molecular mechanisms that underlie the evolutionary innovation of cephalopod appendages we isolated a number of wnt homologs from the Hawaiian bobtail squid Euprymna scolopes and examined their expression by in situ hybridization. We will show that Euprymna wnt paralogues are strongly expressed during the development of the arms and tentacles of the squid, displaying distinct but overlapping expression domains. Our results suggest that the Wnt pathway plays an important role in limb patterning within the cephalopod mollusks.

doi:10.1016/j.ydbio.2009.05.286

Program/Abstract # 263
Diverging functions of Scr between embryonic and post-embryonic development in a hemimetabolous insect, Oncopeltus fasciatus
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Hemimetabolous insects undergo an ancestral mode of development in which embryos hatch into first nymphs that resemble miniature adults. While recent studies have shown that homeotic (hox) genes establish segmental identity of first nymphs during embryogenesis, no information exists on the function of these genes during post-embryogenesis. To determine whether and to what degree hox genes influence the formation of adult morphologies, we performed a functional analysis of Sex combs reduced (Scr) during post-embryonic development in Oncopeltus fasciatus. In strong Scr-RNAi adult phenotypes, the T1 segment (pronotum) is transformed toward a T2-like identity, complete with the formation of ectopic wings. However, structures that were previously established during embryogenesis are either unaffected (T1 legs) or display only minor changes (labium). These observations reveal novel aspects of a hox gene function in insects. There is a temporal and spatial divergence of Scr roles during embryonic (main effect in labium) and post-embryonic (main effect in pronotum) development. While Scr is required during post-embryogenesis for the formation of adult specific features, it has relatively no effect on structures previously established during embryogenesis. Hence, our results of Scr function in a hemimetabolous species provide an important new complement into the evolution of the insect prothorax in general.

doi:10.1016/j.ydbio.2009.05.287

Program/Abstract # 264
Calcium channel MAGUK gene CACNB4 required for mitosis in zebrafish early development
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Voltage-depndant calcium channels (VDCCs) are comprised of a pore forming alpha1 (CACNA) subunit and several auxiliary subunits. The CACNB4 auxiliary subunits chaperone the alpha1 subunit to the membrane and modulate gating properties of the channel. Mutations in the CACNB4 subunit are associated with ataxia and seizures in mice and with epilepsy in humans, but since known mutant alleles of CACNB4 are not embryonic lethal, the developmental functions of the protein are unclear. In studying the functional roles of the CACNB4 gene, we unexpectedly discovered that targeted knockdown of CACNB4 genes in zebrafish led to arrest or delay of epiboly and subsequent death of the early embryo. In CACNB4 knockdown blastula-stage embryos, we localized the phenotypic defects to the extra-embryonic yolk syncytial layer (YSL), a syncytium containing a few hundred nuclei. We find that nuclei in the YSL fail to remain physically separate, and instead form multipolar spindle arrays that fail mitosis. Supernumerary centrosomes appear to contribute to the inappropriate joining of adjacent nuclei. We also report progress on strategies to use transgenic lines to determine the subcellular localization of CACNB4 in the YSL and to identify the CACNB4 domains required for normal epiboly.

doi:10.1016/j.ydbio.2009.05.288

Program/Abstract # 265
Rab-endosome polarization during retinogenesis
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The mature vertebrate neural retina forms from a polarized layer of neuroepithelial cells. Cell-cycle exit of progenitors is precisely timed to control correct number and class of neurons generated. Both intrinsic factors and the extrinsic microenvironment of the retinal progenitors cooperate to regulate cell-cycle exit. Research has demonstrated the importance of Rab-mediated endocytosis on modulating signaling pathways such as Shh, Wnt, FGF, and Notch. The Rab family of small GTPases function as molecular switches regulating membrane and protein trafficking, and through their role in endocytosis, can locally control intracellular signaling activities initiated by extrinsic cues. For example, activation of the Notch-signaling pathway is dependent on endocytotic mechanisms. In the developing zebrafish retina, it was recently shown that Notch activity assessed by the her4^-dRFP reporter is dependent on nuclear position, with high expression of the her4^-dRFP transgene detectable when the nucleus is located near the apical surface. Due to the dependence of Notch pathway activation on endocytosis, this led to the hypothesis that endocytotic components polarize in retinal neuroepithelial cells in a nuclear position dependent manner. We have generated several transgenic lines in zebrafish to dynamically assess endosomes and their role in signaling activity. Analysis of transgenic zebrafish embryos expressing EGFP-Rab5c, marking early endosomes, showed a correlation to nuclear position and the degree of endosome polarization in retinal neuroepithelia. Currently, we are assessing the functional significance of each endosome type in localized signaling during retinogenesis.

doi:10.1016/j.ydbio.2009.05.289

Program/Abstract # 266
Brain development in the annelid Capitella sp. I: Insights into nervous system evolution
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A key step of early central nervous system (CNS) development is fate specification of neural progenitor cells (NPCs) that generate the brain and nerve cord. However, much of our understanding of the mechanisms controlling NPC fate specification comes from detailed studies in two of the three major bilaterian clades, ecdysozoans and deuterostomes. In addition, recent phylogenomic data make compelling arguments that CNSs may have evolved independently in different animal lineages. Consequently, it is of utmost importance that we close the gap in our understanding of early CNS development in the third bilaterian clade, the lophotrochozoans. To address this,