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.

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.

Voltage-dependant 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.

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 GTases 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 endocytic 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.
we have assembled a detailed molecular and cellular description of early brain neurogenesis in the lophotrochozoan annelid Capitella sp. I utilizing several different approaches. In Capitella sp. I brain neurogenesis proceeds by the ingress of single cells from the anterior ectoderm to generate a stratified epithelial layer. Cell divisions are apically restricted, while expression of neural differentiation markers is basally localized. Prior to and during NPC ingress, a proneural achaete-scute homolog, CapI-ash1, is apically expressed in patches of anterior ectoderm with varying levels of intensity. Furthermore, functional analysis indicates that CapI-ash1 may be involved in fate specification of NPCs and/or their differentiation, but not ingestion. These results suggest that the cellular mechanisms of Capitella brain neurogenesis share many elements with arthropods, although the function of CapI-ash1 may be more similar to proneural function during vertebrate neurogenesis.

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Program/Abstract # 267
Central nervous system development and regeneration in ptychoderid hemichordates
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Hemichordates share several characteristics with chordates, a Hox-specified A–P axis, pharyngeal gill slits, a dorsal central nervous system (CNS) and a postanal tail in some species. Ptychoderaids are the more complex of the motile hemichordates and their planktonic larvae share many similarities with echinoderm larvae. We have been describing developmental, morphological and molecular characteristics that distinguish ptychoderid hemichordates (for example, Ptychodera flava) from harrimanid hemichordates, such as Saccoglossus kowalevskii. We show dramatic differences in the development of the larvae and CNS. An additional interesting property of the ptychoderid hemichordates is their amazing capacity to regenerate after being bisected. We have been comparing the development of the central nervous system in ptychoderid hemichordates during metamorphosis and regeneration. Surprisingly, development of the CNS appears to be similar and it forms adjacent to the dorsal vessel, suggesting that there are signaling properties in the dorsal vessel. The proboscis skeleton is then induced to develop from the endoderm more ventrally. The proboscis skeleton serves a structural function, similar to the notochord in chordates. Experiments are ongoing to examine whether the CNS is a convergent or homologous structure when compared to the chordate CNS. These results have important implications for the evolution of the chordate body plan.

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Program/Abstract # 268
Comparative analysis of regulatory elements associated with snail in Parhyale hawaiensis and Drosophila melanogaster
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Parhyale hawaiensis is an amphipod crustacean that has become an experimentally tractable model system for understanding various aspects of arthropod development and evolution. To allow us to further dissect the mechanisms of pattern formation in Parhyale, we have begun to develop techniques to understand the cis-regulation of several candidate genes. One of these is Ph-snail, which is expressed in the mesoderm, CNS, and PNS during development. The snail ortholog in Drosophila melanogaster is well-characterized, and some aspects of expression are the same and others different between Drosophila and Parhyale, therefore comparisons between the two will provide information on both conserved and diverged aspects of gene regulation. We are using comparative sequence information to identify putative regulatory elements, and then experimentally testing these elements through the microinjection of transposable element constructs in which the putative enhancer drives the fluorescent reporter DS-Red. We expect to find separate enhancers for the mesoderm, CNS, and PNS expression of Ph-snail, similar to Drosophila snail, although the mechanisms by which these elements function may be quite different between the two arthropod species.

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Program/Abstract # 269
Myf-5 and the molecular analysis of tail development in the four-toed salamander, Hemidactylium scutatum
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Tail development in the salamander, Hemidactylium scutatum, is interesting because the process of tail elongation occurs throughout larval, juvenile and adult life stages via the development and growth of additional caudal segments. Some vertebrates resorb the embryonic tail; others resorb the larval tail during metamorphosis. Most vertebrates retain and restructure the tail for use throughout later life stages where it may serve a variety of functions including locomotion, balance, energy storage, and predator defense. For those vertebrates that retain a tail throughout adulthood, the process of tail elongation and segment formation usually terminates. To analyze segment formation in H. scutatum, we cloned 47% of the 5’ end of the H. scutatum Myf-5 cDNA. Myf-5 is a myogenic regulatory factor expressed during somitogenesis known to orchestrate the specification and differentiation of skeletal muscles. H. scutatum Myf5 shows 85.5% sequence similarity at the nucleotide level and 91.7% sequence similarity at the amino acid level to Myf5 from Notophthalmus viridescens (red-spotted newt). Unlike the somites of Xenopus and Ambystoma, somites in the trunk and tail of H. scutatum are not visible on the superficial surface of the embryo. Fortunately, in situ hybridization analysis of Myf5 expression in H. scutatum embryos allows the visualization of somites and provides a useful marker for segment formation during axial elongation. We plan to use Myf5 to explore the process of tail segmentation throughout all life history stages in H. scutatum.

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