Pax3. To examine this, neurospheres (NS) were generated from lower lumbar tissues of E10.5 Sp−/− and WT embryos and grown with and without FA. In the absence of FA, the number of NS generated from Sp−/− embryos was minimal compared to WT. However, a addition of FA rescued neural stem cell (NSC) proliferative potential in Sp−/− embryos. Immunostaining and q-RT PCR data showed that Fgfr4-protein and mRNA levels in FA treated Sp−/− NS were close to WT levels. Fgfr4 levels were significantly lower in NS grown without FA. Fgfr4 promoter-luciferase reporter transfection assays in DAOY cells, showed a significant increase in Fgfr4 promoter activity in response to treatment with EGF and FA. These results suggest that FA along with EGF, may fine-tune Fgfr4-signaling, and thereby regulate NS proliferation.

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Program/Abstract # 418
Expression of cell cycle regulators during zebrafish development
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Both extensive proliferation and terminal differentiation are hallmarks of early embryonic development. We believe that many of the factors that promote terminal differentiation of cells also regulate exit from the cell cycle. To investigate this hypothesis we have begun a detailed analysis of expression of cell cycle regulators in both embryonic muscle and primary neurons in the zebrafish embryo. Since we are particularly interested in the timing of cell cycle exit we have initially focused on expression of the cell cycle inhibitors cdkn1b (p27, kip1) and cdkn1c (p57 kip2). We are detailing normal gene expression through 24 h of development and are also documenting gene expression in embryos with altered levels of specific signaling pathways. In particular we have focused on the expression of cell cycle inhibitors in embryos that lack Hedgehog signaling. We have found that cdkn1c, which is normally expressed both in slow muscle and in primary neurons, requires Hedgehog signaling in the slow muscle, but not in the primary neurons. Previous research has shown that slow muscle precursors switch fate to fast muscle in the absence of Hedgehog signaling. We suspect that slow muscle precursors will show a concomitant switch to expression of cdkn1b, the cell cycle inhibitor expressed in fast muscle. We are also in the process of altering other signaling pathways, such as the retinoic acid pathway, to see if they regulate expression of cell cycle inhibitors in primary neurons.

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Program/Abstract # 419
Brambleberry, a novel nuclear envelope associated protein, acts in membrane fusion during cleavage stage development
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Compared to later somatic cells, early cleavage stage blastomeres employ modified cell division mechanics presumably to accommodate the nature of the large cells present following zygote formation. For instance, mouse maternal chromokinesis KiD is required to maintain proper nuclear structure specifically during the early cleavage stage. Using a genetic approach to identify mutants that disrupt normal cleavage in zebrafish, we identified the maternal-effect mutant brambleberry (bmb). bmb blastomeres are multinucleated during cleavage due to a failure of nuclear membrane fusion of karyomeres at the end of mitosis. Karyomeres are chromatin structures that normally form at the telophase-interphase transition in the early embryo of a variety of organisms. Positional cloning reveals that bmb encodes a conserved novel protein with limited homology to Kar5, a protein required for pronuclear fusion in yeast. Bmb contains a predicted N-terminal coiled-coiled domain and two predicted C-terminal transmembrane domains. Immunofluorescence using a polyclonal Bmb antibody reveals dynamic localization throughout the cell cycle during the cleavage stage. Bmb protein is first detected proximal to, but not associated with, metaphase chromosomes. As anaphase progresses, Bmb becomes increasingly associated with chromosomes. During telophase, when karyomeres are formed, high levels of Bmb protein are detected associated with the nuclear envelope with prominent Bmb puncta evident near karyomere-karyomere interfaces. Our studies identify the first molecular factor acting in karyomere fusion and suggest that specialized proteins are necessary for proper nuclear division in the large cells present during early development.

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Program/Abstract # 420
FOG-3/Tob can either promote or inhibit proliferation in the Caenorhabditis elegans germline
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Tob/BTG proteins (for t ransducer o f E R B 2/ B -cell t ranslocation g ene) influence multiple aspects of metazoan development, but the common thread among vertebrate family members is an inhibitory effect on cell proliferation (Jia and Meng 2007; Mauxion et al. 2009; Winkler 2010). C. elegans encodes a single protein predicted to be a member of the Tob/BTG family, FOG-3 (Chen et al 2000). The f og-3 gene (for f eminization o f t he g ermline) was identified in a genetic screen for regulators of sperm fate specification (Ellis and Kimble 1995). We ask if the predicted Tob/BTG protein FOG-3 affects germline proliferation in addition to its known effect on sperm fate specification and find that FOG-3 has antiproliferative and tumor suppressor properties comparable to vertebrate Tob/BTG proteins. However, we find that FOG-3 can also promote proliferation and that