Germ cells and gametogenesis

Program/Abstract # 249
Bad Cop: Good Cop? KGB-1 and CSN-5 control GLH-1, a C. elegans P granule component
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Homeostasis in the C. elegans germline appears to require appropriate levels of the germline RNA helicase, GLH-1, a constitutive component of P-granules. We report that GLH-1 is targeted for proteasomal degradation by its binding partner KGB-1, a JNK MAP kinase. Conversely, CSN-5, a subunit of the COP9 complex, which also interacts with the GLHs, protects GLH-1 from degradation. We propose KGB-1 and CSN-5 team up to maintain GLH-1 levels. In other organisms the COP9 complex protects some and targets other proteins for degradation. In C. elegans loss of CSN-5 results in under-proliferated germlines, while loss of KGB-1 is temperature sensitive and causes germline over-proliferation; both kgb-1 and csn-5 mutants are sterile. GLH-1 levels in kgb-1 worms are up to seven times higher than in wild type. csn-5(RNAi) into kgb-1 mutants partially rescues their sterility and results in GLH-1 levels closer to normal. In oocytes, when P granules leave their peri-nuclear location, KGB-1 and GLH-1 are in dispersed cytoplasmic particles, while CSN-5 is mostly nuclear. Perhaps KGB-1 phosphorylates GLH-1, dispensing and targeting it. In biochemical studies the binding of KGB-1 to GLH-1 requires a MAP kinase docking site; when this docking site is mutated, binding is lost. In addition to binding each other, KGB-1 and CSN-5 bind Drosophila VASA, another germ granule RNA helicase. Thus the regulatory functions reported here may be conserved between worms and flies. We are analyzing other putative targets of KGB-1, including cyclin E. KGB-1 is also being tested in another case of GLH-1 homeostasis; in pro- and anti-apoptotic strains, germ cell numbers vary while GLH-1 remains constant.

doi:10.1016/j.ydbio.2007.03.357

Program/Abstract # 250
Now you see them; now you don’t! Centrosome elimination during oogenesis in C. elegans
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Sexually reproducing organisms must eliminate a pair of centrosomes prior to the first zygotic division to avoid formation of a multipolar spindle. During the development of the C. elegans germline, germ cells undergo several developmental changes following oocyte specification that include the elimination of the centrioles that were present during the earlier meiotic stages. This process requires the activity of a p21/p27-like CDK inhibitor protein called cki-2 and its reduction results in centrioles that perdure during oogenesis that eventually get incorporated into the early embryo. These embryos arrest due to aneuploidy caused by abnormal chromosomal segregation after the first anaphase, presumably caused by the presence of both oocyte- and the normal sperm-derived centrosomes. cki-2 is therefore required to block CDK activity during the onset of oogenesis. Consistent with this, reduction of either cyclin E or CDK2 suppresses the tetrapolar spindle defect and ameliorates the one-cell arrest phenotype. This suggests that a cyclin E/CDK2-dependent phosphorylation is required to stabilize the centrioles and its cki-2-dependent inhibition results in their rapid destabilization/elimination. Many of the proteins required for centrosome duplication possess canonical CDK phosphorylation sites. We are testing these candidate proteins for their potential role as CDK targets for centriole stabilization. Using variants of these proteins that lack canonical CDK phosphorylation sites, we are monitoring effects on oocyte and early embryonic development for phenotypes typical of the centrosomal defects observed in cki-2 compromised embryos.

doi:10.1016/j.ydbio.2007.03.358

Program/Abstract # 251
A two-hybrid screening to isolate proteins that interact with the C. elegans germline DEAD box RNA Helicase VBH-1
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Dead box RNA helicases play important roles in germline development and function. VBH-1 is highly similar to the DEAD box RNA helicases Belle and Vasa from Drosophila, which are essential for germline function. VBH-1 is expressed