Program/Abstract # 104
Investigations of early and late onset scoliotic curvatures in zebrafish
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Familial/idiopathic-type scoliosis affects 3–4% of the pediatric population. This syndrome largely manifests during early infant development due to abnormal vertebral development in utero, but can also present symptoms during adolescence. We provide evidence that three independent leviathan (levm531, vu41, vu105) mutant alleles are mutations in the zebrafish collagen type 8 alpha 1 (Col8a1a) gene. Homozygous mutants display dramatic lateral and dorsoventral folding of the notochord after 1 day post fertilization (dpf). In addition, leviathan mutants display ultrastructural defects of the notochord sheath and exhibit aggregation and misalignment of the notochord sheath cells. We suggest that mutations that affect proper folding of Col8a1a activate the unfolded protein response to degrade mutant proteins. Moreover, a small percentage of leviathan mutants can survive till adulthood and display dramatic scoliotic curvature of the axial skeleton. We hypothesize that the notochord sheath cells regulate the stability of the axial column both early and later in development. We are also investigating a novel heterozygous dominant viral insertion mutant fish, druk. We observe no defects in later in development. We are also investigating a novel heterozygous dominant viral insertion mutant fish, druk. We observe no defects in lateral and dorsoventral scoliotic curves at approximately 25 dpf. The severity of the curves appears to progress throughout adult development. We will test a possibility that a loss of regional bone density contributes to the formation of the vertebral curvatures. The molecular basis of this late stage onset model of zebrafish scoliosis is currently under investigation.

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Program/Abstract # 105
Molecular mechanisms underlying Xenopus somite morphogenesis
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Somitogenesis establishes both the segmented body plan and the progenitors of the skeletal muscle, axial skeleton and dermis of the adult. In the frog, Xenopus laevis, somitogenesis consists of the partitioning of the presomatic mesoderm into somites that undergo a 90° rotation. These somites are comprised of myotome fibers that are aligned parallel to the notochord. Work from our lab reveals that signals present at the lateral edge of the paraxial mesoderm play an important role in the proper alignment of these myotome fibers. A prior study showed that the secreted cytokine, stromal derived factor 1a (SDF1a) played a role in the lateral migration of cells within the anterior compartment of zebrafish somites (Hollway et al., 2007). This lateral movement of cells is reminiscent of the 90° rotation that occurs during X. laevis somite morphogenesis. Using an RT-PCR approach we also found that SDF1a is enriched in this lateral tissue. Subsequent knockdown of SDF1a and its receptor, CXCR4a caused a disruption in myotome alignment. Using Western blot analysis, we show that knockdown of SDF1a leads to a reduction in Rac1 protein. Interestingly, morpholino knockdown of Rac1 shows a very similar morphological phenotype as the SDF1a and CXCR4a morphants. We propose that SDF1a signaling pathway, via Rac1, plays a role in coordinating the cell movements that lead to the proper alignment of myotome fibers within each somite in X. laevis suggesting a possible conserved pathway in the regulation of somite rotation among vertebrates.

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Program/Abstract # 106
Dynamin is required for the maintenance of EVL cell polarity and the progression of epiboly in the developing zebrafish embryo
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Epiboly, the first morphogenetic movement initiated in the zebrafish, involves the thinning and spreading of the blastoderm to engulf the yolk cell. The cellular and molecular mechanisms governing this process are not well understood. One mechanism thought to be important for the progression of epiboly is endocytic removal of exposed yolk cell membrane just ahead of the advancing blastoderm margin. We have found that there is extensive fluid-phase endocytosis at the blastoderm margin prior to and during epiboly, as shown by uptake of the fluorescent dye Lucifer Yellow (LY). During epiboly initiation, LY containing vesicles are enriched in the marginal-most enveloping layer (EVL) cells, but become increasingly localized to the external yolk syncytial layer (E-YSL) as epiboly progresses. Treatment with the Dynamin inhibitor, dynasore, successfully inhibited marginal endocytosis and delayed epiboly in the progression phase. Dramatic defects in EVL cell morphology and polarity were also observed in response to dynasore treatment. Localized expression of dominant-negative Dynamin2 (K44A) in the yolk cell had a mild effect on epiboly, suggesting that endocytosis of the yolk cell membrane is dispensable for epiboly progression. Instead, the epiboly delay caused by inhibition of Dynamin is likely a result of perturbing the morphology or integrity of the enveloping layer.

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Program/Abstract # 107
Alpha-catenin regulates cell cortex stability in zebrafish radial intercalation
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Adherens Junctions (AJs) are essential components for cell–cell adhesion and play a key role in cell migration and epithelial–mesenchymal transition during embryogenesis, wound healing and cancer. The AJs are composed of members of the cadherin family, transmembrane cell adhesion proteins that are connected to the actin cytoskeleton through interaction with the β-catenin/α-catenin (actc) complex. The textbook model described actc as a static molecular