Program/Abstract # 252
Characterization of genes selectively expressed in the developing zebrafish nervous system
Dena R. Hammond, Angela Schmoldt, Ava J. Udvadia
Department of Biological Sciences, UW-Milwaukee, Milwaukee, WI, USA
Great Lakes WATER Institute, UW-Milwaukee, Milwaukee, WI, USA

In this study we describe the initial characterization of two sequences isolated from a subtraction library enriched in sequences that are expressed in developing neurons in the process of axon growth. The first is a novel sequence, DAG4802D06, that does not correspond to any previously documented gene, and the second is a sequence that corresponds to calcineurin binding protein 1 (Cabin1). Cabin1 is a calcineurin and myocyte enhancer factor 2 (MEF2) inhibitor that has been shown to play a role in vesicle endocytosis in the adult mammal, but its role in the developing nervous system has not yet been examined. Based on these known interactions, we propose that Cabin1 may function in axon growth and suggest three possible mechanisms for this action. Cabin1 may indirectly stabilize actin filaments and/or regulate vesicle endocytosis in the growth cone by inhibiting calcineurin. Cabin1 may also act to indirectly promote synapse formation by inhibiting MEF2. We have isolated the full-length messages from both of these library sequences and determined their spatial and temporal expression patterns using mRNA in situ hybridization. Based on our results, we propose that DAG4802D06 may represent previously undocumented 3′UTR of the neuron-specificRNA-binding protein elavl3. We are currently performing functional analysis of Cabin1 using knockdown and overexpression techniques to determine its role(s) in the developing nervous system.

doi:10.1016/j.ydbio.2008.05.268

Program/Abstract # 253
Discovery of selectively expressed genes in the developing vertebrate nervous system
Forecki Jennifer, Hammond R. Dena, Forecki Jennifer, Udvadia J. Ava
Department of Biological Sciences, UW-Milwaukee, Milwaukee, WI, USA
Great Lakes WATER Institute, UW-Milwaukee, Milwaukee, WI, USA

To understand the mechanisms regulating axon growth in the developing vertebrate nervous system, we identified genes enriched in growing neurons of zebrafish. These studies utilize a transgenic line of fish expressing green fluorescent protein (GFP) under the regulation of a gap43 promoter, which promotes gene expression in neurons undergoing axon growth and plasticity. We isolated GFP+ cells from these fish at 48h post fertilization (hpf). From these cells, we have created a suppression subtraction hybridization (SSH) library of genes enriched in growing neurons and have thus far isolated 176 unique sequences that correspond to some known and many novel genes. We have used quantitative PCR (qPCR) to determine the relative expression level change of these sequences in GFP+ and GFP− cells, and have found that over 60% of the sequences analyzed are enriched 2-fold or greater in GFP+ cells. We have also conducted in situ hybridization in developing zebrafish (day 1, 2, and 3) using probes for several known and novel sequences. The sequences tested thus far appear to be expressed selectively in the nervous system. We will begin functional testing of the sequences in the processes of neuronal differentiation, axonogenesis, and synaptogenesis using overexpression and gene knockdown. Since over 75% of the known genes isolated in this screen have documented roles in the nervous system, we anticipate discovery of nervous system functions for the novel genes as well as known genes that have not been characterized in the developing nervous system.

doi:10.1016/j.ydbio.2008.05.269

Program/Abstract # 254
cMet signaling functions during zebrafish hindbrain development
Gina E. Elsen [a], Louis Y. Choi [b], Victoria E. Prince [a,b], Robert K. Ho [b,c]
[a] *Committee on Neurobiology, University of Chicago, Chicago, IL, USA*
[b] *Committee on Developmental Biology, University of Chicago, Chicago, IL, USA*
[c] *Department of Organismal Biology and Anatomy, University of Chicago, Chicago, IL, USA*

During development, cMet signaling regulates a range of cellular processes, including growth, survival and migration (Birchmeier, 2003). Human genetic studies have demonstrated a correlation between a cMET mutation and autism (Campbell et al., 2006). The *cMet* gene encodes a tyrosine kinase receptor, which is activated by Hgf (hepatocyte growth factor) ligand. In this study we are exploiting the advantages of the zebrafish to ask how cMet signaling functions during hindbrain development. We have analyzed *cMet* expression in the developing zebrafish hindbrain. We find expression in rhombomere 1 (r1; future cerebellum), and in migrating facial branchiomotor neurons (FMNs). We are in the process of identifying additional *cMet*-expressing neurons. Database analysis reveals two zebrafish *hgf* isoforms (*hgf1* and *hgf2*). *hgf1* is expressed at low levels throughout the developing hindbrain, and at high levels in migrating neural crest. We are currently analyzing *hgf2* expression. Our functional analysis shows that cMet signaling is required for normal FMNs migration. Morpholino knock-down of cMet function causes major defects in FMNs migration, while Hgf1 function knock-down causes mild defects. We further find that cMet knock-down causes specific cell death, suggesting a role in cell survival. In future experiments we will establish whether cMet signaling plays roles in hindbrain neural proliferation and/or differentiation, or in migration of cerebellar neurons.

doi:10.1016/j.ydbio.2008.05.270

Program/Abstract # 255
Semaphorin3A regulates neural crest migration during ocular development
Peter Y. Lwigale [a,b], Marianne Bronner-Fraser [a]
[a] *Division of Biology, California Institute of Technology, Pasadena, CA, USA*
[b] *Department of Biochemistry and Cell Biology, Rice University, Houston, TX, USA*

Neural crest, a highly migratory multipotent cell population, contributes to numerous ocular tissues including the cornea, ciliary body, and stroma of the iris. Little is known about the nature and source of guidance molecules regulating neural crest migration during ocular development. Usingquail-chick chimeras and in situ hybridization, we characterized the expression of *Semaphorin3A* (*Sema3A*) in the eye and its receptor *Neuropilin-1(Npn-1)* by neural crest cells in the periocular region during cornea development. Our results show that the lens continuously expresses *Sema3A* during cornea development and neural crest cells express *Npn-1* in the periocular region. Interestingly, only the neural crest cells that down regulate *Npn-1* migrate into the eye to form the cornea. We also show that the lens, which immediately underlies the ectoderm, inhibits neural crest migration into the rudimentary eye since lensectomy causes premature entry and malformation of the cornea. Additionally, inhibiting of Sema3A signaling in the lens phenocopies lensectomy. We demonstrate that