VanWoRM June 20th – Meeting Abstracts

TALKS (5:15 – 6:15; Room SSB 7172):

1. **Copy number variants in *Caenorhabditis elegans* mutants and natural isolates detected by Comparative Genomic Hybridization.**

Jason Maydan[1], *Stephane Flibotte*[2], *Mark Edgley*[3], *Joanne Lau*[3], *Christine Lee*[3], *James Thomas*[4], *Donald Moerman*[1,3]. 1) Department of Zoology, University of British Columbia, Vancouver, BC, Canada; 2) Canada's Michael Smith Genome Sciences Centre, BC Cancer Agency, Vancouver, British Columbia, Canada; 3) Michael Smith Laboratories, University of British Columbia, Vancouver, British Columbia, Canada; 4) Department of Genome Sciences, University of Washington, Seattle, Washington.

We are using array comparative genomic hybridization (array CGH) to locate copy number variants (CNVs) in Caenorhabditis elegans. We have designed high-density (385,000 features) oligonucleotide microarrays that probe both the whole genome and specific chromosomes. We have detected heterozygous deletions as small as 141 bp, with breakpoint resolution to within fewer than 50 bp in some experiments (Maydan et al. Genome Res. 2007. 17(3): p.337-47.). This approach is being used by the C. elegans Gene Knockout Consortium (see abstract by Edgley et al.) and has already detected over 25 novel induced deletions, ranging from single-exon deletions to large deletions of several hundred kilobases affecting hundreds of genes. Over 12 balanced homozygous lethal deletions and 14 homozygous viable deletions have been identified in screens for mutations on chromosome II.

We have also used array CGH to detect extensive natural gene content variation in wild C. elegans isolates (Maydan et al. Genome Res. 2007. 17(3): p.337-47.). The degree of variation we observe in C. elegans is comparable to that found in the human genome. The whole genome array consists of probes tiled across 98 percent of annotated exons in the genome. Some of the more divergent isolates carry deletions in roughly 3% of the genes in the canonical N2 genome, implying the likelihood that many novel genes present in these genomes are absent in the sequenced N2 strain. CNVs are non-randomly distributed within and between chromosomes, occurring frequently on autosome arms and rarely in their centers or on the X chromosome. Deletions are strongly enriched in clustered unstable gene families, particularly for MATH-BTB, F-box, C-type lectin and Srz chemoreceptor families. These gene families tend to be involved in immunity, chemosensation, signal transduction and environmental response.

Whole genome CGH results from 10 natural isolates (AB1, CB4853, CB4854, CB4856, CB4858, JU258, JU263, JU322, KR314 and MY2) reveal complex relationships between the strains. Haplotype blocks of shared CNPs should allow the inference of specific outcrossing events that have occurred in the C. elegans lineage. This research should greatly increase our understanding of the natural history and evolution of the species.

2. **The Parkin co-regulated protein PCRG-1 localises to cilia and controls lifespan.**

Nathan Bialas, *Michel Leroux*. Department of Molecular Biology & Biochemistry, Simon Fraser University, Burnaby, BC, Canada.

VanWoRM June 20th – Meeting Abstracts

Caenorhabditis elegans senses environmental cues through ciliated sensory neurons located primarily in the head and tail. Mutants with defective cilia have impaired sensory perception, and often, this correlates with an increased lifespan and dauer defects.

Here we demonstrate that the *C. elegans* orthologue of mammalian *Pacrg*, the gene which shares a promoter element with Parkin, a ubiquitin ligase implicated in Parkinson's disease, is required for specifying normal lifespan. Although the *C. elegans* PACRG protein (PCRG-1) localises specifically to ciliary structures, abrogating its function causes none of the known or canonical ciliary phenotypes, including increased longevity or defects in chemotaxis, osmo-avoidance, mating, or dauer formation. In addition, the mutant animals are morphologically sound, develop normally to the adult stage, and exhibit a typical brood size. Surprisingly, *pcrg-1* mutants have a reproducible and significant reduction in lifespan, a first for a ciliary gene. This defect does not stem from a general or non-specific loss of viability, since animals lacking both *pcrg-1* and the insulin-like receptor gene *daf-2* have a lifespan indistinguishable from that of the very long-lived *daf-2* single mutant. Epistasis analyses reveal genetic interactions between *pcrg-1* and several ciliary mutants, and suggest that PCRG-1 may function in a parallel pathway with the insulin-like signaling pathway that ultimately controls DAF-16 activity and lifespan. Our findings therefore reveal that a previously uncharacterised ciliary protein is required for controlling a specific programme that regulates longevity in *C. elegans*.

3. **Neural excitability of mechanosensory neurons is important for dopamine-dependent short-term habituation but not dopamine-dependent long-term memory for habituation.**

Andrew Giles[1,2], *Katie Kindt*[4,5], *William Schafer*[5,6], *Catharine Rankin*[1,3]. 1) Brain Research Centre, UBC, Vancouver, BC, Canada; 2) Graduate Program in Neuroscience, UBC, Vancouver, BC, Canada; 3) Psychology Department, UBC, Vancouver, BC, Canada; 4) Biomedical Sciences Graduate Program, UCSD, La Jolla, CA, USA; 5) Division of Biology, UCSD, La Jolla, CA, USA; 6) MRC Laboratory of Molecular Biology, Cambridge, UK.

The tap withdrawal response of Caenorhabditis elegans habituates to repeated mechanical stimuli. When exposed to a distributed training protocol of 4 sets (each separated by an hour) of 20 stimuli (with a 60 s interstimulus interval), C. elegans show long-term memory for this habituation. Recently, a dopamine receptor has been identified and is expressed on the sensory neurons involved in this response. We hypothesized that dopamine neurotransmission may modulate these neurons and that this plasticity may play an important role in short-term habituation, and/or long-term memory of this response. Mutant analysis revealed that C. elegans lacking dopamine (cat-2) or the dopamine receptor (dop-1) habituate (short-term) significantly more quickly than wild-type. Further mutant analysis of putative signalling targets downstream of the dopamine receptor suggested that dopamine may be modulating calcium-mediated neural excitability in order to affect short-term habituation. To explore this hypothesis, we expressed a calcium sensitive FRET-based genetic molecule called cameleon in the mechanosensory neurons and recorded calcium responses to repeated mechanical stimuli in vivo. The calcium response of wild-type animals slowly decreased with repeated stimulation; calcium responses of dop-1 mutants decreased significantly more quickly than wild-type, suggesting that dopamine may be modulating the tap withdrawal neural circuit by affecting the neural excitability of the mechanosensory neurons. We have also found that cat-2 and dop-1 mutants both lack the ability to show long-term memory for habituation.

We found that the transgenic strain with cameleon expressed in mechanosensory neurons is capable of forming long-term memory for habituation, so we measured the calcium response to mechanical stimulation for both trained and naive transgenic worms 24 hours after training. Preliminary results suggest that the calcium response in the mechanosensory neurons of trained versus naive worms was the same; this was also observed for dop-1 mutants. This suggests that the dopamine-dependent long-term memory is not mediated by the neural excitability of the mechanosensory neurons. We are currently investigating the calcium response in the command interneurons to help localize the site of plasticity for the long-term memory.

POSTERS (6:15-7:00, SSB 6000 level atrium):

1. ***C. elegans* functional genomics of sensory cilia identifies an evolutionarily conserved gene, DYF-11, as a novel intraflagellar transport protein.**

Chunmei Li[1], ***Peter Inglis***[1], *Evgeni Efimenko*[2], *Peter Swoboda*[2], *Michel Leroux*[1]. 1) Department of Molecular Biology & Biochemistry, Simon Fraser University, Burnaby, BC, Canada; 2) Karolinska Institute, Department of Biosciences and Nutrition, Södertörn University College School of Life Sciences, Huddinge, Sweden.

Primary cilia play important roles in sensing the local chemical and physical environment, and are now linked to several critical signaling and developmental pathways. The building and maintenance of cilia and flagella requires intraflagellar transport (IFT), a conserved kinesin and dynein dependent bidirectional motility of multi-subunit protein complexes along ciliary axonemes. To investigate IFT in *C. elegans*, we employ a multi-disciplinary approach encompassing (1) transcriptomics- and bioinformatics-based experimentation to identify novel IFT genes, (2) fluorescence microscopy and *in vivo* IFT motility assays to examine IFT protein cellular localization and transport, and (3) analysis of mutant alleles to uncover the genetic basis of IFT gene function. In the last several years, we have identified several novel proteins associated with IFT, including a family of proteins associated with Bardet-Biedl syndrome, which in humans is characterised by numerous ailments, including obesity, blindness and polycystic kidneys. Here, we demonstrate that a *C. elegans* protein regulated by the ciliogenic DAF-19 transcription factor is a novel IFT-associated protein required for normal IFT and sensory cilia function. The protein, DYF-11, is encoded by a gene which we show is mutated in the dye-filling mutant strain *dyf-11*, whose phenotype is indicative of cilia dysfunction. Abrogation of DYF-11 results in truncated cilia and phenotypic

behaviours associated with defects in sensory cilia function. *In vivo* analyses of GFP-tagged DYF-11 or other IFT proteins in wt or various mutant strains suggest that DYF-11 is associated with subcomplex B IFT components that were first isolated biochemically in *Chlamydomonas*. Consistent with the notion that DYF-11 is a conserved IFT protein, DYF-11 is present in the membrane and matrix fraction of *Chlamydomonas* cilia, a property exhibited by other IFT proteins. Our results therefore extend the repertoire of components known to be associated with IFT and cilia function.

2. **The ciliary Bardet-Biedl syndrome proteins are required for normal thermotaxis and noxious temperature avoidance.**

Peter Inglis, *Michel Leroux*. Department of Molecular Biology & Biochemistry, Simon Fraser University, Burnaby, BC, Canada.

Under normal conditions, *C. elegans* can 'remember' the temperature at which it is reared. When removed from this familiar thermal environment, the animals will follow thermal gradients to return to their original rearing temperature. At least one neuron has been implicated in this process of thermo-recognition, namely the AFD neuron. Since this neuron possesses a cilium, an organelle ubiquitously responsible for sensing the chemical and physical environment, we investigated the role of cilia function in thermotaxis. To approach this question, we made use of Bardet-Biedl syndrome (BBS) mutants, which have defects in intraflagellar transport (IFT), a process required for building functional sensory cilia. Their functions are highly specific to the function of cilia and as far as we are aware, are unlikely to affect other neuronal properties. In particular, we have found that *bbs* mutants have morphologically normal AFD neuronal endings, which possess many finger-like membraneous protrusions emanating from a small ciliary axoneme. Using a shallow linear thermal gradient, we observed that similar to other mutants with defects in IFT, *bbs* mutants are unable to recognize subtle temperature cues. To further examine the connection between BBS/cilia function and temperature sensation, we tested the ability of *bbs* mutants to respond to noxious temperature levels, employing the established thermal avoidance (TAV) assay. Similar to our results involving physiological temperature levels, we found that *bbs* mutant animals were significantly less capable of recognising, and therefore escaping, localized extreme temperature levels compared to their wild-type counterparts. These data strongly suggest that BBS protein and cilia function is required for the ability of *C. elegans* to sense a wide range of thermal cues.

3. **Chromosomal clustering of tissue-specific genes in the nematode caenorhabditis elegans genome revealed by GFP expression profiles.**

Jeffrey Shih Chieh Chu[1], *Donald G. Moerman*[2], *Tim Swartz*[3], *David Baillie*[1], *Nansheng Chen*[1]. 1) Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, BC, Canada; 2) Zoology, University of British Columbia, Vancouver, BC, Canada; 3) Statistics and Actuarial Science, Simon Fraser University, Burnaby, BC, Canada.

How genes are organized in a genome is not well understood. The recent establishment of about 2,000 transgenic *Caenorhabditis elegans* strains that express green fluorescence protein (GFP) driven by endogenous promoters, and the subsequent identification of high resolution expression profiles (Hunt-Newbury et al., submitted) has provided a solid platform for investigating this problem. We hypothesize

that organ- and tissue-specific genes are not randomly distributed in a genome and instead, they are clustered to form groups. Previous works using microarray have suggested that genes in muscle, intestinal, and germ-line are non-randomly clustered in the *C. elegans* genome. In this study, we would like to explore whether such clustering properties are evident generally across organ systems or tissues. The advantage of using the GFP expression data for this project is the high resolution and high sensitivity of expression pattern identification across many tissues. We analyzed both at the organ level and the tissue level expression profiles. At the organ level, we found genes express in almost all organ systems are significantly clustered at various intervals. Furthermore, different organs show significant clustering at different ranges of intervals suggesting that different organs may show different mechanisms underlying clustering. At the tissue level, we found that genes express in 17 out of 48 tissues exhibit significant clustering at different ranges of intervals with some tissues cluster more narrowly.

In addition to the clustering distribution of organ- and tissue-specific genes in the *C. elegans* genome, with this data set, we also found that some tissue types are correlated because of shared expressed genes. As expected, different types of muscle tissues are correlated and cluster together in a correlation tree, and so are many neuronal cell types. Unexpectedly, we also found that certain groups of muscle cells are more correlated to certain group of neuronal cells, suggesting that the shared genes may play important roles in functionally specialized tissues. Finally, we have examined the similarity of expression profile of different genes by digitizing expression profiles of each gene. Such similarity in gene expression profile may be able to guide functional predictions and annotation of uncharacterized genes.

4. **Examining the role in muscle of low-abundance transcripts detected in *C. elegans* muscle SAGE libraries.**

Mariana Veiga[1], *Adam Lorch*[2], *Donald Moerman*[1,2]. 1) Dept Zoology, Univ British Columbia, Vancouver, BC, Canada; 2) Michael Smith Laboratories, Univ British Columbia, Vancouver, BC, Canada..

At least a third of these genes are expressed at low levels, many of which are uncharacterized in any system. Low-abundance transcripts have typically been overlooked, however in recent studies many of these transcripts have been confirmed (Lee *et al.*, RNA (2005), 11:939-946). Here we focus on the analysis of low expressed transcripts in the muscle SAGE libraries in order to confirm their expression in muscle and elucidate their biological role. We also anticipate that by focusing on these rarely expressed genes we will identify novel muscle components previously missed by more conventional approaches.

To first estimate what fraction of these low abundance transcripts present in the SAGE data are indeed expressed in muscle we performed RT-PCR on RNA isolated from purified muscle cells. We examined 128 genes, of which 84 were represented by a single tag. From this initial list, 38% of the low-expressed genes were confirmed and were investigated for their role in muscle cell formation, development or maintenance via RNAi and knockout strains. Twelve of these confirmed genes were examined using knockouts while twenty-five were examined using RNAi. RNAi results have shown that about 36% of the genes screened show defect in muscle myofilament organization. A total of 8 candidate genes (previously uncharacterized in muscle) appear to play a significant role in muscle cell anatomy as determined by KO and RNAi analysis.

We are currently using GFP translational fusions to deduce the location of these proteins within muscle. It has been suggested that transcripts with lower abundance tend to be from genes with specialized functions (Kim *et al*., FEBS (2006) 580:6721-6729). We expect that this work will help reveal the overall importance of low-abundance transcripts.

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

Adam Warner[1], *Barbara Meissner*[1], *Hiroshi Qadota*[2], *Guy M Benian*[2], *Donald Moerman*[1]. 1) Univ of British Columbia, Vancouver, BC; 2) Dept Pathology, Emory Univ, Atlanta, GA.

Attachment of actin and myosin filaments to structures known as dense bodies and M-lines respectively is necessary to convert the force generated by sliding myofilaments into movement of the worm. These structures in *C. elegans* muscle are both homologous and analogous to Z-lines and M-lines in mammalian muscle cells. 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, has to date not been identified in the worm as a full-length protein. Here, we describe work that demonstrates such a protein is present in the worm, plays an important role in body wall 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 and microarray expression data (McKay et al. 2003; Moerman lab, unpublished; Miller III 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. We have also found that the predicted gene directly upstream, C28H8.13, contains homology to the N-terminal half of paxillin. We believe that these two predicted genes constitute one coding sequence hereby termed cePaxillin.

Our initial analysis has shown that cePaxillin plays a significant role in *C. elegans* muscle. First, we have found that a GFP translational fusion containing the 4 LIM domains of cePaxillin localizes to dense bodies and M-lines, but not the nucleus where other body wall muscle LIM proteins have been found. Secondly, a homozygous gene knockout of cePaxillin provided by the *C. elegans* Knockout Consortium results in uncoordinated animals arrested at the L1 developmental stage. These developmentally arrested worms do not die immediately, but live for a normal lifespan while displaying mild disorganization of the myofilament lattice in their muscle cells. Lastly, yeast two-hybrid data has shown interactions between cePaxillin and other body wall muscle proteins. A genome wide yeast two-hybrid screen (Li et al. 2004) found an interaction between the LIM domain region of cePaxillin and the dense body protein ALP-1, and our own preliminary studies have shown that cePaxillin binds to the dense body protein UIG-1 and the dense body/M-line protein UNC-95. We will continue to characterize the role that cePaxillin plays in body wall muscle, and where it fits in the sarcomere assembly pathway.

6. Neural plasticity in Caenorhabditis elegans during the Dauer developmental stage.

VanWoRM June 20th – Meeting Abstracts

H. Lee Lau*, Andrew C. Giles, Catherine H. Rankin.* Brain Research Centre, UBC, Vancouver, BC, Canada

Caenorhabditis elegans is a microscopic worm that, in unfavourable environmental conditions, will enter an ageless stage called Dauer; which allows them to live 5-8 times their normal lifespan. No one knows if Dauer worms can learn and remember. These experiments were designed to test short-term habituation to mechanosensory stimulation (non-localized Petri-dish taps and localized head touches) during the Dauer stage. The results showed that Dauer worms responded less after both 30 taps and 30 head touches than they did initially. Further investigation into the development of the mechanosensory circuit during Dauer will extend this finding.

7. Effects of ethanol on the development of C. elegans.

Conny H.C. Lin*, Justin R. Davis, Yun Li, Catharine H. Rankin.* Brain Research Center and Department of Psychology, University of British Columbia, Vancouver, BC, Canada.

Nervous system deficits in fetal alcohol spectrum disorder had been investigated extensively in multiple animal models such as rodents, pig, sheep, fish, and insects (Cudd, 2005). However, fundamental molecular and genetic alteration and interaction with alcohol that result in cognitive and behavioral abnormalities are difficult to dissect in higher order animals due to the complexity of their nervous systems and behavior. Here we attempt to develop a model in which both chronic and acute embryonic exposure of ethanol can be effectively investigated at behavioral, system and molecular levels. With a fully mapped genome, a readily accessible mutant library, 302 fully mapped neurons, and simple behavior, C. elegans is a great model to use in the search for ethanol's effect on molecular level which results in nervous system deficiencies. We investigated the effect of chronic or acute ethanol exposure on survival rate, reproductive onset, worm length/size, fecundity and lifespan during various developmental stages in C. elegans. Chronic exposure of ethanol (0.0, 0.1 , 0.2 and 0.4mM) over the course of the lifetime, over larval development, and during adult only was explored. In all three cases, we found that high doses of alcohol significantly decreased length, reproductive onset, decreased total number of eggs laid and shortened lifespan. The effect of acute ethanol exposure during embryonic development was investigated by exposing eggs to ethanol (0.2%v/v) for 1 hour at various times (1-9h after eggs were laid). We report significant effect of 1hr exposure on age of reproductive onset. Pilot data suggests that acute exposure results smaller and more variable response to mechanosensory tap and more rapid habituation at a 10sec inter-stimulus interval. Our current result suggests that both chronic and acute exposure to ethanol during development have long-lasting effects on C. elegans. The development of this model will allow us to examine the effects of embryonic exposure to ethanol on the dynamic relationship of gene expression, neural circuitry development, and behavior. Cudd TA (2005) Animal model systems for the study of alcohol teratology. Exp Biol Med (Maywood) 230:389-393.

8. Elucidating the Role of DSH-2 and CWN-1 in Asymmetric Cell Division.

Kyla Hingwing[1]*, Sam Lee*[1]*, Lani Nykilchuk*[1]*, Tim Walston*[2]*, Jeff Hardin*[2]*, Nancy Hawkins*[1]*.* 1) Department of Molecular Biology & Biochemistry, Simon Fraser University, Burnaby, BC, Canada; 2) Department of Zoology, University of Wisconsin, Madison, USA.

VanWoRM June 20th – Meeting Abstracts

In *C. elegans*, all 302 neurons are generated by asymmetric cell division. To investigate the mechanisms underlying asymmetric neuroblast division we have focused on the lineage that generates PHA, a sensory neuron in the tail. Asymmetric division in this lineage requires *dsh-2* and *mom-5*, which encode Dishevelled (Dsh) and Frizzled (Fz) homologs respectively. We have shown that MOM-5 regulates the distribution of DSH-2 protein to the cell cortex, suggesting that MOM-5 regulates asymmetric cell division by controlling DSH-2 localization. DSH-2 and MOM-5 are components of both canonical and non-canonical Wnt signaling pathways. To elucidate which pathway is controlling asymmetric division, we performed a domain analysis of DSH-2. All Dsh 's contain three highly conserved domains: a N-terminal DIX domain, a central PDZ domain and a C-terminal DEP domain. The DIX domain is essential for canonical Wnt signaling while the DEP domain is required for non-canonical signaling. Deletion of the DIX domain did not disrupt DSH-2 localization to the cell cortex. Deletion of the DEP domain, however, delocalized DSH-2 from the cell cortex to the cytoplasm. We have shown that a DSH-2 construct lacking the DIX domain is able to rescue *dsh-2* asymmetric division defects, while no rescue is observed with DSH-2 lacking the DEP domain. Thus, we propose that DSH-2 and MOM-5 may operate through a non-canonical Wnt pathway. We have evidence that CWN-1 likely functions upstream of MOM-5 and DSH-2. We have generated a CWN-1::GFP fusion construct to analyze the pattern of CWN-1 expression. In addition, we are currently determining if CWN-1 misexpression disrupts asymmetric neuroblast division. Because our attempts to identify genes that function downstream of *dsh-2* by RNAi were unsuccessful, we took a genetic approach. In addition to division defects, loss of *dsh-2* function also results in maternal effect embryonic lethality. Therefore, we performed a genetic screen to isolate suppressors of the *dsh-2* lethality. We isolated over 60 suppressors, the majority of which suppressed the division defects in the PHA lineage. While the majority of the suppressors were dominant, we have isolated 4 X-linked suppressors and 3 autosomal recessive suppressors. We have mapped one of our X-linked recessive to the right of *lin-15* on the far right end of the X-chromosome. There are no known Wnt of PCP pathway genes in this region indicating that we have likely identified a novel gene that functions to modulate *dsh-2* signaling. We are also in the process of mapping a dominant suppressor on the X chromosome.