Lower Mainland's 8th Nematode Regional Research Review

Wednesday, June 14th, 2006

Hosted by the Rose Lab

Michael Smith Building

Lecture Theatre

2185 East Mall, UBC

Sponsored by:

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Itinerary:

(1) 5:30 pm - Introduction

New NRRR Organizing Committee

(2) 5:35 pm – Rose Lab Talks

(A) Monica Sleumer - *De Novo* Detection of Regulatory Modules in *C. elegans*

(B) Jillian Youds - The DOG-1 helicase, genomic instability and Fanconi anemia

(C) Nigel O'Neil - Utilizing TILLING to identify genes involved in genome stability

(3) 6:30 pm - Food/Beverages

Pizza (courtesy of the Rose Lab)

Drinks (courtesy of Invitrogen)

Abstracts:

(A) *De Novo* Detection of Regulatory Modules in *C. elegans*

Sleumer MC, Bilenky M, Dagpinar M, Griffith OL, He A, Pleasance ED, Robertson AG, Siddiqui AS, and Jones SJM

The availability of genomic data from several species of nematodes has provided an opportunity to search for novel functional regulatory elements in *C. elegans* on a genome-wide scale. Orthologues for *C. elegans* genes in *C. briggsae* were obtained from Wormbase, while orthologues in *C. remanei* and *Brugia malayi* were obtained by comparing their preliminary genomic sequences with the *C. elegans* genome using the WABA alignment algorithm5. The upstream region of each gene in *C. elegans* was pooled with the upstream regions of its orthologues to form a motif discovery sequence set. Regulatory motifs were predicted for 4894 *C. elegans* genes for which two or more orthologues were available. Negative control sequence sets were produced from *C. elegans* sequences by modeling neutral evolution.

Potential regulatory elements were generated using existing motif discovery algorithms (MotifSampler and CONSENSUS) to search for over-represented motifs in each sequence set. Discovered motifs were scored using a function that was optimized with known regulatory elements in *C. elegans*. An empirical p-value was assigned to each predicted motif by comparing its score to scores from motifs discovered in negative control sequences. High-scoring motifs were added to the cisRED database. (www.cisred.org), which contains putative regulatory elements for other genomes.

Groups of similar motifs were identified using a pairwise motif similarity metric based on shared information content and a density-based clustering algorithm. Co-occurring patterns of multiple motifs, which are putative regulatory modules, were identified in the upstream regions of *C. elegans* genes. All results are available through the web interface of the database.

(B) The DOG-1 Helicase, Genomic Instability and Fanconi Anemia

Youds JL, O'Neil NJ, and Rose AM

Fanconi anemia (FA) is a cancer susceptibility syndrome in which cells show chromosomal instability and hypersensitivity to DNA cross-linking agents. At least 11 complementation groups have been identified, including *BRIP1*, which was recently shown to be mutated in a subset of patients with FA and was subsequently renamed *FANCJ*. In *Caenorhabditis elegans*, *dog-1* is the gene most similar to *BRIP1/FANCJ*. We are currently investigating the possibility that DOG-1 and BRIP1/FANCJ have functionally conserved roles in DNA repair. Our preliminary data indicate that *dog-1* mutants are sensitive to DNA cross-linking agents, suggesting that *dog-1* and *FANCJ* could be functional orthologs. Previously, DOG-1 was shown to be required for the maintenance of polyG/polyC-tracts (G-tracts). In the absence of DOG-1, it is thought that G-tracts form secondary structures that block replication, leading to deletions that initiate in the G-tracts. Using our assay for deletions forming in the absence of DOG-1, we have assayed the *in vivo* contribution of various repair genes to the maintenance of G-tracts. We show that DOG-1 and the BLM ortholog, HIM-6, act synergistically during replication; simultaneous loss of function of both genes results in replicative stress and an increase in the formation of small deletions that initiate in G-tracts. Similarly, we show that genes implicated in homologous recombinational repair and trans-lesion synthesis are required to prevent G-tract deletions in the *dog-1* background. However, genes essential to the non-homologous end-joining and nucleotide excision repair pathways do not appear to be involved in deletion prevention or formation. In light of the *dog-1* deletion phenotype, it is possible that G-rich DNA secondary structures contribute to the genome instability observed in FA.

This research is funded by the Natural Sciences and Engineering Council and the Michael Smith Foundation for Health Research.

Abstracts (continued):

(C) Utilizing TILLING to identify genes involved in genome stability

O'Neil NJ, Gilchrist EJ, Zetka MC, Haughn GW, Rose AM

The sequencing of the human genome has created opportunities for the understanding of human biology never before possible. One approach to understanding human gene function is genetic analysis of gene orthologues in experimental models such as *Caenorhabditis elegans*. *C. elegans* has been extensively studied using genetic approaches and powerful means for understanding gene function have been developed. One of the factors limiting genetic analysis of human gene orthologues is the availability of mutations. There are several approaches to generating mutations in *C. elegans.* Forward mutagenesis screens for specific phenotypes have been very successful in isolating mutants affecting many different biological pathways. One disadvantage of such an approach is that the mutations must be mapped and correlated with genomic sequence, a process that can take months or years. Another disadvantage is that mutations are limited by the screening criteria, meaning that all the mutations isolated will result in a particular phenotype. Another mutagenesis approach takes advantage of PCR to identify deletions in targeted genes. An international consortium provides gene knockouts using this reverse genetic approach to generate deletions in targeted loci (The *C. elegans* Reverse Genetics Consortium www.celeganskoconsortium.omrf.org). This important resource provides genetic strains, which can be used to study the loss-of-function phenotype, an essential tool for genetic analysis. A disadvantage of this approach is that the majority of the mutations identified in this manner are loss-of-function mutations. We are investigating TILLING (Targeting Induced Local Lesions in Genomes) as a method for identifying mutations in target genes. TILLING provides a range of alleles including missense and nonsense mutations.

We have used the TILLING approach to identify mutations in a clonal EMS mutagenized library of 1500 worms. We have identified 71 mutations in 11 target genes. We have sib-selected several of these mutations and analyzed the phenotypes associated with these TILLed mutations. For each of the six targets we have analyzed, we have observed mutant phenotypes consistent with RNAi knock-down or previously generated mutations in these genes. We will present the results of our pilot project and discuss the efficacy of the TILLING approach in *C. elegans.*

The Lower Mainland Collective of *Caenorhabditis elegans* Researchers

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Dr. Catharine Rankin (UBC)
Dr. Terry Snutch (UBC)
Dr. Don Moerman (UBC)
Dr. Eve Stringham (Trinity Western)
Dr. Ann Rose (UBC)
Dr. Michel Leroux (SFU)
Dr. Nancy Hawkins (SFU)
Dr. Dave Baillie (SFU)
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NRRR organizing committee

Andrew Giles
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Special Thanks to **Jillian Youds** for being the NRRR contact at the Rose Lab and helping organize this event

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