The

Second Annual

Seattle-Vancouver Worm Meeting

Friday, March 28th, 2008

1:00 pm

Michael Smith Building

Lecture Theatre

2185 East Mall, UBC

Sponsored by:

Fisher Scientific

Fermentas LIFE SCIENCES

MACROGEN Advancing through Genomics

Itinerary:

12:50 pm – Introduction

Update from the NRRR Committee

1:00 pm – Session 1

Zhongying Zhao - Studies of gene functions at high spatio/temporal resolution

Martin Jones - High Resolution Analysis of *C. elegans* Deficiencies

2:00 pm – Starbucks Coffee Break

2:30 pm – Session 2

Pavitra Narasimha - A nuclear role for HAM-1 in asymmetric neuroblast divisions

Conny Lin - Developing a model for fetal alcohol spectrum disorder in *C. elegans*

3:30 pm – Fermantas Coffee Break

Coffee and snacks (courtesy of Fermentas)

4:00 pm – Session 3

Max Boeck - Not so Redundant, the Distinct Roles of end-1 and end-3 in Endoderm Formation

Don Moerman - Comparative Genome Hybridization: A versatile tool for exploring the *C. elegans* genome

5:30 pm – Poster Session with Pizza

6:30 pm – Pub Night

Session 1 Abstracts:

Studies of gene functions at high spatio/temporal resolution

Zhongying Zhao

Currently, the study of gene activity during *C. elegans* embryogenesis is largely done at the tissue level with little temporal resolution. Higher temporal and spatial resolution could add useful functional insights. Exploiting the invariant cell lineage of the *Caenorhabditis* species and the 4D fluorescence microscopy, we developed automated tools to analyze gene function with cellular resolution at one minute intervals. Specifically, we developed algorithms and strains to allow automatic tracing of cell identities during embryogenesis and visualization tool to allow manual correction of any errors made by the algorithm. These tools make it possible to trace the embryonic cell lineages routinely, providing a wealth of gene/cell activities at high spatial/temporal resolution. For example, we have compared the embryonic cell lineage between *C. elegans* and *C. briggsae*. We have used these tools to automatically assigning cell identity, onset and quantification of gene expression. For instance, we have characterized the detailed expression patterns between a posterior specific microRNA gene and its putative regulatory transcription factor, allowing us to infer the possible functional relationships. We have also used our methods to examine the developmental control of cell cycle and cell fate determination. Taken together, these tools will help achieve gene/cell activities with high spatial/temporal resolutions which are otherwise difficult or impossible.

Charactersation of genetic deficiencies in *C.elegans*

Martin Jones

rol-3 is an essential receptor tyrosine kinase required for proper cuticle development in *C. elegans.* In my efforts to characterize the function of this gene I have applied whole genome array-CGH to the analysis of genetic deficiencies in an attempt to create a physical deficiency map that will allow me to position known suppressors of *rol-3* with more accuracy. Further to this I have generated, by deletion mutagenesis, eight new mutations that are able to suppress the lethality associated with severe alleles of *rol-3*. By combining a traditional suppressor screening strategy with whole genome array-CGH mapping I have been able to rapidly identify genetic lesions within these new suppressor strains, which will lead to the identification of genes whose altered expression results in suppression of *rol-3* lethality.

Session 2 Abstracts:

A nuclear role for *C. elegans* HAM-1 in asymmetric neuroblast divisions

Pavitra Narasimha

HAM-1 is a protein known to be involved in many asymmetric neuroblast divisions during *C. elegans* embryogenesis, particularly in lineages where a neuroblast gives rise to an apoptotic daughter and a neuronal precursor. *ham-1* encodes a 414 amino acid protein whose only recognizable domain is a winged helix motif near the N-terminus. Although the winged helix domain has been primarily characterized as a DNA binding domain, using HAM-1 specific antibodies, the protein is observed asymmetrically localized to the periphery in many dividing cells during embryogenesis. The mechanism by which HAM-1 becomes asymmetrically localized and regulates asymmetric cell division is unknown.

To identify HAM-1 sequences required for localization and function, we fused *gfp* to the N-terminus of *ham-1* and expressed HAM-1 under control of the pan-neural *unc-119* promoter. In the context of this construct, a series of nested N- and C-terminal HAM-1 deletions were generated. The full-length *gfp::ham-1* construct was functional, and by antibody staining the GFP:::HAM-1 fusion protein was asymmetrically localized in dividing cells. However, by direct GFP fluorescence, strong GFP expression was also detected in the nucleus. Removal of the first 32 amino acids resulted in a non-functional protein that was localized to the cytoplasm and nucleus and no longer detected at the membrane. A further deletion of the first 210 amino acids resulted in complete nuclear localization. Bioinformatics analysis identified two nuclear localization sequences (NLS) in the C-terminal half of the protein. Mutation of these two NLS's in the full length protein prevented nuclear localization and significantly impaired its ability to rescue *ham-1* defects. We are now testing if lack of rescue is due to the inability of the protein to localize to the nucleus. To determine if the nuclear export of HAM-1 is regulated, murine kidney cells transfected with eGFP::HAM-1 were treated with Leptomycin B (LMB), a strong inhibitor of CRM-1 mediated nuclear export. In the absence of LMB, eGFP::HAM-1 was evenly distributed between the cytoplasm and nucleus, while after LMB treatment the fusion protein was predominantly nuclear. Therefore, I am currently testing if HAM-1 localization is regulated by IMB-4, the CRM-1 homologue in *C. elegans* and we are taking a biochemical approach to detect endogenous HAM-1 in the nucleus of wild type embryos. In addition, we are also further defining the sequences required for membrane localization and function.

Developing a model for fetal alcohol spectrum disorder in *C. elegans*

Conny Lin

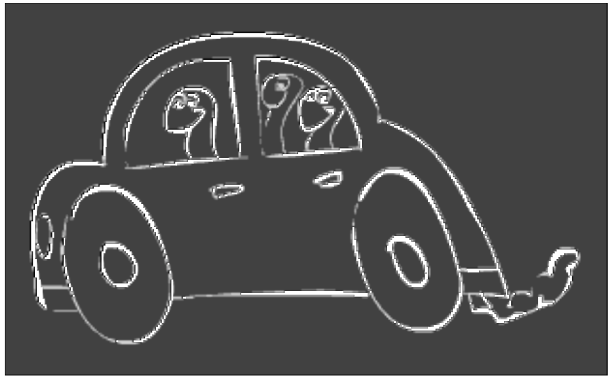
Using a candidate gene approach to study short-term habituation in *C. elegans* has been useful in the past to elucidate clues to understand the molecular mechanisms involved; however, the number of suitable candidate genes is relatively small, and it is very time consuming when the outcome is merely a null result. For this reason, performing a genetic screen to find novel genes that play a role in habituation is very attractive. Until now this has been a daunting task because it takes a long time to analyze an individual genetic line (approx. 40 hours by manual behavioural analysis; 6 hours using an automated system that tracks individual worms). We have developed a system that is capable of simultaneously analyzing multiple worms (as many as 50 reliably). This lowers the time needed to assess an individual strain to 10 minutes, making a genetic screen temporally feasible. The tracking system can score the frequency, magnitude, and duration of responses to tap as well as the average speed of the response. We have validated the system by demonstrating a number of essential habituation characteristics, such as the interstimulus interval dependence on both the rate and asymptotic level of habituation in wild-type worms. We have also tested several previously identified mutants, such as *dop-1* and *cat-2*, which show altered short-term habituation and found that the system is capable of reproducing the same phenotypes as previously reported. We are currently optimizing the system for use in a genetic screen to identify genes that affect different characteristics of habituation.

Session 3 Abstracts:

Not so Redundant, the Distinct Roles of *end-1* and *end-3* in Endoderm Formation

Max Boeck

As the sequences of more genomes becomes available, we increasingly appreciate our ignorance concerning what the majority of genes do or even why they are preserved. As many as 85% of genes in *C. elegans* have been shown to be dispensable for development when knocked out using RNAi or mutagenesis. A large number of these genes are the result of duplication events, with some duplicated genes preserved over 100 million years. The question I want to ask is whether these genes are truly redundantor if they have unique and important roles in development that we have not been able to detect. To address this question I have utilized our laboratory's ability to observe the kinetics of gene expression during *C. elegans* development. Using our 4D imaging technology I analyzed two duplicated GATA transcription factors, *end-1* and *end-3*, important in endoderm formation. Both genes are expressed at identical times during development and apparently activate the same set of genes. When these genes are knocked out *C. elegans* still undergoes apparently wild type development. Despite this apparently wild type development I haven been able to show that when either gene is deleted there is a reproducible delay in the onset of expression of downstream target genes. This delay can lead to low penetrance changes in fate of particular lineages along with extra divisions. Significanly, the phenotype associated with deletion of one redundant gene was not an exact phenocopy of the deletion of its partner: while both deletions caused a delay in the onset of downstream gene expression, only one , *end-3*, causes partial fate transformation or extra cell division. These results point towards a unique and important role of redundant genes during development.



Poster Presenters:

Tiffany Timbers - Piecing together a molecular mechanism for long-term habituation in *C. elegans*

Andreas Steimel - The *C. elegans* Flamingo homologue FMI-1 is involved in pioneer-mediated axon guidance in the ventral nerve cord

Mariana Veiga - Significance of low-abundance transcripts detected in *Caenorhabditis elegans* muscle SAGE libraries

Allan Mah - Modulation of gene expression in the *Caenorhabditis elegans* excretory cell by the octamer DNA motif

Kyla Hingwing - Characterization of a Wnt signaling pathway regulating asymmetric neuroblast division

Heesun Shin - Transcriptome analysis for *C. elegans* based on novel expressed sequence tags (ESTs)

Andrew Giles - A Multi-Worm Tracker for a Genetic Screen for Abnormal Habituation in *C. elegans*

Lee H. Lau - Context Conditioning in *Caenorhabditis elegans*

Shu Yi Chua - Identification and Characterization of let-725 & let-713 on SuperCosmid C05D11

Danyela P. Lee - Characterizing let-56 in the unc-22 Region of Caenorhabditis elegans Chromosome IV

Jessica McLellan - Genetic interactions of select genome stability genes and their relevance to cancer

Kristopher Schmidt - UNC-53/NAV2 is linked to the Arp2/3 Complex through ABI-1

Nancy Marcus - Examining the Role of UNC-53 and VAB-8 in Posterior Migration

Lily Fang - Insulin/IGF-1-like and Steroid Signaling in *Caenorhabditis elegans*

Adam Warner - The *C. elegans* Paxillin homolog and its role in body wall muscle

Nick Inglis - Meckel-Gruber syndrome-related proteins localise at the base of cilia to regulate insulin signaling

Poster Abstracts:

The *C. elegans* Flamingo homologue FMI-1 is involved in pioneer-mediated axon guidance in the ventral nerve cord

Andreas Steimel

The ventral cord of *C. elegans* is the main nerve connection along the anterior-posterior body axis. The two ventral cord axon tracks are established through the sequential outgrowth of pioneer and follower axons. The PVP axon pioneers the left axon track closely followed by the PVQ axon. PVP and PVQ axons are characterized by a tight pioneer-follower relationship. In an EMS screen for animals with defects in ventral cord axon guidance we isolated the *fmi-1* allele *rh308* (1). *fmi-1(rh308)* animals display strong PVP and PVQ axon guidance defects. Interestingly the pioneer-follower relationship between PVP and PVQ axons is disrupted in *fmi-1(rh308)* animals. Independent of the PVP axons the PVQ axons cross the ventral midline, leave the ventral cord or stop prematurely in 98% of *fmi-1(rh308)* animals. HSN axons as PVP-followers are similarly affected. In a separate EMS screen for defects in HSN axon guidance performed by the Garriga Lab two *fmi-1* alleles were isolated. In 68% of *fmi-1(rh308)* animals HSN axons fail to join the ventral cord axon tracks and circle around the vulva. Moreover HSN axons stop outgrowth before reaching the head region in nearly all *fmi-1(rh308)* animals. Interneuron axons that extend along pioneers in the right axon track are affected in 31% of *fmi-1(rh308)* animals.

The non-classical cadherin FMI-1 is the *C. elegans* homologue of *Drosophila* Flamingo and vertebrate CELSR1,2 and 3. FMI-1 is characterized by a unique domain composition with eight cadherin repeats, laminin G and EGF modules and a G-protein coupled receptor domain. *Drosophila* Flamingo functions in the planar cell polarity (PCP) pathway, axon target selection in the eye and establishment and maintenance of dendrites. Similarly CELSR2 and 3 mediate maintenance of dendrites and establishment of axonal tracks in mouse. To characterize the expression pattern of *fmi-1* we generated a 2.6 kb *fmi-1*-promoter GFP reporter construct. This construct is mainly expressed in neurons among them PVP, PVQ and HSN. Expression starts during gastrulation before axons grow out, persists throughout all larval stages and decreases noticeably in adults. To determine the subcellular localization of FMI-1 we fused the *fmi-1* transcript to GFP. FMI-1::GFP is predominantly localized to axons and is able to rescue PVQ defects in *fmi-1(rh308)* animals. Currently we are investigating the function of *fmi-1* in pioneer-follower axon guidance via mosaic analysis and targeted gene expression. We are performing a domain analysis and are searching for downstream effectors of *fmi-1*.

Genetic interactions of select genome stability genes and their relevance to cancer

Jessica McLellan

Chromosomal instability (CIN) is a hallmark of approximately 85% of solid tumors. This phenotype is characterized by an increase in the rate of loss or gain of whole chromosomes or large pieces thereof. Many genes have been identified in *S. cerevisiae* that are required to maintain chromosomal stability (CIN genes). Not surprisingly, many of these genes are mutated in cancer cells exhibiting CIN. An important question then is whether the CIN genetic background of these tumors can be used to specifically target cancerous cells for selective killing? Synthetic lethal genetic interactions in yeast between CIN genes associated with human cancer suggest that this is a valid approach to identify new therapeutic targets. In an effort to investigate this question further, comprehensive genetic interaction networks for the CIN genes mutated in cancers are being elucidated in *S. cerevisiae* using a genomic technology, Synthetic Genetic Array. Interactions that converge on single genes are being assayed in a multicellular animal, *C. elegans*, to determine conservation between species. These interactions are being assayed by feeding *C. elegans* mutant strains with RNAi. It is the interactions that are highly conserved that are most likely to be present in mammalian systems and which would be the most useful in terms of therapeutics.

Poster Abstracts:

The *C. elegans* Paxillin homolog and its role in body wall muscle

Adam Warner

Attachment of actin and myosin filaments to dense bodies and M-lines respectively is necessary to convert the force generated by sliding myofilaments into movement of the worm. Not surprisingly, worm muscle attachment complexes also contain many of the same protein components as vertebrate focal adhesion complexes, which rely on anchoring of actin filaments for movement of migrating cells over the extracellular matrix. One of the major focal adhesion components, paxillin, had previously not been identified in the worm. Here, we describe work that demonstrates such a protein is present in the worm, plays an important role in muscle, and is homologous to full length paxillin in humans and other species. In order to identify novel genes affecting C. elegans body wall muscle, we used tissue specific SAGE data (Moerman lab, unpublished) to compile a list of genes with enriched expression in body wall muscle cells. One of these, the LIM domain protein C28H8.6 is highly muscle enriched and has a high level of homology to the C-terminal half of human paxillin. The predicted gene directly upstream, C28H8.13, contains homology to the N-terminal half of paxillin. Using RTPCR we found that C28H8.6, and C28H8.13 transcripts are in fact one gene, hereby termed *pxl-1*. Our initial analysis has shown that *pxl-1* plays a significant role in *C. elegans* muscle. First, we have found that a full length GFP translational fusion localizes to dense bodies and M-lines in body wall muscle, and to ring shaped structures in the membrane of pharyngeal muscle cells, possibly corresponding to actin attachment sites. Secondly, a homozygous gene knockout of *pxl-1* provided by the *C. elegans* Knockout Consortium leads to animals arrested at the first larval developmental stage that have hindered movement and very weak pumping of the pharyngeal muscle. These developmentally arrested worms do not die immediately, but live for a normal lifespan. Lastly, yeast two-hybrid data has shown interactions between *pxl-1* and other body wall muscle proteins. We have found that the N-terminal half of PXL-1 binds to the dense body protein DEB-1/vinculin, and PXL-1 also binds to the muscle proteins UNC-95, UIG-1, HUM-6, LIM-8, and UNC-96. We will continue to characterize the role that *pxl-1* plays in body wall muscle, and where it fits in the sarcomere assembly pathway.

Meckel-Gruber syndrome-related proteins localise at the base of cilia to regulate insulin signaling

Nick Inglis

Meckel syndrome (MKS) is a developmental disorder associated with central nervous system malformations, cystic kidney disease, liver fibrosis and polydactyly. An identifying feature of MKS1, one of three MKS-associated proteins identified to date, is the presence of a B9 domain of unknown function. Our comprehensive phylogenetic analyses reveal that this domain occurs exclusively within a family of three proteins distributed widely in ciliated organisms. We show that all three *C. elegans* B9 domain-containing proteins, MKS-1 and MKS1 related proteins 1 and 2 (MKSR-1, MKSR-2), localise to transition zones (akin to basal bodies) at the base of sensory cilia. Their subcellular localisation is largely co-dependent, consistent with a functional relationship between the three proteins. Importantly, this localisation is evolutionarily conserved, since the three human orthologues also localise to basal bodies. Single, double and triple *C. elegans* mks/mksr mutants do not display overt transition zone positioning, ciliary structure, intraflagellar transport or chemosensory defects. However, we demonstrate genetic interactions between all double mks/mksr mutant combinations, which manifest as an increased lifespan phenotype often associated with ciliary signaling defects, and that the lifespan changes are mediated through modifications to the insulin signaling pathway. Our findings therefore demonstrate functional interactions between members of a novel protein family, and provide new insights into the molecular etiology of a pleiotropic human disorder.