18th International *C. elegans* Meeting
June 22-26, 2011
University of California, Los Angeles

Program Addendum

Additional Workshop
NSF Grants Workshop, Thursday, June 23
Westcoast Room, Covel Commons, 5:00 pm – 6:30 pm
Chairs: Aixa Alfonso, James Deshler (NSF)

Presenter Changes
Thursday, June 23
Development and Evolution I: Fate, Evolution and Timing
49 Presentation will be given by Steven Gore

Cell Biology I: Cell Division, Chromosome Dynamics and Polarity
64 Presentation will be given by Scott Robertson

Saturday, June 25
Cell Biology II: Cell Polarity and Morphogenesis
#174 Presentation will be given by Martha Soto

Author Corrections/Additions
23 Keith Nehrke
49 Marieke Oldenbroek, Steven Gore, Tugba Guven-Ozkan, Scott Robertson, Rueyling Lin
467C Vaida Juozaityte, Konstantinos Kagias and Roger Pocock
1056A Chonglin Yang

Late Poster Abstracts (complete text of abstracts available at www.celegans.org)

1252B
A new *osm-3* missense allele is a specific genetic modifier of *nphp-4* cilia phenotypes. **Svetlana Masyukova**, Kelly Roszczynialski, Corey Williams, Travis Ptacek and Bradley Yoder. Univ.of AL at Birmingham, AL

Primary cilium dysfunctions cause complex oligogenic disorders called the ciliopathies. Among the ciliopathies are Nephronophthisis (NPHP), and Meckel-Gruber syndrome (MKS). *MKS/NPHP* patients exhibit multiple phenotypes including midgestation lethality, left-right body asymmetry defects, skeletal abnormalities, cystic kidney disease, retinal degeneration and CNS malformation. In *NPHP4* patients phenotypes can be limited to renal dysfunction or may include retinal degeneration, with no apparent genotype-phenotype correlation. Many of the genes identified in NPHP and MKS patients encode proteins that localize to the base of the cilium. Most of these proteins are conserved in *Caenorhabditis elegans.* Compound mutations in *C.elegans nphp* and *mks* genes result in the appearance of more severe cilia phenotypes that are not observed in any of the single mutants, for example inability to absorb lipophilic dye DiI into the sensory neurons (Dyf phenotype). The goal of this study was to identify mutations in new genes producing synergistic Dyf phenotypes with *nphp-4*(tm925) null mutation using *C. elegans*. These genes would be potential targets for analysis in human patients. For this we performed a large scale EMS mutagenesis screen on *nphp-4 (tm925)* mutants and isolated 40 synthetic Dyf mutant lines. SNP mapping was done to place new mutations on chromosomes. Interval mapping and gene rescue experiments performed for one of the lines (YHW66) identified a new missense mutation in *osm-3* gene encoding kinesin motor protein required for building of ciliary distal segments in subset of sensory neurons. Similarly, Zebrafish homolog of *osm-3,* Kif17 is essential for vertebrate photoreceptor sensory outer segment development. Multispecies alignment revealed that the position of the new mutation is highly conserved in humans. Dye-filling and osmotic avoidance experiments showed that new *osm-3* allele does not produce noticeable cilia defects compared to wild type worms. In contrast, when combined with *nphp-4(tm925)* mutation, but not mutations in other transition zone proteins, it caused severe dye-filling and osmotic avoidance defects comparable to *osm-3* null allele. Further, OSM-3::GFP containing the new missense mutation properly localizes to the cilium in wild type background. Interestingly, it abnormally accumulates at the base of the cilium in YHW66 mutant background. Together our data suggest that NPHP-4 may be involved in controlling OSM-3 entry to the cilium. This can potentially explain retinal degeneration in a subgroup of NPHP4 human patients. Collectively, our studies identify a new

genetic modifier of *nphp-4* and help to understand complex genetic interactions contributing to the diversity of phenotypes associated with cilia disorders.

1253C

The unfolded protein sensor UDP-Glc:glycoprotein glucosyltransferase is conserved in *Caenorhabditis elegans* and participates in ER stress tolerance, development and lifespan. **Lucila I. Buzzi**[a], Sergio H. Simonetta[a] , Evelyn Hernández[a], Armando J. Parodi [a,b], Olga A. Castro [a,b,c]. [a]Fundación Instituto Leloir, [b]Instituto de Investigaciones Bioquímicas de Buenos Aires (IIBBA-CONICET), Avenida Patricias Argentinas 435, C1405BWE Buenos Aires, Argentina and [c]School of Sciences, University of Buenos Aires, C1428EHA Buenos Aires, Argentina.

The UDP-Glc:glycoprotein glucosyltransferase (UGT) is the key component of the glycoprotein folding quality control mechanism that takes place in the endoplasmic reticulum (ER). It behaves as a sensor of glycoprotein conformation as it exclusively glucosylates glycoproteins not displaying their native conformations. The addition of this glucose residue enables the interaction of folding intermediates with Calnexin/Calreticulin. An enzymatically active UGT is encoded by a single gene in *Schizosaccharomyces pombe, Drosophila melanogaster, Trypanosoma cruzi* and plants. There are two homologues coding for UGT-like proteins in Euteleostomi and in the genus *Caenorhabditis*. Both UGT homologues (HUGT1 and HUGT2) are expressed in human cells, the former but not the latter displayed GT activity and was upregulated under ER stress conditions. Bioinformatics analysis showed that in *C.elegans* there are two open reading frames (F48E3.3 and F26H9.8 hereinafter referred to as *C. elegans uggt-1* and *uggt-2* genes respectively) coding for UGT homologues. Here we report that *C. elegans* expresses an active UGT protein localized in the ER that is encoded by *uggt-1* gene. We constructed transgenic worms carrying the *uggt-1promoter::gfp* construct and found that UGGT-1 is expressed in cells of the nervous system and is upregulated under ER stress. Real time PCR analysis showed that both *uggt-1* and *uggt-2* are expressed during the entire *C. elegans* life cycle but at very different levels, being *uggt-2* expression at most *3 %* of *uggt-1.* Depletion of UGGT-1 by RNA interference resulted in a reduced lifespan and that of UGGT-1 and UGGT-2 in a delay in development. We found that both CeUGGT-1 and CeUGGT-2 play a protecting role under ER stress conditions since worms arrested at L2/L3 stages, in conditions that produce the accumulation of unfolded glycoproteins.

1254A

High-throughput identification of toxicants that disrupt chromosome segregation during meiosis. **Patrick Allard**, Monica P. Colaiácovo. Harvard Medical School, Boston, MA 02115, USA.

Abnormal chromosome segregation during meiosis, the process by which haploid sperm and eggs are generated, is a major contributor to aneuploidy and therefore to infertility, miscarriages and birth defects. Although environmental exposure plays a significant role in the etiology of these diseases, there is currently no high-throughput approach for the identification of environmental meiotic disruptors. We propose to use the worm *Caenorhabditis elegans* in a novel screening strategy for the identification of toxicants altering meiotic chromosome segregation. *C. elegans* is both a well established meiotic model system that has illuminated our understanding of the genes and pathways governing this process and an emerging animal model used in toxicological studies. Here, we describe a high-throughput method for the identification of environmental aneugens in *C. elegans*. We have developed a dual luciferase/GFP reporter that is specifically induced in aneuploid embryos. First, worms are exposed to environmental compounds and screened for the presence of aneuploid embryos via a standard luciferase assay. The positive hits are then validated by direct or automated presence of GFP positive embryos in the worms' uterus and also by DNA staining for a detailed analysis of the meiotic defects in the germline. In test experiments, we have successfully induced luciferase and GFP expression following exposure to known aneugenic chemicals, such as chemotherapeutic agents, and also detected the expression of the reporters using an automated set-up, indicating that our system is suitable for high-throughput screens. With this novel screening strategy, we address the need for fast and reliable screening of environmental meiotic toxicants and their involvement in the induction of aneuploidies. This work was funded by the Colgate-Palmolive Grant for Alternative Research.

1255B

Density-based regulation of ascr#2 and ascr#4 expression in *Caenorhabditis elegans*. **Fatma Kaplan**[1], Frank C. Schroeder[2], Arthur S. Edison[3], Peter Teal[1], Hans T. Alborn[1]. [1]USDA-ARS, Gainesville, FL. [2]BTI/Cornell University, Ithaca, NY. [3]University of Florida, Gainesville, FL.

The ascarosides are a family of nematode small molecules, many of which induce formation of long-lived and highly stress resistant dauer larvae. More recent studies have shown that ascarosides serve additional functions as social signals and mating pheromones. For example, the male attracting pheromone is composed of a blend of at least four ascarosides, ascr#2, ascr#3, ascr#4 and ascr#8. Although many of the ascarosides have been shown to induce dauer formation at high concentrations, ascr#2 has been shown to be the most potent dauer-inducing compound. By using developmentally synchronized nematodes under starvation conditions we recently showed that ascr#2 content in growth media increased dramatically in conjunction with dauer formation. In addition to being a component of the mating pheromone, ascr#2 may also function as a density pheromone for *C. elegans*. In that case, its concentration in growth media would increase with increasing worm density. To test this hypothesis we grew nematodes in standard media and incubated at densities of 5000, 10000, 20000, 30000 and 40000 worms/ml in water for 1 h. Exudates were analyzed by LC-MS as previously described. As expected, total ascr#2 and ascr#4 content increased; however, ascr#2 release positively and ascr#4 release was negatively correlated with the increasing nematode density. Because ascr#2 is the non-glucosylated form of ascr#4, these data suggested that ascr#4 may be converted to ascr#2. Deuterium labeled ascr#2 or ascr#4 was incubated in water either with nematodes or nematode exudates. Labeled ascr#4 was converted to labeled ascr#2 wheras no ascr#2 was converted to ascr#4 in the presence of nematodes suggesting ascr#4 as a precursor of ascr#2. Furthermore, incubating ascr#4 with

worm exudates did not result in conversion to ascr#2, suggesting that ascr#4-to-ascr#2 conversion does not happen in the media but instead after ascr#4-uptake by the worms. In conclusion, ascr#2 secretion per worm increases with increasing worm density, and this increase may in part result from enzymatic conversion of ascr#4 to ascr#2 by the worms. Ascr#4 might mainly function as an inactive form of low nutrients or high density "alarm pheromone", easily converted to its active form by the nematodes. The function of ascr#4 or the ascr#2/ascr#4 ratio remains unknown. However, the conversion appears to be highly nematode specific, thus knowledge of enzymes that regulate production of nematode density pheromones may reveal new drug targets for controlling nematode parasites in humans and plants.

1256C

PINK-1 functions as an activator of programmed cell death during embryogenesis. **Julia E. L. Palter** and Joel H. Rothman University of California Santa Barbara MCDB/BMSE/NRI, Santa Barbara, CA

Programmed cell death (PCD) plays critical roles in development and its inappropriate activation can result in pathological conditions, including neurodegenerative diseases. Autosomal recessive mutations in PINK1 (PTEN-induced kinase 1), a mitochondrial serine-threonine kinase, are the second most common cause of early-onset Parkinson's disease (PD). Increased cell death and mitochondrial dysfunction are observed in Drosophila and cultured cells in PINK1 knockouts, presumably accounting for the loss of dopaminergic neurons in PD patients. While these studies suggested that PINK1 functions as a PCD suppressor, we have made the surprising finding that it instead appears to activate PCD in *C. elegans*. The pro-apoptotic function of PINK-1 is evident during embryogenesis. *pink-1(tm1779)* and *pink-1(ok3538)* result in survival of cells that are normally destined to undergo PCD during embryogenesis. In these mutants, the sister cells of dopaminergic neurons that normally undergo PCD persist into adulthood and adopt a dopaminergic neuronal fate. These surviving cells are unevenly distributed along the dorsal-ventral axis, with survival of dorsal CEP neurons being significantly more common. Loss of *pink-1* by RNAi or chromosomal mutation results in a reduction in PCD cell corpses during the comma stage, as observed by DIC. Both *pink-1* RNAi and chromosomal mutations suppress the embryonic lethality of *ced-9(n1950n2161)* and *ced-9(n1653)*, which normally arises as a result of ectopic cell death. In both *pink-1(-); ced-9(n1950n2161)* double mutants, embryogenesis is able to proceed farther and more animals survive to hatching. These phenotypes suggest that, in contrast to its proposed anti-apoptotic roles in humans and flies, PINK-1 may function positively in apoptosis.

1257A

The evolution of genetic incompatibility in *Caenorhabditis briggsae*. **Joseph Ross[1]**, Daniel Koboldt[2], Julia Staisch[2], Helen Chamberlin[3], Bhagwati Gupta[4], Raymond Miller[2], Scott Baird[5], and Eric Haag[1]. [1] Univ. of MD, College Park, MD, [2] Washington Univ., St. Louis, MO, [3] Ohio State Univ., Columbus, OH, [4] McMaster Univ., Hamilton, ON, [5] Wright State Univ., Dayton, OH.

The nematode *Caenorhabditis briggsae* is a useful species for conducting evolutionary comparisons with *C. elegans* and other related species. It also has the potential to illuminate the evolutionary genetic basis of intraspecific variation. To facilitate such studies, we produced a high-resolution *C. briggsae* genetic map using the SNP genotypes of advanced-intercross recombinant inbred lines (RIL) derived from reciprocal crosses between two allopatric strains. Using this map, we reassembled the *C. briggsae* genome and conducted a whole-genome synteny analysis with *C. elegans*. The genotypes also revealed widespread segregation distortion, suggesting the action of selection on genotypes of hybrids between particular strains. The preferential fixation of one allele at a locus on chromosome III reflects selection against a hybrid developmental delay phenotype. Biased allele fixation on two other autosomes, each seen only in one cross direction, suggests the existence of epistatic interactions between mitochondrial and nuclear alleles. These effects might be due to mitochondrial polymorphisms in *C. briggsae* strains and possibly reflect ecological adaptation. A second *C. briggsae* genetic map produced with a third strain revealed surprising variation in fine-scale recombination rates between strains of the same species. This is discussed in relation to what is known about the genomic divergence between strains. Together, our findings promote the continued use of *C. briggsae* to study the effect of intra-species genomic divergence on recombination and to explore the genetic basis of the onset of reproductive isolation.

1258B

Caenorhabditis elegans as a model to study selenocysteine decoding. Lucía Otero[1], Laura Romanelli[1], Vadim N. Gladyshev[2], Antonio Miranda-Vizuete[3], **Gustavo Salinas**[1].[1]Universidad de la República, Uruguay, [2]University of Harvard, Boston, MA, [3]Universidad Pablo de Olavide, Spain

Selenocysteine (Sec) is incorporated into proteins through a non-cannonical pathway. A selenocysteine incorporation sequence (SECIS) present in selenoprotein mRNAs reprograms an UGA codon allowing for Sec incorporation. We are using *Caenorhabditis elegans* as a model to study some aspects of Sec incorporation that are poorly understood in eukaryotes. Among other advantages, this organism possesses the whole Sec decoding machinery for the expression of only one selenoprotein: thioredoxin reductase (TRXR-1). We identified by blast the SECIS-binding protein of *C. elegans* (K04G2.11), which is significantly smaller than the SECIS-binding protein described for all other eukaryotes. *C. elegans* SECIS-binding protein contains only the L7ae domain, and lacks the postulated Sec incorporation domain and putatively regulatory domains; these latter are also absent in the entire genome. We performed metabolic incorporation of radioactive ^{75}Se on: i) the Bristol-N2 strain (positive control), ii) a *trxr-1* knock out (negative control) and iii) a *sbp-2* knock out strains. While N2 was able to incorporate Sec, the mutant strains were not. This result indicates that the SECIS-binding protein is not only functional, but also essential for Sec incorporation. We reasoned that the small SECIS-binding protein may be a consequence of the presence of a single selenoprotein gene in *C. elegans* genome (and hence a single SECIS element). To assess whether this feature of the Sec-incorporation trait is idiosyncratic in nematodes, we performed nematode comparative genomic analysis. We found that all nematodes that incorporate Sec possess small SECIS-binding protein; our preliminary results also indicate that TRXR-1 is the single selenoprotein in completely sequenced nematodes. An interesting result of the analysis is that the plant parasitic nematodes *Meloidogyne spp*. have lost the capacity to incorporate Sec. Overall, the results

suggest a reduced reliance on selenium in the nematode lineage, with idiosyncratic adjustments of the Sec incorporation machinery.
Funding: CSIC (Uruguay), ANII (Uruguay), AECI (Spain).

1259C
A conserved network of miRNAs belonging to the lin-4 and let-7 families regulates *C. elegans* development. Jeanyoung Jo, Kimberly Breving, Kenya Madric, and **Aurora Esquela-Kerscher**. Department of Microbiology and Molecular Cell Biology, Leroy T. Canoles Jr. Cancer Research Center, Eastern Virginia Medical School, Norfolk, Virginia.

There is a poor understanding of how miRNAs functionally interact to control basic biological events in animals. These limitations are due in part to the inability of researchers to identify physiologically relevant miRNA targets. Use of the simple but genetically powerful animal model system, *C. elegans*, will address this problem and allow us to study essential miRNA-target interactions in the intact animal. We are focusing on the poorly characterized *lin-4* homologue, miR-237, and *let-7* members, miR-48 and miR-84, in *C. elegans*. Our preliminary data using deletion mutants and RNAi suppressor screens indicate that these miRNAs possess overlapping activities that control the formation of the gonad & egg-laying structures (vulva) and regulate targets associated with cell cycle progression and chromatin remodeling. Although the *lin-4* and *let-7* families are not grouped together in the *C. elegans* genome, we note that a homologue from each of these families resides in a cluster on three specific chromosomal regions in mammals and are similarly expressed during mouse embryogenesis. A detailed interrogation of this conserved miRNA network will further our understanding of the complexities of miRNA function during development and cancer progression. Funding: This work is supported by EVMS start-up funds and a grant from the Thomas F. and Kate Miller Jeffress Memorial Trust (to A.E-K.).

1260A
Genetic analysis of food-associated salt concentration memory. **Yuki Sakurai**, Hirofumi Kunitomo, and Yuichi Iino. Department of Biophysics and Biochemistry, Graduate School of Science, University of Tokyo.

Caenorhabditis elegans is usually attracted to NaCl if grown on standard culture conditions. On the other hand, it avoids the salt after exposure to salt under starvation (salt chemotaxis learning). We have reported that the insulin/PI3-kinase signaling regulates salt chemotaxis learning in ASER, one of the major taste neurons of the organism (Tomioka et al. 2006, Adachi et al, 2010). To examine how preference for salt is modified by experiences, we exploited a novel behavioral test, salt preference assay. Briefly, adult animals grown under standard culture conditions (50 mM NaCl with *E. coli* as food) are transferred to a conditioning plate that contains a defined concentration, 0-100 mM, of NaCl with food. After 6 hours of conditioning, worms are washed out from the plates and tested for chemotaxis to 35-95 mM of NaCl. Through this analysis, we found that wild-type *C. elegans* is attracted to the NaCl concentrations at which it has been fed (food-associated salt preference), indicating that the behavior on salt gradient is based on salt concentration memory. Mutants of PI3-kinase pathway didn't show significant defects in this assay. This result suggests that the mechanisms for salt preference is distinct, at least in part, from that of salt chemotaxis learning. To elucidate the mechanisms of food-associated salt preference, we screened for mutants of food-associated NaCl concentration preference. 30,000 EMS-mutagenized F2 animals were divided into 30 independent groups and screened by the salt preference assay after 0 or 100 mM NaCl conditioning with food. Animals that were attracted to high or low concentrations of NaCl after 0 or 100 mM conditioning, respectively, were collected and bred for next generation. In this screen we isolated several independent mutant strains JN572-JN577. JN574 showed no food-associated salt preference, whereas JN572 and JN577 showed defects only after conditioning at low concentrations of salt. These mutants are not likely chemosensory mutants because they showed normal salt chemotaxis after starvation. Characterization of these mutants will clarify the molecular mechanisms as to how the animals adapt to ambient salt conditions.

1261B
A *C. elegans* RNA interference curriculum for high school and college. **Bruce Nash**, David A. Micklos. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

We developed and field-tested an experiment- and bioinformatics-rich curriculum that explores RNAi using *C. elegans* as the model organism. The curriculum begins with observation of mutant phenotypes and basic worm "husbandry," progresses to simple methods to induce RNAi and explore the mechanism of RNAi using polymerase chain reaction (PCR), and culminates with experimental methods allowing students to silence any gene in the *C. elegans* genome. We are currently completing a summative evaluation of the RNAi curriculum dissemination program, launched in 2005 with 262 faculty trained to date. The accompanying *Silencing Genomes* Internet site (www.silencinggenomes.org), which has received over 120,000 visits, includes all lab protocols and reagent recipes. A free strain library includes all needed bacterial and *C. elegans* strains, as well as 93 vectors developed by workshop participants to silence worm homologs to human genes. To date over 2,000 strain orders have been fulfilled. Two stand-alone kits derived from the program have been released by Carolina Biological Supply Company, with another in production. Longitudinal evaluation included (follow-up surveys conducted 9 and 15 months post workshop show that the majority of faculty trained have used workshop materials in lecuture (71%) and labs (51%) to improve their classroom teaching. Surveys returned by 197 survey respondents represent 75% of the 262 faculty trained to date. 57% of respondents taught units on RNAi, and 36% did student experiments using RNAi by feeding with their classes. 21% of teachers had students develop custom RNAi vectors and 44% had students do complementary bioinformatics exercises. Respondents reported doing the new labs with 3,926 students and sharing them with 156 other instructors. We are currently beginning another round of dissemination to 288 biotechnology educators through collaborations with twelve community colleges nationwide. Extensions to the experiments and new "distributed" experiments will be discussed. Funded by grants from NSF DUE-0341510, 6/2004-12/2006, DUE-0717765, 9/2007-8/2011, and DUE-1104236 4/2011- 3/2014.

1262C
WincG: a cyclic GMP biosensor for worms. **O. Scott Hamilton**, Damien M. O'Halloran, Chantal Brueggemann, Bi-Tzen Juang, Noelle D. L'Etoile. University of California, Davis, Davis, CA.

Cyclic 3',5'-guanosine monophosphate (cGMP) is a second messenger that has many cellular functions including regulating ion channels and protein kinases. cGMP is catalyzed from guanosine triphosphate (GTP) by guanylyl cyclases and hydrolyzed by phosphodiesterases to guanosine monophosphate (GMP). Given the diversity of cellular functions mediated by cGMP, a means of monitoring and measuring *in vivo* cGMP dynamics in *C. elegans* would provide the worm community with better resolution to probe many cellular pathways. Within the past few years a number of cGMP biosensors have been developed for mammalian cell cultures including FRET based reporters and, more recently, a non-FRET based single-fluorophore reporter that combines a circularly permutated EGFP with the cGMP binding sites of the regulatory domain of the *Bos taurus* PKG Iα that the authors (Nausch et al., PNAS, 2008) named FlincG (**Fl**uorescent **in**dicator of **c**yclic **G**MP). Since there are currently no available cGMP biosensors for nematodes, we have expressed a modified version of the FlincG in *C. elegans* that we call WincG (**W**orm **in**dicator of **c**yclic **G**MP). By cloning the FlincG into the worm expression vector pPD95.75, removing a non-coding region of the 3'UTR and enhancing the circularly permutated EGFP portion of the construct, we have been able to successfully express the cGMP biosensor under *C. elegans* promoters. Here we show that, as a proof of concept, when we express the WincG in phasmid neurons and introduce SDS as a repellent stimulus to the tail of a worm (that is immobilized in a worm trap) we are able to consistently measure cGMP changes ($\Delta F/F_0\%$). We are also currently examining cGMP dynamics in amphid neurons.

1263A
Genetic interactions with fzr-1, vab-1 and vab-2 reveal distinct roles for the nhr-6 nuclear receptor gene in regulating cell proliferation and cell differentiation during organ development. Brandon Praslicka, Anna Holloway, Mayur Fagwani, and **Chris R. Gissendanner**. University of Louisiana at Monroe, Monroe, LA.

NR4A nuclear receptors are emerging as increasingly important transcriptional regulators of numerous metabolic and developmental processes. NR4A NRs have been implicated in several human diseases including cancer, atherosclerosis, and neurodegeneration. A core cellular function of NR4A appears to be in regulating the balance between cell proliferation, differentiation, death, and survival. In our previous studies we determined that the NR4A NR ortholog in C. elegans, NHR-6, is required for development of the spermatheca. In nhr-6 mutants, the spermathecae are decreased in size and have ~ half the normal number of cells. To better understand the role of nhr-6 in regulating spermathecal cell proliferation, we performed genetic interaction studies to determine if the loss of function of negative G1/S cell cycle regulators suppresses the nhr-6 cell proliferation phenotype. We found that loss of function of fzr-1, but not lin-35, strongly suppresses the cell number phenotype of nhr-6 mutants. In addition, expression of cki-1::GFP was not altered in nhr-6 mutants, indicating that the cell proliferation defect is not due to premature cell cycle exit. While cell number is restored in nhr-6; fzr-1 double mutants, the spermathecal cells still fail to properly differentiate and the organ remains nonfunctional, causing near sterility of the animal. Thus, loss of fzr-1 function uncouples the cell proliferation and cell differentiation phenotypes of nhr-6 mutants and suggests a role for nhr-6 in both processes. This is also supported by genetic interaction studies with genes encoding components of Eph receptor tyrosine kinase signaling pathway. vab-1, which encodes the Eph receptor, and vab-2, which encodes an ephrin, both synergistically enhance partial loss of function of nhr-6 RNAi. However, these interactions lead to different spermatheca phenotypes, indicating distinct roles for these genes. vab-1(null); nhr-6(RNAi) animals exhibit spermathecae that lack the normal proximal-distal patterning of the organ but the organ still retains the normal or near normal number of cells. In contrast, vab-2(null); nhr-6(RNAi) animals exhibit a much more severe spermatheca development phenotype that is associated with a significant decrease in the number of spermathecal cells. Together, these data suggest a dualistic role for nhr-6 in regulating both G1/S cell progression and cell differentiation during spermatheca development and establishes the spermatheca as an important model system in identifying the molecular mechanisms by which NR4A NRs regulate cellular transitions.

1264B
C. elegans tag-208 encodes a sorbin-family homolog that localizes to sites of integrin-mediated adhesion. **Tim Loveless**, Jeff Hardin. UW – Madison, Madison, WI.

The sorbin family is a class of adapter proteins involved in numerous processes, including cell-cell adhesion, cell-matrix adhesion, and growth factor signaling. Three sorbin family members exist in humans: ArgBP2, CAP/Ponsin, and Vinexin, which are characterized by an N-terminal Sorbin-homology domain and three C-terminal SH3 domains. To date, these sorbin proteins have been primarily studied in cell culture systems. We have begun characterizing *tag-208*, which codes for the single sorbin family member in *C. elegans*. TAG-208 shares 27% identity and 42% conservation with human vinexin, and driving *tag-208::gfp* by 2 kb of endogenous promoter produces fluorescent signal at multiple sites of integrin-mediated adhesion. Beginning at approximately the 2-fold stage of embrogenesis, TAG-208::GFP localizes to dense bodies in the body wall muscle, as well as to muscle arm attachment sites near the nerve ring. TAG-208 also localizes to attachment sites of the enteric and vulval muscles, with additional punctate signal throughout the uterus and gonad sheath. Beginning in L4, signal is also seen in the distal tip cells of the somatic gonad. RNAi-mediated knockdown of *tag-208* produces a low penetrance of cuticle shedding defects, suggesting that it, like the dense-body protein UNC-95, may serve a role in regulating molting (Zaidel-Bar et al., 2010). Future studies will investigate whether loss of TAG-208 produces any effect on the integrity of body wall muscles or the distal tip cells.

1265C
Dopamine autoreceptor DOP-2 interacts with GPA-14 and modulate C. elegans learning and behavior. **Pratima Pandey**, Mahlet Mersha, Rochelle McDonald, Rosaria Formisano, Chandran Sabanayagam, and Harbinder S. Dhillon. Delaware State University, Dover, DE 19901

Dopamine (DA) receptors belong to the large and diverse family of G-protein-coupled receptors (GPCRs). DA receptors play pivotal roles in essentially all neural activities such as cognition, memory as well as neuropsychiatric and neurodegenerative disorders. Recent work in C. elegans has shown that dopamine release in behavioral plasticity is modulated in part through dop-2 that codes for a dopamine auto-receptor primarily expressed in the eight pre-synaptic neurons. DOP-2 is most similar in sequence to D2-like mammalian receptors with characteristic seven trans-membrane domains known to act through G-protein coupled pathways. Experiments conducted in our lab with dop-2 loss-of-function mutants (obtained through CGC) reveal that they display an aberrant habituation to mechanosensation. In order to assign the signaling pathway for DOP-2 we examined the C. elegans expression database and noticed that DOP-2 expression overlaps with a Gα-subunit encoded by gpa-14. Based on this hypothesis that dopamine auto-receptor DOP-2 might act through this identified Gα-subunit, we carried out a split-ubiquitin based yeast two-hybrid screen. Yeast two-hybrid results revealed that about one-third of the interacting clones were represented by gpa-14 sequences. To demonstrate the physical coupling between auto-receptor DOP-2 and GPA-14, in-vitro pull down assays were performed. We have generated truncated DOP-2 constructs in order to characterize the specific domains of DOP-2 required for interaction with GPA-14, through in-vitro and in-vivo interaction studies. Behavioral characterization of loss-of-function mutants in gpa-14 showed that it exhibits habituation deficits remarkably similar to dop-2. Our interaction studies and behavior data suggest that DOP-2 signaling during C. elegans learning is mediated through GPA-14. Funds from NIH-INBRE (2P20RR-016472-10) are gratefully acknowledged.

1266A

MicroRNA predictors of longevity in C. elegans. Zachary Pincus, **Thalyana Smith-Vikos**, Frank Slack. Molecular, Cellular and Developmental Biology, Yale University, New Haven, CT.

Inbred animals in homogenous laboratory conditions often experience a wide spread in lifespans, suggesting that neither genetic nor environmental factors fully determine individual longevity. Here we analyze biometrics of C. elegans observed throughout their lives using novel single-animal solid-media culture techniques. We find that early-to-mid–adulthood variability in several measures of homeostatic ability jointly predicts 62% of longevity variation. Though correlated, markers of growth/muscle maintenance and of metabolic by-products (“age pigments”) report independently on lifespan. We also identify three microRNAs for which single-gene expression patterns predict up to 47% of lifespan differences. Though expression of each increases throughout early adulthood, mir-71 and mir-246 expression in this time range correlates with future lifespan, while mir-239 expression anti-correlates. With upstream roles in longevity pathways, including insulin/IGF-1-like signaling (IIS), these microRNAs are both biomarkers and determinants of future longevity, and are the first genes shown to predict future longevity in un-perturbed, intact individuals. While it has been long assumed, this work is also the first demonstration that endogenous fluctuations in IIS activity act to determine individual lifespan. Taken together, these findings indicate that individual differences in longevity are at least partially the result of epigenetic states set early in life, and not solely the result of differences in stochastic damage accumulation over time.

1267B

TES-1 mediates epithelial morphogenesis. **Mark W. Schramp**, Allison Lynch and Jeff Hardin. University of Wisconsin, Madison, WI.

tes-1 encodes an ortholog of human Testin. TES has been identified as a tumor suppressor(Tobias et al., 2001) and a novel focal adhesion-associated protein that can co-localize with paxillin and zyxin, which it depends on for focal adhesion targeting in mouse fibroblast cells(Garvalov et al., 2003). In addition, TES has been shown to enhance cell spreading and inhibit cell migration, highlighting its potential role as a tumor suppressor. Recently, TES was shown to co-localize with □-catenin at sites of cell-cell contact in fibroblasts(Griffith et al., 2004), corroborating its potential role as a modulator of cell attachments. tes-1 was identified in a screen for enhancers of embryonic lethality observed in worms homozygous for a hypomorphic allele (fe4) of hmp-1/□-catenin. The fe4 mutation results in ~70% embryonic lethality, with most embryos showing elongation defects and varying degrees of defects in circumferential F-actin bundles (CFBs). The knockdown of tes-1 by feeding RNAi in fe4 worms results in >98% embryonic lethality with the vast majority of embryos exhibiting the Humpback phenotype characterized by dorsal humps along the body axis. Using a TES-1::GFP fusion protein driven by the tes-1 promoter, we have found that tes-1 is expressed in the hypodermis and in neurons during embryogenesis. During the process of embryonic elongation, TES-1 expressed in seam hypodermal cells localizes with HMR-1 at sites of cell-cell contact, just apical to the DLG-1/AJM-1 complex. In adult hermaphrodites, TES-1 is expressed in many neurons including the nerve ring, ventral nerve cord and tail ganglia. In addition, TES-1 is expressed in epithelial cells of the vulva and uterus. Worms homozygous for a tes-1 null allele (ok1036) do not exhibit increased embryonic lethality; however, ~5% of adult hermaphrodites show a protrusive vulva (Pvl) phenotype. We conclude that tes-1 is involved in epithelial morphogenesis and may stabilize cell contacts to withstand increased mechanical forces generated during embryonic elongation and egg-laying.

1268C

Identification of substrates of DRE-1/FBXO11 in C. elegans developmental timing**. Moritz Horn**, Christoph Geisen, Adam Antebi. Max-Planck-Institute for Biology of Ageing, Cologne, Germany.

Metazoan development depends on the precise coordination of a vast number of events in both time and space. In Caenorhabditis elegans the heterochronic circuit, controls developmental timing during the different larval stages, conferring temporal identity to cells. Gene activities in the heterochronic circuit include various transcriptional and translational regulators, many of which play evolutionarily conserved roles in stem cell biology and differentiation. We identified the dre-1 locus as an important component of the heterochronic circuits involved in post-translational regulatory mechanisms. Originally found in screens for enhancement of the mild gonadal migration defects of daf-12 null mutants, hypomorphic dre-1 mutants also show precocious seam cell fusion and gaps in the adult alae. Thus, dre-1(+) specifies late larval development

by preventing precocious expression of adult specific fates (Fielenbach et al. 2007). DRE-1 is an ortholog of the human F-box protein FBXO11, and is highly conserved across taxa. F-box proteins function as the substrate recognition component of SCF (Skip-1, Cullin, F-box) E3-ubiquitin ligase complexes, and indeed, knockdown of other components of the SCF complex gives similar phenotypes as dre-1, implying a role for ubiquitin mediated proteolysis in developmental timing. However, the substrate(s) of DRE-1, as well as those of its human ortholog FBXO11, remain unknown. To identify and characterize substrates of the DRE 1/E3-ubiquitin ligase complex, we are carrying out immunoprecipitation of DRE-1 complexes from C. elegans, testing candidate genes, and performing genetic suppressor screens. Interestingly, we have identified candidate substrates that behave as suppressors of dre-1 and whose protein expression levels are dre-1 dependent. Further characterization should clarify if these are bonafide ubiquitylated substrates of the SCF complex. These studies should yield important insights into post-translational mechanisms regulating metazoan developmental timing and stem cell biology.

1269A

lin-61 encodes a histone tail binding protein and acts as a transcriptional repressor. **Lena Kutscher**, Nora Koester-Eiserfunke, Wolfgang Fischle. Max Planck Institute for Biophysical Chemistry, Goettingen, Germany.

Malignant brain tumor (MBT) domain-containing proteins are involved in epigenetic regulation of the eukaryotic genome. These proteins have been implicated in a variety of cellular processes, including gene repression, germ cell formation and organism development. They localize to the nucleus and bind chromatin. MBT proteins in mammals and flies bind monomethylated and dimethylated lysines on histone tail peptides, with little discrimination towards a specific lysine residue. Key amino acid residues and binding mechanisms have been determined in these organisms in vitro, but many studies have struggled to relate MBT protein structure to its function in vivo. In C. elegans, however, one MBT protein, LIN-61, is unique in that it preferentially binds dimethylated and trimethylated lysines, and only in the context of histone H3 N-terminal tail methylated at lysine 9, a post-translational modification indicative of silenced heterochromatin. lin-61 is involved in vulva development, among other developmental pathways, in the nematode, and binding to H3K9me3 appears essential for this process. However, many open questions remain about its biology, and the binding mechanism of LIN-61 to chromatin has not yet been determined. To gain a complete understanding of LIN-61, we investigated its molecular function by implementing a multifaceted approach, including microarray analysis of lin-61 mutant worms to see how gene expression changes in its absence, its effect on the binding of HPL-2, a HP-1 homolog, to histone tail peptides, and a genetic assay to determine the role of H3K9me3 binding in genome instability. We found that lin-61 is a repressor of gene expression, with over 60% of the genes identified in the microarray as being upregulated in the absence of lin-61. Many of the genes that are upregulated include vulva development-related genes, genes involved in RNAi silencing in the germline, and genes involved in germ cell establishment. Additionally, we found that binding of HPL-2 to H3K9me3 histone tails is dependent on the presence of LIN-61, and that HPL-2 and LIN-61 seem to physically interact. Finally, we found that genome stability depends on the physical presence of LIN-61, and H3K9me3 binding appears to have a minimal role in maintaining genome stability. These findings provide essential information in determining the role of chromatin and HPL-2 interaction in regards the function of LIN-61 in C. elegans. L.K. was supported by the German Academic Exchange Service (DAAD).

1270B

Functions of microRNAs in aging. **Alexandre de Lencastre** and Frank Slack, Dept. of Molecular, Cellular and Developmental Biology, Yale University, New Haven, CT, 06511 USA.

MicroRNAs (miRNAs) constitute a class of regulatory elements that control gene expression and development in higher eukaryotes. We have previously shown that mutations to lin-4 and its target lin-14 significantly affect the lifespan of C. elegans (1). In addition, microarray analysis in C. elegans revealed dynamic miRNA expression changes during aging (2). These observations suggested that miRNAs might function during adulthood in pathways that impact lifespan. We have now found that miRNAs can function both to promote or to antagonize longevity in C. elegans. Recently, we demonstrated that four miRNAs of previously unknown function – miR-71, miR-238, miR-239 and miR-246 - affect lifespan and stress resistance in C. elegans (3). We found that these miRNAs are up-regulated in aging and genetically interact with components of the DNA damage checkpoint response pathway and the insulin signaling pathway. In addition, we used deep sequencing to identify 10 novel miRNAs that are expressed in aged C. elegans. These results establish miRNAs as a new class of aging-associated genes, with the potential to interact with a wide range of aging pathways.

Following up on our observation that multiple microRNAs (miRNAs) that are up-regulated in aged C. elegans have functions that affect lifespan, we have begun characterizing the factors downstream as well as upstream of these miRNAs that mediate their function. We have shown by genetic epistasis that the insulin-signalling and the DNA damage response pathways interact with 2 of these aging-associated miRNAs. In order to characterize the detailed molecular identities of pathway genes that may transduce these miRNAs' functions, we have surveyed the mRNA expression levels of multiple genes in these pathways in the genetic background of aging-associated miRNA mutants. We have found significantly de-regulated expression of candidate targets genes in these pathways that are also predicted to be direct targets of these miRNAs. In addition, we have found evidence for at least one negative feedback loop wherein the target gene also down-regulates the expression of the miRNA. We are now trying to understand the mechanisms upstream of these miRNAs that control their expression and to identify other possible targets of these miRNAs. Given the high conservation of aging pathways and miRNAs across species, it is likely that insights uncovered by this research will have high relevance towards our understanding of aging in higher organisms and humans. References: 1. M. Boehm, F. Slack, Science. 310, 1954-7 (2005). 2. C. Ibáñez-Ventoso et al., Aging Cell. 5, 235-246 (2006). 3. A. de Lencastre et al., Curr Biol. 20, 1-10 (2010).

1271C

mRNA degradomes reveal the cleavage targets and functions of endogenous siRNA pathways, **Jin-Wu Nam**, Chanseok Shin, and David Bartel. Whitehead Institute, MIT, and HHMI, Cambridge, MA.

C.elegans has at least two classes of endogenous siRNAs (endo-siRNAs), which associate with different families of

argonautes (AGOs). The most abundant endo-siRNAs, 22G RNAs (~22 nt long, typically beginning with G), are mainly loaded onto either worm-specific AGOs (WAGOs) that lack key catalytic residues responsible for target transcript cleavage, or CSR-1, which retains the residues needed for slicing activity. 26G RNAs (~26 nt long, typically beginning with G) are mainly associated with cell type–specific AGOs that have retained the catalytic residues, ALG-3/4 in sperm and ERGO-1 in oocytes. To find the endogenous cleavage targets of worm endo-siRNAs, we preformed high-throughput sequencing of mRNA cleavage fragments (degradome sequencing) and analyzed these data together with new and published data from small-RNA sequencing and mRNA microarrays. Our analyses indicate that CSR-1 catalyzes canonical mRNA cleavage between residues that pair to nucleotides 10 and 11 of the 22G RNA, but WAGO-1 is associated with noncanonical or exonucleolytic cleavage. CSR-1–associated 22G RNAs mostly target mature mRNA regions, with bias toward the 3'end of these mRNAs. This targeting is accompanied by derepression of mRNAs in the drh-3 mutant, which lacks all 22G RNAs. WAGO-1–associated 22G RNAs match not only mature mRNA regions but also intronic and intergenic regions, suggesting a function in the nucleus. Indeed, a correlation between WAGO-1–associated 22G RNAs and sites of histone-modification suggests a role for WAGO-1 in heterochromatin formation/maintenance.

1272A

Requirements for replication in the gene expression of the early C. elegans embryo. **Peter Insley1**, Shai Shaham1. Rockefeller University1, New York, NY.

A multicellular organism goes during embryogenesis from a single totipotent cell to a collection of differentiated ones. Along the way, certain genes need to be turned on in particular cells at particular times, and not before. One way in which this timing can be managed is if the onset of gene expression is coupled to the timing of cellular replication and divisions. Indeed, asymmetric divisions provide a well-known mechanism by which differentiation is linked to the cell cycle. In addition, there have been several reports demonstrating a requirement for S phase progression in metazoan gene expression. This suggests another mechanism by which organisms could time their transcriptional programs, by linking them to the replication of DNA molecules through a replication requirement for transcription. Despite the potential interest of such a coupling, it appears that links between gene expression and replication have not been thoroughly explored in any system, and the extent of the phenomenon remains unclear. Few experiments to determine factors involved in replication-dependent gene expression have been reported. Using the C. elegans embryo as a model, we are beginning to explore these issues in detail. C. elegans is the ideal system to use, as replication requirements can, in principle, be associated reliably with the activation of particular genes in particular cells during development. In addition, the necessary foundations of such a study have already been laid: prior researchers (Edgar and McGhee, 1988) presented evidence suggesting that replication in the early embryo is required for the differentiation of a variety of tissues, particularly the gut. Although suggestive, these experiments relied on a small number of late differentiation markers and did not identify which upstream regulatory genes may require replication for activation. The ultimate goals of our experiments are: to determine the extent of replication requirements for gene expression by identifying targeted genes, and to enumerate factors necessary for establishing and maintaining these requirements.

1273B

Vulval organogenesis involves an interaction between SMO-1 and the nuclear receptor NHR-25 Jordan D. Ward1, Nagagireesh Bojanala2, Teresita Bernal1, Marek Jindra2, Kaveh Ashrafi1, Keith R. Yamamoto1, **Masako Asahina2**. 1) UCSF, Mission Bay Campus, San Francisco, CA; 2) Biology Centre ASCR and Univ. South Bohemia, Budweis, Czech Republic.

Organogenesis at the cellular level involves a complex interplay of cell fate acquisition, cell cycle control, remodeling of adherens junctions, cell migrations and cell fusions. Thus, this process is tightly regulated temporarily as well as spatially. Tissue-specific regulatory programs governed by nuclear receptors (NRs) are a critical component of metazoan development and homeostasis with aberrations in these programs leading to pathophysiology. We are using the simplicity and powerful genetics of C. elegans to deconvolute how regulatory inputs differentially converge on NRs in specific cell and tissue-types. The single C. elegans NR5A family receptor, NHR-25, coordinates diverse tissue-specific developmental events such as molting, seam cell differentiation, fat metabolism, cell fate decisions and vulva formation. To uncover novel co-regulators of NHR-25 activity we performed a genome-wide yeast two-hybrid (Y2H) screen that uncovered the C. elegans SUMO homolog (SMO-1) as an NHR-25 interacting protein. Both deletion of the SMO-1 C-terminal di-glycine repeat and mutations in the SMO-1 beta sheet, which abrogate covalent sumoylation and non-covalent binding, respectively, prevented NHR-25 binding in Y2H assays. in vitro biochemical assays confirmed the sumoylation of NHR- 25. We have identified three lysines in NHR-25 responsible for the interaction, as well as non-covalent SUMO interacting motifs (SIM) located in the ligand binding domain of NHR-25. These data argue that NHR-25 initially binds SMO-1 non-covalently in order to promote its sumoylation, similar to thymine DNA glycosylase sumoylation. Deletion of the smo-1 gene in C. elegans causes severe protruding vulva (Pvl) and weak multivulva (Muv) phenotypes. When NHR-25 activity was reduced in a smo-1 mutant, Muv induction in P3.p, P4.p and P8.p was enhanced. Interestingly, daughters of P(5-7).p vulval precursor cells (VPCs) were also affected and could not complete the full program of vulval cell divisions, thereby suppressing the smo-1 Pvl phenotype. Our work suggests that SMO-1 and NHR-25 function together during VPC cell division in a cell context-dependent manner. The C. elegans vulva is a paradigm of organogenesis and these data highlight how the sumoylation of nuclear receptors fine-tunes target gene regulation and thus ensures proper organ development. Supported by GACR 204/09/H058, 204/07/0948, NPVII 2B06129, TFF postdoctoral fellowship 700046, NIH grant CA020535.

1274C

Hunting for new genes that function in neuronal maintenance. **James J. Ritch**, Andrea Thackeray, Steven DeGroot, Claire Bénard. Department of Neurobiology, UMass Medical School, Worcester, MA.

Maintenance factors ensure that neuronal structures, connectivity and function are preserved throughout life, well after

their establishment earlier during development. A number of molecules are dedicated to maintain the precise position of neuronal soma, axons and dendrites in the face of the wear and tear of body movements. Two of these molecules are ZIG-5 and ZIG-8, which are secreted two-Ig domain proteins that we have found to function redundantly in maintenance. Numerous related molecules exist in mammals, many of which are expressed in the adult mouse brain, but their function is not understood. While zig-5 zig-8 double mutant animals develop normally, they later exhibit progressive displacement of head neurons ASH and ASI from the L4 stage onwards, and axon flip-over defects in the ventral nerve cord. The biochemical role of ZIG-5 and ZIG-8 is unknown, but our genetic analysis indicates that they interact with SAX-7, the C. elegans ortholog of the L1CAM family of vertebrate adhesion molecules that have been implicated in multiple human neurological disorders. The phenotype of zig-5 zig-8 is very similar to that of sax-7 null mutants, suggesting they may have related roles in maintenance. Moreover, loss of the sax-7 long isoform suppresses the zig-5 zig-8 mutant phenotypes. To elucidate the roles of zig-5 zig-8 in neuronal maintenance, we are hunting for additional genes that interact with zig-5 and zig-8. We are carrying out a modifier screen to identify genes involved in promoting neuronal maintenance (enhancers) as well as those that antagonize it (suppressors). Progress on the screen will be presented.

1275A

Exploring mechanisms of dietary restriction in C. elegans. **Natalie Moroz1**,2,3, Juan J. Carmona3, Edward Anderson3,4, David A. Sinclair3, Anne Hart4 and T. Keith Blackwell2,3. 1. Harvard School of Public Health, Boston, MA. 2. Joslin Diabetes Center, Boston, MA. 3. Harvard Medical School, Boston, MA. 4. Brown University, Providence, RI.

Genetic variability and environmental conditions influence health and lifespan. A key component of our environment, our food intake, can dramatically affect longevity. The reduction of food consumption without malnutrition, called dietary restriction (DR), increases lifespan in essentially all eukaryotes, including yeast, Drosophila, C. elegans and mice. In C. elegans there are several methods of performing DR, including: diluting bacteria, using bacteria that are rich in different nutrients, using a chemically defined medium, or using a genetic mutant that has reduced pharyngeal pumping. Surprisingly, many of the current DR methods work independently of major longevity pathways. To further address how DR affects lifespan we developed a liquid DR protocol in C. elegans that 1) establishes bacteriostatic conditions, allowing bacteria to be kept at set concentrations for extended periods of time, and 2) is easy to maintain, in comparison to other methods that require daily maintenance and perturbation of the worms' environment. We will discuss our findings regarding the role of key longevity factors in our protocol, including: all four homologs of the yeast NAD+-dependent histone deacetylase SIR2, the nicotinamidase PNC-1, the low energy sensing kinase AMPK/AAK-2, the FoxO/DAF-16 transcription factor, and the Nrf/SKN-1 transcription factor. While some factors were found to be required, others play a partial role. Our results show that some key nutrient sensing pathways that are important for longevity do play a role in DR.

1276B

Leveraging existing genomic and microarray data to find direct targets of the C. elegans transcription factor TBX-2. **Tom Ronan**, Lynn Clary, and Peter G. Okkema. University of Illinois at Chicago.

Direct targets of transcription factors are time-consuming to find biologically and difficult to predict accurately solely using bioinformatics methods. Predictions can be significantly improved by utilizing the large repository of existing genomic and structural data in combination with select newly-generated gene expression data. Here we combine a single microarray experiment with classic transcription factor binding site prediction and novel uses of existing genomic and expression data sets to predict direct targets of the C. elegans transcription factor TBX-2. Binding data is not available for TBX-2, but is available for related T-box transcription factors. Alignments and structural data are used to distinguish among key characteristics and relevant regions of existing T-box binding motifs to produce a novel binding motif for predicting TBX-2 binding sites. This new motif is used to search five-prime cis-regulatory regions in five nematode genomes to identify potential direct targets. An alignment-independent definition of regulatory conservation is implemented to limit potential targets to binding sites conserved in multiple species. Genome-wide profiles of transcription factor binding characteristics, recently published by the modENCODE project, are used to narrow the field to targets most likely to be biologically significant. As TBX-2 is hypothesized to be a repressor, only potential targets which are up-regulated in a microarray comparing gene expression in wild-type and tbx-2(bx59) mutant embryos are considered. And finally, using an existing embryonic time-course mRNA expression data set, the subset of genes which anti-correlate with tbx-2 at various time-lags are used as evidence that they are directly targeted by TBX-2. This results in a small set of 18 biologically testable genes which are likely to be enriched for direct targets of the C. elegans transcription factor TBX-2, representing a 99% reduction over the microarray and a 91% reduction over bioinformatic predictions alone.