAi) technology is being carried out currently.

# ISOLATION OF GENETIC SUPPRESSORS OF *DAF-16*, A FORKHEAD CLASS TRANSCRIPTION FACTOR MEDIATING INSULIN-LIKE SIGNALING

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Insulin and insulin-like signaling pathways are phylogenetically conserved in animals. Biochemical analysis done in mammalian cells and genetic analysis in worms indicated that insulin (-like) signals are transduced via a lipid and protein kinase cascade that negatively regulates specific forkhead class transcription factors: DAF-16 in *C. elegans* and AFX, FKHR, FKHRL1 in mammals. In worms, reduced insulin signaling causes pleitropic effects including constitutive dauer larval development, metabolic shift towards fat storage, and extended adult lifespan. We are taking a genetic approach to identify targets of *daf-16.*

Loss-of-function *daf-16* mutants have a short adult lifespan. We sought long-lived animals among F3 progeny of (semi) clonal lines of EMS-mutagenized *daf-16(null)*. In a pilot screen of 320 cohorts, each established from 10 F2 parents, we recovered two suppressor mutants. We are expanding the scale of the screen.

We are also screening for suppressors of phenotype associated with forced expression of *daf-16*. Animals carrying transgenes that express DAF-16 under the control of the hsp16 heatshock promoter (*hsp::daf-16*) show a slow and kinky uncoordinated movement phenotype (Unc) even without any heat shock treatment. In a screen for suppressors of Unc, we recovered five mutants among 30,000 EMS-mutagenized F2s. Four of them showed reduced expression of a GFP-encoding co-injection marker. The remaining mutant is still unable to move normally but is significantly more active than the starting strain. Furthermore, whereas *hsp::daf-16* transgenic animals inappropriately form dauers at high temperatures, the suppressed animals do not. Nonetheless, normal dauers do form on starved plates. Because *daf-16(null)* mutants do not form normal dauers under similar conditions, this result suggests that the suppressor affects *daf-16* function only partially. Alternatively, the suppressor affects specifically the transgene expression of *daf-16*. We are testing these possibilities by asking if the suppressor affects the dauer-constitutive phenotype caused by either reduced insulin signaling or a different *daf-16* transgene.

# CHEMOSENSORY MODULATION OF THE *DAF-2*/INSULIN SIGNALING PATHWAY

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The *daf-2* insulin-like, *daf-7* TGFb-like, and *daf-11* guanylate cyclase signaling pathways regulate dauer formation, whereas *daf-2* signaling also controls longevity. Although environmental cues are critical in regulating dauer formation, not much is known about how such environmental signals are integrated into the downstream signaling components. Ciliated sensory neurons play important roles in sensing the environment and epistasis studies have placed cilia mutants upstream of *daf-2* and *daf-7*, but downstream of *daf-11*, in controlling dauer arrest. Recently, mutations affecting the sensory cilia have also been shown to extend adult lifespan (Apfeld & Kenyon, 1999), a phenotype likely mediated through the *daf-2* signaling pathway. Interestingly, double mutants harboring defects in both *daf-2* signaling pathway and sensory cilia function exhibit synthetic lethality, possibly due to enhanced *daf-2* defects in the mutant animals. We reasoned that genetic screens which identify suppressors of this synthetic lethality would likely further our understanding of the signaling pathway between sensory cilia and the DAF-2 insulin-like receptor and identify outputs from chemosensory neurons which modulate the *daf-2* signaling. In a screen of 10,000 EMS-mutagenized *daf-2;*cilia mutant F2s, we identified ~20 suppressors which exhibited reduced lethality. Progress on the characterization of these mutants will be reported.

# A DELETION OF *C. ELEGANS HCF-1* CAUSES SMALL BROOD SIZE AND A COLD-SENSITIVE HATCHING DEFECT.

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*C. elegans hcf-1* was found because of its similarity with human *HCF-1*. Human *HCF-1* encodes a cellular component of a viral transcription activation complex required for herpes simplex virus (HSV) gene expression. A mutation in *HCF-1* can arrest cell cycle progression in a mammalian cell line, but the mechanism of HCF-1 function in mammalian cells has not yet been elucidated. The product of *C. elegans hcf-1* (CeHCF) shares some known functional properties of HCF-1 including association with the HSV viral protein VP16. The goal of our research is to determine the cellular function of HCF proteins using *C. elegans* as a model organism.

A chemically mutagenized *C. elegans* deletion library of R. Plasterk and colleagues (The Netherlands) was screened by PCR and an *hcf-1* deletion mutant was found. The isolated mutant has a 1465bp deletion which causes a frameshift in the *hcf-1* open reading frame. The absence of WT CeHCF protein in the homozygous deletion mutant was confirmed by western blot. These worms are viable and do not show any gross morphological or developmental defect. Analysis of the brood size at 15, 20, and 25°C showed that the brood size of the mutant is about 50% of that of the WT at all three temperatures. At 15°C, about 10% of mutant embryos also do not hatch. A further decrease of the temperature to 12°C resulted in a further reduced mutant brood size (25% of WT) and only a 50% hatching rate. Thus, the *hcf-1* deletion mutant has a small brood size and a low temperature-sensitive embryonic arrest phenotype. This cold temperature-induced phenotype can be enhanced by propagating worms at low temperature (12°C) for more than two generations. These results suggest a role for CeHCF in germ line and/or embryonic development.

# ISOLATION OF MUTANTS DEFECTIVE FOR THE INITIATION OF POSTEMBRYONIC DEVELOPMENT IN *CAENORHABDITIS ELEGANS*

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Normal postembryonic development of *C. elegans* depends on precise cellular proliferation and differentiation. Cells must respond to cues which govern proliferation, arrest and differentiation in synchrony with the organism's development. Environmental signals also play a major role in controlling these processes particularly during dauer formation and also at the transition from embryonic to larval development. Although the initiation of dauer development is well understood, little is known about the mechanism by which these environmental signals activate the onset of the postembryonic developmental program in *C. elegans* remains largely misunderstood.

To understand the molecular basis that underlies the onset of larval development and the control of cell division at initiation of postembryonic development, screens were performed to identify temperature-sensitive mutants that are unable to initiate larval development and cell divisions typical of postembryonic development.

Exposure of L1 larvae to a food source and hydroxyurea (HU), a potent inhibitor of DNA replication, causes worms to grow up to become uncoordinated and sterile adults. However, HU does not affect worms that harbor temperature sensitive mutations blocking them from initiating cell division.. We used this observation to screen for mutants that do not enter cell cycle at 25C (and hence are unaffected by HU) but activate postembryonic development and cell divisions normally at 15C.

After a 48-hour culture in the presence of HU at 25C, worms were transferred to permissive temperature and allowed to grow to the adult stage. Healthy worms were isolated and maintained at 15C for further characterization.

Two temperature sensitive mutants have been isolated which are normal at 15C but show complete developmental arrest as L1 larvae at 25C. Following this arrest, these animals can be shifted to 15C and development will proceed correctly. One mutant has been mapped to right arm of chromosome IV by STS mapping while mapping of the second mutant is presently under way.

# EVIDENCE FOR A *DAF-16*/FH-INDEPENDENT *DAF-2*/IR SIGNAL

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TGFb -like and insulin--like pathways regulate dauer entry and recovery. Since the Daf-c (dauer formation constitutive) phenotype associated with *daf-2(lf)* (insulin receptor-like) can be fully suppressed by *daf-16(lf)* (fork-head-like transcription factor), *daf-16* was considered to be the terminal dauer-promoting gene of the insulin-like pathway and the sole output of the *daf-2*/IR signal. Likewise, *daf-3* (smad transcription factor) is considered to be the terminal dauer-promoting gene of the *daf-7*/TGFb -like pathway. These two signaling pathways may converge by regulating the *daf-12* (nuclear hormone receptor) activity.

A third signaling pathway for dauer arrest is suggested by the finding that *daf-16(0); daf-3(0)* animals can still form partial dauers in response to pheromone. In order to identify the components in the putative third signaling pathway, we screened for Daf-c mutations in a *daf-16(0); daf-3(0)* background. No Daf-c mutations in either the TGFb or insulin-like pathways were expected to be isolated from this screen. Surprisingly, *mg293*, one of the two alleles isolated from this screen, is likely to be an allele of *daf-2* based on its map position and a complementation test. The Daf-c phenotype caused by *daf-2(lf)* was expected to be suppressed by *daf-16(0).* But the result suggested that a *daf-3(0)* mutation can relieve this suppression. To directly test this possibility, we constructed a *daf-16(0); daf-2(0); daf-3(0)* strain ,using a known *daf-2(0)* allele. At all temperatures tested, this triple mutant gives rise to transient partial dauers. This result indicates a *daf-16*-independent *daf-2* output that is normally masked by the *daf-3(+)* function. This *daf-2* output affects dauer entry but not recovery and it may well be the third signaling pathway. Since *daf-12* is the most downstream dauer-promoting gene and can mutate to Daf-c (likely *gf*) alleles, which also give rise to transient partial dauers, we hypothesize that *daf-12* and its co-factors may be the targets of the *daf-16*-independent *daf-2* signal. We performed a suppressor screen on the Daf-c *daf-12(rh273)* strain and have obtained 12 suppressors. We are currently analyzing these suppressors and testing whether they (and *daf-12* itself) are the targets for the *daf-16*-independent *daf-2* signal.

# INITIAL CHARACTERIZATION OF *APH-1* SUPPRESSORS

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The maternal-effect-lethal gene *aph-1* is essential for early embryonic development. Mutations in *aph-1* were isolated in the Priess lab and found to cause an embryonically arrested phenotype that is remarkably similar to that of *glp-1* mutant embryos. The phenotype of aph-1 mutants suggests that the *aph-1* gene product might be involved in the same specific cell induction events that are mediated by the GLP-1 receptor. As a way of learning more about the specific role of *aph-1* in these cell communication events, we have carried out a screen for suppressors of the weak *aph-1(zu147)* allele. We have identified 6 independent mutations that suppress the partial maternal-effect lethality caused by *aph-1(zu147).* One of these mutations appears to map to the *aph-1* gene locus (or at least very close), and is likely to be a revertant of the *aph-1(zu147)* allele. The other four mutations are unlinked to *aph-1*. Complementation and mapping analysis indicates that at least three different genes are represented by these mutations. These suppressor mutations do not appear to have phenotypes of their own other than suppression of *aph-1(zu147).* Allele-specificity and interactions with other genes are currently being tested. Our latest mapping information for these suppressors will also be presented.

# THE *SPE-10* AND *SPE-21* GENES ENCODE SPERM TRANSMEMBRANE PROTEINS THAT CONTAIN A DHHC-CRD ZINC FINGER MOTIF

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Spermatogenesis in *C. elegans* is a good model for studying asymmetric partitioning of organelles during cellular differentiation. This one-way differentiation pathway begins with a stem cell and ends with a terminally differentiated cell, the spermatozoa. Like most organisms, *C. elegans* spermatids are compact cells that eliminate unnecessary components during their differentiation. This occurs because certain components are actively retained within spermatids while others are eliminated by placement within the acellular cytoplast (the residual body). We are interested in how asymmetric partitioning participates in the development of this specialized cell and how this process is genetically controlled.

The *C. elegans spe-10* and *spe-21* genes affect spermatogenesis so that abnormally small nonfunctional spermatozoa are found in these mutants. Mutations in *spe-10* disrupt the proper function of ER/Golgi derived, fibrous body-membranous organelles (FB-MOs) that are known to play a central role in the proper segregation of cytoplasmic components into spermatids (Shakes, D.C., and Ward, S. 1989. Dev. Biol. 134, 307-316.). The details of the mutant *spe-21* phenotype are currently being investigated by electron microscopy. We have positionally cloned both of these genes, and they both encode 3-4 pass transmembrane proteins that contain a cysteine-rich domain (CRD) with a DHHC zinc finger motif (DHHC-CRD), and mutantations in either gene appear sperm specific in their phenotype. The DHHC-CRD zinc finger motif is a recently identified motif that is present in organisms from yeast to man, and this motif is thought to be involved in protein-protein interactions. This is the first known instance of two genes encoding a DHHC-CRD zinc finger motif that affect the same cellular pathway. An intriguing speculation is that Spe-10 and Spe-21 interact via this motif to form a heterodimer.

Presently, we are continuing our analysis of *spe-10* and *spe-21* by generating allelic series, electron microscopy, and raising peptide-specific antibodies to look at the subcellular localization of the proteins. Such analysis will broaden our understanding of asymmetric partitioning and allow us to further elucidate the spermatogenesis pathway.

# PATTERNING OF NEUROTRANSMITTER PHENOTYPE AMONG MALE RAY SENSORY NEURONS

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The male sensory rays provide an opportunity for exploring how neuronal diversity can be generated among lineally related cells. Our objective is to understand how diversity in ray neurotransmitter phenotype is generated and how the genetic programs that underlie these fates are coordinated with pathways that define other aspects of neuron and ray identity.

Dopamine is made in the A-type neurons of rays 5, 7 and 9 and is correlated with expression of *cat-2*, which encodes the dopamine biosynthetic enzyme tyrosine hydroxylase. This pattern of dopaminergic (DA) fate is established, at least in part, through the interaction of a TGF-beta signaling pathway (the DBL-1 pathway) and the HOX transcription factor, EGL-5. In the absence of DBL-1 signaling, DA fate is expressed at low frequency in rays 5, 7 and 9 and in two rays that are not dopaminergic in wild type, rays 4 and 6. Ubiquitous expression of the pathway ligand, DBL-1, induces ectopic expression of DA fate in rays 3, 4, 6 and 8. EGL-5, which is expressed in rays 3-6, is required for normal expression of DA fate in ray 5 and for ectopic expression of this fate in rays 3, 4, and 6.

Serotonin is made in the B-type neurons of rays 1, 3 and 9 (see abstract by Jia and Emmons) and this pattern is also defined, in part, by the DBL-1 pathway and EGL-5. DBL-1 signaling induces serotonergic fate in ray 9 and suppresses expression in the B-type neuron of ray 5. Serotonin expression in rays 1 and 3 is independent of DBL-1. EGL-5 appears to be required for serotonergic fate in ray 3 and for ectopic expression in ray 5 in the absence of DBL-1 signal. Taken together, the data suggest that DBL-1 signaling refines prepatterned expression of DA and serotonergic fate. Whether fates are induced or suppressed in response to DBL-1 appears to be a function of ray and of neuron type. EGL-5 confers competence to adopt either fate in rays 3-6.

To identify additional genes involved in neurotransmitter patterning we have initiated a genetic screen for mutants with altered patterns of CAT-2::GFP expression. We have isolated two mutants characterized by ectopic expression of DA fate in several rays. Neither mutation appears to affect ray serotonergic patterning, although in one mutant 2-3 additional CP neurons of the ventral cord express serotonin.

# EVOLUTION OF CYCLIN-DEPENDENT KINASES (CDKS) AND CDK-ACTIVATING KINASES (CAKS): DIFFERENTIAL CONSERVATION OF CAKS IN YEAST AND METAZOA

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Cyclin-dependent kinases (CDKs) are central regulators of both the cell cycle and transcription. CDK activation depends on phosphorylation by a CDK-activating kinase (CAK). All known CAKs belong to the extended CDK family. The sole budding yeast CAK, *CAK1*, and one of the two CAKs in fission yeast, *csk1*, have diverged considerably from other CDKs. Cell cycle regulatory components have been largely conserved in eukaryotes, however, orthologs of neither *CAK1* nor *csk1* have been identified in other species to date. In particular, recent experiments suggest that there are unidentified CAK(s) in metazoa. To search for metazoan CAKs, we performed a phylogenetic analysis of the extended CDK family in the sequenced genomes of budding yeast, *Caenorhabditis elegans,* and *Drosophila melanogaster,* as well as fission yeast and humans. We observed that there are ten clades for CDK-related genes, of which seven appear ancestral, containing both yeast and metazoan genes. The four clades that contain CDKs that regulate transcription did not undergo gene expansion from yeast to metazoan. In contrast, the ancestral cell cycle CDK gave rise to a number of genes in metazoa, as did the ancestor of budding yeast PHO85. Interestingly, *CAK1* and *csk1* branch together with high bootstrap support values. Divergent taxa sometimes group together solely due to their divergence relative to other taxa, producing a long-branch attraction artifact. We determined that the *CAK1*/*csk1* association was real and was not due to long-branch attraction by using the Relative Apparent Synapomorphy Analysis (RASA) method in combination with the S-F method of sampling reduced character sets, as well as gamma-corrected distance methods. Our results suggest that *CAK1* and *csk1* are orthologs and that a central aspect of CAK regulation has been conserved in budding and fission yeast. Although there are metazoan CDK-family members for which we could not define ancestral lineage, our analysis failed to identify metazoan *CAK1*/*csk1* orthologs, suggesting that if the *CAK1*/*csk1* gene existed in the metazoan ancestor it has not been conserved. This result allows us to propose a model for CAK1 evolution encompassing known CAK regulatory mechanisms in budding yeast, fission yeast, and vertebrates.

# THE *CYCLIN B* AND *B3* GENES HAVE DISTINCT FUNCTIONS DURING CELL DIVISION IN *C. ELEGANS*

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In eukaryotes, A- and B-type cyclins are thought to be required for mitotic events such as chromatin condensation, reorganization of the microtubule cytoskeleton into a bipolar spindle and degradation of the nuclear envelope. However, whether cyclins exert specific functions and what cellular targets are regulated remains unknown. To address cyclin specificity during cell division, we are examining the role of the B-type cyclins in *C. elegans*.

We have identified four B-type genes (*cyb-1, cyb-2, cyb-3, cyb-4*) in *C. elegans*. Sequence analyses showed *cyb-2* and *cyb-4* share 96% nucleotide identity with each other and 65% identity with *cyb-1*. These B-type cyclins are significantly divergent from either the *cya-1* or *cyb-3* genes.

To address the roles of B-type cyclins during cell division we used RNA interference and followed the first divisions of these RNAi embryos by Nomarski microscopy. Injection of *cyb-1*, *cyb-2*, *cyb-3* or *cyb-4* dsRNA each resulted in an embryonic lethal phenotype, indicating distinct essential functions for B-type cyclins in *C. elegans*. The *cyb-3(RNAi)* phenotype was most severe. In *cyb-3* defective embryos cells entered mitosis, however, sister chromatids failed to separate, cytokinesis was initiated in the absence of a completed nuclear division and cell division arrested. In contrast, in *cyb-1(RNAi)* embryos chromosomes were separated successfully but mitosis often resulted in the formation of multinucleated cells. Interference of both *cyb-1* and *cyb-3* resulted in an arrest upon meeting of the pronuclei at the one-cell stage, following abnormal meiosis, pseudocleavage and/or pronuclear migrations. This more dramatic phenotype suggests that *cyb-1* and *cyb-3* exert overlapping functions in addition to their distinct functions. The high degree of sequence identity among *cyb-2* and *cyb-4* prevents conclusions about specific functions. However, injection of either *cyb-2* or *cyb-4* dsRNA resulted in multinucleated cells, clearly distinct from *cyb-3(RNAi)*. We conclude that *cyb-3* is specifically required for an essential step in chromosome segregation.

# CANDIDATE GENES THAT GOVERN MOTORNEURON SYNAPTIC SPECIFICITY

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The *unc-4* gene of *C. elegans* encodes a homeodomain protein, which when mutated changes the synaptic inputs to a single type of neuron, the VA motor neurons. We have identified several genes whose expression decrease (*und-1-4*, *kin-8*) or increase (*ruf-1*) in *unc-4* mutants. These genes encode several novel secreted and membrane-bound proteins and a receptor tyrosine kinase similar to those implicated in the agrin-induced formation of the neuromuscular junction in vertebrates.

We have examined the expression patterns of these genes and showed that at least three of these genes are expressed in the ventral cord motorneurons and the levels of expression decreased in both the *unc-4(e120)* and *unc-37(e262)* backgrounds. We also examined the synapses formed by the interneurons(AVA, AVD, AVE) and the A-type motorneurons in the ventral cord using a *vamp::gfp* construct (provided by M. Nonet) driven by a *sek-1* promoter (the *sek-1* gene is expressed in AVA,AVD,AVE , VCs and some other tissues, M. Tanaka et al. WBG 15(4):30). The *sek-1::vamp::gfp* is expressed in a similar pattern as reported by Tanaka et al. and punctated staining can be seen along the ventral cord. In the *unc-4(e120)* and *unc-37(e262)* backgrounds, the ventral cord staining become weak and diffuse, indicating loss of synapse formation. Similar results were obtained when *kin-8* RNAi was injected into wild-type animals carrying the *sek-1::vamp::gfp* construct. Initial injection of RNAi for *und-1, und-2* and *und-3* did not yield mutant progeny. Therefore, we made hairpin constructs of these genes under the control of the heat shock promoter. Such hairpin constructs have been shown by Monica Driscoll's lab to be effective in inducing the RNAi effect especially in neurons (Tavernarakis, N. et al. *Nat Genet*. 24(20):180-183). We co-injected these hairpin constructs with the *sek-1::vamp::gfp* construct using *lin-15* as the selectable marker. In the stable lines generated after injection, we could induce a high percentage of mutant uncoordinated progeny (35-40%) by heat -shocking parent L4 animals. Furthermore, the expression of the *sek-1::vamp::gfp* construct was decreased and became diffuse similar to the *kin-8* RNAi case. We are currently testing the *let-858* promoter-driving hairpin constructs to see whether we can induce a similar phenotype.

# *PAT-4* MEDIATES INTEGRIN SIGNALING EVENTS DURING ASSEMBLY OF THE MYOFILAMENT LATTICE

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Integrin-mediated signaling events are essential for cell migration, differentiation, and cytoskeletal organization

In *C. elegans*, null alleles of the a-integrin (*pat-2*) and b-integrin (*pat-3*) genes result in lethality. Mutant embryos are **P**aralyzed and **A**rrest at the **T**wo-fold stage of embryogenesis. This Pat phenotype is attributed to failure of the myofilament lattice in body-wall muscle cells to organize and become contractile. Therefore, mutational and functional characterization of sarcomere assembly provides a model for analysis of integrin-mediated signaling pathways. *pat-4* was identified in a screen for genes required for this assembly process. Transformation rescue of *pat-4* reveals it is the homologue of the vertebrate gene *integrin-linked kinase* (ILK). *pat-4* contains four ankyrin repeats, a PH-domain, and a C-terminal kinase domain. *In vitro* studies show the kinase domain of ILK binds b-integrin (*pat-3*), and the ankyrin repeats bind PINCH, an *unc-97* homologue. Interestingly, *unc-97(RNAi)* was recently shown to generate the Pat phenotype.

We constructed a *pat-4*::*GFP* fusion gene which completely rescues the mutant phenotype. The fusion gene product is expressed in body-wall muscle cells, and co-localizes subcellularly with PAT-3 and UNC-97. *pat-4::GFP* is also expressed in the touch neurons PLM and ALM, the vulva muscle cells, the spermatheca, and the distal tip cells. Ectopically expressed mouse *ILK::GFP* localizes to dense body and M-line attachment sites as well.

In *pat-4* mutants, both UNC-52 and PAT-3 are able to localize properly, but vinculin (DEB-1) and the MH-42 antigen (an M-line component) are disorganized. This suggests *pat-4* is required for the transduction of downstream integrin-mediated signaling events required for assembly. In order to determine if PAT-3 and PAT-4 physically interact *in vivo* we have begun an experiment which will measure fluorescent resonance energy transfer (FRET) between PAT-3::CFP and PAT-4::YFP fusion proteins using fluorescent lifetime imaging microscopy (FLIM). So far, we have been able to make lifetime measurements of PAT-4::GFP and are currently generating the strains required for the FRET analysis.

# THE LATE ACTING GENES *LET-7* AND *LIN-41* INTERACT TO CONTROL TEMPORAL DEVELOPMENT IN *C. ELEGANS.*

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*let-7* and *lin-41* are late acting genes in the *C. elegans* heterochronic pathway. *let-7*, a small RNA is predicted to bind to the mRNA of its target *lin-41*, which encodes an oncogenic-like RBCC protein. Together they regulate the timing of a proliferation versus differentiation decision by seam cells. We would like to understand the molecular mechanisms of regulation employed by *let-7* and *lin-41*. One approach we are taking is to find other genes interacting at this stage of development.

Preliminary results from a screen that sought mutations causing the characteristic *let-7* heterochronic phenotype indicate that we have found at least one new heterochronic gene (Slack, Pasquinelli, and Ruvkun, unpublished). Two mutations, *mg281* and *mg283* map near *lin-29* on chromosome II (Pasquinelli and Ruvkun, personal comm.), but complement *lin-29* mutations (Rougvie, personal comm.) Complementation tests are being carried out to determine if these two mutations are in the same gene. We are also utilizing *MH27::gfp* (*jcIs1*) which tags cell junctions allowing for the visualization of cell fusion and division, to further characterize these mutant phenotypes. These mutations may define a gene that works with *let-7* to control temporal patterning.

In a screen to find genes negatively regulated by *let-7*, multiple alleles of the gene *lin-41* were found. In the same screen another mutation, *lin(mg188),* was isolated. *lin(mg188)* causes cold sensitive sterility and a heterochronic phenotype on its own. We are characterizing the mutant phenotype and mapping *lin(mg188)* to determine the gene it mutates. We postulate that this gene may interact with *lin-41* and *let-7* in some way late in development.

# THE FUNCTION AND EVOLUTION OF LATROPHILINS AND CELSR, G-PROTEIN COUPLED RECEPTORS OF THE SECRETIN FAMILY THAT ARE CONSERVED IN *C.ELEGANS*, *DROSOPHILA* AND VERTEBRATES

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Latrophilins and Celsr (Cadherin, EGF-like, LAG-like Seven pass receptor) are members of the secretin family of G-protein coupled receptors. The function of these proteins is unknown. They are conserved in *C. elegans*, *Drosophila* and vertebrates. In vertebrates, latrophilin-1 is the receptor for alpha-latrotoxin, a neurotoxin that causes a massive increase in neurotransmitter release at the presynaptic terminal, suggesting a role for latrophilins in mediating exocytosis. Celsr, F15B9.7 in *C. Elegans*, is a protein related to latrophilins with homologous regions in the transmembrane and membrane proximal extracellular domains.

There are currently three known latrophilins in vertebrates, two in *C. elegans*, and one in *Drosophila*. The vertebrate latrophilins most likely arose from a single latrophilin in this lineage because they share a high degree of homology. The two latrophilin homologues in *C. elegans*, B0457.1 and B0286.2, may have arisen prior to the split of the *C. elegans* and vertebrate lineages or soon after the split because they show a high degree of divergence. The vertebrate latrophilins show a high degree of alternative splicing and so far we have identified two different splice variants of B0457.1 by looking at ESTs in the Kohara database.

We are currently trying to study the expression, function, and evolution of these proteins. We have made a C-terminal B0457.1::GFP construct. Embryonic expression starts in a few cells at the coma stage and then progresses throughout the pharyngeal and head regions at the two fold and three fold stages. The expression becomes more fainter and localizes to the regions around the anterior and posterior bulbs of the pharynx at hatching. There is variable expression in the adult with faint staining also in the anterior and posterior bulb regions. We are currently attempting to characterize this pattern in more detail.

# LIN-13 UPDATE

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The "SynMuv" genes appear to be involved in providing a signal that inhibits vulval precursor cells from adopting vulval fates in C. elegans. These genes are called "SynMuv" genes because a Multivulva phenotype is only visible if mutations in two different genes, one from Class A and one from Class B, are combined (a "Synthetic Multivulva phenotype) (see Ferguson and Horvitz 1989). Class B includes a gene encoding a protein related to the tumor suppressor Rb. Class A includes certain components of the nucleosome remodelling and histone deacetylase (NURD) complex (Solari and Ahringer 2000). Other genes related to Rb and NURD have both Class A dna Class B activity (Solari and Ahringer 2000). The lin-13 gene has genetic properties consistent with function as a SynMuv gene. We have found that null alleles of lin-13 are temperature-sensitive and maternally rescued, resulting in phenotypes ranging in severity from L2 arrest (when both maternal and zygotic activities are removed at 25o), to sterile Multivulva (when only zygotic activity is removed at 25o), to sterile non-Multivulva (when both maternal and zygotic activities are removed at 15o), to wild-type/Class B SynMuv (when only zygotic activity is removed at 15o). The predicted LIN-13 protein contains multiple zinc fingers and a motif (LXCXE) that has been implicated in Rb binding. In addition, LIN-13 is a nuclear protein and is consistently expressed in many cell types, including hyp7, at the time of VPC specification. Our results are consistent with a role for LIN-13 in Rb-mediated and NURD-mediated transcriptional control processes that lead to repression of vulval fates.

We have begun a genetic mosaic analysis to determine the cellular focus of lin-13 in vulval development. We will report on our progress and we will also speculate about the connection between lin-13 and the class B synMuv gene lin-35Rb.

Ferguson, E.L. and Horvitz, H.R. (1989) Genetics 123, 109-121

Solari, F. and Ahringer, J. (2000) Current Biology 10, 223-226.

# *SNS-10(OY42)* IS INVOLVED IN THE DEVELOPMENT OF THE AWA CHEMOSENSORY NEURON

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How is the specific fate of a cell determined? How does it become different from other cells? We are studying the development of the chemosensory neurons in an attempt to answer these kinds of questions. The chemosensory system of *C. elegans* enables its response to a wide variety of chemicals. The AWA and AWC neuron pairs mediate chemotaxis towards volatile attractants, while the AWB neuron pair mediates repulsion. Each neuron pair responds to a different subset of chemicals. Although their identities are different, i.e. they express different transcription factors, receptor molecules etc., some aspects of their development are similar. Recent work has shown that these three olfactory neurons share a common developmental default state, an AWC fate (Sagasti *et al*., 1999. *Genes Dev*. **13**: 1794-806). Expression of cell specific transcription factors in the AWA and AWB neurons overrides this basal fate, allowing AWA and AWB development. We would like to understand the mechanisms that give rise to the similar as well as the distinct characteristics of these cells.

The nuclear hormone receptor transcription factor *odr-7* is required for the functional specification of the AWA neurons. In the absence of *odr-7*, all AWA mediated responses are lost and AWC characteristics are exhibited. In order to identify factors that initiate and maintain *odr-7* expression and thus AWA specification, I conducted a screen looking for mutants showing altered expression of *odr-7*. In this screen I isolated *sns-10*(*oy42*). This mutant mostly exhibits a variable loss in *odr-7* expression and rarely shows ectopic *odr-7* expression. Behavioral studies indicate that AWA function is defective suggesting that AWA is either lost or not specified correctly. Mutant animals exhibit other pleiotropies suggesting that *sns-10* is involved in the formation and/or function of other cells as well. I mapped *sns-10* to LG X and have recently obtained single cosmid rescue of its mutant phenotype. I am now examining candidate genes. Identification of the molecular nature of *sns-10* will allow me to further investigate its role in the development of the AWA neurons. In addition, its interaction with other identified genes in this process including *odr-7*, *unc-130* and *lin-11* will be studied (see abstract by Sarafi-Reinach and Sengupta).

# DISSECTING THE SIGNALING PATHWAYS REGULATED BY THE PTEN TUMOR SUPPRESSOR HOMOLOG DAF-18 IN *C. ELEGANS*

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PTEN is a tumor suppressor protein and mutations in its gene have been found in a variety of human cancers and in cancer predisposition syndromes. At the molecular level, recent studies suggest that PTEN is a phosphatase for phosphatidylinositol 3,4,5-trisphosphate (PIP3). PIP3 is produced by phosphatidylinositol 3-kinase (PI 3-kinase). We have previously shown that in *C. elegans*, the PTEN homolog *DAF-18* functions as a negative regulator for the signaling pathway controlled by DAF-2, an insulin receptor-like molecule and by AGE-1, a PI 3-kinase homolog (1). We have established that a deletion mutation in the catalytic domain of CePTEN/DAF-18, *daf-18(nr2037)*, completely suppresses the dauer-constitutive phenotype caused by mutations in *daf-2* or in *age-1*. In addition, we have demonstrated that *daf-18(nr2037)* mutant dramatically shortens life-span, both in a wild-type strain background and in a *daf-2* mutant background that normally prolongs life-span. The fact that inactivation of *daf-18* suppresses the null mutation in *age-1* PI 3-kinase suggest that there may be other enzymes involved in the production of PIP3 in a mutant strain that lacks AGE-1 activity. In the *C. elegans* genome database, we found that there is a type II PI 3-kinase homolog encoded by the *F39B1.1* locus. AGE-1 belongs to the type I PI 3-kinase subfamily. While the type I PI 3-kinases are known to be activated by association with the growth factor receptor tyrosine kinases, very little is known about the function and regulation of the type II PI 3-kinases. To determine if the type II PI 3-kinase homolog in *C. elegans* is involved in generating PIP3, we undertook the approach of the reverse genetics and obtained a mutant strain that carries a deletion in the *F39B1.1* gene. We are in the process of characterizing this mutant and determining the functional relationship of this gene with *age-1* and *daf-18/CePTEN* in regulation of dauer formation and life-span processes.

1. V. Mihaylova, C. Borland, L. Manjarrez, M. Stern and H. Sun (1999) Proc. Natl. Acad. Sci. USA 96, 7427-7432.

# A SCREEN FOR GENES THAT CONTROL PROGRAMMED CELL DEATH IN THE GERM LINE

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Although we know a great deal about the apoptotic program in *C. elegans*, we know very little about how the program is regulated. Since this cell fate is responsible for the demise of about 10% of the somatic cells, and as many as half of the germ cell, we are interested in defining the genes that may be involved in the decision of a cell to die.

In order to find such genes, we developed a screen using the vital dye acridine orange, which specifically stains apoptotic cells in the germ line. Using this screen we have looked at approximately 40,000 genomes and have identified 21 mutants that have increased levels of germ cell death. The ten mutants that have thus far been mapped represent eight complementation groups, only one of which has multiple alleles. Of these I am currently cloning two by single nucleotide polymorphism mapping, *gla-1(op212)* and *gla-3(op234).*

In order to determine if these mutants are specifically defective in the regulation apoptosis, or if they are pathologically compromised, and thus have damage that causes the cells to undergo apoptosis, we made double mutations with *ced-3* and *rad-5*. Most of the mutations examined are suppressed in the *ced-3* background, suggesting that the damage is non pathological (we would have expected necrosis or some other gross morphological consequence if this were the case). In the *rad-5* background, a mutation that we have shown to render worms insensitive to DNA damage, these mutations are not suppressed. This again suggests specificity since mutations that somehow result in DNA damage would be suppressed.

# EXPRESSION AND FUNCTIONAL ANALYSIS OF GENES CONTAINING RING FINGER DOMAINS IN *CAENORHABDITIS ELEGANS*

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Using microarrays and RNAi (RNA interference) we hope to better characterize proteins containing a RING motif in the nematode *Caenorhabditis elegans*. We have printed an array that consists of 1kb coding rich segments of 112 genes encoding for proteins containing the zinc RING domain along with a number of control genes. Beginning with L1 synchronized worm populations we collected samples every 6 hours from which we isolated total RNA. Total RNA was also extracted from the embryos of the adult hermaphrodite. The total RNA samples were reverse transcribed in the presence of $^{33}$P-dCTP to create a radiolabeled cDNA. After hybridization of these samples to the array, the expression patterns across the embryo to adult time course were analyzed to determine common expression patterns. Recent literature suggests that proteins containing the RING motif may play a role in the ubiquitin degradation pathway. By clustering the array data according to levels of expression across the time course, we found a cluster which includes genes suspected to be involved in ubiquitin degradation as well as some previously uncharacterized genes. We are currently using RNAi to block the functional expression of these genes in order to investigate their function. By analyzing the expression patterns and RNAi phenotypes of uncharacterized genes with those of previously characterized genes we hope to provide evidence of their function.

# PROGRESS TOWARDS CLONING *EGL-47*, A PUTATIVE COMPONENT OF A G PROTEIN-COUPLED SIGNALING PATHWAY

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Our goal is to understand how neurotransmitters act through G protein-coupled receptors to modulate the activities of neurons. Egg-laying behavior in *C. elegans* is regulated through G proteins and is an excellent model to study G protein regulation. A screen to identify mutations that affect the function, but not morphology, of the HSN neurons that control egg laying identified five genes: *egl-10*, *egl-42*, *egl-47*, *egl-49* and *egl-50*[1]. Mutants in these genes are both egg-laying defective and insensitive to the serotonin reuptake inhibitor imiprimine, and yet these mutants have anatomically normal egg-laying systems and wild-type sensitivity to serotonin. These characteristics are thought to be the result of functional defects in the HSN neurons that control egg laying. EGL-10 has been identified as a regulator of G protein signaling (RGS) protein, while the remaining genes have not been cloned. Of these remaining genes, *egl-47* was chosen for further study as it has the strongest egg-laying defective phenotype. Both of the originally isolated *egl-47* alleles are dominant gain-of-function mutations. We isolated loss-of-function mutations as revertents of a dominant allele; these have no discernible defects.

Traditional genetic mapping located *egl-47* on chromosome V in the interval between *unc-42* and *egl-3*, a distance of over 400kb containing almost one hundred genes. We are using single nucleotide polymorphisms (SNPs) to further map *egl-47*. This mapping procedure involves crossing a marked *egl-47* mutant with CB4856, a strain with frequent SNPs. Analyzing recombinants from this cross allows the position of *egl-47* to be determined with respect to the SNPs. The resolution of this mapping technique is only limited by the number of recombinants generated and the frequency of SNPs in CB4856. By sequencing CB4856, we have identified SNPs approximately every 40kb throughout the region containing *egl-47.* Once *egl-47* is mapped to a 40kb interval, PCR products spanning this interval, generated from *egl-47(gf)* mutant animals, will be injected into wild-type worms. PCR products containing the mutant *egl-47* gene should confer the mutant phenotype, thereby identifying *egl-47*.

1. Desai, C. and Horvitz, H.R. (1989). Genetics, **121**:703-721.

# REGULATION OF THE HETEROCHRONIC GENE *LIN-28* INDEPENDENTLY OF THE *LIN-4* RNA

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The heterochronic genes act in a temporal cascade to permit the normal succession and synchrony of developmental events through the larval stages. Two of these genes, *lin-14* and *lin-28*, act early in development to cause L1- and L2-specific events to occur. Both must be repressed to allow subsequent stage-specific events to occur in the L3 and later. *lin-4*, which encodes a small RNA, is needed for the repression of both of these genes. When *lin-4* is absent, both *lin-14* and *lin-28* expression remain high in late stages, causing a reiteration of early larval fates and retarded development. However, if either *lin-14* or *lin-28* is removed by mutation in the absence of *lin-4*, the other is gene is repressed. We have referred to this as the mutual positive feedback loop between *lin-14* and *lin-28*. The existence of this feedback loop indicates that repression of *lin-14* and *lin-28* can take place independently of the *lin-4* RNA. We have characterized the *lin-4*-independent repression of *lin-28*. The repression of *lin-28* by the *lin-4*-dependent and *lin-4*-independent mechanisms appears to occur in the same time frame. LIN-28 decreases after the L1 in wildtype and in a *lin-4; lin-14* double mutant, but not in a *lin-4* mutant. Repression of *lin-28* fails to occur in a *lin-14(gf)* mutant that is insensitive to *lin-4* due to a rearrangement of its 3' UTR -- this is true even in with a functional *lin-4* gene. Therefore, the *lin-4* RNA is neither necessary nor sufficient to cause the temporal repression of *lin-28*. We have determined that the *lin-4*-independent repression of *lin-28* is mediated through the gene's 3' UTR through a site that is distinct from the *lin-4*-complementary element. We have also determined that the *lin-28* mRNA is polysome-associated in the repressed state, as is the case for the *lin-4*-dependent repression, suggesting the two mechanisms of repression are similar, if not identical. It may be that *lin-4* exerts its effect by acting simultaneously on *lin-14* and *lin-28* to enhance the feedback loop repression mechanism. Using a new genetic screen we are seeking mutations in trans-acting factors that are needed for the repression of *lin-28*.

# FUNCTIONAL STUDIES ON THE FAX-1 NUCLEAR RECEPTOR

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The *fax-1* gene encodes a nuclear hormone receptor that is related to the human RNR and *Drosophila tailless* proteins (see abstract by Reinert et al.). *fax-1* is required for normal axon pathfinding and FMRFamide-related neurotransmitter expression. We are pursuing three lines of research directed toward understanding the function of *fax-1* in nervous system development.

First, we are attempting to identify genes that are regulated by FAX-1. These may include mediators of axon pathfinding and neurotransmitter expression. The close relationship between FAX-1 and RNR DNA-binding domains suggests that the two proteins may bind the same DNA sites. RNR has been shown to bind to dimeric AAGTCA Tailless binding sites (Kobayashi et al., 1999), suggesting that FAX-1 may also bind this sequence. The *C. elegans flp-1* gene, which could be regulated by FAX-1, has two copies of a TCGTCA sequence immediately upstream of the transcribed region. We are evaluating the DNA-binding properties of FAX-1 by gel-shift assays. We are also identifying downstream targets of FAX-1 by employing the "One-Hybrid" strategy in yeast.

Second, we are evaluating whether the human RNR can functionally substitute for FAX-1. While RNR is closely related to FAX-1 in the DNA-binding domain, it is quite divergent in the ligand-binding domain. We are placing a human RNR cDNA under the control of the *fax-1* promoter. The resulting fusion gene will be introduced into *fax-1* mutants and the transgenic progeny evaluated for rescue of Fax-1 phenotypes. Partial or complete rescue of *fax-1* by RNR would establish the functional equivalence of the two proteins.

Third, we are evaluating the consequences of FAX-1 ectopic expression. Loss of *fax-1* results in defects in axon pathfinding and FMRFamide-related neurotransmitter expression. We are creating fusions of *fax-1* cDNA and genomic clones to the *hsp16-2* promoter. Transgenic animals that bear *hs::fax-1* DNA will be examined for Fax-1-related phenotypes following a heat pulse. Ectopic FMRFamide immunoreactivity would suggest that FAX-1 expression is sufficient. We will also examine neurons that do not normally express FAX-1 for axonal anatomy. Defects in axon pathfinding may reflect reprogramming of pathway choice by the ectopic presence of FAX-1.

# STRUCTURE-FUNCTION CHARACTERIZATION OF BAR-1 AND OTHER B -CATENINS IN *C. ELEGANS* 

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*C. elegans* vulval development is co-ordinated by conserved RTK/Ras and Notch signaling pathways, so that six Vulval Precursor Cells (VPCs) adopt cell fates in the pattern 3/F° 3° 2° 1° 2° 3° (F stands for Fused fate). In a screen for mutants affecting vulval development, *bar-1* was identified. *bar-1* mutants show defects in VPC and P12 cell fate specification and $Q_L$ progeny migration. In each case, *bar-1* is required to maintain Hox gene expression in the affected cells. *bar-1* encodes a *C. elegans* b -catenin/Armadillo homolog. These proteins function in both cell adhesion and Wnt signal transduction. Wnt pathways regulate Hox genes in other systems and Wnt pathway mutants in *C. elegans* show similar phenotypes as *bar-1* mutants which suggests that *bar-1* acts in a Wnt pathway in the vulva.

We would like to further understand BAR-1 structure and function. To identify transcriptional activation domains in BAR-1, regions of BAR-1 were made as GAL4 DBD fusions and tested in yeast. N-terminal and the C-terminal regions of BAR-1 possess transcriptional activating function, similar to other b -catenins. The effects of deletion of different BAR-1 domains on BAR-1 activity in the worm will be analyzed. A single region containing Armadillo repeats (1-9) did not activate transcription on its own and was used to identify BAR-1 interacting factors (143) by the yeast two hybrid method. The roles of potential interactors in vulval development are being investigated. BAR-1 is also being tested for interaction with other known Wnt pathway components in *C. elegans*. APR-1 and POP-1 interact with BAR-1 and reducing the activity of these genes results in vulval defects (work from our lab and Hajnal lab). BAR-1 does not interact with EGL-27, LIN-25 and HMP-1 (a -catenin) in yeast.

*C. elegans* has three b -catenins, HMP-2, WRM-1 and BAR-1, as opposed to one (Armadillo) in Drosophila. WRM-1 functions in Wnt signaling in embryogenesis and HMP-2 in cell adhesion/morphogenesis. We are testing if BAR-1 function in the vulva can be substituted by fly Armadillo and other b -catenins in *C. elegans*. We would also like to know if BAR-1 interacting proteins can interact with WRM-1 and HMP-2. These experiments will help us understand if the functions of b-catenins in *C. elegans* have been conserved.

# CONTRIBUTIONS OF WRN-RELATED HELICASES TO *C. ELEGANS* AGING

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Four genes in the *C. elegans* genome exhibit sequence similarity to the RecQ family of DNA helicases which include the human Werner (*WRN*), Bloom (*BLM*) and Rothmund-Thomson (RTS) syndrome genes. Mutation of human *WRN* and *RTS* can confer features of accelerated aging. Both WRN and RTS patients have a high rate of genome instability, which is also a key feature of patients with Bloom syndrome. Similarly, mutation of the yeast RecQ homolog, *SGS1,* results in a high rate of genomic instability and a lifespan 40% shorter than wild type[1, 2]. To determine if the RecQ family of helicases share similar functions in *C. elegans*, we generated deletions within the *C. elegans* gene most closely related to the Werner's gene (F18C5.2) and in another homolog (E03A3.2) and have tested these strains for lifespan and genomic instability.

Preliminary results indicate that animals lacking F18C5.2 or E03A3.2 are viable and fertile. They have normal lifespans and do not have a high rate of genomic instability. Since *C. elegans* has four related helicases, there may be redundancy in the activity of these helicases. To test this hypothesis, we are now analyzing animals that carry mutations in multiple DNA helicase family members. It has recently been determined that *him-6* mutant animals, which have a high rate of chromosome nondisjunction, contain mutations in the *C. elegans* DNA helicase gene T04A11.6, which is most closely related to the human *BLM* gene[3]. We are also testing mutants for UV radiation or X-ray hypersensitivity, since it is possible that these helicases play a role in DNA repair. We are testing the expression pattern of these genes and are screening for a deletion in the fourth remaining *C. elegans* DNA helicase family member, K02F3.1. By establishing a nematode model for accelerated aging disorders, we hope to extend understanding of the action of helicase family members in senescence and learn more about mechanisms of aging overall.

1. Watt, P.M., I.D. Hickson, R.H. Borts and E.J. Louis. 1996. *Genetics* **144**: 935.
2. Sinclair, D.A., K. Mills and L. Guarente. 1997. *Science* **277**: 1313.
3. Wicky, C., A. Rose and F. Mueller. 1998. European Worm Meeting Abstract.

# FOOD-DEPRIVATION AND MODULATION OF LOCOMOTORY BEHAVIOR: *MOD-6* AND A SCREEN FOR NEW GENES

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Upon entering a bacterial lawn, well-fed hermaphrodites exhibit a basal slowing response while acutely food-deprived worms exhibit enhanced slowing (see abstract by Ranganathan et al.). A number of cloned genes define a molecular pathway in which serotonin signaling is critical for this enhanced slowing response. Food-deprived worms are no more sensitive to exogenous serotonin than well-fed worms, suggesting that food-deprivation induces a physiological change that modulates serotonin release rather than alters the sensitivity to endogenous serotonin. *mod-6(n3076)* (modulation of locomotion defective) was isolated in a screen for mutants that failed to exhibit enhanced slowing after food-deprivation. Mutations in *mod-1,* which encodes an ionotropic serotonin receptor, and in *goa-1*, which encodes a G protein coupled to serotonin signaling, result in a Mod phenotype. These animals are resistant to immobilization by exogenous serotonin, indicating that they define components that act postsynaptically to serotonin. By contrast, *mod-6* animals are modulation defective, serotonin positive by immunostaining, sensitive to exogenous serotonin, and flouxetine (Prozac) resistant. These characteristics suggests that *mod-6* may be involved in modulating serotonin release in response to food-deprivation. We have mapped *mod-6* to a small interval on chromosome I and are presently attempting to clone the gene by cosmid rescue.

We are also performing a screen to isolate more mutants that fail to modulate their locomotory rate after food-deprivation. Specifically, we are looking for suppressors of *n3314,* a deletion allele of the *mod-5* serotonin re-uptake transporter. *mod-5(n3314)* sensitizes animals to endogenous serotonin release, resulting in immobilization of food-deprived animals upon re-entering a bacterial lawn. We hope to find mutations involved in detecting food-deprivation, storing this information, modulating the release of serotonin, and signaling downstream events directly involved in slowing. Our primary focus will be on new genes involved in changing the internal state of the animal in response to food-deprivation. These mutants would likely be modulation-defective and flouxetine-resistant but still sensitive to exogenous serotonin.

# ARRAYS CONTAINING THE *CKI-1* PROMOTER REGION CAUSE INAPPROPRIATE DAUER FORMATION IN *C. ELEGANS*

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*Cki-1* is a p21/p27-like cyclin dependent kinase inhibitor in *C. elegans*. It plays an important role in arresting cells at the G1 to S phase transition in response to numerous developmental cues (Hong *et al.*, 1998). Two *cki-1* transcriptional constructs were used to prepare transgenic worms in order to determine the spatial and temporal expression pattern of *cki-1* during development. Constructs containing either 2 kb or 8 kb of promoter sequence upstream of GFP were microinjected and arrays were integrated to yield *maIs109* and *maIs113* respectively. Different expression patterns were observed for each strain suggesting complex transcriptional regulation of this gene. During dauer formation, strong GFP expression was observed in the hypodermis with *maIs113* compared to *maIs109* (Hong *et al.*, 1998). While examining the expression pattern of these reporters in dauer we noticed an unusual number of dauers present at permissive temperature in *daf-7*(e1372) mutants that harbored *maIs113*. *daf-7*(e1372) is a temperature sensitive mutant that develops normally at 15° C but constitutively forms dauers at non-permissive temperature (25° C), even under conditions that promote normal development (Ren *et al.,*1996). 22% of the *maIs113;daf-7*(e1372) worms form dauers at permissive temperature compared to about 5% in *daf-7* and 0% with *maIs113* alone. This effect is only seen with *maIs113* and not *maIs109*. It is also specific to the TGFb -like pathway since we did not observe inappropriate dauer formation at permissive temperature when *maIs113* was crossed into a *daf-2*(e1370) mutant background. It is possible that factor(s) important for normal development that bind to the *cki-1* promoter may be sequestered by this concatamerized array thereby causing animals to execute dauer inappropriately. Our future work will consist of characterizing deletion constructs of the 8 kb promoter region (generously provided by Rosalind Lee and Victor Ambros) to identify promoter sequences involved in dauer-specific expression of *cki-1* in addition to regions that mediate this dauer effect. A genetic modifier screen will also be performed to obtain suppressors and enhancers of this unusual dauer formation phenotype.

# SCREENS FOR TRANSGENIC EXPRESSION OF MATERNAL GENES

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U Mass Medical Center

Protein tagging with GFP is very useful for analyzing the spatial and temporal distribution of a protein in living cells. This approach, however, has limited use in studying maternal proteins in *C. elegans* embryos since germline transgenes are rapidly silenced leaving only a short time window for observation. We are developing a strategy to improve maternal transgene expressions in the germline. The strategy involves 1) the expression of the transgene from a YAC array and 2) a genetic screen for second site mutations that improve transgene expression.

We found that yeast artificial chromosome (YAC) containing a *pie-1::gfp* fusion can confer high levels of fluorescence from the PIE-1::GFP protein. However, silencing still occurs; after five generations, only 5-10% of transgenic animals continue to exhibit detectable GFP fluorescence. The integrated version of the *pie-1::gfp* transgene can rescue *pie-1* mutants very well as long as the transgene is expressed. However, most animals produce only a few viable progeny due to partial silencing.

A genetic screen was then performed to search for mutants with improved *pie-1* rescue which meant better expression of the *pie-1::gfp* transgene. The rescue strain was crossed into a *lin-2* background and 100,000 rescued animals were mutagenized. Mutants were scored for better viability. We have so far isolated two mutants, KE1 and KE6, that rescue *pie-1* mutation almost completely. All of the embryos from these strains show high fluorescence signal for the PIE-1::GFP fusion protein. The expression pattern of the fusion protein is identical to that of endogenous PIE-1; i.e. mutations do not cause ectopic expression. As secondary screen, we are testing these desilencing mutants for their ability to increase expression from an independent transgene (*lit-1::gfp*). These mutations may define the genes required for transgene silencing and hence will be very useful for expressing germline transgenes in the future.

# CHARACTERIZATION OF CALRETICULIN (*CRT-1*), A CALCIUM-BINDING PROTEIN, IN *CAENORHABDITIS ELEGANS*

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Calreticulin is a ubiquitous, multi-functional and well-conserved calcium binding protein. The calreticulin gene *crt-1* in *C. elegans* is mapped to the chromosome V and encodes 395 amino acid protein, which has three domains and a C-terminal ER retention sequence, HDEL. The *crt-1* shows overall 60% amino acid sequence identity with mouse and human calreticulins. We characterized the spacio-temporal pattern of the *crt-1* gene expression by northern, *in situ* hybridization and western analyses. The mRNA transcripts were abundant during early embryonic stages and mostly restricted to intestinal precursor cells during later stages. These results were further confirmed by whole-mount immunostaining with anti-recombinant calreticulin antibody. We obtained a crt-1 mutant by screening deletion mutants using TMP/UV mutagenesis. The DNA sequencing of deletion mutation revealed that the deletion spans from the first intron through 3' UTR of the *crt-1* gene. Western analysis showed that no calreticulin is made in this mutant. The mutant shows slow growth and significantly reduced brood size. We are currently analyzing the function of *crt-1* gene by characterizing this mutant animal in detail.

# FOLLOWING THE *IN VIVO* FATE OF THE DOUBLE-STRANDED TRIGGER RNA DURING RNA-MEDIATED INTERFERENCE (RNAI)

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We are investigating the molecular mechanisms underlying the process of RNA-mediated interference (RNAi). In *C.elegans*, introduction of double-stranded RNA (dsRNA) results in the post-transcriptional silencing of genetic loci containing homologous sequences. To achieve RNAi, only a few molecules of dsRNA are required to trigger an effect. Importantly, similar homology-dependent silencing mechanisms have been documented in several diverse systems, such as plant, fungal, protozoan, planarian, and insect systems. To expand our understanding of RNAi and its possible relationship to other post-transcriptional silencing mechanisms, we are following the fate of the dsRNA trigger molecule during RNA interference.

Two intriguing apects of RNAi are the requirement for both sense and anti-sense strands in the trigger and the ability of a double-stranded trigger to function at very low concentrations. Our data indicate that one role of the incoming sense strand is to provide biological stability for the complementary strand.

Evidence from plants and *Drosophila* suggests that the trigger molecule may be cleaved into 23-25 nucleotide fragments during post-transcriptional gene silencing. Through the development of an *in vivo* assay which allows us to trace the fate of the trigger molecule, we have begun to address whether the dsRNA molecule undergoes similar processing during RNAi. By pursuing the characterization of the fate of the trigger molecule, we hope to acquire some insight into the mechanism of RNA interference.

# TWO CONSERVED DOMAINS OF THE EGL-10 RGS PROTEIN COOPERATE TO INHIBIT G PROTEIN SIGNALING IN THE *C. ELEGANS* NERVOUS SYSTEM

## Georgia A. Patikoglou, Dan Chase, Michael R. Koelle

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Regulator of G protein Signaling (RGS) proteins negatively regulate heterotrimeric G proteins by functioning as potent GTPase Activating Proteins (GAPs). Thus, they accelerate the slow intrinsic rate of GTP hydrolysis by G-alpha proteins and convert them to their inactive GDP-bound forms. While all RGS proteins contain an "RGS domain", which has GAP activity, a subset of RGS proteins also contain an additional N-terminal domain of unknown function. In *C. elegans*, one member of this subset, EGL-10, inhibits the neural G-alpha protein GOA-1 to control egg-laying behavior.

To analyze the function of the two conserved RGS protein domains, we expressed full-length EGL-10 and its subdomains in *C. elegans* and assessed their effects on egg laying. Although full-length EGL-10 rescued the *egl-10* egg-laying defect, the N-terminal domain alone did not, and an RGS domain fragment gave only partial rescue. Surprisingly, the N-terminal and RGS domain fragments completely rescued the *egl-10* egg-laying defect when coexpressed as separate polypeptides. Therefore, the N-terminal domain appears to assist the RGS domain in inhibiting G-protein signaling, and the two domains need not be covalently attached to function together.

EAT-16 is another *C. elegans* RGS protein that, like EGL-10, contains both the conserved N-terminal and RGS domains. However, EAT-16 appears to inhibit a different neural G-alpha protein, EGL-30, a close homolog of mammalian $G_q$. Transgenic expression of EAT-16 does not rescue the *egl-10* egg-laying defect but does rescue the defects seen in an *eat-16* mutant. To understand where the G-alpha protein target specificity determinants in EAT-16 and EGL-10 lie, we generated chimeras between EGL-10 and EAT-16 in which the N-terminal domain, the RGS domain region, or the linker between the two domains were swapped. These chimeras were expressed in *egl-10* and *eat-16* mutant backgrounds and tested for rescue activity. While these experiments found that no single region tested entirely determines G-alpha specificity, our results suggest that the linker region between the N-terminal and RGS domains plays an important role. To extend our genetic findings we are purifying GOA-1, EGL-10, EGL-30, EAT-16, and various EGL-10 protein fragments for use in biochemical assays.

# AN RNAI SCREEN FOR GENES INVOLVED IN PIE-1 LOCALIZATION

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During embryonic cleavages, the maternal factor PIE-1 is segregated to the germ lineage, where it inhibits mRNA production and maintains germline fate. PIE-1 is uniformly distributed in oocytes and newly fertilized eggs, but becomes localized to the posterior of the embryo prior to the first cleavage. As a result, PIE-1 predominantly segregates to the germline blastomere P1. This asymmetric pattern of segregation is repeated at each unequal P cell cleavage, with PIE-1 consistently segregating to the germline precursor blastomeres.

We have previously identified two domains of the PIE-1 protein required for its localization. A first domain near the carboxyl terminus of the protein is necessary and sufficient for localizing PIE-1 to the posterior prior to cell division. A second domain is necessary and sufficient for degrading left over PIE-1 in somatic blastomeres after cell division. Further, we have shown that segregation of PIE-1 to the posterior depends on the presence of an intact actin cytoskeleton, but does not absolutely depend on PIE-1 binding to P granules, which also segregate with the germ lineage.

Collectively, these findings provide clues about the nature of the mechanisms underlying PIE-1 localization. Nevertheless, the molecular identities of the factors involved in these mechanisms remain unknown. In an effort to attain further insight into this area, we are screening for genes that cause mislocalization of a PIE-1:GFP fusion when they are subjected to RNAi. Candidate genes being screened include 258 genes defined as oocyte-enriched in DNA microarray experiments (1) as well as genes that have functions consistent with our previous observations regarding the regulation of PIE-1 localization.

# CHARACTERIZATION OF PROXIMAL PROLIFERATION (PRO) MUTANTS

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In many organisms, undifferentiated germ cells proliferate prior to entering meiosis, but the factors that govern the temporal and spatial aspects of the earliest meiotic entry events are not well understood. We are using proximal proliferation mutants in *C. elegans* as an entry point to investigate these factors.

Genetic screens in our lab have identified mutants that display a "proximal proliferation" (Pro) germline phenotype but do not appear to affect somatic gonad development. In Pro mutants, instead of the normal adult distal-to-proximal germline pattern of "mitosis, meiosis, gametogenesis", a pattern of "mitosis, meiosis, gametogenesis, mitosis" is observed. Therefore these mutants contain an ectopic region of proliferating cells in the proximal region of the gonad (proximal to mature gametes), yet maintain their ability to adopt normal germ cell fates. Normally, the proximal-most cells are the first cells to enter meiosis in the L3. Therefore, the Pro phenotype could arise from a disruption in mechanisms that control the spatial and/or temporal regulation of the proximal mitosis/meiosis switch in the L3. In this model, the Pro phenotype results from abnormal proliferation of proximal-most germ cells, while more distal germ cells undergo normal differentiation. Three of our Pro mutants proved to be *glp-1* alleles (see abstract by Lo and Hubbard). Several other mutations were identified that cause a similar Pro phenotype but are not *glp-1* alleles. These mutations likely identify genes involved in the regulation of the spatial or temporal patterning of meiotic entry.

We are in the process of characterizing the non-*glp-1* Pro mutants genetically, phenotypically and molecularly to determine their role in patterning of meiotic entry. Two-factor, three-factor and deficiency mapping are being used to refine the map positions of the Pro alleles. Recent results indicate that one of the alleles maps within a defined region of chromosome II, while another maps to the X chromosome. Further progress on characterization of these alleles will be presented at the meeting.

# CHARACTERIZATION OF *SUP-11* AND *SUP-18*, TWO REGULATORS OF THE SUP-9/SUP10/UNC-93 TWO-PORE POTASSIUM CHANNEL COMPLEX

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Rare altered-function mutations in the genes *unc-93*, *sup-9*, and *sup-10* result in the abnormal regulation of muscle contraction. These mutants move sluggishly, are unable to lay eggs, and exhibit the rubber-band phenotype: when worms are prodded on the head, they contract and relax along their entire bodies without moving backwards. Genetic studies suggest that these three genes act at the same step and likely encode subunits of a protein complex. We have shown that *sup-9* encodes a two-pore $K^+$ channel subunit with similarity to mammalian TASK. *unc-93* and *sup-10* encode novel putative transmembrane proteins.

Loss-of-function mutations in a fourth gene, *sup-18,* completely suppress the rubber-band phenotype caused by a *sup-10(gf)* mutation and partially suppress the *unc-93(gf)* and *sup-9(gf)* rubber-band phenotypes. We have shown that *sup-18* encodes a single-pass transmembrane protein. Its intracellular domain shares sequence similarity with a family of bacterial NADH oxidases. A *sup-18::gfp* fusion is expressed in muscle and is localized to dense bodies, along with SUP-9 and UNC-93::GFP. We are making deletions in the *sup-18::gfp* reporter to identify regions of SUP-18 required for colocalization with SUP-9. In addition, we are assaying recombinant SUP-18 for nucleotide binding and dehydrogenase activities *in vitro*.

Rare gain-of-function mutations in *sup-11* cause a small scrawny phenotype, completely suppress the *unc-93(gf)* rubber-band defects and act as partial suppressors of the *sup-10(gf)* and *sup-9(gf)* rubber-band phenotypes. Loss-of-functions mutations in *sup-11* cause embryonic lethality. While *sup-11(gf)* and *sup-18(lf)* mutations each only partially suppress the *sup-9(gf)* rubber-band phenotype, *sup-11(gf)*; *sup-9(gf)*; *sup-18(lf)*animals are completely suppressed for the rubber-band phenotype, suggesting that *sup-11* and *sup-18* act in parallel. Since *sup-11* is an essential gene, SUP-11 may interact with channels in addition to SUP-9, with the gain-of-function mutations affecting mainly its interaction with the SUP-9/SUP-10/UNC-93 complex but not with other channels. *sup-11* has been mapped to a small interval on LGI. We are further mapping *sup-11* against polymorphisms in the region and determining the sequences of candidate genes from *sup-11* mutants.

# CHEMOSENSORY CONTROL OF SURFACE ANTIGEN SWITCHING IN *C. ELEGANS.*

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Nematodes restrict the expresssion of specific surface molecules to a particular time or developmental stage and can switch surface molecules in response to environmental changes. Our study of surface antigen switching in *C. elegans* has led to the discovery that it is modulated in response to environmental signals, and this response requires functional chemosensory neurons.

Previously, we identified a surface antigen switch in which wild type *C. elegans* is induced to display an L1 surface epitope at a later larval stage (inducible larval display or ILD) when grown under special conditions. We also identified mutations that result in nonconditional display of this epitope on all four larval stages (constitutive larval display or CLD). These include mutations in a new gene, *srf-6*, and in previously identified dauer-constitutive (*daf-c*) genes involved in signal transduction during dauer larva formation.

Surface antigen switching and dauer formation are controlled differently. For example, *srf-6* mutations apparently do not affect dauer formation. Examination of double mutants combining *srf-6(yj13)* with ts mutations in *daf-c* genes suggested that *srf-6* acts in parallel with the TGF-b signaling pathway defined by some *daf-c* genes, but might act in the same pathway with *daf-11.*

ILD requires intact sensory cilia. Cilium structure mutations such as *che-3* and *osm-3* resulted in greatly reduced ILD. All sensory cilia are abnormal in *che-3* mutants, while *osm-3* mutations affect only the chemosensory neurons that detect water-soluble substances (taste). A *che-3; srf-6* double mutant showed no CLD, indicating that intact sensory cilia are required for *srf-6* to affect phenotype. By contrast, mutations that affect olfaction, but not taste, had no effect on ILD. Taken together, these results suggest that ILD requires some ciliated sensory nerve endings, but not the olfactory neurons.

In chemotaxis assays, *srf-6(yj13)* showed greatly reduced attraction to both volatile and nonvolatile substances that attract wild type. Taken together, our results are consistent with a model in which *srf-6* activity is required in a chemosensory neuron to inhibit a downstream component that activates expression of the L1 surface epitope at later larval stages.

# *INS-1,* ONE OF MANY INSULIN-RELATED GENES IN *C. ELEGANS*, CAN REGULATE DAUER FORMATION

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The activity of the DAF-2 insulin/IGFI receptor homolog is required for non-dauer development and normal adult life span, and is likely to be regulated by one or more insulin-like ligands. A comprehensive search of the *C. elegans* genome identified 37 *ins* genes predicted to encode insulin-like peptides. *ins-1* on F13B12 is most closely related to mammalian insulins, based on structural predictions and likely C-peptide cleavage sites typical of mammalian insulins. To test whether the *ins* genes could play a role in dauer formation, several were overexpressed by injecting worms with the appropriate PCR-amplified genomic regions. We hypothesized that overexpression of putative DAF-2 ligands might suppress the Daf-c phenotype of weak *daf-2* mutants. None of the tested genes suppressed *daf-2*(*e1365*) at 26°C. Unexpectedly, the *ins-1*, but not the *ins-9, ins-22* or *ins-19/31*, transgene strongly enhanced *daf-2(e1365)* and *daf-2(e1370)*at 20°C. Reducing the injected *ins-1* DNA by 10-fold decreased the enhancement but never caused suppression of *daf-2(e1365),* suggesting an endogenous role for INS-1 as a DAF-2 antagonist rather than saturation of DAF-2 to a non-signaling state by excess INS-1. The *ins-1* transgene also enhanced a mutation in *daf-7*, which encodes the ligand for a parallel TGF-ß pathway that synergizes with the *daf-2* insulin-like signaling pathway. An *ins-1* transgene in which the coding region was replaced with the human insulin cDNA had similar effects. Like many other *ins* genes, an ins-1::GFP transgene is expressed in many neurons, as well as intestine, and vulval muscles. Lastly, a deletion mutant, *ins-1(nr2091)*, lacking the entire coding region, did not enhance or suppress dauer formation in wild type, *daf-2*, or *daf-7* backgrounds. Thus, although overexpression of *ins-1* can enhance dauer arrest in these backgrounds, *ins-1* is not required for dauer arrest. Other *ins* genes may act redundantly as agonists or as antagonists of DAF-2.

# FUNCTIONAL ANALYSIS OF PKN-1, AN EFFECTOR OF RHO GTPASE, IN *C. ELEGANS* MUSCLE

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The Rho family GTPases (Rho, Rac, Cdc42) are involved in the regulation of actin reorganization, cell polarity, cell growth, and cell-cell adhesion. The Rho family GTPases have two interconvertible forms; the GTP-bound active and GDP-bound inactive forms. The GTP-bound form of Rho family GTPases interacts with their specific effectors. The GTP-bound form of RhoA binds preferentially to Protein Kinase N (PKN), Rho-kinase (also called ROK), myosin binding subunit of myosin phosphatase (MBS). Besides proceeding of functional analysis of Rho-kinase and MBS, the physiological functions of PKN remain to be clarified.

Previously, we reported the identification of the *C. elegans* homologue of PKN, PKN-1, and its expression in the muscle cells (Qadota, *et al*., 12 th *C. elegans* meeting). Here we examined physiological functions of PKN-1 in *C. elegans*. To investigate effects of activated PKN-1, we expressed PKN-1 under the control of heat shock promoter. Overexpression of a catalytic domain of PKN-1 caused abnormal, loopy, movement of worms. Since the heat shock promoter can drive the expression of the downstream gene in all tissues, we next determined the tissues in which the catalytic domain of PKN-1 functions by two methods. First, mosaic analysis revealed that the expression of the catalytic domain of PKN-1 in neurons is dispensable for loopy movement and that the expression in all body wall muscle cells is required for loopy movement. Second, the expression of the catalytic domain of PKN-1 under the control of the body wall muscle specific promoter caused slightly loopy movement. These results suggest that the activation of PKN-1 in the muscle cells affects balance between contraction and relaxation in the *C. elegans* body wall muscle. Now we have a plan to examine response of transgenic worms expressing the catalytic domain of PKN-1 to drugs to inhibit muscle excitation and will discuss the function of PKN-1 in the muscle cells from pharmacological results.

# *MOD-1* AND *MOD-5* CONTROL SEROTONERGIC NEUROTRANSMISSION AND EXPERIENCE-DEPENDENT MODULATION OF LOCOMOTORY BEHAVIOR

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Hermaphrodites respond to the presence of a bacterial lawn by slowing their rate of locomotion. Animals deprived of bacteria for 30 minutes exhibit enhanced slowing when they are reintroduced to a bacterial lawn. This modulatory response is mediated by serotonin. Mutations in *mod-1* and *mod-5* (modulation of locomotion defective) affect both the modulatory response and serotonergic neurotransmission.

*mod-1* mutants display reduced slowing in the modulatory response and resistance to serotonin in assays of locomotion in liquid (exogenous serotonin inhibits the locomotion of wild-type animals). We cloned *mod-1* and performed electrophysiological studies using *Xenopus* oocytes to show that *mod-1* encodes an ion channel that is gated specifically by serotonin. *mod-5* mutants are defective in serotonin loading of the NSM neurons (C. Trent and B. Horvitz, unpublished observations), display more pronounced slowing than the wild type in the modulatory response, and are hypersensitive to exogenous serotonin in assays of locomotion in liquid. *mod-5* encodes a protein similar to mammalian serotonin reuptake transporters (SERTs), which are the proposed sites of action of the tricyclic antidepressants and the selective serotonin reuptake inhibitors (SSRIs), such as Prozac. We have confirmed that MOD-5 is a functional SERT by performing uptake assays using mammalian cells.

We did a non-clonal F2 screen for suppressors of *mod-5(n3314)* (a deletion allele) by seeking mutants no longer hypersensitive to serotonin. From a screen of 18,300 genomes we obtained 61 independent isolates suppressed for the serotonin hypersensitivity to varying degrees. Fifteen of these 61 isolates also suppress to varying degrees the Mod phenotype caused by *mod-5(n3314)*, i. e., after food-deprivation and re-introduction to bacteria, these isolates move faster than *mod-5(n3314)* mutants. We are mapping *n3461* and *n3488,* two suppressor mutations that strongly suppress both the serotonin hypersensitivity and the Mod phenotype.

# THE CED-2 CRKII, CED-5 DOCK180, CED-10 RAC PATHWAY CONTROLS CELL-CORPSE ENGULFMENT AND CELL MIGRATION, AND CED-10 FUNCTIONS REDUNDANTLY WITH THE MIG-2 RHO-TYPE GTPASE TO CONTROL AXON GUIDANCE

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The engulfment of cells undergoing programmed cell death is controlled by at least seven genes that define two parallel pathways. Mutations in genes of one pathway, which includes *ced-2, ced-5, ced-10,* and *ced-12*, result in defects both in cell-corpse engulfment and in the migration of the gonadal distal tip cells. *ced-5* encodes a protein similar to human DOCK180. DOCK180 interacts with the oncoprotein Crk and the GTPase Rac. We have cloned the *ced-2* and *ced-10* genes and found that they encode proteins similar to CrkII and Rac, respectively. That *ced-2*, *ced-5*, and *ced-10* act together in a pathway strongly indicates that mammalian CrkII, DOCK180, and Rac functionally interact *in vivo* as well. We have obtained genetic and biochemical evidence supporting a model in which CED-2 recruits CED-5 to the membranes of engulfing and migrating cells, thereby activating CED-10 to control the polarized extension of cell surfaces in both cell-corpse engulfment and distal tip cell migration.

Rac is a member of the Rho-family of GTPases, which includes Rac, Rho, and Cdc42. How Rho-family GTPases interact during animal development is largely unknown. We found that three *C. elegans* members of this family, *ced-10*, *mig-2*, and *rac-2*, function redundantly to control axon guidance. First, ultrastructural studies revealed that *ced-10; mig-2* double mutants show severely reduced axonal numbers and increased fasciculation defects in both the ventral and dorsal nerve cords; by contrast, neither *ced-2; mig-2* nor *ced-5; mig-2* double mutants show such defects. Second, *ced-10; mig-2* double mutants show defects in CAN cell migration and axonal extension and guidance, as determined using a *ceh-23::gfp* reporter that expresses in the CAN cell. Third, RNAi of *rac-2* shows synthetic CAN cell defects in animals mutant in either *ced-10* or *mig-2*. We also have analyzed how these three Rho-family genes, along with the *unc-73* GEF (guanine nucleotide exchange factor), interact during the processes of cell-corpse engulfment and distal tip cell migration.

# REGULATION OF FMRFAMIDE-RELATED EXPRESSION BY *FAX-1* AND *UNC-42,* AND THE TAILLESS SUBFAMILY OF NUCLEAR HORMONE RECEPTORS

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The *fax-1* and *unc-42* genes both function in regulating axon pathfinding and aspects of neuron identity (Baran et al., 1999; Much et al., 2000). *fax-1* encodes a nuclear hormone receptor and *unc-42* encodes a homeodomain protein. Thus, both genes are likely to regulate the transcription of genes that function in nervous system development. Both *fax-1* and *unc-42* mutations disrupt axon pathfinding by the AVK interneurons. Both genes are expressed in the AVK neurons, suggesting cell-autonomous function. *fax-1* is required for normal expression of FMRFamide-related peptides in the AVK's. FMRFamide expression in *fax-1* mutants is compromised in both AVK neurons: only 70% of *fax-1(gm83)* AVK's display detectable FMRFamide immunoreactivity. Expression of *flp-1::gfp* in *fax-1(gm83)* AVK's is similarly compromised (62% of neurons). *flp-1* encodes one of several *C. elegans* FMRFamide-related protein precursors (Nelson et al., 1998). In contrast, FMRFamide immunoreactivity in *unc-42* mutants appears normal, but *flp-1::gfp* expression is entirely absent. The two phenotypes are additive in *unc-42; fax-1* double mutants: animals display compromised FMRFamide immunoreactivity, like *fax-1*, and an absence of *flp-1::gfp* expression, like *unc-42*. We are currently testing whether *fax-1* expression is dependent on *unc-42*, and vice versa.

We are also beginning an analysis of *C. elegans* nuclear receptors that are members of the *fax-1/tailless* subfamily. In the DNA-binding domain, FAX-1 is most similar to the human RNR protein and an uncharacterized *Drosophila* gene predicted by the genome sequencing effort. Thus, the FAX-1 group likely diverged from other receptors prior to the vertebrate-invertebrate split. A second, but closely related subfamily, includes *C. elegans* NHR-67, *Drosophila* TLL, and vertebrate TLX. A more divergent *C. elegans* nuclear receptor is the predicted F44G3.9 protein. However, unlike the other receptors in this class, F44G3.9 shows a relationship with FAX-1 in the ligand-binding domain. This may suggest a closer evolutionary relationship with FAX-1 or convergent evolutionary events. We are initially focusing our efforts on determining the expression of the F44G3.9 receptor by making *gfp* reporter fusions.

# CHARACTERIZATION OF MUTANTS DEFECTIVE IN POSTEMBRYONIC MORPHOGENESIS OF THE MALE TAIL TIP

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Morphogenesis is an interesting process that occurs throughout animal development. We are interested in the genetic and molecular mechanisms that control the assembly and function of male tail tip morphogenesis. The male tail tip of *C. elegans* undergoes morphogenesis during the fourth larval stage (L4 stage) of post-embryonic development. The four hypodermal cells that form the tail tip coordinately fuse and retract in the males, generating a blunt-ended (peloderan) tail tip in the adult. In some species related to *C.elegans*, the tail tip cells fail to retract, producing a pointy (leptoderan) tail tip. This suggests that tail tip morphogenesis can be regulated independently from general male tail morphogenesis.

To further understand the genetic mechanisms that underlie mail tail tip morphogenesis in *C.elegans*, we are characterizing mutants in which the tail tip cells fail to retract. The tail tip of these mutants resembles that of leptoderan nematodes ("Lep" phenotype). Fourteen mutants defective in male tail tip morphogenesis have been isolated in our lab. We are in the process of characterizing some of these mutants by genetic and phenotypic analysis. The position of one of these mutants within LGIII is being determined by deficiency mapping as well as by three-factor crosses. Similar strategies will be used for LG V.

We are also currently working on the phenotypic characterization of the Lep mutants. The dynamics of the four tail tip cells in living organisms can be visualized using worms carrying a GFP marker fused to an adherens junction molecule (JAM-1::GFP, kindly provided by Drs. Simske and Hardin). Preliminary results with one of the mutants (*ny4*) suggest that, unlike wild-type males, the tail tip cells in most of these adults fail to retract. We are in the process of analyzing these *jam-1::GFP*; *lep* strains further. Eventually, the isolation of genes involved in tail tip morphogenesis will allow us to identify components involved in the regulation and evolution of this process.

# ANALYSIS OF BODY SIZE REGULATION IN *C. ELEGANS* BY TGF-BETA 

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Two distinct TGF-beta signalling pathways have been elucidated in *C. elegans*, the dauer pathway and the Small/Mab (male abnormal) pathway. Small/Mab mutants show a characteristic small size and have male tail defects such as abnormal ray fusions and crumpled spicules. In an attempt to uncover previously unknown components and modulators of the pathway, a screen was performed to isolate mutations that lead to a small body size. One such mutation is sma-12.

Sma-12 animals are small in size but appear to have no male tail defects. This could indicate that sma-12 is involved specifically in the body size regulation branch of the pathway. The mutation has been mapped to chromosome V in the region between 1.7 and 1.9 on the genetic map. Further attempts to map and clone sma-12 will be made using SNP (single nucleotide polymorphism) mapping techniques as well as more traditional methods. By cloning and characterizing sma-12, we hope to provide additional insight into the mechanism with which TGF-beta signalling functions to regulate growth and development in nematodes as well as higher organisms.

Further efforts to elucidate downstream targets of the pathway are being undertaken using cDNA microarray technology. The global pattern of gene expression in body size mutants can be determined in this way, and changes in expression may illuminate possible downstream targets of the pathway.

# *SEM-3* ENCODES A CIS-PRENYLTRANSFERASE HOMOLOG, AN ENZYME REQUIRED FOR PRODUCTION OF AN ESSENTIAL LIPID

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In *C. elegans*, the egg-laying muscles are descended from a pair of M-derived cells known as the sex myoblasts (SMs). The SMs are born in the posterior of the animal during the L2 stage and migrate anteriorly, in response to multiple cues, to flank the center of the developing gonad.1 The development of functional egg-laying muscles requires their proper differentiation, attachment, and innervation in addition to the proper migration of the SMs.

*sem-3(n1655)* was identified as a spontaneous Egl mutation with defects in SM migration and vulval muscle attachment. The final positions of the SMs in *sem-3(n1655)* hermaphrodites lack the precision found in wild type, but are very close to normal. The sex muscles derived from these SMs are normal both in number and in their expression of appropriate reporter constructs, but often fail to attach properly. The subtle nature of the SM migration defect makes it likely that the vulval muscle attachment defects are the cause of the Egl phenotype of *sem-3(n1655)* mutants.

*sem-3* was mapped to linkage group IV and found to be allelic to *let-654.* Standard germline transformation rescue of the Let and Egl phenotypes was used to identify the *sem-3* open reading frame from among cosmids in the region; the presence of lesions in multiple *sem-3* alleles confirmed the identification of the *sem-3* gene. By sequence analysis, *sem-3* encodes a member of the cis-prenyltransferase class of enzymes. This class of enzymes is responsible for the production of dolichol, a lipid molecule with essential functions in N-linked glycosylation, formation of GPI anchors, and regulation of membrane fluidity.2 BLAST analysis shows that SEM-3 is the only member of this class of enzymes in *C. elegans* and suggests that the lethality associated with *sem-3* mutations may be due to a lack of dolichol. The bases for the vulval muscle attachment defects, as well as the subtle SM positioning defects, in *sem-3(n1655)* animals are not currently known. Experiments to address the role of SEM-3 in vulval muscle attachment, as well as the attachment of other muscles, will be discussed.

1. Chen, E.B. and Stern, M.J. (1998) TIGS 14, 322-327

2. Chojnacki, T. and Dallner, G. (1988) Biochem. J. 251, 1-9

# UNC-43 CAMKII REGULATES THE DENSITY OF CENTRAL GLUTAMATERGIC SYNAPSES IN VIVO

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Synaptic connections undergo a dynamic process of stabilization or elimination during development. To determine how central synapses change during development, we observed neuron-neuron synapses in C. elegans that contain the AMPA-type GluR GLR-1. We previously showed that chimeric receptors tagged with the GFP (GLR-1::GFP) can be used to visualize glutamatergic synapses. In late L1 animals, GLR-1::GFP becomes localized to synapses along the lengths of axons in both the ventral cord and nerve ring processes. During larval growth, the density of GLR-1 synapses is held constant despite dramatic increases in neurite length.

Here we describe the role of the UNC-43 type II calcium and calmodulin dependent protein kinase in regulating formation of glutamatergic synapses in C. elegans. The coupling of synapse number to neurite length requires both UNC-43, and the UNC-2 and EGL-19 voltage-gated calcium channel subunits. Mutants lacking UNC-43, UNC-2, or EGL-19 accumulate high levels of perinuclear GLR-1::GFP in their cell bodies. Constitutive activation of UNC-43 also resulted in reduced numbers of GLR-1-synapses; however, GLR-1::GFP did not accumulate in the cell bodies of neurons. A GFP::UNC-43 protein containing a mutation that generates a constitutively active kinase was diffusely distributed in axons, unlike wild-type GFP::UNC-43, which was localized to punctate structures in axons. Thus, constitutively activated CaMKII fails to localize to synaptic sites and decreases the density of GLR-1 synapses, suggesting that synaptically localized CaMKII is required to add or maintain GLR-1 at synaptic sites. Our results suggest that CaMKII regulates GLR-1 by two distinct mechanisms: regulating transport of GLR-1 from cell bodies to axons, and regulating the addition or maintenance of GLR-1 to synapses.

# MOLECULAR GENETICS OF $LI^+$ SENSITIVITY IN *C. ELEGANS*

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Lithium has multiple developmental effects. *Xenopus* embryos injected with 10 mM lithium are dorsalized because lithium inhibits glycogen synthase kinase (GSK). This inhibition blocks phosphorylation of beta-catenin, which prevents B-catenin from activating target genes in the nucleus. Mouse oocytes treated with lithium have cytokinesis defects and are induced to undergo a second meiotic cleavage. In *Dictyostelium*, lithium alters gene expression and development by affecting proliiooligopeptase and therefore altering the breakdown of $IP_5$ to $IP_4$ . Little is known about the effects of lithium in *C. elegans* (Y. Tabuse 1997 Int. Worm Meeting 1997 abstract # 586). Here, we report that lithium has a dosage dependent effect on *C. elegans* embryos. Depending on the concentration of lithium, ranging from 20mM to 10mM, animals exhibit cytokinesis defects, spindle orientation defects, and morphogenic effects that lead to eggs that are unable to hatch. We demonstrate that lithium has an effect on larval development in a dosage-dependent manner. Animals laid on plates containing 10-20mM lithium have delays in the timing of developmental events proportional to the concentration of lithium. They then go on to become progressively paralyzed. To learn more about the biology of $Li^+$ sensitivity, we screened for $Li^+$ sensitive mutants. We isolated one mutant in a screen of 22,000 haploid genomes that is resistant to both the larval and embryonic effect caused by 16mM lithium. We also isolated three mutants that are resistant to late embryonic lethal effects caused by 10mM lithium. To understand the importance of phosphatidylinositol metabolites and the potential role they play in lithium sensitivity we are using *in vivo* RNAi to disrupt gene function at different times in development. We targeted *C. elegans* homologues of the genes *rdgB* (*Drosophila* phosphatidyl inositol transfer protein), OCR (Human 4,5-phosphatase), and skittles (*Drosophila* 5-kinase) for dsRNAi disruption to examine if the null phenotype of these genes mimic any aspects of worms reared on lithium.

# *ELM-1,* A NEGATIVE CELL CYCLE REGULATOR

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We are using *C. elegans* genetics to identify negative regulators of cell division. To date, several *C. elegans* loci have been identified as functional homologs of negative cell cycle regulators in mammals. For example, *cki-1*, a member of the CIP/KIP family, has been shown to restrict the number of post-embryonic cell divisions in the hypodermal, intestinal and gonadal lineages (Hong *et al.* 1998). We have made use of the *cki-1(RNAi)* phenotype in designing a screen for extra cell divsions.

Our screen uses a genetic background in which mutations that cause additional cell divisions during vulval development are easily detected. During wild-type development, *lin-12*, a Notch receptor family member, signals two vulval precursor cells (VPCs) to adopt the secondary cell fate. In the *lin-12(gf)* animal, all six VPCs adopt this cell fate resulting in the formation of up to 6 pseudovulvae. Loss of a negative regulatory activity, such as *cki-1,* allows an ectopic cell division within the VPC lineage and up to 12 VPCs are formed. In the *lin-12(gf)* animal the twelve VPCs can give rise to 12 pseudovulvae that are easily observed. We have screened 2750 haploid genomes following EMS-mutagenesis of *lin-12(gf)* animals and have obtained two independent mutant strains with this "SuperMuv" phenotype.

Mutation of *elm-1* (enhancer of *lin-12(gf)* multivulva) specifically affects the VPC lineage. In the wild-type worm, the VPCs are formed in mid-L1 and remain quiescent until they undergo further divisions in L3. In the *elm-1* mutant strain, several VPCs undergo an additional division during L2, resulting in up to twice the wild-type number. We did not observe ectopic cell divisions in the intestinal or gonadal lineages. *elm-1* has been mapped to LGII. Since ectopic VPC divisions have been observed in *lin-31* mutants (Miller *et al.* 1993), we determined that *elm-1* can complement *lin-31(lf)* for both the ectopic VPC division and lineage defects. Since the *cki-1* gene is also located on LGII and *cki-1(RNAi)* results in supernumerary VPC divisions, we will determine if *elm-1* is required for expression of a *cki-1::GFP* transgene (a kind gift from V. Ambros) or is a tissue-specific allele of *cki-1*.

# GENETIC ANALYSIS OF NEURAL INPUTS TO THE DAUER PATHWAY

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*C. elegans* uses sensory information about its environment to decide whether to proceed with development or arrest at the dauer larval stage. Signalling through converging TGF-b/DAF-7 and insulin-like/DAF-2 neuroendocrine pathways is required for non-dauer development. Animals mutant in *daf-7*, a TGF- b super family member (Ren at al. Science 274, 1996), arrest development inappropriately at the dauer larval stage. A study to identify the neurotransmitters used in the neural inputs into the dauer pathways revealed that *daf-7* animals can return to normal reproductive development by the application of the acetylcholine muscarinic receptor agonist oxotremorine (Tissenbaum et al. PNAS, 2000). Muscarinic agonists do not induce dauer recovery in animals defective for an insulin-like receptor DAF-2 which are daf-c. This suggests a role for muscarinic signalling in an insulin-like pathway.

We have taken two approaches to further investigate the role of muscarinic signalling in the dauer pathway. We have identified three potential muscarinic receptor homologues (*acm-1, acm-2 and acm-3*) by sequence comparison in the *C. elegans* genome sequence database. We have analysed the expression of a GFP promoter fusion of each of these three genes. All constructs are expressed in a small set of neurons with one construct also exhibiting expression in intestinal cells. The pharynx also exhibits differential expression with *acm-2* showing expression in the pharyngeal neurons and *acm-3* in the pharyngeal muscle cells. Currently, deletion mutants of *acm-1* (from Kate Steger, Leon Avery) and *acm-2* (from Stefan Eimer, Ralf Baumeister ) are being analysed for dauer phenotypes and genetic interactions with genes in the dauer pathway.

To further address the role of acetylcholine signalling in the dauer pathway we made double mutants between daf-c genes and animals with mutations that have decreased cholinergic signalling. We observed an L1 arrest in the double mutant of the insulin-like receptor *daf-2 (e1370)* and a vesicular transporter of acetylcholine *unc-17 (e245)* (Alfonso, A. et al. Science 261, 1993) but not with *daf-7(e1372); unc-17(e245)* . To isolate other components in this signalling pathway we have done a screen to suppress this L1 arrest and isolated *daf-2* and *unc-17* suppressors. We are in the process of characterising and mapping these mutants.

# SPECIFICATION OF THE AWA CHEMOSENSORY NEURONS

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We are interested in the developmental specification of chemosensory neuron fate and function. The AWA neurons sense a subset of volatile attractants. To identify genes required for the development of the AWA neurons, we have isolated mutants in which the expression of the AWA-specific gene *odr-7* is altered.

We have identified a gene, *unc-130,* which is required to restrict AWA fate. *unc-130* mutants show ectopic expression of all AWA genes examined, including the ODR-10 olfactory receptor, the GPA-5 G protein, and the ODR-7 nuclear hormone receptor. Ectopic expression is seen in a single additional neuron pair, the ASG neurons (AWA's lineal sisters), which appear to fully adopt an AWA-like fate. We have cloned *unc-130* and shown that it encodes a member of the forkhead class of transcription factors. (*unc-130* has been independently cloned by B. Nash and J. Culotti based on other pleiotropies.) We generated antibodies to non-conserved portions of UNC-130, and find broad embryonic staining. Expression is severely reduced by the adult stage. At approximately 260 min. of embryonic development, UNC-130 is expressed in the ABpl/raapapa cells, which are the immediate precursors to the AWA/ASG neurons. UNC-130 does not appear to be inherited by the AWA neurons; we are currently investigating whether the ASG daughters inherit UNC-130. Misexpression studies have shown that UNC-130 can repress ODR-7 expression in the AWA neurons. We are testing whether UNC-130 is also sufficient to specify ASG fate. Taken together, our results indicate a role for UNC-130 in establishing or executing the asymmetry of the AWA/ASG precursor division, either by functioning within the precursor, and/or by acting in the ASG neurons to repress AWA fate.

We have also identified a LIM homeobox gene, *lin-11*, which is required for the establishment of AWA fate. In *lin-11* mutants, expression of AWA-specific genes is frequently lost in one or both AWA neurons, and these neurons adopt an AWC olfactory neuron fate. We find that *lin-11* acts upstream of *odr-7* to specify AWA fate. We are examining the expression pattern of LIN-11 in further detail to determine its site of action in the specification of AWA fate.

# CDC27, AN ANAPHASE PROMOTING COMPLEX SUBUNIT, IS REQUIRED FOR THE METAPHASE TO ANAPHASE TRANSITION DURING MEIOSIS

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The metaphase to anaphase (met-ana) transition in mitotic cells is controlled by the activity of the Anaphase Promoting Complex (APC). APC is an E3 ubiquitin ligase that targets proteins for destruction in order for sister chromatids to separate at anaphase. The substrates of APC include cyclins, anaphase inhibitors, and chromosome cohesins. The APC is composed of at least 12 subunits in *S. cerevisiae*; mutations in these genes result in metaphase arrest.

Temperature sensitive mutations in 6 genes disrupt the met-ana transition during meiosis I in *C. elegans* embryos. These genes are *mat-1, -2, -3* (for met-ana transition defective), *emb-1*, *-27*, & *-30* (Miwa et al., 1980; Cassada et al., 1981; Furuta et al., 2000; Nishiwaki & Miwa, WBG 10; Golden et al., 1999 Int. WM). Homozygous mothers shifted to 25°C produce broods of 1-cell arrested embryos in which the oocyte chromosomes congress and set up a meiotic spindle after fertilization. However, the first meiotic division fails to occur, anaphase is never observed, polar bodies are never extruded, and pronuclei never form. The sperm chromosomes remain condensed and the sperm centrosomes fail to nucleate microtubule asters. The *emb-30* gene was recently shown to encode an *apc4* homolog (Furuta et al., 2000) and *emb-27* a *cdc16* homolog (another APC subunit; Sadler & Shakes, pers. comm.).

Genetic mapping of *mat-1* places it on LG I near another APC subunit gene, *cdc27*. RNAi of this gene results in the production of 1-cell arrested embryos that fail to progress through meiosis I. We have sequenced this *cdc27* gene from a number of *mat-1* alleles and found mutations in the *cdc27* coding region for each.

Based on 2-cell embryo and L1 shift-up experiments, the 6 *mat-1* alleles make up an alleleic series. Two alleles are >95% lethal even at 15°C and are maintained as balanced heterozygotes. Another 2 alleles result in sterility when embryos or L1s are shifted to 25°C. The last 2 alleles are maternal-effect embryonic lethal; these animals, when shifted to 25°C as 2-cell embryos, develop normally and produce 1-cell arrested embryos. We will show the sequence alterations and the phenotypic characterization of these 6 *mat-1* alleles.

# A SCREEN FOR MUTANTS DEFECTIVE IN THE SPECIFICATION OF THE PROGRAMMED CELL DEATHS OF THE MALE-SPECIFIC CEM NEURONS

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

We have performed a genetic screen for hermaphrodites in which the male-specific CEM neurons fail to undergo programmed cell death. The CEM neurons die during normal hermaphrodite development, but survive and differentiate in males. The reporter *pkd-2*::*gfp* (kindly provided by Maureen Barr and Paul Sternberg) expresses in the CEMs of males and in the CEMs of *ced-3* hermaphrodites, which are defective in programmed cell death. By using the *pkd-2::gfp* reporter as a marker for CEM survival, we were able to screen efficiently for survival of a single cell using a dissecting microscope fitted with fluorescence optics. We expect this screen to yield mutations in the sex determination and programmed cell death pathways and hope it will also yield mutations in genes specifically required for the deaths of the CEM neurons in hermaphrodites.

A screen of 60,000 mutagenized haploid genomes yielded at least 135 independent mutations that cause survival of the CEMs, including at least 50 that cause sexual transformation and at least 29 alleles of known cell-death genes. We are currently mapping the uncategorized mutations and placing them into complementation groups.

# SEARCHING FOR FACTORS RESPONSIBLE FOR PAR-2 CORTICAL LOCALIZATION

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In *C. elegans*, six *par* genes are involved in establishing the anterior-posterior axis of the zygote. PAR-2 protein is localized to the posterior cortex of the one-cell embryo and is segregated to the posterior of the P1 cell in the two-cell embryo. Throughout the early cleavages, PAR-2 is found primarily in the P-cell lineage. We are interested in understanding how PAR-2 is localized to the cortex in these cells and are performing a genetic screen to identify factors responsible for PAR-2 cortical localization. Our screen is based on ectopic expression of genomically integrated PAR-2:GFP expressed from the inducible heat shock promoter. PAR-2:GFP expressed in these strains is cortical in embryonic cells. Expression of the transgene is lethal to the embryos, however expression of just GFP is not. After construct integration, worms were mated into a *par*-6 mutant background. This was done because in a heterozygous *par*-6 mutant, the embryonic lethality of *par*-2 mutations is suppressed. Thus, after mutagenesis with EMS, we should be able to recover viable strains with mutations affecting PAR-2 localization. So far, we have screened over 7000 haploid genomes and may have recovered several strains that survive heat shock expression of PAR-2:GFP. Of these, none appear to affect the localization of PAR-2:GFP. We plan to continue screening to identify factors involved in PAR-2 localization.

# POST-TRANSCRIPTIONAL CONTROL OF C. ELEGANS LIN-28 EXPRESSION THROUGH ITS 3'UTR

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Many developmental genes are controlled through elements in the untranslated regions of their mRNA. The heterochronic genes lin-4, lin-14 and lin-28 are involved in controlling the proper timing of events during the larval stages of development of C. elegans. Genetic and biochemical evidence indicates that lin-4 encodes a small RNA that down-regulates the expression of LIN-14 and LIN-28 proteins. Both lin-14 and lin-28 contain putative lin-4 complementary elements in their 3' untranslated regions, suggesting that the control involves a direct RNA-RNA interaction between lin-4 RNA and the target mRNA. However, the mechanism of lin-14 and lin-28 control by lin-4 remains unknown. Our studies suggest that the down-regulation of lin-28 expression occurs after translational initiation and elongation.

RNase protection assays show that lin-28 mRNA levels remain constant while LIN-28 protein diminishes. This suggests that lin-28 control is post-transcriptional. Assays of sucrose gradient fractionated extracts from early and late stage C. elegans larvae show that lin-14 and lin-28 mRNA associate with the polysomal fraction. The presence of these mRNAs in the polysomal fraction indicates that the message is either being translated by ribosomes or is frozen in a non-translating polysomal complex. We have obtained cycloheximide sensitive translational run-off of polysomes isolated from early and late stage C. elegans extracts by incubating the polysomes with reticulocyte lysate. We observe a shift in lin-14 and lin-28 mRNAs out of the polysomal fraction after run-off has occurred. This suggests that lin-14 and lin-28 mRNAs associated with polysomes in late stages are actively translated, while repression by lin-4 is taking place. These results suggest that the down-regulation of lin-28 expression takes place after translation initiation and elongation. We are further exploring the mechanism of control of lin-28 by measuring the rate of protein production by purified polysomes, and developing an in vitro system which recapitulates the down-regulation of LIN-28 protein through the 3'UTR of lin-28 mRNA.

# A *C. ELEGANS* GENE RELATED TO *S. POMBE POM1* IS REQUIRED FOR MICROTUBULE-BASED PROCESSES IN THE *C. ELEGANS* EMBRYO

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Mutations in the *Schizosaccharomyces pombe* gene *pom1* result in the misplacement of septa, as well as misplacement of microtubule organizing centers during cell division (1). In addition, *pom1* mutants are synthetically lethal with mutations affecting either the actin or microtubule cytoskeleton; and the distribution of *pom1* protein, Pom1p, within the cell depends on intact microtubules. These observations suggest that Pom1p has a role in positional regulation of the cytoskeleton during division in *S. pombe*. Pom1p belongs to a protein kinase family with related members in *S. cerevisiae*, *C. elegans*, *Drosophila*, and humans. The functional role of a *C. elegans pom1*-like gene was examined using RNA interference. 100% lethality was observed among embryos laid by hermaphrodites injected 24 hours prior with dsRNA corresponding to the *C. elegans pom1*-like gene. A reduced number of severly aneuploid cells were observed in terminal stage embryos. Observations of pronuclear-stage embryos revealed defects in pronuclear migration, spindle orientation, DNA segregation, and cytokinesis. However, actin-based processes such as pseudocleavage formation, cytoplasmic flow, and cleavage furrow formation appeared normal. Therefore, we hypothesize that the *C. elegans pom1*-like protein is required for microtubule dynamics in the early embryo and, given the similarities between the *S. pombe* and *C. elegans* mutant phenotypes, suggest that the yeast and worm genes are functionally conserved.

1. Bahler, J., and J.R. Pringle, Genes and Development, 12(9):1356-1370.

# SPECIALIZED ROLES OF DRIP/TRAP/ARC/MEDIATOR TRANSCRIPTION COMPLEX COMPONENTS IN *C. ELEGANS*.

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In humans and *Drosophila*, nuclear receptors and many other transcription factors have been shown to interact with multiprotein co-factor complexes that share common subunits, and are known as DRIP, TRAP, SMCC, hMediator, and ARC. These complexes include components of the yeast Mediator co-activator, as well as multiple metazoan-specific subunits. They are generally similar if not identical to each other, although some correspond to negatively-acting subcomplexes. *In vitro* these complexes are essential for ligand-dependent activation by nuclear receptors, and transcription activation in a chromatin environment. The questions raised by these findings include whether these complexes have corresponding functions *in vivo*, whether different subunits are required specifically to regulate different classes of genes, and whether the metazoan-specific subunits have more specialized functions than the components that are present in yeast, and might be predicted to be required more broadly for transcription.

To address these questions, we have begun to study *C. elegans* homologs of subunits of these complexes, including some that are metazoan-specific, and others that are counterparts of yeast Mediator components. Our preliminary RNAi experiments suggest that different complex components may have different specific functions, and that metazoan-specific subunits may have more specialized functions than components that are present in all eukaryotes.

# VERIFICATION OF GENE PREDICTION IN THE CAENORHABDITIS ELEGANS GENOME

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Theoretical methods for gene finding in genomic DNA significantly accelerate analysis of experimental data by providing almost instant insight into their biological meaning. The danger of the error contamination of databases is increasing as more theoretically annotated genomes are becoming available and genes and proteins annotated by computer are used for subsequent annotation of new genomes. The complete C. elegans genome was annotated with an aid of computer program GeneFinder (P.Green and L. Hillier, unpublished). Assuming that the accuracy of GeneFinder is high we, nevertheless, attempted to get more precise evaluation of this method as a part of our project of creating experimentally verified training and test sets of genes in several eukaryotic genomes. This direction of our work has been started yet in 1996 in a project of using both GeneMark [1] and experimental verification of its prediction in finding exact exon-intron structure of C. elegans unc89 [2]. Another goal of our project has been improving the accuracy of the gene finding method GeneMark.hmm [3] that was demonstrated to be highly accurate for eukaryotic genome of Arabidopsis thaliana [4]. To assess the accuracy of gene prediction method we have generated a database of experimentally verified genes by matching genomic DNA with recently sequenced mRNA sequences available in GenBank. In C. elegans case this new set is used for comparison with original annotation (GeneFinder) as well as for training and testing of GeneMark.hmm. The results will be given in our presentation.

[1] Borodovsky, M and McIninch, J. 1993. GeneMark: gene prediction of both DNA strands. Computers & Chemistry 17:123-133.

[2] Benian, G., Tinley, T., Tang, X., and Borodovsky, M. 1996. The Caenorhabditis elegans gene unc-89, required for muscle M-line assembly, encoded a giant modular protein composed of Ig and signal transduction domains. Journal of Cell Biology 6: 835-848.

[3] Lukashin A. and Borodovsky M. 1998, GeneMark.hmm: New solutions for gene finding, Nucl. Acids Res. 26, 1107-1115.

[4] Pavy N., Rombauts S., Dehais P., Mathe C., Ramana D.V.V, Leroy P. and Rouze P. Evaluation of gen prediction software using a genomic data set: application to Arabidopsis thaliana sequences, Bioinformatics, 15, 887-899

# KINESIN MOTORS MOVING CHROMOSOMAL CARGO

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Kinesins form a large family of microtubule based motors, which mediate intracellular transport including vesicle delivery, ciliary growth, and chromosomal movement during cell cycle. We have brought to light genes encoding for all known kinesin subfamilies found in mammals in the nematode *C. elegans* (*viz. klp-1-klp-20*), by a combination of genetic and molecular approaches. The simple nematode provides an excellent model to elucidate the relationship between structure and function for all kinesin motors within a simple model animal. Our results suggest that the nematode kinesins perform highly specialized cellular functions in ciliary and axonal growth, neurotransmitter vesicle transport, and chromosomal movement during meiosis and mitosis. Most of these *in vivo* functions are conserved in species as divergent as the nematode and humans. Elucidating kinesin motor function during cell division will unravel how the spindle precisely segregates chromosomes, and may offer insights into the molecular basis of disease states that arise from abnormal spindle dynamics. For example, chromosome non-disjunction during meiosis causes defects as **Klinefelter, Down, and Turner Syndromes**. Chromosome non-disjunction during mitosis is critical for tumor progression. Chromosome specific kinesin motors provide novel targets for intervention into cell division cycle, and strategies that allow specific blockage of motor function during mitosis may have powerful chemotherapeutic potential.

# WORMBASE; A MODEL ORGANISM DATABASE

## John Spieth, WormBase Consortium

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ACeDB, has served the *C. elegans* community as a genome and genetic database. However, because it was mainly funded as part of the *C. elegans* sequencing project, its scope of active curation has been essentially limited to genetic maps and to genome sequence annotations. Funding is being sought to extend and expand ACeDB to make a more complete Model Organism Database, called WormBase, with complete coverage of core genomic, genetic, anatomical and functional information about *C. elegans*.

The two top priorities will be data curation and user interface. WormBase will include up-to-date annotation of the genomic sequence, the current genetic and physical maps and experimental data on the function and interactions of cells and genes, as well as development and organismal behavior. Direct links to the sources of biological material, such as the strain collection of the Caenorhabditis Genetics Center, and to data sets maintained by others will be provided. Data will be recovered from the literature and direct contribution of the individual laboratories. While WormBase will act as a central forum through which every laboratory will be able to contribute, WormBase professional curators will ensure detailed attribution of data sources and check consistency and integrity.

The standard access to WormBase will be Web based, both for consultation and for data submission. The Web site will center on five pages providing users with entrée via Gene, Cell or Process pages, and Sequence and Genetic Map Viewers.

Coordination of the project and the main curation site will be at Caltech. Curation and annotation of genomic sequence will take place at the two sequencing centers, the Sanger Centre and Washington University. The Montpellier team will develop interfaces to new large-scale projects and development of new user interfaces will take place at Cold Spring Harbor.

# BIOCHEMICAL PURIFICATION OF MITOTIC LIN-5 PROTEIN COMPLEXES

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We use *C. elegans* as a genetic model for studying the events of cell division. Phenotypic characterizations of *lin-5* mutants indicated an essential role for *lin-5* in chromosome and spindle movements during mitosis. LIN-5 localizes to spindle microtubules, centrosomes and the cell cortex. Together, these results suggest LIN-5 is involved in spindle force generation (Lorson *et al*, 2000). To reveal the molecular mechanisms that underlie the mitotic functions of *lin-5*, we have initiated biochemical analyses of LIN-5 and its associated proteins.

Many mitotic proteins are regulated by mitotic kinase activities. Western blots of LIN-5 from embryonic lysates reveal a protein with an apparent molecular weight of 100 kD and a minor, more slowly migrating form. LIN-5 contains several putative Ser/Thr phosphorylation sites, three of which conform to the consensus phosphorylation sites of the mitotic cyclin-dependent kinase, *Cdc2/Cdk1*. Experiments to determine if LIN-5 is phosphorylated are currently underway.

Identification and study of LIN-5-associated proteins may reveal the molecular function of *lin-5*. To determine the size of LIN-5 protein complexes, taxol-treated lysates were fractionated by gel filtration. This analysis showed that LIN-5 is part of a large protein complex greater than 1 megadalton in size. This LIN-5 protein complex will be affinity purified using antibodies recognizing endogenous or epitope-tagged LIN-5, and interacting proteins will be identified. In addition, two classes of candidate LIN-5 interacting proteins are being tested for coimmunoprecipitation from embryonic lysates: interactors identified in two-hybrid screens and proteins involved in spindle force generation. Genes identified as LIN-5 interacting proteins will be tested genetically for their role in mitosis. Analysis of the functions of LIN-5 and its associated proteins should contribute to our understanding of the processes of chromosome segregation.

# GENETIC CHARACTERIZATION OF OSMOSENSATION: STRUCTURE-FUNCTION ANALYSIS OF OSM-10

## Hana Sugimoto, Anne C. Hart

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Osmosensation has been molecularly characterized in unicellular organisms but is poorly understood in multicellular organisms.

When the nematode *C. elegans* encounters high osmolarity, it reverses direction to avoid the stimulus. Laser ablation studies demonstrated that the ASH sensory neurons are primarily responsible for the detection of high osmolarity, as well as nose touch and volatile repellents (1-octanol). *osm-10* is specifically required for osmosensation as *osm-10(nr2076),* a null allele disrupts osmosensation but not mechanosensation or chemosensation. *osm-10* encodes a novel protein of 419 amino acids which is expressed by the ASH, ASI, PHA and PHB sensory neurons. The OSM-10 protein contains 38 putative serine and threonine phosphorylation sites. *osm-10(n1602),* a recessive allele which changes an E to a K codon in a putative tyrosine phosphorylation site is completely defective for osmosensation. This suggests that phosphorylation of OSM-10 may be critical for signal transduction. The OSM-10 protein localizes to the cytoplasm, supporting this hypothesis. *osm-10(n1602)* protein expression is normal by western blot and immunohistochemistry.

We are currently investigating the molecular function of OSM-10. First, the potential role of tyrosine phosphorylation in OSM-10 function will be addressed. In order to determine the role of tyrosine in OSM-10 function, tyrosine 199 (Y199) will be converted to a phenylalanine (Y202F) in the *osm-10* rescue construct. In addition, a construct will be generated which replicates the *n1602* mutation (E199K), perturbing the consensus tyrosine phosphorylation site. Both the Y202F and E199K encoding constructs should disrupt osmosensation if phosphorylation is important. We will also create a series of constructs encoding truncated OSM-10 proteins to identify the domains important for the function of OSM-10. Some of the mutant constructs may have a dominant negative effect on osmosensation.

By studying the function of OSM-10, we hope to elucidate the molecular mechanism of osmosensation in *C. elegans*, which may shed a light into the molecular mechanism underlying osmosensation in multicellular organisms in general.

# THE WHOLE ENCHILADA (DEGENERIN FLAVOR)

## Nektarios Tavernarakis, Beate Gerstbrein, Yun Ning, Ami Modi, Monica Driscoll

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The database compiled by the *C. elegans* Genome Sequencing Consortium includes a total of 23 additional degenerin-related genes. Our characterization of these genes has led the determination of the expression pattern of eight previously uncharacterized degenerins: C24G7.2, C24G7.4, F23B2.3, T28B8.5, T28D9.7, T28F2.7, T28F4.2 and ZK770.1. This analysis revealed that members of the degenerin family function in a variety of cell types ranging from neurons to muscles and epithelia. Some of these degenerin-like genes are expressed in nose touch neurons and could thus be candidates for the elusive mechanosensory channel in those cells. In support of this notion, dsRNA mediated interference with the expression of these degenerins largely decreases response to nose touch. In addition, we have found degenerin-like genes to be expressed in body touch neurons and motorneurons of the ventral nerve cord where they could co-assemble with known degenerins to mediate the mechanosensory properties of these cells. Specific degenerins are expressed in the excretory canal cell which, in the nematode, is the functional equivalent of the kidney. It is intriguing that in mammals, degenerin homologues function in this organ to regulate electrolyte balance. We will present detailed expression patterns at the meeting. Our observations indicate, that contrary to what is probably expected for such a multi-gene family, closely related in sequence members of the degenerin group are not functionally redundant. In an effort to assign any of these genes to known genetic loci, we are currently attempting to complement closely linked candidate mutations and we are also screening deletion libraries for null alleles.

# PROTEASE-RELATED FEATURES OF THE INTRACELLULAR AMINO-TERMINI OFDEG/ENAC ION CHANNELS

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The DEG/ENaC superfamily is a recently identified ion channel class which includes members that mediate touch transduction (MEC-4 and MEC-10) and proprioception (UNC-8) in *C. elegans* and play critical roles in lung fluid clearance, $Na^+$ homeostasis (ENaCs), and possibly pain sensation (ASICs) in humans. Despite pivotal contributions to exciting areas of biology, relatively little is known of structure/activity relationships in the channel class. Computational analysis has revealed several conserved blocks of sequence in DEG/ENaC subunits, but understanding structure/function relations in this channel class is in its infancy. To identify potential DEG/ENaC N-terminal domain functions, we searched the Prosite database for similarities within this domain to characterized protein motifs. Unexpectedly, our analysis uncovered the presence of a thiol-protease histidine active site motif within the conserved N-terminal region. The conserved region in DEG/ENaC channels corresponds to one domain of a bi-partite active site in thiol proteases, which in the cathepsin family folds together with another domain to create the functional active site. The motif is intact in *C. elegans* MEC-4 and MEC-10 and is highly conserved in several additional degenerins. A histidine residue corresponding to the critical active site core histidine and the flanking small sidechain amino acid is 100% conserved in all DEG/ENaC family members. Although the motif is more divergent in the ENaC subfamily, it is intriguing that a Kunitz type protease inhibitor motif precedes the conserved N-terminal region in alpha ENaC. Several channel inactivating mutations cluster within this domain, underlining its functional significance. In addition, we have detected a striking and significant sequence similarity to cathepsin protease prodomains within the extreme amino-terminus of MEC-4 and MEC-10. The sequence similarities have allowed us to build a three-dimensional homology model of the intracellular, N-terminal region in MEC-4. We are now in the process of evaluating this model by obtaining structural information using NMR on a bacterially expressed MEC-4 N-terminal segment.

# REGULATION OF HOX GENES IN MALE TAIL RAY DEVELOPMENTAL PATHWAY

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Establishment of individual ray identities is a complex process involving Hox genes *egl-5* and *mab-5*. The regulation of Hox gene expression in cell lineages, however, is not well understood. For example, in the lineage leading to the rays, MAB-5 turns on *egl-5* transcription in V6.ppp, but for reasons unknown, not in V6, V6.p, or V6.pap, where MAB-5 is also present. To understand this regulation, we are now doing promoter dissections of *egl-5*, trying to find the regulators of *egl-5* by identifying the regulatory motifs in the promoter. We have been concentrating on the sequences in the *egl-5* promoter that are conserved between *C. elegans* and *C. briggsae*, on the assumption that the conservation in DNA sequence implies functional significance. Our approach is to introduce deletions of conserved sequences into *egl-5::gfp* reporters, and try to interpret the changes in expression pattern. So far we were able to localize a B lineage enhancer to a highly conserved region of about 200 bp, and are currently searching for regulatory motifs that can account for specific expression of *egl-5* in seam cells and the ray lineages.

Two other candidate genes that might affect the expression pattern of Hox genes are *ceh-20*, which is a *Drosophila* Exd/human Pbx homolog, and *ceh-25* (*unc-62*), which is a *Drosophila* Hth/human Meis homolog. EXD/PBX amd HTH/MEIS are noncanonical homeobox proteins that physically interact with Hox proteins to enhance their DNA binding specificity. If *ceh-20* and *ceh-25* act in *C. elegans* as they do in *Drosophila* and humans, they should be implicated in the processes of self and cross regulation among Hox genes. Weak alleles of *ceh-20* and *ceh-25* produce males with abnormal ray morphology. RNAi experiments also result in male tail defects, suggesting that *ceh-20* and *ceh-25* do fuction during male tail development. We are now making reporters and constructing dominant-negative forms of *ceh-20* and *ceh-25* to analyze their roles in detail.

# INVESTIGATING THE PEROXISOME IN *C. ELEGANS*:GFP ANALYSIS AND FUNCTIONAL KNOCKOUT OF 5 PEROXIN GENES

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The peroxisome is a ubiquitous subcellular organelle that functions in both catabolic and anabolic metabolism. Enzymes within the peroxisome catalyze the b-oxidation of long chain fatty acids and related substrates. Peroxisomes also catalyze the initial reactions in the biosynthesis of plasmalogens, which are abundant in myelin, and have a role in cholesterol and bile acid biosynthesis. Disruption of peroxisome biogenesis underlies several lethal human disorders. The numerous clinical manifestations of the disorders of peroxisome assembly include a characteristic defect in neuronal migration and degeneration in the CNS.

At least 23 proteins (peroxins) are required for peroxisome biogenesis in yeast and humans. We have identified the homologs of 12 of these proteins in the *C. elegans* genome database. dsRNA-mediated interference with 5 of these genes suggests these proteins are essential to normal nematode development. In all cases, the RNAi phenotype is an arrest at the L1/L2 stage. We are also testing cell-specific *in vivo* RNAi in touch neurons to investigate peroxisomal function in individual neurons.

In mammalian systems, a tripeptide sequence (S/A/C - K/R/H - L/M) located at the C-terminal end of a protein has been shown to function as the major targeting signal for directing proteins to the peroxisome. This peroxisome targeting signal (PTS1) is also present in many predicted *C. elegans* peroxisomal proteins. In addition to PTS1, a second targeting signal, PTS2 is utilized in both yeast and mammals for a distinct subset of peroxisomal proteins. Evidence suggests that there has been evolutionary switching of targeting signals since the known PTS2 containing proteins have PTS1 signals in the nematode. We constructed a GFP expression vector carrying the PTS1 targeting signal under control of a heat shock promoter. This fusion enables us to visualize peroxisomes in the worm and can follow their subcellular distribution through development, which has not been possible in other eukaryotic systems to date. We are currently investigating whether the PTS2 signal functions in peroxisomal import in the worm.

# POTENTIAL DOWNSTREAM TARGETS OF TGF-BETA SIGNALING IN DAUER FORMATION

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Depending on environmental conditions, *C. elegans* enters a developmentally arrested dauer larva stage or proceeds through larval development to adulthood. Several genes have been identified that are involved in dauer formation, and molecular analysis of some of these genes revealed that a TGFb-related pathway influences the dauer versus non-dauer decision. Components of this pathway are encoded by *daf-7* (a TGFb ligand), *daf-1* (a type I receptor), *daf-4* (a type II receptor), *daf-8* and *daf-14* (two Smad proteins). Mutations in any of these genes lead to a dauer constitutive (Daf-c) phenotype, which can be suppressed by mutations in *daf-3* (another Smad protein) or *daf-5* (a homolog of the Sno oncogene). This epistasis analysis suggests that TGFb signaling promotes reproductive development by negatively regulating *daf-3* and *daf-5*, which are required for dauer formation.

Although much insight has been obtained into how and when this TGFb-related pathway functions, little is known about its downstream targets. In order to identify some of these downstream factors, a screen was performed to isolate suppressors of the dauer constitutive phenotype of a *daf-7* mutation. These suppressors are necessary for dauer formation, and indeed, several alleles of known *daf-7* suppressors, such as *daf-3*, *daf-5*, and *daf-12*, were isolated. However, this mapping and complementation testing also suggests that at least 4 new loci have been identified. We are currently mapping and analyzing these potential downstream components of the TGFb pathway.

# UNC-89 IS A PUTATIVE MUSCLE SPECIFIC ACTIVATOR OF RHOA

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*unc-89* has a myofilament lattice that is thinner than normal, with disorganization of thick filaments, and for most alleles, no M-lines. UNC-89 is located throughout the depth of the myofilament lattice, in the middle of A-bands. UNC-89 is a 732 kD polypeptide composed of Ig domains, as well as SH3, DH (guanine nucleotide exchange factor) and PH domains (Benian et al., JCB 132: 835, 1996). We hypothesize that *unc-89* receives a signal from outside the muscle cell and recruits molecules to organize the myofilament lattice. Genetic data indicates interaction between *unc-89* and two mutationally defined Rho-GTPases, *mig-2* and *ced-10*. *mig-2* encodes a Rho family member and when mutant results in the failure of many migrating cells to complete their journeys. *mig-2*::GFP is expressed in many cells, including muscle, and is localized at or near the cell membrane (Zipkin et al., Cell 90: 883, 1997). When a null allele of *mig-2, mu28,* is combined with two hypomorphic alleles of *unc-89*, the Unc-89 phenotype is enhanced. When *mu28* is combined with an *unc-89* null allele, the Unc-89 phenotype remains the same. Similar experiments with an activated allele of *mig-2* gave the same results. *ced-10* encodes a Rac1 homolog that when mutant results in defects both in the engulfment of apoptotic cells and in the migrations of distal tip cells (Reddien & Horvitz, Nat. Cell Biol. 2: 131, 2000). When loss of function alleles of *ced-10* are combined with hypomorphic alleles of *unc-89*, the Unc-89 phenotype is enhanced. *ced-10*, *mig-2*, or *ced-10;mig-2* double mutants all have normal appearing muscle. By use of a novel method (yeast exchange assay; De Toledo et al., submitted), we can demonstrate guanine nucleotide exchange activity by the UNC-89 DH domain for mammalian RhoA, but not for nematode MIG-2 or CED-10, nor a number of other mammalian Rho-GTPases. There are no known mutations in *C. elegans* RhoA. RNAi for RhoA results in embryonic lethality (Malone, Orital & Han, pers. comm.; our unpub. data). We plan to use the yeast exchange assay on *C. elegans* RhoA (88% identical to human RhoA), localize RhoA in *unc-89* mutants, and determine the phenotype of an *unc-89* mutant in which antisense RhoA is driven by a muscle promoter.

# FURTHER PROGRESS IN UNDERSTANDING *UNC-98*, A GENE IMPORTANT FOR INTERMEDIATE FILAMENT PROTEIN ORGANIZATION IN NEMATODE MUSCLE

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The gene *unc-98* was orginally identified by Zengel and Epstein (Cell Motil. 1: 73, 1980). *unc-98*, represented to date by a single allele, *su130*, was described as having wild type movement. Our analysis, however, shows its movement to be significantly decreased from wild type in both L4s and adults. As first described by Zengel and Epstein, by polarized light microscopy, *su130* has highly birefringent "needles" near the ends of muscle cells, without clearly defined A and I bands. By EM, *su130* has a disorganized, thinner myofilament lattice with misshapened, shorter dense bodies. On some sections, masses of filaments can be seen, and these were originally described as consisting of thin filaments. However, these clumps more likely consist of intermediate filaments, as is suggested by immunoreactivity with the anti-intermediate filament monoclonal MH13 (as was first described by R. Francis, pers. comm. and confirmed by us). The staining pattern is similar in appearance to the "needles" revealed by polarized light. By immunofluorescence microscopy, these aggregates do not contain actin, but do contain UNC-89, and possibly myosin A. Myosin organization is more severely affected than actin organization in *su130* muscle. The organization of dense bodies also appears abnormal, with greater disorganization in the pattern of anti-a-actinin, than anti-vinculin staining. We mapped *unc-98* to the right of the left breakpoint of *stDp2* and to the left of the left breakpoint of *stDf5 / stDf6*. We then placed *unc-98* between *dpy-7* and *unc-18* by three factor mapping. Both *dpy-7* and *unc-18* have been cloned and are spanned by 5 overlapping cosmids. We were able to achieve transgenic rescue with one of these cosmids, F08C6. None of the genes predicted for this cosmid encode an intermediate filament protein. We are in the process of narrowing down *unc-98* to a single transcriptional unit and obtaining additional mutant alleles.

# CHARACTERIZING THE TGF-BETA SMALL MUTANTS

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We are interested in studying TGF-beta signaling pathways. In C elegans, a type II receptor, *daf-4*, is a common mediator of two distinct TGFbeta signaling pathways, the Dauer pathway, and the Small/Mab pathway. The Small/Mab pathway mutants have reduced body size, approximately 50% that of the wild type in adults, as well as defects in the male tail. We are interested in characterizing the Sma phenotype of the Small mutants. To describe the Sma phenotype more precisely, we've constructed growth curves of N2 and representative mutants of known genes in the Sma/Mab pathway. As a negative control, we measured mutants in *sma-1*, which is not a TGF-beta signaling component, but rather a gene whose mutants have an embryonic defect.. The experiment showed that the TGF-beta Sma mutants possess a post-embryonic growth defect. These mutants all hatch at about the same size as N2 and then proceed to grow at a reduced rate. *sma-1* differs from the TGF-beta Sma mutants. It hatches smaller than N2, but then has a normal growth rate. After measuring the body size of the worms, we characterized the Sma phenotype at the cellular level. Although the Sma mutants are smaller than wild type, it is believed that the cell number is the same. We will confirm that using DAPI staining. If the Small phenotype is not caused by reduced cell number, it must be caused by reduced cell size in some or all of the cells. We asked whether the Sma pathway exhibits the same effect on all tissues, and if all or just some cells are small in the Sma mutants. Using a strain with gfp expressing at sites of MH27 antigen localization, we visualized cell boundaries of the seam cells. We measured the length of the cells in the L3 stage prior to their fusion with the hypodermis, as well as the length of the worms, and their pharynx size. We performed this experiment with N2 as well as the alleles we used in the growth curves. Our results showed that the seam cells in the wild type strain are longer than in the sma mutants. The difference in cell length is similar to the difference in overall body size. Body length and cell length in Sma mutants is about .83 of wild type length at this stage. The area of the cells is also smaller in Sma mutants. The pharynx length of Sma mutants, however, is almost wild-type (.95). We conclude that not all cells are smaller by the same proportion.

# THE *APH-2* GENE OF *C. BRIGGSAE*

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The APH-2 protein is associated with cell surfaces in the early *C. elegans* embryo. This protein is essential for embryonic development as it appears to be required for early cell inductive events that are mediated by the GLP-1 receptor. Mutations in the *aph-2* gene cause a maternal-effect-lethal phenotype that is indistinguishable from that caused by mutations in the *glp-1* gene. The cellular localization of APH-2 suggests that the role of APH-2 in mediating cell interactions is at the level of extracellular events such as the preparation of the receptor or its ligand, or direct facilitation of the receptor-ligand interaction. The predicted amino acid sequence of APH-2 does not reveal any recognizable structural or functional motifs that might shed light on its molecular function. In an attempt to highlight which parts of the APH-2 protein are critical for its function, we have carried out a comparative analysis between the *C. elegans aph-2* gene and the *aph-2* gene of *C. briggsae*. We cloned the *C. briggsae aph-2* gene by building a size-selected library of *C. briggsae* genomic DNA, and screening this library with the *C. elegans aph-2* cDNA.

There is 80% amino acid identity between the predicted APH-2 proteins of *C. elegans* and *C. briggsae*. Some downstream untranslated sequences also show significant conservation, while the intronic sequences are generally not conserved, and much smaller in *C. briggsae*. We have used RNAi to show that the function of APH-2 in early embryos has also been conserved.

# CBP-1 IN *C.ELEGANS* DEVELOPMENT

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Mammalian p300 and CBP are closely related histone acetyltransferases (HAT's) serving as transcriptional integrators of multiple positive and negative signaling pathways. Recently, we have shown that a closely related *C.elegans* gene, *cbp-1*, is required to specify multiple differentiation events during embryogenesis. *cbp-1*(RNAi) embryos display substantial hyperproliferation and a complete lack of tissue specification with the exception of ectopical neural differentiation, implicating that CBP-1 is involved in specifying hypodermal as well as endodermal and mesodermal cell fates (1).

To further investigate the mechanisms underlying the differentiation-promoting activities of CBP-1, we isolated a *cbp-1* deletion mutant using a PCR-based approach. Embryos homozygous for the *cbp-1* deletion die around the 2-fold stage, suggesting that maternally provided CBP-1 is sufficient for most differentiation events. D*cbp-1* gives rise to a truncated CBP-1 that suffers a large internal deletion including the HAT domain. Using RNAi specifically targeting the *wt* CBP-1version of the protein in D*cbp-1* heterozygous animals, we could show that the isolated deletion represents a functional null allele, underscoring the central role of the HAT domain for the functionality of CBP-1.

By injecting animals heterozygous for D*cbp-1* with a YAC as a source for an intact copy of *cbp-1* we have achieved maternal as well as zygotic rescue. Embryos losing maternal expression closely resemble the phenotype observed in the *cbp-1*(RNAi) embryos. A detailed analysis of these embryos as compared to the *cbp-1*(RNAi) and D*cbp-1* embryos will be presented. Using this YAC rescue approach, we will carry out structure-function studies of CBP-1 *in vivo*. These experiments will help to identify domains of CBP-1 that are important for cell fate determination and differentiation of various tissues during embryogenesis. To understand the role of CBP-1 in differentiated tissues in postembryonic animals, we employed tissue-specific and hs-inducible RNAi to interfere with CBP-1 expression. An analysis of the phenotypes will be discussed.

Mello, C. and Shi, Y. (1998), 'A CBP/p300 homolog specifies multiple differentiation pathways in *Caenorhabditis elegans*.' Genes Dev., 12(7), 943-55.

# REGULATION OF *LIN-39* ACTIVITY BY RAS AND WNT SIGNALING PATHWAYS IN VULVAL DEVELOPMENT

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We are studying how extracellular signaling regulates cell fate specification during vulval development. The vulva arises from a group of six vulval precursor cells (VPCs). During the L3, an RTK/Ras pathway induces vulva formation. The Kenyon lab showed that the Hox gene *lin-39* is expressed in the VPCs and that LIN-39 levels are upregulated by activation of the Ras pathway. Our lab showed that the *bar-1* gene is also required for LIN-39 expression in the VPCs. *bar-1* encode a b -catenin homologue which we believe functions in a Wnt pathway to regulate *lin-39* expression. It is not known how either the Ras or Wnt pathway regulate LIN-39 levels. The *lin-39* genomic sequence contains recognition sites for TCF transcription factors, which are involved in Wnt signaling. Also the LIN-39 protein has a single consensus phosphorylation site for MAP- kinase, suggesting that LIN-39 might be regulated at the transcriptional and post-translational level.

Two *lin-39*::GFP reporters, a transcriptional and a translational fusion, are under construction. By injecting them into WT, *bar-1*, *let-23* (*sy1*) and *let-60* (*gf*) mutants we hope to differentiate between transcriptional and post-translational regulation of *lin-39*. Also, we are determining if LIN-39 can serve as a substrate for MAPK in an *in vitro* phosphorylation assay. We will then test if phosphorylation of LIN-39 is required for DNA binding and interaction with the extradenticle homologue CEH-20. We will introduce a LIN-39 with the MAPK phosphorylation site mutated into a GFP background to see if the Ras pathway regulates LIN-39 levels i*n vivo*.

We are also testing if LIN-39 acts as a transcriptional activator or repressor. We have made fusion proteins between the LIN-39 homeodomain and either VP16 or the Engrailed repression domain. If the VP16 construct gives a *lin-39* mutant phenotype when injected into WT animals, this would suggest that *lin-39* functions as a transcriptional repressor. If the Engrailed construct gives a *lin-39* mutant phenotype, this would suggest that *lin-39* acts as a transcriptional activator. Finally, to find the targets of LIN-39, we are examining differences in transcript levels between WT and HS-lin-39 strains using microarrays. Preliminary results of all these approaches will be presented.

# PROTEIN INTERACTION MAPPING IN *C. ELEGANS*

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The emerging field of functional genomics aims at characterizing the function of large numbers of unannotated ORFs predicted from nearly complete genome sequences by developing standardized functional assays that can be applied in high-throughput settings. Mostly gene-based functional genomics approaches have been developed so far. These typically include large-scale gene knock-outs and microarray or chip analysis. However, it is also important to develop protein-based approaches such as protein interaction mapping, protein localization mapping, and biochemical and structural genomics.

Most proteins require physical interactions with other molecules to function appropriately. Therefore, it should be informative to identify potential interaction partners for large numbers of predicted gene-products. We have started such a protein interaction mapping project in *C. elegans*, the first multicellular organism for which a nearly complete genome sequence has been available. As a standardized functional assay, we are using a semi-automated version of the yeast two-hybrid system that we developed.

Using proteins kown to be involved in vulval development, we have shown that ~50% of previously reported interactions can be detected in our two-hybrid assay, indicating that our current version of the system should allow a reasonable first coverage of protein-protein interactions. In addition, we have identified ~150 new potential protein-protein interactions which together provide a functional annotation for approximately 100 uncharacterized gene products. Several interactions seem to cluster in closed loops (X/Y/Z/Wn/X), indicating that they might belong to either macromolecular complexes. Finally ~10% of interactions corresponded to "interologs", which we define as protein-protein interactions conserved between model organisms. We have made our data available through a website on the Internet and in a version of ACeDB.

We will present the data obtained with vulval proteins and data we recently obtained on other systems.

# A CONSERVED TRANSCRIPTION MOTIF SUGGESTING FUNCTIONAL PARALLELS BETWEEN*C. ELEGANS* SKN-1 AND CAP 'N' COLLAR-RELATED BZIP PROTEINS

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In *C. elegans* embryogenesis, the maternal factor SKN-1 plays a pivital role in the specification of gut, some body wall muscle and pharynx. The acitivity of SKN-1 also requires mutliple inputs from transcriptional co-factor interactions as well as defined signal transduction pathways. To understand how SKN-1 responds to different blastomere enviroments and coordinates distinct developmental outcomes, we have begun to analyze SKN-1 structure and function.

The SKN-1 DNA binding region is related to those of the Cap 'n' Collar (CNC) family of basic leucine zipper (bZIP) proteins, which bind DNA as dimers, but SKN-1 is unique in that it binds DNA as a monomer. The similarity between SKN-1 and CNC proteins, such as NF-E2, NRF-1 and NRF-2, might simply reflect overlapping DNA binding strategies, but bZIP containing CNC proteins are not present in *C. elegans*, and no momermeric SKN-1 like protein has been identifed outside of nematodes. However, the involvement of CNC proteins, such as NRF-1, in endoderm and mesoderm development suggests that they may be close functional counterparts to SKN-1.

With a cell culture assay, we have determined that SKN-1 induces transcription through three potent activation domains. The functional core of one of these domains is a short sequence motif, the DIDLID element, which is highly conserved in NRF-1 and NRF-2. The DIDLID element is important for SKN-1-driven transcription, suggesting that it is also likely to be significant in NRF proteins. In addition, SKN-1 binds to and activates transcription through the p300/CBP coactivator, supporting the genetic prediction that SKN-1 recruits the *C. elegans* p300/CBP ortholog, CBP-1. The role of CBP-1 in SKN-1 target gene activation is currently under investigation. The DIDLID element appears to act independently of p300/CBP, however, suggesting that it has a distinct conserved target. Thus, the evolutionary preservation of the DIDLID transcriptional element suggests that SKN-1 and NRF proteins interact with analogous co-factors, and may have preserved functional similarities despite diverging in their DNA binding domains.

# *IN VIVO* ANALYSIS OF *C. ELEGANS* $TAF_{II}$ HOMOLOGS IN GENE EXPRESSION AND EMBRYOGENESIS

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Transcriptional regulation depends on communication between DNA binding factors and the general transcription machinery. This machinery is well defined biochemically and in yeast, but little is known about how its components contribute to metazoan gene regulation *in vivo*, particularly to regulation of tissue-specific genes. We have begun to examine the functions of *C. elegans* TATA binding protein-associated factors ($TAF_{II}$s) in embryonic gene regulation. Biochemical assays predict that $TAF_{II}$s contact individual activators and core promoters, and whole genome anaysis in yeast suggests that some may influence the expression of modules or sets of genes.

In yeast, the homolog of human $TAF_{II}$31/32 is required for most, if not all, transcription. Accordingly, RNAi inhibition of the *C. elegans* $TAF_{II}$31/32 homolog causes an early undifferentiated embryonic arrest like that seen with *ama-1* (RNA Pol II) RNAi. In addition, in Ce$TAF_{II}$31/32 RNAi embryos a Pol II phosphoepitope (H5) that marks embryonic transcription is dramatically reduced, indicating a broad transcriptional block. However, the developmentally regulated gfp reporters *end-1*, *med-1* and *pha-4* are expressed robustly in Ce$TAF_{II}$31/32 RNAi embryos, even as the *end-1*-dependent lengthening of the E2 cell cycle is abrogated. A similar early embryonic arrest coupled with robust *end-1* expression is associated with RNAi of other Ce$TAF_{II}$s that are conserved in yeast. Ce$TAF_{II}$31/32 and other TAFs that are conserved in yeast thus appear to be essential for core biological functions such as cell cycle control, and in some cases for most transcription. They are not necessarily limiting for expression of many developmentally regulated genes, however, supporting the idea of modularity within the general machinery.

In contrast, *end-1*, *med-1* and *pha-4* are not expressed after RNAi of Ce$TAF_{II}$130, which appears to be metazoan specific. Ce$TAF_{II}$130 RNAi also causes an early undifferentiated arrest and a more complete diminution of the H5 phosphoepitope. Our data indicate that Ce$TAF_{II}$130 may be necessary for nearly all early embryonic mRNA transcription, presumably due to an essential regulatory role or mechanistic function.

# BIOCHEMICAL CHARACTERIZATION OF *C. ELEGANS* RNA HELICASE A AND MALATE DEHYDROGENASE

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In my laboratory, we are overexpressing and biochemically characterizing two different proteins from *C. elegans*. RNA helicase A (RHA-1) is essential for proper development in many organisms, including *C. elegans*. Human RHA is involved in RNA metabolism. For example, it is capable of mediating an association between CREB binding protein (CBP) and RNA polymerase II and is suggested to be required for the activation of transcription by CBP (Nakajima *et al.* (1997) *Cell 90*, 1107-1112). Human RHA is also required for RNA export from the nucleus (Tang *et al.* (1999) *MCB 19*, 3540-3550). We are attempting to determine the RNA binding targets of RHA-1 in the cell in order to elucidate its function in *C. elegans*. In another project, malate dehydrogenase (MDH), the last enzyme in the citric acid cycle, has been overexpressed and purified. This enzyme is active, and the enzyme kinetics of *C. elegans* MDH are being studied. Recent results from both projects will be presented. We are grateful to Yuji Kohara for providing the cDNA clones of these enzymes.

# CEMI-2, A COMPONENT OF A HISTONE DEACETYLASE COMPLEX, IS ESSENTIAL FOR *C. ELEGANS* DEVELOPMENT

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Dermatomyositis is an autoimmune inflammatory disease. Antibodies against Mi-2 are a specific marker for dermatomyositis. Mi-2 is a 218 kd protein of 1912 amino acids belonging to the SNF2/RAD54 helicase family. It contains 2 PHD-zinc finger domains, 2 chromodomains and a helicase/ATPase domain. Biochemical studies demonstrated that Mi-2 is a component of a complex that has histone deacetylase and nucleosome remodeling activities. Homozygous mutants of *Drosophila* dMi-2 die as first or second instar larva.

We identified two Mi-2 homologs in the *C. elegans* genome, F26F12.7 and T14G8.1. Mi-2 and F26F12.7 are 44% identical; Mi-2 and T14G8.1 are 44.8% identical. The helicase/ATPase domain is the most highly conserved region in these proteins.

To study the *in vivo* function of these two*C. elegans* Mi-2 homologs, we screened for gene knockouts. We isolated a deletion of 1616 bp between the third exon and the forth exon of F26f12.7, that also generates a premature stop codon. The truncated protein is only 528 amino acids and terminates between the two chromodomains. Homozygous mutants (derived from heterozygotes) can develop into adults but are completely sterile. 80% of homozygous mutant worms are defective in vulval morphogenesis such that the vulva extrudes and uterus is deformed. There is a low percentage of multi-vulva worms (1-2%) observed. The gonads of homozygous mutants turn prematurely and the DTC appears abnormal. *In vivo* RNAi experiments showed that the loss-of-function mutant worms of T14G8.1 gene arrest at L1 stage.

Both genes are expressed from the egg to adult. F26F12.7 is expressed in hypodermis and in uterus at L4 stage specifically. In adult worms, the expression in the uterus is greatly decreased. T14G8.1 is expressed strongly in the pharynx and also in hypodermis, intestine and some neurons. Biochemical studies demonstrated that the human Mi-2 has DNA dependent ATPase activity.

# SCREENING FOR NEW ALLELES OF *GON-11*

## Ian White, Marc Ginsburg, Eric Lambie

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*gon-11* is defined by a single temperature sensitive allele, *dx5*. At restrictive temperature, *dx5* homozygotes derived from a heterozygous mother exhibit an incompletely penetrant proximal germline proliferation phenotype. We suspect that this is due to minor defects in gonadogenesis that permit signals from the anchor cell to reach the germ cells during L2 (1). *dx5* homozygotes that are derived from a homozygous mother show an incompletely penetrant Gon phenotype that resembles that seen in *gon-2* mutants (2). Early divisions of the gonadal precursors and their immediate descendants are delayed, incomplete or altogether absent, resulting in very small gonad size.

We are currently searching for additional (stronger!) alleles of *gon-11,* with the hope that they will facilitate mapping and provide us with a better understanding of the function of this gene. Since *dx5/Df* animals produce Gon progeny at high frequency, we have been able to establish an efficient noncomplementation screen that should enable the isolation of an allelic series. The mechanics of this screen and a progress report will be presented.

1. Seydoux, Schedl and Greenwald, 1990. Cell 61:939

2. Sun and Lambie, 1997. Genetics 147:1077

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# A CHEMICAL GENETIC SCREEN FOR LETHAL COMPOUNDS IN *C. ELEGANS*

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The use of small molecules provides the power to conditionally alter biological processes in ways that may not be amenable to traditional genetic analysis. Although temperature sensitive alleles allow some conditional control, screens for temperature sensitive alleles are unlikely to identify all gene products necessary for viability. In contrast, a small molecule that activates or inhibits an essential process allows both dose dependent and temporal control of the process.

To identify small molecules that affect *C. elegans* development and physiology, we screened 16,320 diverse compounds for those that cause adult lethality. In this high-throughput screen using 384-well microtiter plates, we identified 130 small molecules that effectively killed adult worms after one round of screening. Of these, 89 fell into 19 clusters of related compounds. Using a minimal set of representative compounds we are testing the dose and stage specificity of these molecules and are analyzing phenotypes in addition to adult death. Because lethality was scored after two days of treatment, these compounds may have more immediate observable effects. In order to identify potential pathways affected by these compounds, different strain backgrounds can be treated, and the effects of these compounds can be observed in other organisms (yeast, zebrafish, mammalian tissue culture, etc.). Finally, screens for resistance may yield the identity of the molecular targets of these compounds or other pathway components.

# THE *DAF-2*/INSULIN-LIKE PATHWAY FUNCTIONS FROM SECRETORY CELL TYPES TO CONTROL LIFE SPAN.

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Aging, development and metabolism are controlled by the *daf-2*/insulin-like signaling pathway. Mutations in *daf-2*, a homologue of vertebrate insulin and IGF-I receptors, or in *age-1*, a subunit of phosphoinositide 3-kinase, cause animals to arrest development as dauer larvae, double the adult life span and cause enhanced fat accumulation, as judged by Sudan Black staining. To identify the cells where *daf-2* signaling controls aging, development and metabolism, we studied animals in which *daf-2* pathway signaling was restricted to specific cell types. Transgenes expressing an *age-1* or *daf-2* cDNA from cell-type restricted promoters were constructed. *age-1* and *daf-2* were expressed in neurons from *Punc-14*, in muscles from *Punc-54*, in the intestine from *Pges-1*, or, as a positive control, ubiquitously from *Pdpy-30*. In addition, *age-1* was expressed in a subset of neurons from *Pmec-7*. Extrachromosomal arrays derived from these transgenes were introduced into *age-1(mg44)* or *daf-2(e1370)* mutants, respectively, and assayed for rescue of the aging, Daf-c and metabolic phenotypes.

We found that the *daf-2* signaling pathway controls aging most potently from neurons, and weakly from intestinal cells, another secretory cell type. However, restoring *daf-2* pathway signaling to the subset of neurons that express *mec-7* was not sufficient for proper control of aging. Dauer arrest is also controlled from neurons or intestine, as well as weakly from muscle cells, a cell-type not specialized for secretion. Most of the restricted patterns of *daf-2* or *age-1* expression rescued fat accumulation in comparison to *daf-2(e1370)* and *age-1(mg44)* dauers. However, intestinally-restricted *daf-2* pathway signaling had little effect on fat accumulation, a surprising result since the intestine is a major fat storage site. Our results are consistent with *daf-2* mosaic analyses showing that *daf-2* functions non-cell autonomously in control of dauer arrest and aging (Apfeld and Kenyon, 1998). Furthermore, we have shown that neurons are a potent site for aging control by the *daf-2* pathway.

# A GENETIC SCREEN FOR PATERNAL EFFECT MUTATIONS IN *C. ELEGANS*.

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We have undertaken a genetic screen for recessive paternal effect mutations in *C. elegans*. While our understanding of *C. elegans* early embryo development has been facilitated by the analysis of maternal effect mutations whose gene products are present in the oocyte, less attention has been given to the role of the sperm and its contents in fertilization and embryogenesis. It is known that the sperm contributes a haploid genome and centrosomes, specifies the anterior-posterior axis, and activates the oocyte to complete meiosis and secrete an eggshell. Currently only one strict paternal effect mutation, *spe-11*, a novel protein involved in egg activation, has been identified and characterized (Browning and Strome, 1996). Because of the sperm's critical role in multiple embryonic processes, it is reasonable to assume that other gene products can be mutated to cause strict paternal effect lethality. To identify these paternally required gene products, we are screening for recessive parental effect mutations that can be rescued by wild-type sperm. If subsequently isolated homozygous mutant males beget dead embryos when crossed to *fog-2* females, the mutant is deemed a paternal effect. So far we have isolated two paternal effect mutations from 4000 F2s screened. The poster will supply a detailed screening strategy, an update on progress, and discuss future plans.

# SCREENS FOR NEW COMPONENTS IN TGF-BETA SIGNALING PATHWAY FROM A LON-2 SUPPRESSOR SCREEN

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The transforming growth factor b (TGFb) plays critical roles in many important cellular processes. These include the control of cell growth, fate determination, cell cycles regulation, and other developmental processes. In nematode *C.elegans*, at least two TGFb-like signaling pathways exist: the Sma/Mab and the dauer pathways. The Sma/Mab pathway appears to control nematode body length as wells as ray formation in the male tail. In *C.elegans*, mutations that result in small body size and abnormal male tail structures have identified components of a putative Sma/Mab pathway. The daf-4 and sma-6 genes encode type II and type I receptors, respectively. The sma-2, sma-3 and sma-4 genes encode Smad proteins. The dbl-1 gene encodes a putative ligand for this pathway.

In previous work, we have shown that the small genes, as well as dbl-1, are epistatic to the gene lon-2. To identify new members of the Sma/Mab pathway, we have conducted an F2 genetics screen designed to suppress the lon-2 mutation. 26,000 genomes have been screened and several mutations capable of suppressing the long phenotype to either that of wild type or small body size have been identified. Complementation tests show that we have identified several existing components of the pathway including alleles of sma-2, sma-3, sma-4 (Smads), sma-6 (type I receptor) and dbl-1 (ligand). We are currently in the process of mapping two mutants from this screen: sma-17 and sma-18. From STS mapping and deficiency mapping results, we are able to localize those two mutants to a small region. Given the results of genes already characterized from this screen, it is likely that some of these genes will play important roles in TGFb signaling.

# *TLP-1* ENCODES A PROTEIN SIMILAR TO *DROSOPHILA* NOCA, A ZINC FINGER PROTEIN

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Mutations in the *tlp-1* (**T** cell **l**ineage defects with le**p**toderan male tail) gene result in a pointy (leptoderan) tail tip in adult males with additional defects in the fan and T cell lineage causing defects in phasmid dye-filling. *tlp-1* maps to linkage group IV and is deleted by *nDf27*. We isolated two new alleles, *ny14* and *ny15*, in a UV/TMP screen. Using PCR polymorphism mapping we determined that *tlp-1* is in the T23G4 region. Sequence analysis suggests that *ny14* and *ny15* might entirely delete open reading frame T23G4.1. Transformation with a 10 kb genomic fragment containing T23G4.1 rescues the *tpl-1* phasmid dye-filling defect. By sequencing the alleles *bx85* (a DEB-induced allele kindly provided by Dr. Scott Emmons) and *mh17*, we found that *bx85* contains a 5 base-pair deletion (resulting in an early termination codon) and that *mh17* is a nonsense mutation (Glu to Ter). T23G4.1 encodes a protein most similar to a *Drosophila* zinc finger protein, *NocA* (approx. 18% amino acid identity). There are two putative zinc finger motifs near the C-terminus in *NocA*. The downstream one is a CCHH motif, which is highly conserved in TLP-1. The upstream one is a CCHC motif, lacking in TLP-1. Another highly conserved motif of unknown function (KSPLALLAKTC) occurs near the N-terminus. *NocA* is required embryonically and postembryonically for patterning ocelli (Cheah et al., 1994 ). A TLP-1::GFP fusion construct is expressed in the nuclei of the posterior intestine cells, the tail hypodermal cells and the T cells. This suggests that *tlp-1* may be a transcription factor required for patterning the *C. elegans* tail region, a hypothesis we aim to test in the future.

# GENETIC ANALYSIS OF COELOMOCYTE SPECIFICATION AND PATTERNING

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We are interested in studying events which are responsible for patterning the late *C. elegans* embryo. We are using coelomocytes as a model system to study such events since both molecular and functional markers for the coelomocytes are available, since the organism tolerates deviations in the number of coelomocytes, and since the small number of these cell facilitates screening for mutants. Coelomocytes are highly endocytic mesodermal cells that reside in the pseudocoelomic space. Four of the six coelomocytes arise late in embryogenesis from MS progeny; the other two arise from the post-embryonic divisions of M. The lineages and timing of coelomocyte specification present several questions about cell fate decisions. In particular, we are interested in understanding how cells choose between muscle and non-muscle fates, how mesodermal cell fate choices are linked to the cell cycle, and how two unique lineages can give rise to cells with the same terminal phenotypes.

To address these questions, we have been screening for changes in the number of coelomocytes by assaying for changes in tissue-specific GFP expression. This screen was faciliated by a strain with extra-bright GFP expression in these cells (provided to us by Dr. J. Fares). Using this strain, we have isolated over 20 mutations. These fall into several phenotypic categories based on (1) which cells are affected, (2) the number of cells, and (3) the timing of changes in cell number. We are currently mapping the mutations and performing more detailed phenotypic characterization.

# IDENDIFICATION OF NEW RDE (RNAI DEFICIENT) LOCI IN *C. ELEGANS*

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In a number of organisms, the introduction of double-stranded RNA into cells causes the post-transcriptional silencing of the corresponding gene. This experimental phenomenon is called RNA interference (RNAi). We are particularly interested in molecular mechanism of this phenomenon. In order to elucidate the mechanism of RNAi, we screened for rde (RNAi deficient) mutants and identified four complementation groups (*rde-1, -2, -3, -4*). In this study, we report the identification of three more rde loci. *ne321* has been mapped on LGV and is named *rde-5*. *rde-6* complementation group consists of *ne322* and *ne323*, and has been mapped on LGI. *rde-7* complementation group consists of *ne302*, *ne307*, *ne317*, *ne328* and *ne334*, and has been mapped on LGV. To clone the *rde-6* gene we are now trying to rescue this mutant by the injection of cosmids and YACs.

# TGF-BETA SIGNALING AND EGG LAYING.

## Ling Yu, Rafal Tokarz, Cathy Savage-Dunn

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A TGF-beta -related signaling pathway regulates dauer larval development and egg laying in *C. elegans*. Mutations in *daf-7* ligand, *daf-1* type I receptor, *daf-4* type II receptor, and *daf-8* and *daf-14* Smads result in Dauer-constitutive and Egg-laying defective animals. These mutations are suppressed for both defects by mutations in *daf-3* Smad. We are interested in the role of this pathway in egg laying. Two other genes that affect egg laying, *egl-4* and *egl-32*, are also implicated in this pathway by their suppression by *daf-3*. In addition, *egl-4* mutants have defects in chemotaxis (S.A. Daniels and P. Sengupta, pers. comm.). Because egg laying and dauer formation are influenced by environmental cues, it is possible that all of these phenotypes have a common cause in defective chemosensory neuron function. We first determined whether other egg-laying defective mutants could be suppressed by *daf-3*, but found no additional genes of this class. We then undertook a molecular analysis of *egl-32*. We narrowed down the *egl-32* interval using chromosomal deficiencies to the region between *unc-29* and *mec-8* on LGI. One cosmid from this interval, C26G6, which overlaps the sequenced cosmid T08G11, rescued the *egl-32* phenotype. Among several subclones of this cosmid, one containing two predicted ORFs T08G11.2 and T08G11.3, but not one containing only T08G11.3, also rescued *egl-32*. Therefore, *egl-32* likely corresponds to predicted ORF T08G11.2. We further tested this hypothesis by RNAi, and found that injection of L4 animals by dsRNA from this gene results in the Egl defect. Injection of dsRNA into adults gave no phenotypes in the offspring. The ability of late dsRNA injection to phenocopy the egg-laying defect is consistent with a defect in chemosensory function rather than a developmental defect. The predicted ORF encodes a small (282 aa) protein with a putative SH2 domain. How such a protein fits in with TGF-beta signaling is an intriguing problem.

# PHENOTYPIC AND GENETIC ANALYSIS OF MUTATIONS REQUIRED FOR FERTILIZATION

## Sonia Zannoni, Pavan Kadandale, Brian Geldziler, Andrew Singson

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Mutant hermaphrodites that are spermatogenesis-defective (*spe*) are self-sterile and lay unfertilized oocytes. However, when these otherwise heathly worms are mated to wild-type males they can produce cross-progeny. In the lab, we are focusing on the *spe-9, spe-13, spe-19, spe-36* and *spe-38* genes that produce sperm with normal morphology and motility that cannot fertilize oocytes even after contact between gametes. We believe that these mutants disrupt sperm-egg interactions during fertilization. The *spe-9* gene encodes a sperm transmembrane protein with an extracellular domain that contains ten-epidermal growth factor (EGF)-like repeats. We hypothesize that *spe-9* functions in the specialized cell-cell interactions required for fertilization. We will report on our phenotypic characterization of *spe-13, spe-19, spe-36* and *spe-38*. In anticipation of the molecular cloning of these genes we are mapping and screening for additional alleles. Understanding these mutants will help fully define the steps leading to fertilization in the worm and could complement studies of fertilization in other organisms.

# *SOP-3*, A NOVEL COMPONENT IN THE TRANSCRIPTIONAL REGULATION OF HOX GENES

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We use *C. elegans* male ray development as a model to study how the transcriptional machinery uses developmental information to regulate the expression of Hox genes. Hox genes *mab-5* and *egl-5* are expressed in and required for seam cell V6 to generate 5 rays. The expression of *mab-5* in V6 is activated by the *caudal* homolog *pal-1* during embryogenesis. The expression of *egl-5* in the V6 lineage is dependent on *mab-5*. In *pal-1(e2091)* mutants, V6 rays are missing due to the lack of expression of *mab-5* in V6.

To identify new genes regulating Hox gene expression we performed a screen for suppressors of the V6 ray loss phenotype of *pal-1(e2091)*, which has a mutation in a putative V6 enhancer element. *sop-3* is one of several suppressors we identified. *sop-3* activates the expression of *mab-5* in V6 by restoring the activity of *pal-1(e2091)*. *sop-3* encodes a novel protein with characteristics of transcriptional repressors, including Ala-rich, Pro-rich, and highly charged domains. *sop-3* interacts with a Wnt pathway to regulate the expression of the Hox genes *mab-5* and *egl-5* in the V6 lineage. Mutation of *sop-3* results in the ectopic expression of *mab-5* and *egl-5* and bypasses the requirement of *mab-5* for the activation of *egl-5* in the V6 lineage. The inappropriate expression of *mab-5* and *egl-5* in *sop-3* mutants requires the activity of *bar-1*/b-catenin.

*sop-3* interacts genetically with *sop-1*, *sur-2* and *unc-37*. *sop-1* and *sur-2* encode the human TRAP230 and hSur-2 homologs, respectively, which are components of the transcriptional Mediator complex. The multiprotein Mediator complex is required for relaying signals from transcriptional regulators to RNA Pol II. *unc-37* encodes the homolog of the general corepressor Groucho. A *sop-3* mutation causes a synthetic lethal phenotype with mutations in *sop-1*, *sur-2* and the weak allele of *unc-37(e262). sop-3* also functions with *sop-1, sur-2* and *unc-37* to play a positive role in the generation of V6 rays. Our results suggest *sop-3* interacts with components of the transcriptional mediator complex and the *C. elegans* general corepressor Groucho homolog to regulate gene expression, including Hox genes *mab-5* and *egl-5*.

# A *MEC-3*-DEPENDENT GENE ISOLATED BY REVERSE GENETICS

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The LIM homeodomain protein MEC-3 controls the differentiation of ten neurons: the six touch cells, the two FLP cells and the two PVD cells. Several genes (*mec-2,4,6,7,9,10,14,18*), all needed for the function of touch cells, are expressed in a *mec-3* dependent fashion. These genes are likely to be a subset of the *mec-3*-dependent genes for several reasons: 1) these genes were identified by mutations that cause touch insensitivity, genes that mutate to supersensitivity have not been sought; 2) genes with redundant functions would not have been identified in past screens; 3) genes that are needed for touch cell development or function but do not affect touch sensitivity (e.g., genes needed for chemical synaptic transmission or exclusively for AVM and PVM function) would not be identified in past screen; and 4) genes required for FLP or PVD functions have not been identified. To identify these other genes, we used Representational Difference Analysis (RDA). 10% and 8% of clones from the difference library generated by four rounds of subtraction and PCR represent fragments of *mec-7* and *mec-18*, respectively. Up to now, one new *mec-3*-dependent gene has been isolated.

This new gene encodes a novel GPI-anchored membrane protein and have four potential glycosylation sites. We examined the expression pattern of the new gene using both *gfp* and *lac-Z* reporters and showed that it is expressed in the six touch cells and the expression level decreased in both *mec-3(u6)* and *mec-3(e1498)*. Initial injection of RNAi for the new gene into wild type worms did not yield any obvious phenotype. Therefore, we injected RNAi for the gene into sensitized background and showed that RNAi of the new gene enhanced temperature-sensitive allele of *mec-6 (u247)* at 15° C, suggesting a protein interaction with MEC-6.

# A NEW MUTATION DISRUPTING MEMBRANE MORPHOGENESIS DURING *C. ELEGANS* SPERMATOGENESIS

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Our lab has studied a number of mutants affecting membrane morphogenesis during spermatogenesis, including *spe-4* and *spe-5*. *eb9* is the best known phenocopy of *spe-5* and, to some extent, *spe-4*. *eb9* mutant has defects in spermatid formation. The mutation usually arrests spermatogenesis at the spermatocyte stage, but the division that forms spermatids (budding) is often attempted. Occasionally, budding can be successful and spermatid-like cells result. However, these cells are abnormally small and rarely become motile spermatozoa. DAPI staining shows that the arrested spermatocytes have four condensed nuclei, suggesting that meiosis is not affected by the mutation.

During wild-type spermatogenesis, division of spermatocytes is associated with segregation of ER-Golgi-derived fibrous body-membranous organelle (FB-MO) complexes. The FB-MO's prepackage macromolecules required for spermatozoon formation, and their correct segregation is required for completion of cellular division. Electron microscopy indicates that *eb9* spermatocytes have abnormalities in the morphogenesis of FB-MO's. The FB's in *eb9* mutant are not surrounded by any membranous structures, while FB's in wild-type spermatocytes are surrounded by two layers of membranes extended from the closely associated MO's. For spermatocytes that attempt to bud, these naked FB's are not always segregated into the spermatids as seen in wild type. Instead of normal-appearing MO's, there are a large numbers of vesicles present in *eb9* spermatocytes.

*eb9* is in linkage group V. Genetic data have put *eb9* between *dpy-11* and the right end of *nDf32*, an interval of about 400 kb on the physical map. Presently, transgenic rescue is being used to positionally clone *eb9*.

# DEFINING NEW COMPONENTS OF TGF-BETA SIGNALING PATHWAYS IN C. ELEGANS

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Transforming growth factor-beta is a multi-functional growth and differentiation factor responsible for regulating many diverse biological processes in both vertebrate and invertebrate species. Among the most dramatic of TGF-beta's effects are those associated with specification of cell fates during development and inhibition of cell cycle progression. The core TGF-beta signaling pathway has now been described using a synergistic combination of genetic and biochemical approaches. Transmembrane receptors with intrinsic protein serine kinase activity bind ligand in the extracellular milieu and then phosphorylate intracellular proteins known as Smads. Phosphorylated Smads form heterooligomers and translocate into the nucleus where they can modulate transcriptional responses. To identify additional components of this pathway, including potential regulators and target genes, we have carried out a genetic screen in the nematode, *Caenorhabditis elegans*. Disruptions in the TGF-beta signaling pathway in *C. elegans* result in worms with either small body size or dauer phenotypes. The success of the screen has been confirmed by the identification of each of the known components of the TGF-beta signaling pathway including ligands, receptors, Smads, and schnurri. In addition, we are now cloning and molecularly characterizing other genes identified in this screen that either mediate TGF-beta signaling or modify pathway activity. We will present the most recent data regarding these mutants.