

**1013C**

Identification of Genes Required for P0 Spindle Alignment. **James Carter**, Aleena Garner, Encalada Sandra, Bruce Bowerman. Inst Molecular Biol, Univ Oregon, Eugene, OR.

After fertilization of the *C. elegans* oocyte, the fused maternal and paternal nuclei must rotate 90 degrees in order for the mitotic spindle to align correctly parallel to the anterior-posterior axis of the embryo. If this spindle rotation does not occur, P0 can instead divide perpendicular to the normal axis of division. This results in a failure of the embryo to hatch, or an embryonic lethal phenotype. In order to identify genes that are required for this event, we conducted a screen for temperature-sensitive embryonic lethal mutants. We collected several hundred mutants, and made nomarski (DIC) movies of the first mitotic division of all of the mutant lines. Twenty-one of these had some degree of failure in P0 spindle rotation. We used genetic complementation and DNA sequencing to determine if any of these had mutations in genes that had previously been shown to be required for spindle rotation. We found seven alleles of *zyg-9*, (or624, or625, or628, or630, or634, or635, and or637) two alleles of *tac-1*, (or490 and or602) two alleles of *tba-1*, (or599 and or629) and one allele of *dnc-1* (or680) among this group. In addition, we have three mutants that map to chromosome I (or660, or491, and or358) and two alleles that map to Chromosome IV (or618 and or633). These mutants complement genes known to be required for P0 spindle rotation. They are candidates to interact with the other genes known to be involved in setting up the spindle in the first mitotic division, or they may be new components of the mitotic spindle assembly. We will report on our efforts to further characterize the phenotypes of these mutants, in addition to 3-factor and SNP mapping of the remaining alleles to determine their genetic identity.

**1014A**

The Perturbation of Genes that Regulate the Microtubule Cytoskeleton Causes the Appearance of Extra-Embryonic Anucleate Bodies. **Dan Denning**, Bob Horvitz. HHMI, Dept. Biology, MIT, Cambridge, MA 02139.

Embryos defective in the execution of programmed cell death or the engulfment of corpses produce cells that detach from the developing animal and remain within the egg. We screened for mutant embryos with this "shed cell" phenotype. From a screen of 4,500 haploid genomes, we isolated two alleles of *ced-3*, one allele of *ced-4*, and three alleles of *ced-8*. In addition, we recovered three allelic mutations (*n4874*, *n4911* and *n4991*) that generate extra-embryonic bodies similar to shed cells. These mutants are not defective in the execution of programmed cell death or the engulfment of cell corpses. The extra-embryonic bodies in *n4874*, *n4911* and *n4991* embryos lack nuclei, unlike the shed cells in *ced-3* embryos. Thus, these "anucleate bodies" are fundamentally distinct from shed cells.

We mapped the *n4874* mutation to a 70 kb region on LGII. This region contains 17 genes, including *zyg-9*, a gene for which mutant alleles have been characterized previously. We observed that the canonical *zyg-9* allele *b244ts* causes the generation of anucleate bodies and that *n4874* fails to complement *b244* for this defect. *zyg-9* encodes a homolog of XMAP215, a microtubule-binding protein that promotes (+)-end growth of microtubule filaments. Previous studies showed that *zyg-9(b244ts)* embryos exhibit temperature-sensitive defects in microtubule spindle size and orientation during meiosis and early mitoses. We tested other regulators of the microtubule cytoskeleton for roles in preventing the formation of anucleate bodies. A semidominant allele of *mei-26*, a gene that is required for the degradation of the microtubule-severing protein MEI-1/katanin, causes the appearance of anucleate bodies. Additionally, *tac-1(RNAi)* and *evl-20(RNAi)* embryos contain anucleate bodies. *tac-1* encodes a binding partner of *zyg-9*, and *evl-20* encodes an ARF-like GTPase with an evolutionarily conserved role in the biogenesis of microtubule filaments. Thus, the anucleate body phenotype appears to be a consequence of defects in the microtubule cytoskeleton.

During mitosis, the microtubule spindle assembly provides signals that position the cleavage furrow prior to cytokinesis. Interestingly, specific cell lineages in *evl-20* mutants were reported to have improperly positioned cleavage furrows that resulted in cytokinesis defects and the creation of binucleate cells. We hypothesize that anucleate bodies are caused by improper cell divisions that generate a binucleate daughter and an anucleate daughter, which separates from the developing embryo.

**1015B**

Protein Phosphatase 4 in MEI-1 Regulation. **Xue Han**<sup>1</sup>, Cheryl Birmingham<sup>2</sup>, Asako Sugimoto<sup>3</sup>, Paul Mains<sup>1</sup>. 1) Dept Biochem & Molec Biol, Univ Calgary, Calgary, AB, Canada; 2) Molecular and Medical Genetics, University of Toronto, Toronto, Ontario, Canada; 3) Laboratory for Developmental Genomics, RIKEN Center for Developmental Biology, Kobe, Japan.

*mei-1* encodes the worm "katanin" microtubule-severing protein. It is required during meiosis to regulate the shape and dynamics of meiotic spindles (McNally et al., 1993) but must be degraded before mitosis. MEL-26 recruits MEI-1 to the E3 ubiquitin ligase complex for post-meiotic ubiquitin-mediated degradation.

In a screen of the chromosome I RNAi library, we identified F16A11.3 as a suppressor of a *mei-1(gf)* allele that is refractory to MEL-26 mediated degradation. RNAi against F16A11.3 in a *mei-1(gf)* background increased hatching from 1% to 14%. Lethality of a *mei-26(lf)* allele was also suppressed while a *mei-2(lf)* mutant with limited meiotic rather than excess mitotic MEI-1 was not affected. These findings suggested that F16A11.3 regulated *mei-1* only after meiosis.

F16A11.3(*ppfr-1*) encodes the R1 regulatory subunit of Protein Phosphatase 4 (PP4). Protein phosphatase 4 is a member of the PPP family of protein serine/threonine phosphatases. Like ectopic MEI-1, it localizes to centrosomes in mammals and flies (Brewis et al., 1993). One catalytic subunit, *pph-4.1* plays an essential role in spindle formation in both mitosis and sperm meiosis in *C. elegans* (Sumiyoshi et al., 2002).

We are investigating the mechanism of *ppfr-1* suppression of *mei-1(gf)*. In my work, I demonstrate that *pph-4.1* and another regulatory subunit, alpha 4, act in the same pathway as *ppfr-1* to suppress ectopic mitotic MEI-1. Other PP4 subunits (PPH4.2 and R2) do not suppress ectopic MEI-1. I also test several models to investigate how *ppfr-1* suppresses *mei-1(gf)*. It is unlikely that reduction in PP4 activity results in general increase in microtubule stability, making them resistant to ectopic MEI-1 severing. Another model, which proposes loss of PP4 phosphatase results in higher levels of MEI-1 phosphorylation, leading to its degradation, also does not seem to be the case. Reciprocal co-immunoprecipitation experiments indicate PP4 may regulate MEI-1 activity via direct binding.