

### **3. Downstream of DAF-16**

#### **Genetic and biochemical identification of DAF-16 regulated genes** **Scott Kennedy, Weiqing Li, and Sylvia Lee**

The increase in *C. elegans* longevity that is caused by decreases in insulin-like signaling is completely dependent on DAF-16 function. Thus key to the regulation of longevity in *C. elegans* are the transcriptional targets of DAF-16. Finding the downstream targets of DAF-16 is not trivial. For example, even though for nearly 20 years the Hox genes have been known to regulate spatial patterning across phylogeny at a transcriptional level, the downstream genes that mediate this function are only now beginning to emerge. There are genetic strategies we are using to identify important *daf-16* downstream genes. We have generated mutant and transgenic strains with DAF-16-related phenotypes that can easily be suppressed or enhanced. From such genetic screens, we are now mapping a number of mutants that are likely to encode genes regulated by DAF-16. In addition, the almost complete genome sequence of *C. elegans* allows very efficient biochemical studies of *daf-16* regulated genes. First, we are screening for genes regulated by DAF-16 using the gene array technology developed at Stanford. Because such expression analysis is dependent on mRNA expression levels, we are also seeking DAF-16-regulated genes using the yeast one hybrid approach. We expect that the expression of many of the downstream genes will be modulated during aging, generating molecular markers of normal aging that will assist in future genetic analyses of aging. We expect to find that free radical protective enzymes will be regulated by DAF-16. We expect to find that fat and sugar metabolism genes are regulated. We expect to find novel downstream genes that may have been previously unsuspected in regulation of longevity. There is much to be learned.

#### ***daf-2/ age-1* insulin pathway is involved in osmoregulation** **Duo Wang**

Osmoregulation may be important for *C. elegans*. To study the genetics factors which control osmoregulation we changed the osmolarity of the culturing plates for *C. elegans* and tested the different survival rates at high osmolarity among worms which carry various mutations. We used NaCl, KCl, Na<sub>2</sub>SO<sub>4</sub> or sorbitol to change the osmolarity. The mutants we have tested include mutants in *daf-2/age-1* insulin pathway (*daf-2* (e1370), *age-1* (mg 305), *pdk-1* (mg 291) and *akt-1* (mg 309)), *daf-7* TGF $\beta$  pathway (*daf-7* (e 1372)), and *daf-11* pathway (*osm-3* (p 802)). We found that worms with mutations in *daf-2/age-1* insulin pathway are specifically sensitive to high osmolarity compared with wild type animals (N2). For example, [NaCl]  $\geq$  200 mM L1 lethality in *daf-2* (e1370) or *age-1* (mg305); while 0% lethality in other mutants as well as N2 at 25 C. Interestingly *pdk-1* (mg 291) is more resistant to high osmolarity than *daf-2* (e1370) or *age-1* (mg 305) but are still sensitive compared with N2. Worms carrying another mutation in the insulin pathway -- *akt-1* (mg 309) are as resistant to high osmolarity as wild type animals are. The lethality of *daf-2* or *age-1* in response to high osmolarity can be rescued by *daf-16* (*daf-2* (e1370; *daf-16* (mgDf 47) double mutant) or neuronal-specific expression of *age-1* transgene, and partly rescued by *daf-18* (*daf-2* (e 1370) *daf-18*(e 1375) double mutants). Currently, we have mutagenized 50,000 half genomes of *daf-2* (e1370) and screened for suppressors for the early embryo/ L1 lethality caused by high osmolarity. We have obtained 13 fertile mutations. Eleven are allelic to *daf-16* and the genetic characterization of the other 2 is still under investigation.

#### **Role of cytochrome P450 enzymes in *C. elegans* development** **Ho Yi Mak**

Loss of function mutation in the *daf-9* gene, which encodes a P450, gives rise to a dauer constitutive phenotype. Sequence analysis suggests that DAF-9 is most closely related to P450s involved in degradation of lipophilic ligands. There are 80 genes encoding cytochrome P450

enzymes in *C. elegans*. In higher organisms, P450s are involved in the synthesis and degradation of lipophilic signalling molecules like steroid hormones. In addition, P450s play a role in the modification and subsequent inactivation of toxic chemicals in metazoans. We have generated animals carrying an integrated *daf-9::gfp* transgene. Expression of *daf-9::gfp* is detected in a pair of IL1 neurons in L1 larvae which persists in all larval stages and in adults. Notably, *daf-9::gfp* in IL1 neurons appears to be up-regulated in dauers. *daf-9::gfp* expression is also evident in the hypodermis of L2 and L3 larvae and in the spermatheca of adult animals. We are currently investigating *daf-9::gfp* expression in different genetic backgrounds in order to establish whether *daf-9* is under the control of known signalling pathways in dauer formation. The dynamic expression pattern of *daf-9::gfp* leads us to speculate that DAF-9 may play a more general role in attenuating signalling cascades triggered by lipophilic ligands in multiple developmental processes. To this end, we will carry out phenotypic analysis in animals subjected to *daf-9* RNAi at various larval stages.

## **A genetic screen for new players in *C. elegans* lifespan regulation**

**Siu Sylvia Lee and Raymond Lee**

Multiple genetic pathways play a role in worm lifespan control. Most notably, mutations leading to reduced *daf-2* insulin-like signaling extend the lifespan of *C. elegans* up to 4-fold. The longevity phenotype associated with *daf-2(lf)* is completely suppressed by loss-of-function mutations in the forkhead transcription factor *daf-16*, suggesting *daf-16* likely regulates genes that control *C. elegans* lifespan. To identify *daf-16* targets, as well as genes in other parallel pathways, which regulate lifespan, we performed a genetic screen to identify mutants that exhibit an extended lifespan phenotype in a *daf-16* null background. From a pilot screen of 1600 haploid genomes, we recovered three independent mutants with lifespans significantly longer than the starting *daf-16(mgDf47)* strain. Among the three mutants, *age-3(mg312)* displayed the greatest lifespan extension, up to 3-fold that of control strain. *age-3(mg312)* is pleiotropic, including slight uncoordinated movement, arrested germ line development and sterility. These pleiotropies allowed rapid mapping of this mutation to a small region around +2 on chromosome I. Cosmid rescue and sequencing of genes in the region suggested that *age-3(mg312)* resulted from a nonsense mutation in the only *C. elegans* leucyl-tRNA synthetase gene. Single gene rescue experiments and further characterization of *age-3* are underway. While it is not clear how such a fundamental player of gene expression specifically regulates lifespan, other basic cell machinery components have been previously implicated in longevity, including the WRN DNA helicase in human, the Indy Krebs cycle transporter in *Drosophila*, and the SIR2 histone deacetylase in worm and yeast. On the other hand, given the lifespan effects of germ line ablations, the sterility associated with *age-3* could indirectly contribute to its lifespan phenotype.

Phenotypic analysis and mapping of the other two longevity mutations is also in progress. In addition, a large-scale genetic screen to isolate more mutations with an extended lifespan phenotype is ongoing. Taking into account that mutations which affect global metabolism may modulate *C. elegans* lifespan, as well as other developmental processes, mutants with significant extension in lifespan, but no other pleiotropies, will be of the highest priority for mapping and cloning. Furthermore, we are also using the recently generated chromosome I RNAi library (A. Fraser, *et. al.*, Nature, 2000.) to survey for genes which, when inactivated, result in long lifespan.