**867A**

DRE-1: An Evolutionarily Conserved F Box Protein that Regulates *C. elegans* Developmental Age. **Nicole Fielenbach**[1], Dongling Li[1], Daniele Guardavaccaro[2], Kerstin Neubert[3], Tammy Chan[1], Qin Feng[1], Harald Hutter[4], Michele Pagano[2], Adam Antebi[1]. 1) HCOA, Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX; 2) Department of Pathology, NYU Cancer Institute, NYU School of Medicine, New York, NY; 3) Max-Planck-Institut fuer Molekulare Genetik, Berlin, Germany; 4) Department of Biological Sciences, Simon Fraser University, Burnaby, British Columbia, Canada.

The heterochronic loci are global regulators of larval temporal fates. Most encode conserved transcriptional and translational factors, which affect stage-appropriate programs in various tissues. dre-1 is a new heterochronic gene that encodes an F-box protein, suggesting another level of regulation, that of ubiquitin mediated modification or proteolysis. dre-1(+) works in the larval to adult (L/A) switch. Mutant phenotypes include precocious terminal differentiation of epidermal seam cells and altered timing of gonadal outgrowth (Mig phenotype). In the seams, epistasis experiments place dre-1 upstream of the lin-29/ZnF protein and downstream or parallel to let-7/miRNA. In the gonad, dre-1(+) promotes L3 reflexion of the distal tip cells (dtcs). Notably, dre-1;daf-12/NHR, dre-1;lin-29, and daf-12;lin-29 double mutants exhibited penetrant Syn-Mig phenotypes, in which the dtcs fail one or both their L3 turns, interpreted as a heterochronic delay. RNAi of lin-42/Period induces precocious dtc reflexion at the L2m, which is completely suppressed by lin-29 (Tennessen, 2006). Similarly, dre-1, daf-12, and dre-1;daf-12 mutants partially suppressed lin-42 phenotypes at the L2m. However, lin-42 RNAi partially restored gonadal reflexion of dre-1;daf-12 at the L3m. Mutual suppression suggests that dre-1/daf-12 work downstream or parallel to lin-42. Conceivably, regulation by transcription (daf-12, lin-29, lin-42) and protein stability (dre-1) may be important for regulating gonadal developmental timing.dre-1's molecular identity as an F-box protein suggests that it works in an SCF E3 ubiquitin ligase complex. Accordingly, RNAi knockdown of the skp1-like homolog SKR-1, the cullin CUL-1, and ring finger RBX homologs yielded similar heterochronic defects. In addition, DRE-1 and SKR-1 form a complex, as do the human orthologs. Of critical interest are DRE-1 targets. One potential target is LIN-29, since the mutant shows strict epistasis within the seams. Indeed, LIN-29 was expressed precociously in a low percentage of dre-1 mutants. However, physical interaction was undetected by yeast 2-hybrid or immunoprecipitation. Conceivably, DRE-1 targets something else or requires specific modifications for recognition not present in these assays. Presumably, an analysis of dre-1 suppressors will identify novel targets within the heterochronic pathway.

**868B**

*mab-10*, a Heterochronic Gene Required For the Terminal Differentiation of Hypodermal Cells and the Cessation of Molting, Encodes a Member of the NAB Family of Transcription Factors. **David Harris**, Bob Horvitz. HHMI, Dept. Biology, MIT, Cambridge, MA 02139 USA.

The study of heterochronic mutants has revealed a complex genetic pathway that regulates the timing of many developmental events in *C. elegans*. Heterochronic mutants fall into two classes: precocious mutants, which prematurely express developmental fates, and retarded mutants, which reiterate developmental fates. One abnormality often associated with the retarded heterochronic mutant phenotype is the execution of supernumerary molts. Supernumerary molts are observed in strains with loss-of-function mutations in *let-7* or *lin-29*. In an attempt to further understand the regulation of developmental timing, we have cloned *mab-10*, a gene required for the terminal differentiation of hypodermal cells and the cessation of molting.

The *mab-10(e1248)* allele was originally identified by Jonathan Hodgkin in a screen for mating-defective males (Hodgkin J, Genetics 103: 43-64, 1983). *mab-10* mutant males have a swollen bursa, are mating-defective and undergo a supernumerary molt approximately 18 hours after the L4-to-adult transition (Chris Link, Worm Breeder's Gazette 10: 92, 1988).

We have shown that *mab-10* hermaphrodites often enter lethargus as adults and perish from a ruptured vulva prior to the completion of a supernumerary molt. The seam cells of *mab-10* mutants normally transition from larval to adult states, as evidenced by the fusion of the seam cells, the expression of an adult-specific *col-19::gfp* reporter and the generation of an adult cuticle. However, the seam cell nuclei of *mab-10* mutants inappropriately undergo extra rounds of division.

We determined that *mab-10* encodes the only *C. elegans* member of the conserved NAB (NGFi-Alpha Binding) family of transcription factors. Recent mammalian studies have implicated the NAB family of proteins in the regulation of terminal differentiation of specific stem cell lineages (Le N et al., Nature Neuroscience 8: 932-940, 2005). A rescuing MAB-10::GFP fusion protein localizes to hypodermal nuclei (including seam cells) beginning in the fourth larval stage and remains present throughout adulthood. This tissue- and temporally specific pattern of expression and localization suggests *mab-10* may play an analogous role in *C. elegans*. We are currently investigating a possible link between the heterochronic pathway in *C. elegans* and the regulation of terminal differentiation in mammals by NAB family members.

**869C**

A novel *lin-14* allele revealing novel function? **Ryan Johnson**, Helen Chamberlin. Dept Molecular Genetics, Ohio State Univ, Columbus, OH.

Heterochronic genes function to ensure the precise timing of stage-specific developmental events. Mutations in these genes can cause certain developmental programs to be executed in a precocious or retarded fashion. For instance, distinct precocious (loss-of-function) and retarded (gain-of-function) mutations in the *lin-14* gene lead to elimination or reiteration of L1-specific cellular events, respectively. However, there are *lin-14* mutations that display a more complex phenotype, affecting the timing of different events in opposing ways. Recent work has also shown that another heterochronic gene, *cog-3*, preferentially affects somatic gonad development. Together, these observations suggest that different tissues may have distinct requirements for *lin-14* and other genes to properly control their developmental timing.

We have identified a novel missense allele of *lin-14*. *lin-14(sa485)* hermaphrodites exhibit a previously undescribed asynchrony between vulval and gonadal maturation. *lin-14(sa485)* worms often begin oocyte formation and fertilization prior to vulval eversion. Further work is required to confirm whether vulval maturation is truly retarded or gonadal development is precocious in these animals. In either case, the *sa485* allele appears to behave distinctly from previously characterized *lin-14* alleles, either affecting gonad development specifically, or producing a retarded vulval progression phenotype from an allele which shares no molecular or genetic similarities with known *lin-14(g.o.f.)* alleles. Interestingly, this phenotype is manifest during late larval development, at a time not thought to be directly influenced by *lin-14*. We plan to test whether this phenotype is simply an indirect result of an early disruption in synchrony between the vulval and gonadal tissues, or evidence for a novel, late function of *lin-14*.

In addition to the phenotype described above, we have characterized other defects of *lin-14(sa485)* animals. Hermaphrodites exhibit an abnormal uterus, which is often distended to one side of the vulva and compacted to the other. We are currently working to determine the underlying defect(s) in this tissue. Finally, *lin-14(sa485)* males have defective spicule production as a result of abnormalities in the asymmetric division of the postembryonic blast cell, B. Curiously, the B cell of *lin-14(sa485)* males does not appear to divide with grossly abnormal developmental timing. We are examining whether these B cell abnormalities result from a more subtle heterochronic defect or represent a timing-independent *lin-14* function.