

33.4

**Quantitative Mapping of Intracardiac Blood Flow in Embryonic Zebrafish**

Jay Robert Hove<sup>1</sup>, Reinhard W Koster<sup>2</sup>, Arian S Forouhar<sup>1</sup>, Gabriel Acevedo-Bolton<sup>1</sup>, Scott E Fraser<sup>2</sup>, Mory Gharib<sup>1</sup>. <sup>1</sup>Bioengineering, California Institute of Technology, 1200 E. California Blvd, Pasadena, CA 91125, <sup>2</sup>Biology, California Institute of Technology, Pasadena, CA. Using real-time *in vivo* imaging and digital particle image velocimetry (DPIV) we quantitatively described the intracardiac flow environment of early zebrafish (*Danio rerio*) embryos. Gross cardiac dynamics were defined for two embryonic stages: 4.5 days post fertilization (dpf) and 37 hours post fertilization (hpf) using high-speed transmitted light microscopy with valve dynamics visualized through high-speed laser-scanning microscopy on transgenic embryos expressing GFP. Blood flow patterns were mapped out as velocity vector fields generated from the motions of circulating red blood cells. In the 4.5 dpf embryos we discovered a highly dynamic flow environment with high-speed (0.5 cm·s<sup>-1</sup>) jets. Viscous flow at these speeds, within such small structures, results in enormous wall shear stresses (>75 dyne·cm<sup>-2</sup>). 37 hpf hearts produced jets with velocities approaching 1.5 mm·s<sup>-1</sup> and wall shears stresses of 2.5 dyne·cm<sup>-2</sup>. Surgical blockage of the inflow or outflow tracts resulted in severe regurgitation between chambers and shear forces in occluded embryos were reduced 8-10 fold over controls. Alteration of normal blood flow patterns resulted in arrested cardiac looping, absence of valves and malformation of the bulbus arteriosus.

**ZEBRAFISH CNS DEVELOPMENT AND FUNCTION: TRANSGENIC APPROACHES (34.1-34.4)**

34.1

**Dissecting visual pathways in zebrafish**

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Our group is interested in the genetic underpinnings of visually mediated behaviors. We have devised quantitative assays for two innate behaviors, the optomotor response (OMR) and the optokinetic response (OKR). The neural substrates for these behaviors are largely unknown. Furthermore, no systematic attempt has been made to discover the genes required to specify neuronal fate and connectivity in this system. We are using transgenic reporter lines in three lines of investigation. First, using forward genetic screening, we are isolating mutations affecting retinal ganglion cell (RGC) differentiation and connectivity. This approach takes advantage of a stable transgenic line, Brn3c::GFP, in which membrane-targeted GFP is expressed by 50% of the RGCs. We have discovered several mutants in which axon guidance, synapse formation and function, or other processes are disturbed. Second, using GFP-guided laser ablation, we have selectively ablated two of the ten retinorecipient nuclei to determine their roles in OKR and OMR. Third, using an amacrine-specific Pax6::GFP transgenic line, we are investigating the formation of synaptic layers in the inner plexiform layer in the presence and absence of pre- and postsynaptic partners (in collaboration with R. Wong's laboratory at Washington University). Transgenic lines will continue to provide unprecedented access to the study of both development and function of the zebrafish visual system.

34.2

**Transgenic reporters and modulators of Wnt/beta-catenin signaling in the zebrafish CNS**

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Wnt genes and their downstream effectors are expressed throughout the developing central nervous system (CNS), but their functions there are largely unknown. We are using the zebrafish embryo as a model system to examine Wnt/beta-catenin-activated transcription *in vivo*, due to its accessibility for both embryonic and genetic manipulations. Our laboratory is testing the hypothesis that Wnt/beta-catenin signals directly control CNS cell fate through transcriptional activation.

To identify the cellular targets of beta-catenin signaling during normal development, we are analyzing the expression of a Wnt/beta-catenin reporter, TOPdGFP, in transgenic fish. This reporter is expressed in

cells that respond to beta-catenin by activating transcription, and we observe expression in multiple cell populations throughout the brain and spinal cord. We are now characterizing these GFP-expressing populations using cell type-specific markers. In addition, we are examining how transgene expression corresponds with the transcriptional mediators of pathway activity, encoded by genes of the *lef/tef* family.

In addition, we are generating transgenic fish lines that allow manipulation of the Wnt pathway *in vivo*. Using a heat-shock-inducible promoter, Wnt/beta-catenin signaling can be activated or inhibited at specific times during development. We will use these tools to perturb the pathway and examine resulting effects on CNS cell fate specification, proliferation, and differentiation.

34.3

**Analysis of gene function during nervous system development using transgenic zebrafish**

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Genetic screens have been critical for examining loss-of-function phenotypes in zebrafish. To complement loss-of-function studies we have generated inducible, transgenic zebrafish based on the *hsp70* promoter in order to examine gain-of-function phenotypes. Our experiments have examined the function of two semaphorins, *Sem4E* and *Sem3A1*, and chemokine signaling by *SDF1* and its *CXCR4* receptor for their role in guidance of axons and cell migration in transgenic zebrafish. We generated and analyzed transgenic zebrafish that misexpress *Sem4E*, *Sem3A1*, and *SDF1a*. Furthermore, we showed that expression of *hsp70* promoter regulated transgenes can be induced in single cells by focussing a sublethal laser microbeam onto cells. Using these methods in conjunction with morpholino antisense knockdown experiments, we demonstrated that the semaphorins guide motor axons to their targets via a repulsive action and that *SDF1/CXCR4* signaling is important for guidance of retinal ganglion axons and sensory cell migration. (Supported by NINDS.)

34.4

**Using genetic and chemical screens to study retinal development in zebrafish**

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Morphological screens for retinal defects in zebrafish have yielded a number of interesting mutations, but these methods rely on the detection of gross abnormalities that may overlook more subtle defects. We have developed a transgenic line of zebrafish that expresses a GFP fusion protein specifically in the rod photoreceptors. We have used this transgenic line in a mutagenesis screen in order to find more subtle recessive mutations involved in rod development and maturation. Using a traditional F3 recessive screen, we identify mutant animals by fluorescence microscopy. These mutants display reduced GFP expression and/or small eyes. We are also currently attempting to identify small molecules that affect zebrafish retinal development and rod differentiation using chemical genetic screens with this transgenic line. Small molecules are transferred from arrayed libraries to transgenic fish that are arrayed in 96-well plates. Treated fish are examined visually under light and fluorescence microscopy for morphological defects in the eye and/or significant changes in GFP expression. By utilizing a transgenic line expressing a fluorescent reporter gene in a single cell type, we can identify cell-specific and temporal-specific defects in retinal development, and identify more subtle defects and phenotypes in a non-invasive manner.