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Cell Proliferation

Program/Abstract # 415
Notch Mediates a Genetic Switch in Neural Lineage Topology

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During nervous system development, neural progenitor cells divide asymmetrically, renewing themselves and budding off daughter cells with more restricted potential. Daughter cells can either differentiate directly, or divide to expand a certain branch of a lineage tree. However, how the mitotic potential of any given daughter cell is controlled during neural lineage progression is not well understood. In the Drosophila embryo, neural progenitor cells, neuroblasts, generate the CNS by series of asymmetric cell divisions. Daughter cells from such divisions, ganglion mother cells (GMCs), typically divide once to generate two neurons or glia. However, in certain lineages, such as thoracic neuroblast 5–6, there is an intriguing switch in division mode, such that the four last-born daughter cells never divide, instead differentiating directly into the Apterous (Ap) neurons. In a genetic screen scoring for affects upon NB5-6 lineage progression, we have identified two loci that fail to execute the lineage topology switch. Here, Ap cells undergo one ectopic round of division before differentiating, resulting in a doubling of the number of Ap neurons. These two loci map to kuzbanian and neuralized–two components of the Notch signaling pathway. We find that the Notch pathway is not critical for the specification of Ap neurons, but acts exclusively to control the switch in daughter cell division potential. The reason why Ap neurons only divide ectopically one round in Notch mutants is that each Ap neuron is converted into a GMC, and GMCs are prevented from dividing by pros. To our knowledge, this is the first identified mechanism for a genetic switch in neural lineage topology.

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Program/Abstract # 416
Arx regulates proliferation of cortical progenitor cells

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Aristaless-related homeobox gene (Arx) is mutated in many epilepsy and mental retardation syndromes, including West syndrome, Partington syndrome, and a spectrum of X-linked mental retardation disorders. Many different mutations in Arx have been documented and patients with the same mutation can show phenotyptic heterogeneity suggesting that Arx may have multiple functions in development. During development Arx is expressed in progenitor cells throughout the forebrain. In the pallium Arx is expressed in the proliferative ventricular zone (VZ) where the excitatory neurons of the cortex are born. Arx-/Y mice show a decreased proliferation in the VZ of the cortex resulting in smaller brains (Kitamura et al., 2002). Friocourt et al. (2008) have shown that overexpression of Arx in the VZ of the cortex increases cell cycle length, however, the effect on the cell cycle has not been examined. In addition, how Arx regulates the cell cycle and therefore the pattern of differentiation of the neuronal progenitor cells in the VZ is not well understood. To further understand how the loss of Arx in the cortical VZ affects the progenitor cell proliferation we performed EdU/BrdU pulse labeling experiments to look for a change in the cell cycle and found none. We also labeled with BrdU and mitotic markers to look for increased cell cycle exit and found that it was similar to wildtype. We labeled with SVZ and mitotic markers and found a decrease in proliferating intermediate progenitor cells in the SVZ. Finally, we examined layer formation in the cortex and found that deeper layers were more affected than superficial layers by this loss of progenitor cells.

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Program/Abstract # 417
Folic acid regulates Fgfr4 in neural stem cells

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Folic acid (FA) supplementation prevents spina bifida in 60-70% of human cases. The Pax3 mutant Splotch (Sp −/−) mouse has been used to study FA-dependent prevention of neural tube defects (Gray and Ross, 2009). Absence of functional Pax3 in Sp −/− embryos impairs stem cell proliferation and causes premature neurogenesis by affecting Hes1 and Neurog2 expression (Nakazaki et al., 2008). E pigenetic marks associated with Hes1 and Neurog2 promoters are altered in Sp −/− embryos. These marks are reversed in FA rescued embryos (Ichi et al., 2010). The question that we want to address is how does FA rescue Sp −/− phenotype? Lagha et al., (2008) showed direct activation of fgfr4 by Pax3. We therefore hypothesized that FA plays a role in neuronal progenitor cell maintenance in FA-rescued Sp −/− embryos by up-regulating Fgfr4 in the absence of functional

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. I n the absence of FA, the number of NS generated from Sp −/− embryos was minimal compared to WT . However, a ddition of FA rescued neural stem cell (NSC) proliferative potential in Sp −/− embryos. I mmunostaining 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 NSC 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 cdk1nb, 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 chromokinesin 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 multi-micronucleated 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 # 422

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 ERB 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 fog-3 gene (for f eminization o f the 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