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Germ Cells and Gametogenesis

Program/Abstract # 288

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Program/Abstract # 289

Chemical control of protein stability in *C. elegans*

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Our laboratory focuses on molecular controls of germline stem cell maintenance and differentiation in the nematode *C. elegans*. One technical obstacle has been to control regulatory proteins both spatially and temporally. Tissue-specific promoters are commonly used for spatial control and temperature-sensitive mutants are sometimes but not always available for temporal control. We have coupled the use of tissue-specific promoters for spatial control with a chemically sensitive destabilization domain (DD) for temporal control of protein stability. Briefly, a DD-fusion protein is normally degraded, but it is stabilized by addition of a small molecule called Shield1 (Banaszynski et al. 2006). We have now tested this technology in nematodes. Specifically, we generated transgenic lines expressing a DD-GFP protein under control of a Distal Tip Cell (DTC) niche-specific promoter. In the absence of Shield1, GFP fluorescence was barely detectable in any cell, but when incubated with Shield1, the DD-GFP was stabilized and GFP fluorescence was bright specifically in the DTC. We conclude that Shield1 can control the stability of DD-fusion proteins in *C. elegans*.

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Program/Abstract # 290

Germ granules extend the nuclear pore complex environment in the *C. elegans* germ line

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During animal development, somatic cells display ever more restricted developmental potential, while germ cells must retain the ability to produce all of the cell types of each subsequent generation. A key to understanding how germ cells are regulated resides in the germ plasm. Ribonucleoprotein (RNP) aggregates called germ granules are found in the germ plasm of many species, but the importance of their assembly into granules has yet to be determined. In *C. elegans*, a number of germ-granule (P-granule) components, including the Vasa-related proteins GLH-1, GLH-2, and GLH-4, contain a phenylalanine-glycine (FG) repeat domain similar to those found in many nuclear pore complex (NPC) proteins. Within the NPC, these FG-rich domains form a cohesive meshwork of filaments through hydrophobic interactions, creating a size-exclusion barrier that prevents diffusion of large molecules between the nucleus and the cytoplasm. We have demonstrated that P granules, like NPCs, are held together by weak hydrophobic interactions and establish a size-exclusion barrier within the germ plasm. We show that GLH-1 and its FG domain are not sufficient to form granules, but require factors like PGL-1 to nucleate their localized concentration. Our results suggest that P granules extend the NPC environment in the germ line to create a specialized hydrophobic microenvironment that may facilitate post-transcriptional processing events while selectively excluding large protein complexes from gaining access to mRNAs and endogenous siRNAs.

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Program/Abstract # 291

Characterizing Blimp1 expression and PGC migration in *M. domestica*

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Primordial germ cells are the first population of the germ cell lineage that gives rise to oocytes and spermatozoa. Because of this, PGCs are responsible for the transmission of genetic information from one generation of multicellular organisms to the next. In mice PGCs have been extensively characterized by an alkaline phosphatase staining protocol which, for unknown reasons, fails in opossum embryos. In this study, PGCs were identified using a locked nucleic acid (LNA) probe specific for Blimp1—a highly conserved gene that represses the somatic program during PGC specification. In situ reactions were performed on opossum embryos ranging from nine to eleven days old. Results indicate that Blimp1 is first active in *M. domestica* between nine and ten days post fertilization. At this time, faint staining, resembling a fingerprint, is observed in the anterior half of the embryo and its posterior edge. As development continues, dark staining is detected at the anterior of the epiblast bordering extraembryonic tissue. Interestingly, this arch of strong Blimp1 expression corresponds spatially to PGC localization in the germinal crescent of avian and reptilian embryos. In day 11 opossum embryos, gene expression is most prominent in the genital ridges signifying the end of PGC migration. Because not all cells expressing Blimp1 are primordial germ cells, complications arise in inferring PGC