from mutant blastocysts. Here we show that Smad4 null TS cells cultured under undifferentiated stem cell conditions fail to maintain a typical epithelial morphology, display abnormal junctional complex organization and up-regulate the expression of some key mediators of epithelial-mesenchymal transition. Smad4 null TS cells are also more prone to differentiation into trophoblast giant cells upon withdrawal of mitogenic signals. Additional experiments are underway to examine the cellular properties of these mutant cells and Smad4-dependent changes in gene expression that could potentially underlie the observed changes in the mutant TS cells.

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Program/Abstract #374
Exploring the evolutionary loss of regeneration: A comparative genomics study in planarians
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Planarians possess extraordinary abilities to regenerate complete animals from small tissue fragments. However, in contrast to most flatworm species, the planarian Dugesia fluviatilis is limited in its ability to restore lost structures, failing to regenerate heads when amputated in posterior tissues. To identify the critical mechanistic failure in P. fluviatilis regeneration, the early stages of regeneration following amputation have been compared in tissues with different regeneration potentials. While the earliest regenerative phases, such as wound healing and cell proliferation, appear to occur in regeneration-deficient tissues, later signaling processes fail to happen. To examine and contrast global changes in gene expression at this critical time point, we have conducted comparative transcriptomic analyses using RNAseq. Genes upregulated in regeneration-proficient tissues yet not expressed in regeneration-deficient tissues have been identified as candidates with putative functions important to the regeneration process. The specific roles of these genes are currently being determined using RNA interference in P. fluviatilis as well as in the related regeneration-competent planarian Schmidtea mediterranea in an attempt to identify the permissive and/or inhibitory factors involved in planarian regeneration.

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Program/Abstract #375
A neuronal calcium channel mediates posteriorizing cues during planarian regeneration
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The planarian flatworm is capable of regenerating an entire animal from as little as 1/279th of a body fragment. This process requires that pluripotent stem cells (neoblasts) throughout the worm migrate, proliferate and differentiate into multiple cell types. The molecular cues that control the expansion and differentiation of neoblasts into a complete body plan remain largely unknown. Recent reports have implicated ion transport, gap junctions and morphogen signaling in the posterior patterning of the primary axis. Here, we have identified a role for a neuronal voltage-operated Ca 2+ channel, Ca v 1B, in regulating anterior–posterior polarity at an early time point during regeneration of the planarian Dugesia japonica. First, loss of Dj-Ca v 1B function by RNAi was shown to potentiate the efficacy of multiple agents in anteriorizing regeneration. For example, the ability of the drug praziquantel to yield two-headed regenerants increased within a Ca v 1B RNAi background in all types of fragment examined. Second, the effects of Ca v 1B RNAi quantitatively phenocopied results derived from Hedgehog RNAi assays, suggesting functional coupling with the same posteriorization pathway. This linkage was further supported by co-localization data. Finally, the timing of this Ca 2+ dependant posterior determination was resolved to occur within the first 3–6 h of regeneration, underscoring the importance of Ca 2+ regulation of posterior patterning at an early time point. In summary, the neuronal Ca 2+ channel, Ca v 1B, functions at a regulatory nexus impacting early posteriorization signaling during D. japonica regeneration.

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Program/Abstract #376
A screen to identify genes involved in regeneration of the planarian nervous system
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Planarians are excellent organisms to examine the molecular mechanisms underlying tissue regeneration. These animals possess a large population of adult pluripotent stem cells (called neoblasts) that allow them to replace any tissue type following amputation. Following any type of injury, the neoblasts proliferate and form a regeneration blastema, where these cells differentiate to replace the lost tissues. Remarkably, these animals are capable of regenerating their central nervous system (CNS) and regaining normal function. We are capitalizing on the regenerative capacity and experimental tools available for the planarian Schmidtea mediterranea to investigate genes involved in CNS regeneration. Using custom microarrays representing approximately 17,000 unique S. mediterranea transcripts (Wang et al., Genes Dev. 2010 24 : 2081–92), we have identified more than 1000 genes that are differentially expressed at various stages of head regeneration. To determine which of these genes are expressed in the intact and regenerating nervous system, we are currently performing a high-throughput whole-mount in situ hybridization screen. The expression screen has served to validate microarray results and has revealed genes robustly expressed in the CNS, neoblasts, and/or the regeneration blastema. We have recently initiated RNA interference experiments to evaluate the function of genes with expression patterns of interest. Our aim is to identify genes with a role in neuronal differentiation, maintenance, or patterning of the differentiated CNS. [This work was supported by a CIRM New Faculty Award II RN2-00940-1 to R.M.Z.]

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Program/Abstract #377
Characterizing the role of Eg5 kinesin on mediating neural stem cell division in the developing zebrafish neural tube
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Stem cell proliferation must be tightly regulated, especially during early developmental stages such as neurogenesis. In a genetic screen designed to identify essential genes required for astroglial (neural stem cell) development in zebrafish, we found that a loss of the kif11 gene