Zebrafish can regenerate an impressive array of organs including the heart, spinal cord and fins. While much work has been done to elucidate the signaling molecules that initiate regeneration, little is known about the transcriptional changes that are necessary for repair to occur. In order to understand the molecular mechanisms driving the regeneration of complex tissues, we are attempting to identify transcriptional gene networks that regulate caudal fin regeneration in the adult zebrafish. We have carried out a candidate-based quantitative RT-PCR screen to discover transcriptional regulators which are expressed after caudal fin injury. A detailed time course of expression analysis revealed several novel genes induced during regeneration. We have confirmed these results using in situ hybridization, and determined the expression domains of these genes within the regenerating fin. Currently, we are creating a variety of tools in order to understand the function of these transcriptional regulators. We have made transgenic lines that express GFP-tagged versions of the genes expressed either from their native promoters or with the hsp70 heat shock overexpression system. In addition, we are using morpholinos to knock down the expression of these genes at several time points during regeneration. We are also optimizing chromatin immunoprecipitation and high thoughput sequencing (ChIP-Seq) from adult regenerating tissues so that we may ultimately place these transcription factors into regulatory networks.

doi:10.1016/j.ydbio.2009.05.440

Program/Abstract # 410
Comparative analysis of satellite cells from blastema and adult tissues of the electric fish *S. macrurus*

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The vertebrate *S. macrurus* can regenerate muscle and electric organ (EO) after repetitive tail amputations. Our ultrastructural and immunolabeling studies demonstrated that satellite cells (SCs) in muscle and EO contribute to blastema formation and regeneration of myogenic tissues (Weber, unpub). To understand some of the cellular mechanisms responsible for its robust regeneration, we have begun to compare SCs derived from muscle, EO and regeneration blastema tissues regarding their proliferation and differentiation potential. We modified a protocol for isolating satellite cells in rainbow trout by Fauconneau et al. (2000) to establish primary SC cultures from muscle, EO and regeneration blastema (Archer et al., 2008). BrdU incorporation studies using cells in growth medium (9 d) showed no obvious differences in the proliferation capacity of SCs from the different tissues. A fraction of all SCs in differentiation medium (5 d) were multinucleated. Some mono- and multinucleated cells were immunolabeled with antibodies against muscle markers. Blastema SCs seemed to form a greater number of myotubes than SCs isolated from either muscle or EO. Myotubes formed from blastema SCs were also generally longer and thinner than those derived from muscle or EO SCs. Further in vivo and in vitro analyses will determine whether the current findings reflect biological differences between distinct populations of SCs in their competence to differentiate into myofibers and contribute to adult tissue regeneration. Establishment of a myogenic SC line from *S. macrurus* will facilitate our developmental studies on the origin of the EO.

doi:10.1016/j.ydbio.2009.05.441

Program/Abstract # 411
Intestinal renewal and regeneration in the planarian *Schmidtea mediterranea*

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In planarians, pluripotent somatic stem cells called neoblasts continuously replenish tissues during normal cellular turnover and regenerate organ systems after injury, but these processes are not well understood at the cellular or molecular level. We have characterized the dynamics of cell renewal and regeneration in the intestine of the planarian *Schmidtea mediterranea*, utilizing a variety of immunohistochemical methods and live animal imaging in detailed time course analyses. As expected, intestinal epithelial cells do not actively cycle, but rather neoblasts differentiate into intestinal cells both in uninjured animals and during regeneration. Additionally, differentiated intestinal tissue undergoes significant remodeling/repatterning in amputated fragments as polarity and symmetry of the intestinal branches are restored. We have developed a novel method for purification of intestinal phagocytes, allowing the generation of a panel of monoclonal antibodies as well as microarray-based identification of over 1000 genes that are differentially expressed in the intestine. An RNAi screen is underway, focused on candidates that may regulate intestinal renewal, remodeling/regeneration, metabolism, and neoblast proliferation or differentiation. (This work was supported by NIH R01 HD043403 to PAN, and NIH-NIDDK F32 DK077469 to DJF.)

doi:10.1016/j.ydbio.2009.05.442

Program/Abstract # 412
Withdrawn

doi:10.1016/j.ydbio.2009.05.443

Program/Abstract # 413
Dynamic expression of planarian Wnt genes reveals complex response to amputation

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Wnt signaling via β-catenin is known to play crucial roles throughout animal life, from early development to adulthood. In planarians, animals with astounding regenerative abilities, β-catenin functions to maintain and specify AP identity in adults and regenerating animals. While Wnt ligands are expressed in a complex posterior to anterior gradient in intact animals, expression during early phases of regeneration remains unknown. We report that Wnt genes are expressed in dynamic, location-specific bursts after amputation. We show that these transcriptional responses are mounted by pre-existing tissues, and are separate from the expression patterns observed as stem cells proliferate to replace missing structures. As the internal anatomy remodels to establish proper size and proportion, we find an interdependence between new and old tissue to establish proper spatial control of Wnt expression. Thus, cells distributed throughout the planarian body plan appear to evaluate and respond to their new position in an amputated fragment. We suggest that the Wnt expression patterns described in intact planarians represent only a small slice of the complex and dynamic signaling that occurs early during a regenerative event.

K.A.G., F32GM082016 A.S.A., HHMI Investigator and RO-1 GM57260.

doi:10.1016/j.ydbio.2009.05.444