1061C

Mutations that Restore Symmetry in a Left-Right Asymmetric Cell Lineage. **Shunji Nakano**[1], Ronald E. Ellis[2], Victoria Hatch[1], Bob Horvitz[1]. 1) HHMI Dept. Biology, MIT, Cambridge, MA. 02139 USA; 2) Dept. Molecular Biology, UMDNJ, Stratford, NJ 08084 USA.

The body plan of *Caenorhabditis elegans* is mostly bilaterally symmetric. Much of the symmetry arises from pairs of bilaterally symmetric homologous blastomeres, which through similar cell lineages give rise to sets of left-right paired cells. To create either asymmetric cells or three-fold symmetry, the bilateral symmetry in cell lineages must be broken.

The pharyngeal epithelial cells form a three-fold symmetric structure that consists of nine cells. The nine cells are divided into three groups of three cells, e1, e2, and e3, on the ventral left (VL), ventral right (VR), and dorsal (D) regions of the pharynx. e3VL and e3VR are generated as lineal homologs, whereas e3D is generated by breaking left-right symmetry in a specific cell lineage.

The blastomeres ABaraapa and ABaraapp are homologous cells that give rise to identical sets of left-right paired cells, except that the ABaraapaaaa cell becomes the e3D cell and its lineally homologous cell ABaraappaaa becomes the MI neuron. The differential determination of cell fate by these two cells breaks left-right symmetry in these cell lineages.

We sought mutations that transform MI into an e3-like cell or e3D into an MI-like cell, thereby regenerating symmetry in these cell lineages. From screens of 10,000 mutagenized haploid genomes and unrelated screens performed previously, we identified four independent mutations that cause transformation of MI into an e3-like cell fate.Three of the four mutations, *n1921*, *n5020*, and *n5052*, define a single complementation group. We have mapped *n1921* to a 153 kb interval on LG IV. The other mutation, *n5053*, semidominantly transforms MI into an e3-like cell fate and maps to LG I. *n5053* or a mutation closely linked to *n5053* appears to recessively cause embryonic lethality.

Thus, we have identified four mutations that restore symmetry in a left-right asymmetric cell lineage, and these mutations define at least two genes.

1062A

Does zyg-8 display functions after the first embryonic division? **Jean-Michel Bellanger**, Nga Nguyen, Anne Debant. CRBM-CNRS, Montpellier, France.

Microtubules (MTs) constitute an essential cytoskeletal compartment that is broadly required for cell division, cell and organelles translocations, vesicular transport and neuronal morphology for instance. Their biology is tightly controlled by trans-acting factors that bind to the lattice and regulate its intrinsic dynamic instability. Microtubule-associated proteins (MAPs) stabilize MTs whereas several destabilizers promote MTs shortening or instability. Doublecortin (DCX) is the founding member of a family of MAPs that control neuronal migrations in the developing mammalian brain. Mutations in DCX result in a severe human disease called the “double-cortex syndrome”, characterized by multiple cortical heterotopia. The Doublecortin-like kinases (DCLKs) display a bipartite structure, with a Ser/Thr kinase domain associated to the family-defining DCX domain. In mice, DCLK1 acts redundantly with doublecortin during neurocortical migrations but also displays unique roles in the early generation of neuroblasts, presumably by ensuring proper mitotic spindle integrity of glial progenitor cells. Some DCLK1 isoforms as well as DCLK2 are enriched in the mature nervous system, suggesting other unknown functions of these proteins in adult mice. The unique C. elegans member of this family of MAPs, ZYG-8, displays the structure of DCLKs, strongly supporting the hypothesis of an ancestral association of the DCX module with a Ser/Thr kinase domain, with later (Vertebrates) duplication/mutational loss of the kinase domain in the Doublecortin gene. ZYG-8 binds and stabilizes microtubules through its DCX domain but importantly, mutational analysis revealed that both domains are required for proper anaphase spindle behavior during the first embryonic division. Therefore, millions of years of evolution seem to have conserved the DCX/kinase association within the same protein, for the control of spindle integrity/behavior and related biological processes. The molecular mechanisms by which DCLK/ZYG-8 proteins “stabilize” mitotic spindles, the role of the kinase domain, the extent of their physiological functions as well as the way they are regulated remain largely unknown. We decided to investigate these questions in C. elegans, through the use of zyg-8 ts mutants to “bypass” the early zyg-8 requirement and the combination of two-hybrid, transcriptomic and phenotypic data to infer signaling pathways into which zyg-8 may be involved. Our results indicate that zyg-8 may function after the first embryonic division and cross-talk with pathways involved in development, morphogenesis and potentially ER stress and longevity.

1063B

RNAi screen identifies suppressors of *pha-4/FoxA*. **Trisha J Brock**, Susan E Mango. Huntsman Cancer Institute, University of Utah, Salt Lake City, UT.

The Forkhead box A (FoxA) family of transcription factors play key roles in digestive tract development in many animals. In *C. elegans*, *pha-4* encodes the sole FoxA transcription factor, which is required for pharynx development (Mango et al., 1994; Horner et al., 1998; Kalb et al., 1998). The timing, location, and level of PHA-4 expression are vital for its function; however, little is known about how *pha-4* or other FoxA proteins are regulated.

To discover genes that interact with PHA-4, we conducted an RNAi screen for suppressors of lethality caused by the loss of *pha-4*. Because complete inactivation of *pha-4* results in loss of pharynx and larval arrest, we used a conditional strain, *smg-1(ts);pha-4(zu225)*, which relies on a temperature-sensitive allele of the nonsense mediated decay (NMD) component *smg-1* and a nonsense mutation in *pha-4* (Kaltenbach et al., 2005; Gaudet et al., 2002). At 24C, the NMD pathway is inactive and *pha-4* is expressed and worms live, but at 20C, the NMD pathway is active, which causes degradation of *pha-4* transcripts and larval arrest. We call this configuration *pha-4(ts)*.

Our initial screen used an RNAi library of almost 17,000 clones (Kamath et al., 2003) and identified close to 1000 potential suppressors. We expected two classes of suppressors: informational suppressors and suppressors that were more specific to the *pha-4* pathway. We identfied three of the four known NMD pathway components represented in the RNAi library, as well as other genes that likely play a role in RNA metabolism. We also discovered four previously discovered *pha-4* suppressors, indicating that our screen could successfully find *pha-4(ts)* suppressors. The remaining candidate genes are being retested to determine if they are true suppressors. The list of candidates includes genes involved in signaling and in transcription. Our future goals will be to determine the nature of the interactions of these factors with *pha-4* and the roles these genes play in promoting pharynx development.