Program/Abstract # 383
Role of glia in the organization and function of the visual nervous system of Drosophila
Rosa E. Mino 1, Johanna Palacio 1, Margarita Kaplow 1, Jorge Morales 1, Peter O’Day 2, Tadmiri R. Venkatesh 1
1 Dept. of Biol., City College of New York, New York, NY, USA
2 Inst. of Neurosi., Huestis Hall, University of Oregon, Eugene, OR, USA

In recent years, it has become increasingly clear that glia are pivotal for proper neuronal development and function. Glia mediate a remarkable array of cellular functions including axon ensheathment, establishment of blood brain barrier, trophic response, ionic equilibrium, synaptogenesis, axon pruning, engulfment, and neuronal plasticity. Studies made in our laboratory have demonstrated that Rap/Fzr, an activator of the ubiquitinating ligase complex APC (Anaphase Promoting Complex), regulates gliogenesis in the developing nervous system. Loss-of-function mutations of rap/fzr lead to an increase in number of glia and conversely, rap/fzr overexpression results in the drastic reduction of the number of glial cells. We have investigated the role of glia in the structural organization and function of the Drosophila visual nervous system using rap/fzr loss-of-function and gain-of-function mutations. We will present anatomical, physiological and behavioral data which suggest that glia play an important role, not only in the proper organization of the nervous system, but also in establishing a functional synaptic connection in the adult brain. Increase in the number of glia cells leads to abnormal axon projections in the optic ganglia, while loss of glia leads to abnormal phototaxis, electrophysiological responses, and neurodegeneration. Together, these results indicate that normal number and organization of glia are critical to prevent neurodegeneration in adult flies.

Supported by NIH-SCORE RCMI, MBRS-RISE grants and CCFELL.

doi:10.1016/j.ydbio.2007.03.452

Program/Abstract # 384
Visualising the dynamics of retinogenesis in a live vertebrate embryo
Aikaterini Bilitou, Shin-ichi Ohnuma
Department of Oncology, University of Cambridge, Hutchison/MRC Research Centre, Hills Road, Cambridge, CB2 0XZ, UK

Retinogenesis, the generation of a layered functional structure of neurons and glia in the eye, is a multistep developmental process characterized by dynamic morphogenetic events. It involves the sequential formation of seven different cell types from multipotent progenitor cells under the effect of extrinsic and intrinsic factors that coordinate the cell cycle, differentiation and migration to the laminal layers. Live imaging is a powerful tool that allows monitoring of the spatial and temporal parameters of histogenetic events occurring during development. We have established an in vivo cell tracing system in combination with time-lapse confocal microscopy to study retinogenesis in a vertebrate model system, Xenopus laevis. Fluorescent Quantum Dots are used as vital cell tracers to label progenitor retinal cells in the intact embryo allowing direct visualization of the differentiation through embryonic stages. This approach, combined with overexpression and knock-out analyses of candidate genes, aims to monitor and characterise the key events that regulate retinal lineage and cell fate determination.

doi:10.1016/j.ydbio.2007.03.454

Program/Abstract # 385
Wnt-β-catenin signaling blocks retinal progenitor differentiation in a Sox2- and Notch-dependent manner
Kathryn B. Moore 1, M. Agathoclous 2, I. Iordanova 2, W.A. Harris 2, M.L. Vetter 1
1 Neurobiol & Anat, Univ. of Utah, SLC, UT, USA
2 Dept. of Anat, Univ. of Cambridge, CB2 3DY, UK

During development, retinal progenitors undergo a series of transitions to become differentiated post-mitotic neurons. Canonical Wnt signaling is active in the stem cell/progenitor niche in the Xenopus retina, and we have shown that it drives Sox2 expression, which is required for neurogenesis (VanRaay et al., 2005). However, sustained pathway activation prevents retinal neuron differentiation and maintains progenitors as neuroepithelial cells. To analyze this effect we generated
transgenic embryos expressing stabilized β-catenin in retinal progenitors and showed that proneural gene expression was normal but proneural target genes and markers of differentiated retinal neurons were lost, suggesting a block in proneural function. Sox2, normally downregulated as progenitors differentiate, persisted in the undifferentiated cells, suggesting that Sox2 may prevent retinal neuron differentiation. Consistent with this, overexpression of Sox2 mimicked the effects of Wnt signaling on gene expression, and the cells differentiated as non-neural Müller glia. Inhibition of Notch signaling reversed the ability of both Wnt/β-catenin and Sox2 to suppress neuronal differentiation and proneural target gene expression. We conclude that Wnt/β-catenin regulates the process of retinal progenitor differentiation via Sox2, which is required for proneural gene expression but inhibits proneural function through Notch signaling. We propose that differentiation does not proceed until Sox2 is downregulated through feedback inhibition of Wnt signaling. NIH EYI-4954 and Wellcome Trust.

doi:10.1016/j.ydbio.2007.03.455

Program/Abstract # 386
Sbt1 is required downstream of proneural bHLH factors for neurogenesis in the developing retina
Monica L. Vetter, M.A. Logan, M.R. Steele, I. Al-Diri, W. Chen, C. Dooley, B. Moore
Dept of Neurobiol & Anat, U of Utah, Salt Lake City, UT, USA

Proneural basic helix–loop–helix (bHLH) transcription factors are key regulators of retinal neurogenesis, and they function by activating the expression of target genes that execute a program of neuronal differentiation within progenitors. In a previous screen for proneural target genes we identified a novel gene called sbt1 (shared bHLH target 1). The purpose of this study is to investigate the expression and function of sbt1 and determine whether it plays a role in regulating retinal neurogenesis. sbt1 is conserved across vertebrate species and encodes a novel protein with no conserved functional motifs. In situ hybridization analysis showed that sbt1 is transiently expressed in late proliferating or early differentiating cells in both the Xenopus and mouse retina. Epitope-tagged SBT1 protein localizes to both the plasma membrane and the nucleus in Xenopus animal caps, and the N-terminal region is required for membrane localization. Inhibition of SBT1 translation in Xenopus retinal progenitors by injection of morpholino into cleavage-stage blastomeres prevented or delayed retinal neuron differentiation. Conversely, overexpression of either mouse or Xenopus sbt1 by RNA injection promoted differentiation of early born retinal neurons, and also enhanced the ability of proneural bHLH factors to promote retinal neurogenesis. We conclude that sbt1 is expressed in retinal progenitors as they initiate neuronal differentiation, and appears to function as a conserved component of the neuronal differentiation program downstream of proneural bHLH factors during retinal development.

doi:10.1016/j.ydbio.2007.03.456

Program/Abstract # 387
Examining early retinal progenitor multipotency by Mash1 misexpression in the Math5-lineage
Robert B. Hufnagel, Malgorzata Quinn, Nadean L. Brown
Division of Developmental Biology, Cincinnati Children’s Hospital Research Foundation and Departments of Pediatrics and Ophthalmology, University of Cincinnati College of Medicine, Cincinnati, OH, USA

Proneural basic helix–loop–helix (bHLH) transcription factors influence neuronal determination and fate specification throughout the developing nervous system. In the mouse retina, bHLH factors are required for the normal development of the seven major cell types, six neuronal and one glial. Math5 is the first proneural bHLH factor expressed in the embryonic retina and is required for the development of retinal ganglion cells (RGCs), the first retinal neuron specified. Mash1 expression initiates three days later and is required for the proper generation of bipolar neurons, specified last. To test the multipotency of retinal progenitors in the Math5-lineage, we performed a gene swap experiment to generate a Math5<sup>Mash1</sup> knock-in allele, wherein the endogenous Math5 coding sequence was replaced with Mash1 by homologous recombination. As a result, Mash1 is precociously expressed in the Math5-lineage in these mice. The Math5<sup>Mash1</sup> knock-in allele also expresses an IRES-dsRed reporter to distinguish those progenitors that misexpress Mash1. Adult Math5<sup>Mash1/Mash1</sup> mice lack RGCs and optic nerves, indicating that Mash1 cannot rescue the Math5 phenotype. We have assessed these mice embryonically for fate alterations in the Math5-lineage, namely the precocious differentiation of bipolar neurons, and for the relative distribution of the seven retinal neuronal and glial cell types.

doi:10.1016/j.ydbio.2007.03.457

Program/Abstract # 388
Mechanism of early neural stem cell lineage specification in the mouse epiblast
Lan Dang, Vincent Tropepe
Dept. of Cell & Systems Biol., Univ. of Toronto, Toronto, ON, Canada

Mouse definitive neural stem cell (NSC) lineages are derived from a population of primitive neural stem cells (PrNSC) in the epiblast or from embryonic stem cells (ESC) in vitro, yet details on the signaling and transcriptional mechanisms that control this lineage transition are lacking. Data from chick and Xenopus in vivo experiments suggest that FGF and Wnt signaling play a