Cell Fate Specification

Program/Abstract # 243
Generation of zebrafish transgenic lines to study centrosome inheritance
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Centrosomes consist of a pair of cylindrical centrioles oriented perpendicularly to each other and function as the main microtubule organizing center of the cell. Centrosomes also play roles in intracellular trafficking, cell polarity, organization of the mitotic spindle, and organization of primary cilia. The two centrioles within a centrosome each self-duplicate once during the cell cycle, but are asymmetric in their maturation state. This results in two differently aged centrosomes, an older mother, and a younger daughter, which are then separated upon cell division so that each new cell inherits one centrosome. Centrosome inheritance, therefore, is asymmetric and has been shown to influence cell fate in Drosophila neuroblasts and male germline stem cells [Yamashita and Fuller, 2008]. A recent study of mouse cortex neuroepithelium found that inheritance of the mother centrosome correlated with progenitor fate, while inheritance of the daughter the centrosome segregated with differentiated progeny (Wang et al., 2009). To address the question of whether centrosome inheritance influences cell fate in vivo, we have generated a zebrafish transgenic line, \(\beta\)-actin:Kaede-Centrin, that takes advantage of the photoconvertable Kaede protein fused to the centrosomal protein Centrin, to label differently aged centrosomes. Importantly, we found that Centrin protein stably binds to centrosomes, thus photoconverted Kaede-Centrin protein will remain steadily bound so that a centrosome labeled with Kaede-Centrin can be followed through several cell cycles. Currently we are using the transgenic line to study the role of asymmetric centrosome inheritance in cell fate decisions in the retina.

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Poky/Ikk1/Ikka promotes Ripk4 function in zebrafish epidermal differentiation
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Epidermal differentiation in the zebrafish begins with formation of the enveloping layer (EVL). Rapid differentiation of the EVL is required to protect the developing embryo from the hypotonic environment. The EVL differentiates directly from the external cells of the blastoderm. These cells dramatically slow their cell cycle at the onset of zygotic transcription, form a permeability barrier and express differentiation markers, including many homologs of genes expressed in differentiating mammalian epidermis. The EVL differentiates prior to formation of the p63 positive epidermal basal layer raising questions as to the degree of conservation of pathways regulating differentiation of the EVL and mammalian epidermis. Zebrafish poky mutants have defective EVL differentiation and the blastoderm clears and lyces as control embryos are initiating gastrulation. poky encodes the zebrafish homolog of mammalian Ikk1/Ikka, a kinase required for epidermal differentiation in the mouse. To examine the extent of molecular conservation in the pathways that regulate epidermal differentiation between zebrafish and mammals we have examined the expression and activities of additional zebrafish homologs of mammalian epidermal differentiation genes. Among these we have identified the zebrafish homolog of Ripk4, another kinase required for epidermal differentiation in the mouse. Zebrafish ripk4 is expressed maternally and in the EVL. Expression of ectopic wild type Ripk4 does not affect early development. However expression of an activated form of Ripk4 can induce ectopic EVL gene expression indicating regulated function of this kinase. Ripk4 distribution limits Poky/Ikk1 activity and co-expression of wild type Ripk4 greatly potentiates the activity of an activated form of Ikk1. Furthermore, expression of activated Ripk4 can induce EVL differentiation markers in poky mutant embryos indicating that it acts downstream of Ikk1 to promote EVL differentiation. Together these results support a model whereby spatial regulation of Ikk1 and Ripk4 control differentiation of the zebrafish epidermis.

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Program/Abstract # 245
FGF20 is required for differentiation of cochlear outer hair cells and normal hearing function
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Congenital hearing loss is one of the most common hereditary diseases, affecting 2–3 infants per 1000 live births. A large proportion of age-related hearing loss is sensorineural and is caused by loss or damage to outer hair cells (OHC) in the organ of Corti (OC). The OC is the sensory transducing apparatus in the cochlea and is composed of one row of inner hair cells (IHC), three rows of OHCs and associated underlying supporting cells (SC). Although there has been progress in understanding mechanisms of hair cell (HC) and SC differentiation, the cellular signals that specify the distinct phenotypes of cochlear IHCs and OHCs are not known. Here we show that FGF20 is essential for hearing in mice and functions to regulate differentiation of cells in the lateral cochlear compartment (OHCs and outer SCs) within the OC, but has little effect on cells in the medial compartment (IHCs and inner SCs). Specifically, FGF20 is a permissive factor that operates within a specific