Indiana University-Purdue University Indianapolis Center for Developmental and Regenerative Biology presents

Regenerative Mechanisms and Therapeutic Interventions
Regenerative Mechanisms and Therapeutic Interventions  
IUPUI September 16-17, 2016

Day 1 Campus Center Theatre (Downstairs)  

9:30-10:20  Registration  
10:00 Snacks and Coffee  
10:20 Welcome and Remembrance of Takis Tsonis: Teri Belecky-Adams  
10:30 Opening Remarks: David Broecker (Indiana Biosciences Research Institute; IBRI)  

Session I: Mechanisms of Regeneration  

Stem Cells  
10:45  Keynote: Merv Yoder (IUSM)  Progenitor cells for vascular regeneration  
11:20 Teresa Mastracci (IBRI)  Polyamine and hypusine biosynthesis is required for pancreatic beta cell formation and regeneration  
11:40 Karl Koehler (IUSM)  Inner ear organoids and sensorineural circuit regeneration  
12:00 Jason Meyer (IUPUI)  Human pluripotent stem cell-derived retinal organoids and their application for the study of optic neuropathies  

12:20 Lunch  

Pre-Existing Differentiated Cells (De-differentiation)/Transdifferentiation  
1:30 Keynote: David Hyde (Notre Dame)  Stimulating Muller glia dedifferentiation and proliferation to regenerate the damaged adult zebrafish retina  
2:05 Ryan Thummel (Wayne State U. School of Med.)  Coordinating Reactive Gliosis and Regeneration Following Damage to the Adult Zebrafish Retina  
2:25 Guoli Dai (IUPUI)  Hepatocyte differentiation and dedifferentiation  
2:45 Ryan Anderson (IUSM)  Functional feedback from the pancreatic islet regulates its development and regeneration  

3:05-4:55 Coffee Break and Poster Session (See attached poster abstracts)  

Regeneration of Cellular Appendages  
5:00 Keynote: Ben Perrin (IUPUI)  Actin regulation in stereocilia length regeneration  
5:35 Keynote: Nick Berbari (IUPUI)  Mutation of growth arrest specific 8 reveals conserved roles for motile cilia function  

6:30 Dinner  
Jazz Fest/Irish Fest!
7:00AM Breakfast

8:00AM Welcome Remarks: Dean Simon Rhodes (IUPUI)

Session II: Strategies of Regenerative Medicine

Induction of Regeneration
8:15 Keynote: Gary Krishnan (Eli Lilly) *Immune Surveillance and Satellite Stem Cell activation in Muscle Regeneration*
8:50 Jim Marrs (IUPUI) *Small molecule Wnt signaling agonist rescues retina stem cell activity after embryonic ethanol exposure induced defects*
9:10 Steve Trippel (IUSM) *Growth Factor-Induced Articular Cartilage Repair*
9:30 Agustin Madrigal (Miami University) *Re-patterning of histone modifications and DNA methylation during RPE reprogramming towards retina regeneration*

Immune System and Scarring Response
9:50 Keynote: Jennifer Simkin (U. Kentucky) *Inflammation: Setting the stage for tissue regeneration in mammals*
10:25 Teri Belecky-Adams (IUPUI) *Retinal gliosis triggered by BMP7 is mediated through the innate immune cells*
10:45 Ellen Chernoff (IUPUI) *Osteoclast-like multinucleated giant cells, meningeal fibrosis and axolotl spinal cord regeneration*

11:05 Coffee Break

Bioartificial Tissues/Scaffolds
11:25 Keynote: Mike Hiles (Cook Biotech) *Cells, Scaffolds, and Signals in Regenerative Design for Tissue Repair*
12:00 Derek Milner (U. Illinois) *Scaffolds for Long Bone Repair: From Frogs to Pigs and Hopefully Beyond*

12:45 Closing Remarks

Boxed lunches will be available following the symposium for an informal networking session.
Characterization of midkine-a (Mdka) function during caudal fin regeneration in adult zebrafish
Nicholas Ang1, Travis D’Cruz2, Alfonso Saera Vila2, Peter F. Hitchcock2, Ryan Thummel1
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Purpose: Midkine is a heparin-binding growth factor that plays pleiotropic roles in inflammation, angiogenesis, and cell proliferation and is highly up-regulated immediately following tissue injury. Zebrafish possess two midkine genes, midkine-a (mdka) and midkine-b (mdkb). Previous studies have shown that mdka expression is upregulated following damage to the adult zebrafish heart, retina, and caudal fin and is required for the proper regeneration of the adult zebrafish retina. This study aims at determining the role of Mdka during caudal fin regeneration using a mdka knockout zebrafish model. These results support our overarching hypothesis that Mdka is required for the regeneration of multiple tissue types.

Methods: Animals lacking Mdka were generated using Crispr/Cas9-mediated disruption of exon 2 of mdka. Immunoblot and immunohistochemical analysis confirmed the absence of Mdka protein in mdka mutant animals. Fin regeneration was assessed by comparing wild-type adult zebrafish with adult mdka mutant zebrafish. Specifically, we amputated the caudal fins under a light microscope and then imaged fin outgrowth at 4, 6, 12, 19, and 32 days post amputation (dpa). The percent area of the fin outgrowth was calculated using NIH Image J. In a separate experiment aimed at determining the progenitor rate between groups, we labeled proliferating cells in amputated fins with BrdU from 3-4 dpa. Harvested fin tissue was cryosectioned and BrdU immunolocalization was quantified within the distal-most 400 microns of the blastemal compartment. Statistical differences between groups were determined by a student’s T-test.

Results: At 32 dpa, we observed that wild-type fish fully regenerated their caudal fins, whereas mdka mutants regrew to 90% of pre-amputation levels. However, due to a large variability observed in fin outgrowth between individual mdka mutant animals, statistical analysis revealed that the only significant difference between wild-type and mdka was observed at 4 dpa. Consistent with these findings, we observed a significant reduction in BrdU immunolocalization at 4dpa.

Conclusion: Together, these data show that loss of mdka results in reduced proliferation and regenerative outgrowth at 4 dpa, but that mdka mutants partially recovered to regenerate their fins to near-normal levels.
Inhibition of amputation-induced reactive oxygen species blocks salamander tail regeneration

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Purpose: Very little is known about signaling mechanisms that initiate and sustain tissue regeneration responses after injury. Here, we investigated the requirement of reactive oxygen species (ROS) and NAPDH oxidase (NOX) activity for tail regeneration using a Mexican axolotl (*Ambystoma mexicanum*) embryo model.

Methods and Results: *In vivo* imaging using dihydroethidium (DHE) revealed a marked increase of ROS and superoxide anions O$_2^-$ only minutes after tail amputation. Surprisingly, ROS production was observed at 48 hours post amputation (hpa), long after wound healing. To establish that ROS production is dependent upon early NAPDH oxidase activity, we treated embryos with NOX inhibitor VAS2870 and observed a significant reduction in ROS at 6 hpa. We also observed that VAS2870 strongly inhibited tail regeneration at 72 hpa and 168 hpa.

Conclusions: Our findings show that axolotl tail amputation induces ROS production at the amputation site and NOX activity is required for successful tail regeneration. Given the requirement of ROS production for *Xenopus* tadpole tail and zebrafish fin regeneration, our results suggest that ROS is a conserved mechanism of appendage regeneration.

Figure 1. Association of ROS production with axolotl embryo tail regeneration. Representative images of few time points showing ROS/ O$_2^-$ production at the plane of amputation. Scale bar= 500 um.
Understanding the role of hypothalamic primary cilia in Hh signaling pathway

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Primary cilia are microtubule based cellular appendages that play a critical role in mammalian development by coordinating different cell signaling pathways such as the Hedgehog (Hh) pathway. Loss or dysfunction of primary cilia in the proopiomelanocortin (POMC) neurons in the hypothalamus is known to alter feeding behavior and cause obesity. Our aim is to determine if primary cilia on hypothalamic primary cultures of neurons are capable of coordinating the Hh pathway, as has recently been reported for cultured hippocampal neurons. Currently, we are looking for cilia formation in conjunction with different neuronal and glial markers biomarkers such as β-Tubulin III and GFAP. We are also assessing the cilia status and biomarkers of hypothalamic specific neurons such as POMC, β-endorphin, NPY and AgRP. Experiments testing whether hypothalamic primary neurons respond to Hh pathway specific agonists like SAG and purmorphamine and antagonists such as cyclopamine are ongoing. Ultimately, we will use this system as a model with cultured neurons possessing conditional alleles of cilia loss to assess the impact on POMC and AgRP neuronal signaling. Results from these experiments will help to elucidate mechanisms through which cilia affect neuronal development and cell signaling. These studies will not only provide a better understanding of the defects underlying ciliopathy associated obesity but may also reveal more common mechanisms of centrally mediated obesity.
Mammalian blastema formation in the African spiny mouse, *Acomys cahirinus*

Shishir K. Biswas, Thomas R. Gawriluk, Jennifer Simkin, Jeramiah J. Smith, Ashley W Seifert

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Although regeneration is widespread across metazoan taxa, it is severely curtailed in most mammals that have been examined. However, our recent discovery that spiny mice can regenerate complex musculoskeletal tissue raises the prospect that we can uncover the genetic mechanisms regulating tissue regeneration. To this end, our lab has been developing the African spiny mouse (*Acomys cahirinus*) as a novel model of mammalian regeneration. Spiny mice are evolutionarily separated from lab mice (*Mus musculus*) by only 30 million years, and unlike lab mice, can regenerate skin, cartilage, nerves and adipose tissue following injury in the ear. Thus, *Acomys* provides us a model with which we can use comparative methods to investigate regeneration biology. Transcriptomic analysis revealed that *Acomys* create a pro-regenerative extracellular matrix, initiate a peripheral nerve response and form a specialized wound epidermis in response to injury, all of which are characteristics of epimorphic regeneration. This suggests that ear regeneration in *Acomys* is accomplished through formation of a blastema, which has not been previously observed in mammals. We further examined cellular proliferation within the context of blastema formation. A 4mm ear punch assay was performed in mice of both species and proliferating cells were tracked by injection of EdU at D10, D15, D20 and D30 post-injury. Using immunohistochemistry, cellular proliferation was quantified with respect to cell cycle re-entry and progression. While cells in both species were able to re-enter the cell cycle, only cells in *Acomys* maintained progression through the entire cell cycle. Taken together, these data demonstrate that spiny mice form a blastema in response to injury and also help elucidate the role of proliferation during blastema formation in *Acomys cahirinus*.

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Endothelial-derived Foxf1 is required for post-pneumonectomy lung regeneration and cell proliferation.

Craig Bolte, Logan Fulford, Yuqi Cai, Arun Pradhan, Hannah Flood, Jagannathan Sajjeev, Artem Barski, Vladimir V. Kalinichenko

**Purpose:** Foxf1 is critical for lung development as Foxf1-deficient and conditional deletion mice have shown dramatic phenotypes including embryonic fatality and lung malformation. Recently it was shown that endothelial-derived Foxf1 is critical for lung maintenance as deletion of Foxf1 from endothelial cells of adult mice resulted in rapid and unilateral death. Foxf1 had also been shown to be critical for liver regeneration. However, whether Foxf1 mediates postnatal lung regeneration has not been studied. Thus, we sought to determine whether diminished lung Foxf1 expression would result in impaired lung regeneration in adult mice.

**Methods:** This study employed a mouse line in which Foxf1 is selectively deleted from endothelial cells using the tamoxifen-inducible PDGFB-iCre and Foxf1-floxed allele. Mice homozygous for the Foxf1-floxed allele and carrying PDGFB-iCre died shortly after tamoxifen administration, thus mice heterozygous for the Foxf1-floxed allele were used in this study (PDGFb-Foxf1fl/+ and control, Foxf1fl/fl). Left partial pneumonectomy was performed on adult (8-12 week old) male and female mice. Mice were harvested at 2, 4, 7, 14, or 42 days post-pneumonectomy and evaluated for lung regeneration using the Flexivent small animal ventilator to measure lung volume. Immunohistochemistry, immunofluorescence, and qRT-PCR were used to investigate efficiency of Foxf1 deletion, changes in pulmonary morphology, and proliferation following pneumonectomy. Paired RNAseq and ChIPseq analyses were performed to investigate alterations in gene profile between control and experimental mice following pneumonectomy, as well as to determine direct transcriptional targets of Foxf1. Gel zymography was also used to investigate MMP14 activity. Data are represented as mean±SEM and Student’s t-test used to determine significance.

**Results:** Mice deficient for endothelial-derived Foxf1 demonstrated delayed lung regeneration following pneumonectomy. Paired RNAseq/ChIPseq analysis demonstrated Foxf1 regulated numerous genes involved in remodeling of the extracellular matrix (Adamts9, Timp3), cytokinesis (Tubb4a, Cenpj), and cell cycle regulation (Ccnd3, Cdkn1a, Cdkn2b). PDGFB-Foxf1fl/+ mice showed less proliferation, particularly in endothelial cells.

**Conclusion:** Foxf1 has been repeatedly shown to be essential for lung development and maintenance. This study shows that endothelial-derived Foxf1 is critical for post-pneumonectomy lung regeneration. Furthermore, this study strengthens claims of Foxf1 as a master regulator within the lung; mediating functions including tissue remodeling, cell cycle progression, and cytokinesis in addition previously determined mechanisms including organogenesis, vasculature integrity, vasculogenesis, and cytokine release.
The Role of miRNAs in chick Retina Regeneration

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**Purpose:** Embryonic chicks possess the ability to regenerate their retina at embryonic day (E) 4.5 after retina removal through reprogramming of the retinal pigmented epithelium (RPE) with the addition of an exogenous source of Fibroblast Growth Factor 2 (FGF2). RPE reprogramming includes the dedifferentiation of the RPE into progenitor-like cells that proliferate and eventually differentiate into neural retina. MicroRNAs (miRNA) are short segments (20-25 nucleotides long) of non-coding RNA (ncRNA) that are able to function as post-transcriptional regulators by binding to mRNA. They play major roles in many processes of embryogenesis, including retina development, and have also been found to be important for stem cell proliferation, differentiation, and regeneration. Our hypothesis is that different miRNAs are essential to regulating RPE reprogramming. To begin to elucidate their role during retina regeneration, we performed miRNA-seq on RPE undergoing reprogramming, and, using a recently developed a bioinformatics approach (mirPro), that detects variations in miRNA expression levels which found several miRNAs that are upregulated during regeneration and the identification of several potential target genes. The goal of this study is to determine if the miRNAs play a role in the regenerating chick retina.

**Methods:** At E4, the retina was removed and the RPE was collected 6 hours post-retinectomy in presence or absence (injury only) of FGF2, which was added directly following retinectomy., to be used for total RNA extraction. miRNA-seq was performed and the regulated miRNAs were determined. qRT-PCR using TaqMan miRNA Assays was done in order to verify the miRNA-seq data and the targets for those miRNAs were determined using miRDB and TargetScan. qRT-PCR analysis was then done on the expected targets to verify their up/downregulation which would coincide with the opposite regulation of the miRNAs targeting them. The 3’ UTR of the targets was then cloned into a pMIR-Report Luciferase vector to perform subsequent Luciferase assays in HEK-293 cells to verify the miRNAs do indeed target the predicted targeted genes.

**Results:** Bioinformatics analysis using miRDeep2 and mirPro demonstrated differential regulation of miRNAs in response to injury only and treatment with FGF2. Each miRNA targets different mRNAs that could be implicated in the process of RPE reprogramming. We selected two miRNAs that are upregulated post-retinectomy for biological analysis based on their significance and the targets’ role in regeneration. miR-21-5p was found to be upregulated post-retinectomy in response to FGF2 treatment while miR-205b was found to be upregulated post-retinectomy in the presence or absence of FGF2. This has also been validated through qPCR. Additionally, we have discovered several potential targets through miRDB, which have been validated by qPCR analysis as well. The 3’ UTRs have also been cloned into the pMIR-Report Luciferase vector and are now being used for Luciferase tests.

**Conclusions:** miRNAs play a significant role in the regenerative process of a variety of organisms including the embryonic chick. We have identified upregulated and downregulated miRNAs as well as several targets for two of those miRNAs. Further analysis will be done to validate the miRNA targets with Luciferase assays. To test them in vivo, miRNAs will be injected post-retinectomy in order to determine their effect on regeneration through histological analysis and the downregulated gene expression will be verified through immunohistochemistry and western blot analysis.
Osteoclast-like Multinucleated Giant Cells, Meningeal Fibrosis and Axolotl Spinal Cord Regeneration.

Ellen A.G. Chernoff¹, Hidehito Takenaka¹, Hai V. Nguyen-Salfity², Sarah T. Scott¹, Deborah A. Sarria¹, Maia P. Kirk¹, Teri Belecky-Adams¹, Denise Slayback-Berry¹. ¹IUPUI-Department of Biology/IU Center for Developmental and Regenerative Biology. ²Indiana University-Department of Surgery

Purpose.

Intro. Meningeal fibrosis and invasion occurs in salamander spinal cord penetrating wounds as in mammals, but meningeal cells and extracellular material (ECM) is removed during regeneration, so no permanent meningeal scar is formed. Reactive ependymal cells play a key role in this process, but are not the only factor in fibrosis removal. Multinucleated cells sealed to the reactive meninges that may be another source of fibrosis degrading enzymes and scar prevention.

Hypotheses. (1) An unusual lesion cell type is an osteoclast-like/multinucleated giant cell (MNGC) like those found in MNGC tumors and granulomas, but used in a previously undescribed role. (2) the MNGCs produce enzymes actively involved in meningeal ECM turnover. (3) The MNGC precursors are mononucleated foam cells of macrophage origin that are attracted into the lesion site by meningeal and/or ependymal cytokines.

Goal. Characterization of fibrotic salamander (axolotl) meningeal ECM. Localize the presumptive MNGCs in the lesion site, and characterized in terms of nuclear number, functional sealing zone, ruffled border, cytoskeletal composition and presence of the osteoclast/MNGC matrix degrading enzyme cathepsin K. Identify possible MNGC precursor population.

Methods. Adult axolotl (the salamander Ambystoma mexicanum) spinal cords are transected in the lower lumbar region. 23-26 cm wild type and leucistic animals are used, either sex. Control and 2-3 week regenerating cords are isolated, preserving the lesion site and meninges. Fixed tissues are stained in whole mount or paraffin sections with antibodies to Types I and IV collagen, Chondroitin-sulfate, Cathepsin K, the lipid dye Dil, and ECM dyes metanil yellow, alcian blue, aniline blue. Tissue is isolated steriley for cell culture on fibronectin in defined medium, Control and regenerating samples are fixed for transmission and scanning electron microscopy. The cells of interest are examined in situ and in vitro.

Results. Reactive meninges contain Type I collagen and chondroitin-sulfate proteoglycan (Fig.1). In vivo multinucleated cells cluster in the fibrotic material of the lesion site (Fig. 1). MNGCs are found in reactive meninges in situ (Fig. 2A arrow) and in vitro (2B). Actin localization (Fig.2) and TEM shows podosomes arranged in complete sealing zones and ruffled borders characteristic of osteoclast-like MNGCs. Localization beneath meningeal and ependymal explant in vitro correlates with multinuclearity: Tables I,II. MNGCs cells express osteoclast-related cysteine protease cathepsin K and are laden with lipid droplets (Fig. 3).

Conclusions. MNGCs functionally seal to fibrillar collagen and sulfated proteoglycan-containing lesion meninges. They produce osteoclast-like ECM degrading enzyme. Contact effects on multinuclearity suggest that MNGC fusion and/or maturation factors may come from reactive meninges and/or ependymal cells. MNGCs and mononucleated cells clustered on lesion ECM are lipid-laden. This suggests a mononuclear precursor related to CD 36+, M2-macrophage-derived Foam Cells.
COMPARATIVE ANALYSIS OF RETINAL GLIA AND CELLS OF THE VASCULATURE IN TWO DIABETIC MOUSE MODELS

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Purpose: Diabetic retinopathy is the impairment or loss of vision due to complications in the retinal vasculature in people suffering from diabetes. These complications arise due to the loss of the integrity of the blood retinal barrier. The disease typically is a progressive disease that starts with microaneurysms, loss of pericytes, inflammation, and gliosis. Increased severity is frequently seen with hemorrhaging, macular edema, loss of vasculature, and neuronal cell death. In the most severe cases, loss of vasculature is followed by neovascularization and retinal detachment caused by formation of scar tissue. There are, at present no therapeutic treatments to aid regenerative capacity in diabetic retinopathy. Many studies have used model systems that mimic type 1 diabetes and generalize findings to type 2 diabetes. However, no studies have directly compared the disease process using the same metrics at similar stages in model systems of type 1 and type 2 diabetic retinopathy. We propose that, due to differences in the underlying causes, that both mechanisms and progression of retinopathy may not be the same in type 1 and 2 diabetic retinopathy.

Methods: Retinas from Ins2Akita (Akita), Lepr db/db (Db) and wild type (WT) control mice aged 6, 12 and 18 week were isolated. Flatmounts prepared from retinas were labeled for IBA1, NG2, IB34 and SOX2. RNA isolated from retina was used for RT-qPCR analysis of inflammatory markers. Labeled tissues were imaged using confocal microscopy and images analyzed via ImageJ for cell counts. Total vasculature area indicated by IB4 label was analyzed via AngioTool software.

Results: Cell counts of labeled flatmounts revealed retinal macroglia (SOX2+ cells) and microglia (IBA1+ cells) were similar at the three timepoints analyzed in both models. The pericyte (NG2+ cells) numbers did change over time in the diabetic mice. Both the Akita and the Db mice, showed decreased NG2+ cells compared to the WT controls in the 12 and 18 week timepoint. However, the Akita retinas showed a significant rebound in the number of pericytes at the 18 week timepoint. RT-qPCR analysis revealed inflammatory markers to be highly upregulated at 12 and 18 weeks, with the 18 week Db mice showing a much greater upregulation of inflammatory markers than the Akita mice.

Conclusion: The temporal differences observed in the loss of pericytes and inflammatory response in the type I (Akita) and type II (Db) mouse suggests the mechanisms of retinopathy may also be different. These results indicate that therapeutic aids designed to slow progress of the disease or increase regenerative capacity may need to be different for the two forms of diabetic retinopathy.
Comparative Transcriptomics of Limb Regeneration: Identification of Conserved Gene Expression Changes Among Three Species of Ambystoma

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Purpose
Transcriptomic approaches are beginning to reveal the gene expression basis of limb regeneration in the primary salamander model – the Mexican axolotl (Ambystoma mexicanum). To leverage these data, there is need to extend transcription analyses to additional salamander species. Through a comparative approach using close and distant relatives of the axolotl, we reasoned that it would be possible to identify and parse species-specific expression differences and identify a core set of genes that are commonly expressed among salamanders that elicit a natural regenerative response. Here we report results of a study that identified commonly expressed genes among A. mexicanum, A. andersoni, and A. maculatum during the first 24 hours of limb regeneration.

Methods and Results
Three replicate RNA samples were isolated from distal limb tissues that were collected at the time of amputation and 24 hours post-amputation from larval A. mexicanum, A. andersoni and A. maculatum. Technical replicate samples for A. mexicanum were analyzed by microarray and RNA-Seq to identify 656 transcripts that showed the same pattern of differential expression (FDR <=0.1, absolute log2 fold change>=0.58) between these platforms. Using RNA-Seq and the same significance and fold change thresholds, 1,364 and 2,130 significant transcripts were identified for A. andersoni and A. maculatum, respectively. We identified a significantly greater proportion of shared differentially expressed transcripts between sister taxa A. mexicanum and A. andersoni than between the former and the more distantly related A. maculatum. A total of 130 transcripts (122 upregulated, 8 downregulated) were commonly differentially expressed (CDE) among all three species (Fig. 1); 118 of these were also identified in a recent microarray analysis of A. mexicanum limb regeneration. DAVID analysis of the CDE list identified significantly enriched gene ontology terms; including keratinocyte differentiation, cytoskeleton organization, wound healing, inflammatory responses, anti-apoptosis, and angiogenesis. Using geneMANIA to query CDE genes from mammalian co-expression and genetic interaction networks, we generated a filtered network with high connectivity (Fig. 2).

Conclusion
Our study identified limb regeneration genes with conserved expression patterns among three ambystomatid salamander species. These genes represent robust, targets of a transcriptional program that is likely conserved among all ambystomatids, if not all salamanders. The observation of high connectivity among CDE genes supports the idea that limb regeneration is regulated by transcriptional networks shared between salamanders and mammals.
Role of primary cilia on POMC expressing cells in obesity

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Obesity affects over one third of the US adult population and is associated with co-morbidities such as diabetes. Rare genetic disorders that result in extreme morbid obesity have revealed a profound role for genetics in the biology of appetite and satiety. For example, some genetic disorders in an emerging class of syndromes, collectively called the ciliopathies, present with obesity and type II diabetes. However, it remains unclear how cilia dysfunction in the general population may contribute to obesity. Interestingly, directly adjacent to the most common candidate in GWAS studies on obesity, FTO, is a ciliary gene RPGRIP1L. Previous work has shown that conditional cilia ablation, using a constitutive POMC Cre results in hyperphagic and obese mice. However, it is unclear what developmental stage proper cilia or Rpgrip1l function in POMC expressing cells is necessary to maintain normal adult feeding behavior and body weight. Here we present data on conditional loss of cilia (IFT88) or Rpgrip1l from POMC neurons in adult mice. Surprisingly, loss of these genes in terminally differentiated POMC neurons does not appear to result in hyperphagia and obesity. Thus, we have expanded our studies to include early postnatal and embryonic induction of POMC-CreER and subsequent analysis of body weight, feeding behavior, glucose and insulin tolerance, and hypothalamic neuronal patterning. These future studies will address the role of cilia and transition zone proteins, like Rpgrip1l, in hypothalamic development and may reveal unrecognized roles for cilia that can impact feeding behavior and obesity in adults.
Transplantation of Endothelial Progenitor Cells Improves Survival of FOXF1-deficient Mice

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Purpose

During lung homeostasis, the endothelial barrier regulates the passage of nutrients and fluids. After Acute lung injury (ALI), endothelial permeability is increased which causes edema and inflammation. ALI, with the complication of acute respiratory distress syndrome (ARDS) is the annual cause of approximately 75,000 deaths and 3.5 million hospitalization days. ARDS is additionally the cause of death in more than 35% of ALI patients. We have utilized a recently published murine model of lung injury that occurs after endothelial-specific deletion of FOXF1, a transcription factor critical for endothelial barrier function (Cai et al. 2016). Our lab demonstrated that FOXF1 acts through the S1P/S1PR1 signaling pathway to promote normal lung homeostasis and repair. The current study introduces endothelial progenitor cells (EPCs) generated in vitro into the bloodstream of FOXF1-deficient mice with the purpose of improving endothelial barrier function. Our hypothesis was that EPCs would integrate into the lung endothelium of injured mice to reduce the incidence of pulmonary injury and prevent mortality in FOXF1-deficient mice. Treatment of ALI patients is possible, and the goal of our study is to determine novel mechanistic avenues which could lead to new ALI treatment approaches to increase patient survival rate.

Methods

To induce pulmonary edema and inflammation in adult mice, we utilized transgenic C57BL/6 mice containing a tamoxifen-inducible PDGFb-Cre-ER transgene and two Foxf1-floxed alleles (PDGFbCreER;Foxf1fl/fl), which inactivated Foxf1 in lung endothelial cells and resulted in lethality of 100% of mice by day 24 post-tamoxifen administration. At days 0 and 3, tdTomato-labeled endothelial progenitor cells (EPCs) or control media were injected into tail vein of Foxf1fl/fl (control) or PDGFbCreER;Foxf1fl/fl mice. To collect survival data, mice were observed daily. Protein and RNA samples were isolated from the left lobe of the lung, and samples from the remaining lung were analyzed by immunofluorescence (IF) and immunohistochemistry (IHC).

Results

The survival study demonstrated a 53.8% increase in survival of FOXF1-deficient mice after EPC transplantation, which was associated with a marked decrease in lung injury as shown by H&E staining. Additional analysis demonstrated a decrease in inflammatory response and a maintenance of normal endothelial architecture after EPC-injection. Surprisingly, the introduction of EPCs did not result in EPC integration into pulmonary blood vessels, and tdTomato-labeled cells were not detected in lung parenchyma.

Conclusion

Endothelial progenitor cells protect FOXF1-deficient mice from lung inflammation and improve survival. This is not due to the integration of EPCs into the lung structure, but is likely due to secreted factors. The precise mechanism by which the introduction of EPCs reduced the inflammatory response, lung injury, and death of FOXF1-deficient mice is currently unknown.
Acomys exhibit a distinct immune response to injury that is associated with regeneration.

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Why many mammals cannot regenerate following injury remains an unanswered question in biology. Studying animals that regenerate provides an opportunity to understand the cellular and molecular mechanisms that govern the process naturally. Thus, it stands to reason that using such an approach among mammals could uncover conserved genetic and cellular pathways in regenerating mammals that are directly translatable to mammals that heal by scarring. We recently reported that African spiny mice (Acomys spp.) undergo blastema-mediated, epimorphic regeneration in response to a 4 mm circular injury in the ear pinna similar to appendage regeneration in salamanders and zebrafish. In contrast, Mus musculus and sympatric African rodents heal the same injury by scarring. Using a comparative framework (Acomys spp., M. musculus and M. brockmani), we now test the long-standing hypothesis that a bias towards an innate immune response over an adaptive response regulates epimorphic regeneration.

To evaluate the innate response, we compared humoral killing ability of serum using bacterial killing assays. To understand the overall physiological response, we profiled local and systemic cytokines and chemokines throughout the injury response using a sandwich ELISA. Lastly, we characterized the immune cells present in the wound bed during healing by FACS and IHC. Our data show that Acomys spp. have stronger humoral killing capacity compared to non-regenerating species. Remarkably, our cytokine data strongly suggest that regeneration and scarring induce different pro-inflammatory responses. Scarring is associated with a classic and acute IL-6/IL-8 response. On the other hand, regeneration is associated with an IL-5/IL-12 response. This result indicates that regeneration is concurrent with an immune-cell-mediated response. Our cellular characterization shows that there are temporal and magnitude differences between regeneration and scarring. Corroborating the IL-5/IL-12 pro-inflammatory response in Acomys, T cells infiltrate the wound earlier and persist longer compared to M. musculus. One conclusion drawn from this work is that spiny mice have a strong bias toward the innate immune response. Furthermore, inflammation is necessary for regeneration. These data provide groundwork to appreciate how the immune response controls mammalian regeneration. Future studies will modulate the immune response in M. musculus to be similar to Acomys and antagonize lab mouse pathways in spiny mouse.
Knockdown and knockout of dnmt3a affects chick eye morphogenesis
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Purpose:
Morpholinos are used to knockdown targeted proteins of interest by blocking the translation the gene. Because this is not a change to the actual genome, however, this knockdown will only persist for a short amount of time due to cell division.
Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) along with CRISPR Associated Protein 9 (CAS9) technology is now widely used in genome editing. CRISPR/CAS9 mediated gene editing guided by a single guide RNA (sgRNA) can generate double stranded break in the target gene sequence in the result of gene-lose-of-function. The effects could be passed to all subdivided cells.
So we are testing and comparing the two methods to target DNA (cytosine-5)-methyltransferase 3A (DNMT3a) – an enzyme that can epigenetically regulate gene expression by adding methyl groups to DNA – during early chick eye formation.

Methods:
We are targeting DNMT3a on five different sites with CRISPR/CAS9. The sgRNA vectors for CRISPR/CAS9 and DNMT3a morpholinos will be injected into the embryonic chick followed by electroporation at developmental stage 10-11. The morphogenic defects are apparent in the developing eye four days post-injection. The down regulation of DNMT3a can be assayed in the developing eye of the embryonic chick through western-blot and immunohistochemical staining three days postinjection in the case of CRISPR/CAS9 sgRNA and the morpholino treated groups.

Results:
Using DNMT3a morpholinos, we have observed severe defects including microphthalmia (small eye) and ventral defects that result in failure to close the choroid fissure. Additionally, we have shown that the CRISPR/Cas9 constructs are able to successfully generate the double strand break on the target site.

Conclusions:
DNA Methyltransferase 3a eye development in the embryonic chick. Knocking down this enzyme using morpholino technology results in ventral defects and microphthalmia. Using CRISPR/Cas9 technology in chicken cells, we have successfully made the desired disruption in the DNMT3a gene. We are now planning to target DNMT3a in the embryonic chick eye to assess for morphological defects. Immunohistochemical and Western Blot analysis will also be performed to measure the reduction in the amount of DNMT3a.
Chondrogenic Pellet Cultures for Cartilage Tissue Engineering Studies Grow by Deposition of Matrix and Not by Cellular Proliferation

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**Purpose:** Pellet cultures are used to induce chondrogenesis of mesenchymal stem cells. We have demonstrated chondrocyte pellets grow in size during culture and produce cartilage matrix, but pellets of adipose-derived mesenchymal stem cells (ASC) grow only slightly, producing sparse matrix. The objective of this study was to determine if differences in chondrocyte and ASC pellet growth are explained by differences in cell proliferation, or in deposition of extracellular matrix.

**Methods:** Primary chondrocytes and ASC from adult Yorkshire pigs were cultured in Dulbecco’s Modified Eagle’s Medium (DMEM) with 10\% fetal bovine serum. To determine baseline proliferation, cells were grown on coverslips and treated with 10 \(\mu\text{M}\) bromodeoxyuridine (BrdU) for 24 hr and immunostained for BrdU labeling. For pellet cultures, cells were placed in 15 ml conical tubes at 500,000 cells/tube, pelleted by centrifugation in 1.0 ml of chondrogenic base media (CBM: DMEM + 40\(\mu\)g/ml proline, 50 \(\mu\)M ascorbic acid-2-phosphate, 100 nM dexamethasone, and 1X insulin-transferrin-selenium), and cultured in CBM for 1, 2 and 4 weeks. To detect proliferation in pellets, 1 and 2 week samples were labeled with 10 \(\mu\text{M}\) BrdU for 24 hr prior to harvest. Pellets were fixed with 4\% paraformaldehyde, embedded, and cryosectioned on a Leica CM1900 cryostat. To assess chondrogenic differentiation and matrix expression, sections were stained with antibodies for collagen II, keratin sulfate and chondroitin sulfate. Images were then captured and the distance between adjacent nuclei in 1 week and 4 week pellets were measured using Zeiss imaging software.

**Results:** Monolayer cultures showed BrdU labeling, with higher labeling in ASC cultures indicating faster proliferation (\(n=5\), 77.3\(\pm\)3.74\% chondrocyte vs. 92.1\(\pm\)2.88\% ASC, \(\alpha=0.05\), \(P<0.0001\), Student’s t-test). In contrast, nuclear BrdU labeling was not seen in sections from either ASC or chondrocyte pellets (\(n=5\)), at either 1 or 2 weeks. Absence of proliferation in pellets was verified by negative staining for the mitotic marker Aurora Kinase B (AurKB). Cartilage matrix staining was strong in chondrocyte pellets at all timepoints, and absent in ASC pellets. Cell nuclei were closely packed in both ASC and Chondrocyte pellets at 1 week, but a significant increase in distance between adjacent nuclei with interspersed matrix staining is observed in chondrocyte pellets at 4 weeks (\(n=4\), 11.88\(\pm\)0.67\(\mu\)m 1wk vs. 26.85\(\pm\)2.06\(\mu\)m 4wk, \(\alpha=0.05\), \(P<0.0001\), Student’s t-test). As TGF\(\beta\)3 has been shown to induce chondrogenesis, a set of ASC pellets were cultured in CBM+10ng TGF\(\beta\)3 for 1 and 2 weeks (\(n=4\)). TGF\(\beta\)3 treatment did not induce proliferation as sections were negative for BrdU and AurKB. However, expression of keratan sulfate and chondroitin were noted, but not collagen II.

**Conclusions:** Based on the data, neither ASC nor chondrocytes proliferate in pellet culture, and chondrocyte pellet growth is due to matrix deposition.
Meninges-Derived Retinoids In Axolotl Spinal Cord Regeneration.
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Purpose.
Intro. Retinoids from the mammalian meninges maintain CNS health. Retinoid production has also been implicated in the regeneration-permissive interaction of the meninges and ependyma in the spinal cord of regenerating Axolotl (the aquatic salamander *Ambystoma mexicanum*) after penetrating injury, causing re-epithelialization of the reactive ependymal cells. Others have reported CRBP (RBP) in juvenile axolotl in the ependymal cells surrounding the central canal of the spinal cord. CRABP is reported in juvenile axolotl cord white matter. Adult axolotl tissue was not examined. Our prior studies found retinol and all-trans-retinoic acid mediated re-epithelialization of ependymal cells *in vitro* as did co-culture of ependymal and meningeal cells. To determine which components of the retinoid signaling pathway act in urodele spinal cord regeneration, we employed antibody/horseradish peroxidase staining of intact and regenerating adult Axolotl spinal cord tissues in parallel with cell culture to determine expression of three of the pathway proteins: Cellular Retinol Binding Protein 1 (CRBP1/RBP1), Cellular Retinoic Acid Binding Protein 2 (CRABP2), and Retinaldehyde Dehydrogenase 2 (RALDH2) and the nuclear retinoic acid receptor-beta (RARβ).

Hypotheses. (1) Axolotl meninges is site of retinoid metabolism. (2) The meninges is a source of retinoic acid for regenerating spinal cord cells. (3) In the lesion site, there is a mutual interaction between the meninges and the ependymal outgrowth that includes stimulation of ependymal cell re-epithelialization following mesenchymal outgrowth.

Goals. Localize retinoid-binding proteins RBP and CRABP2 in the lesion site and reactive meninges of the reactive cord stump. Localize expression of retinoic acid producing enzyme RALDH2 to specific meningeal layer and reactive cord cell populations. Determine which populations of cells are responding to retinoids using localization of the nuclear retinoic acid receptor RARβ.

Methods. Adult axolotl (the salamander *Ambystoma mexicanum*) spinal cords are transected in the lower lumbar region. 23-26 cm wild-type and leucistic animals are used (either sex). Control and 2-3 week regenerating cords are isolated, with subsequent preservation of the lesion site and meninges. Fixed tissues are stained in whole mount or paraffin sections with antibodies to: RBP1, CRABP2, RALDH 2 and RARβ, for immunoperoxidase localization. The cells of interest are examined *in situ* and *in vitro*. Regenerating tissue is isolated sterilley for cell culture on fibronectin in defined medium. Explants containing reactive meninges and ependymal cells are cultured and reacted with antibodies to CRABP2 and RALDH2.

Results. In the intact cord: 1) CRBP1/RBP1 is expressed in the pia and arachnoid mater meningeal layers, grey matter neurons, and the ependymal cell radial processes producing the glia limitans; 2) CRABP2 is expressed in arachnoid and/or dura mater meningeal layers; and 3) RALDH2 is expressed in the arachnoid as well as cytoplasm of grey matter neurons and some sub-ependymal cells. Following penetrating injury the reactive meninges expands and invades the lesion site (Figure 1). In regenerating cord, CRBP1/RBP1 and CRABP2 are expressed in reactive ependymal cells (Figure 2). Strong RALDH2 staining is observed in reactive meninges and is also upregulated in the cytoplasmic and perinuclear regions of reactive grey matter neurons *in situ*; in addition, meningeal production of RA is present, and following injury, expression is upregulated (Figure 3). *In vitro*, the reactive meninges appears to modulate the behavior of reactive ependymal cells by driving re-epithelialization of reactive ependymal cells, as do exogenous retinoids in ependymal cell-only cultures. The meninges remain CRABP2 and RALDH2-positive *in vitro*. RARβ is localized in the nuclei of gray matter neurons, some ependymal nuclei and nuclei of a population of cells in the arachnoid layer of the reactive meninges.

Conclusion. The control and reactive axolotl cord meninges are a source of retinoic acid. A boundary layer between arachnoid and dura, gray matter neurons and some ependyma show RA responsiveness in the form of nuclear receptor expression.
Title: Engineering Human Induced Pluripotent Stem Cells to Assay for Retinal Pigmented Epithelium to Neural Retina Transdifferentiation

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Purpose: Retinal pigmented epithelium (RPE) forms a monolayer of cells that surrounds and supports the retinal photoreceptors. Worldwide, over 30 million people suffer from permanent vision loss due to the dysfunction and/or death of RPE. In particular, RPE loss precedes the death of photoreceptors in Age-Related Macular Degeneration, a common blinding disease for which there exists no effective treatments to reverse the condition. The loss of Neural Retina (NR) cells is irreversible in humans. However, adult newts can spontaneously regenerate lost or damaged NR through transdifferentiation of RPE. Given appropriate biochemical manipulation, human induced pluripotent stem cells (hiPSCs) can differentiate into RPE in vitro. The long term goal of this research seeks to define conditions required to induce human RPE to regenerate damaged NR, as the newt does spontaneously. Genetic manipulation of hiPSCs will create cell lines appropriate for use as sentinels to easily identify conditions promoting RPE to NR reprogramming.

Methods: Homology directed repair (HDR), following CRISPR/Cas9 mediated double strand DNA breakage (DSB), facilitated the insertion of a viral P2A peptide followed by a cyan fluorescent protein (CFP) reporter gene to replace the stop codon in the endogenous VSX2 gene. VSX2, an important, well-characterized transcription factor, specifically identifies early NR progenitor cells. A dsDNA HDR template, and a single gRNA in a high fidelity Cas9 vector, were electroporated into hiPSCs using the Gene Pulser Xcell system. Cells were selected with G418 antibiotic, manually picked, and screened for reporter integration by PCR using primer sets spanning each side of the homologous recombination junctions.

Results: Following electroporation and G418 selection, 48 resistant clones were screened by PCR. Seven out of 48 clones exhibited the expected PCR fragment sizes on each side of the HDR junction. These clones were further validated by DNA sequencing. These clones are currently being treated with FLP recombinase to remove the Neo selection cassette. Simultaneously, we have successfully adopted working protocols for both hiPSC to RPE differentiation (Zhang et al. 2014, Protein & Cell, 5(1):48-58) and hiPSC to NR differentiation (Zhong, Gutierrez et al. 2014, Nature Communication 5:4047).

Conclusion: A CRISPR-Cas9 based genome editing strategy successfully generated a VSX2 reporter cell line in hiPSCs. These cells will form the basis of high throughput assays to identify growth factors and growth conditions promoting RPE to NR reprogramming.
Nearly 20 million people in the United States suffer from some form of vision impairment. Over half of these people from a retinopathy, including macular degeneration and diabetic retinopathy. One promising source of treatment is regeneration from stem cells. Most organs have a pool of stem cells. These cells are crucial for healing after injury, maintenance of the organ, and regeneration capability. In the embryonic chick eye, retinal regeneration can be induced after complete removal with the addition of outside factors. Our lab has shown that fibroblast growth factor (FGF) is able to induce regeneration by activation of the ciliary margin (CM) as well as transdifferentiation of the retinal pigmented epithelium (RPE). Recently, we have also found that the antioxidant N-acetyl Cysteine (NAC) is also able to induce regeneration from the CM as well as transdifferentiation of the RPE. **Our hypothesis is that NAC is able to induce regeneration in the embryonic chicken via the glutathione pathway.** We explored three key roles of NAC in the cell: its role as a free radical scavenger, a precursor of glutathione, and its thiol-disulfide exchange activity. Regeneration was measured via histology. Cell markers were measured using immunohistochemistry. We found that NAC is able to induce regeneration via an increased pool of progenitor cells marked by coexpression of Chx10 and Pax6, a marker for retinal progenitor cells. There is a delay in cell differentiation when compared to developmental eyes as well as eyes treated with FGF. We found that activation of the GSH pathway is not essential for regeneration. Instead, regeneration is modulated through the MAPK pathway independent of the FGF receptor.
A Porcine Model for Repair of Segmental Long Bone Defects Using Three-Dimensionally Printed Scaffolds

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Purpose: Approximately 10% of bone injuries in the United States result in non-union, costing an estimated $10.4-26.1 billion annually for treatment. While there are several orthopedic strategies to address non-unions in long bones, all have drawbacks and risks of complications. To advance new tissue engineering treatments that are improvements over current options, we have developed a porcine long bone segmental defect model for testing osseous non-union repair using 3D printed biodegradable scaffolds.

Methods: A 3.5 cm mid-diaphyseal defect was generated in the radius of the right forelimb in 6-month old pigs. A polycaprolactone (PCL) scaffold implant designed from radius CT scans and produced using solid form fabrication was implanted into the defect and stabilized with a stainless steel plate.

Results: Animals are able to stand and place weight on the limb at six hours post-surgery, and ambulating without a noticeable limp by one month. CT imaging post-surgery show bone fill in the defect space by 2 weeks, significant defect closure by 8 weeks, and full union by six months. This healing has taken place on a PCL scaffold with no other treatments in conjunction with the implant.

Conclusions: The porcine radius segmental defect model provides a viable platform for testing printed scaffolds and biological composites for efficacy of healing segmental long bone defects.
Development and Characterization of a Model for Studying Retinal Regeneration in Larval Zebrafish
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Purpose: The cellular changes that occur during retinal regeneration in adult zebrafish have been well characterized. Immediately following damage, Muller glia respond with an upregulation of the intermediate filament GFAP, a key indicator of reactive gliosis. This phenotype persists even as the Muller glia reenter the cell cycle. However, as large numbers of retinal progenitors are established, Muller glia downregulate GFAP and switch from a gliotic phenotype to a regenerative phenotype. Interestingly, administration of 5-Fluorouracil (5-FU), which inhibits Muller glial cell proliferation, results in the Muller glia remaining persistently reactive. This phenotype mimics the mammalian response to damage and suggests that we can model the mammalian gliotic phenotype in zebrafish to screen for compounds that may overcome the gliotic response. However, performing these studies with adult zebrafish is not cost-effective due to the large volumes of drugs that would be needed. Therefore, this study aims to 1) develop an intense light-damage model using larval zebrafish, 2) characterize the regenerative potential of the larval fish retina, and 3) determine whether 5-FU also inhibits Muller glial proliferation and regeneration in the larval fish.

Method: To induce photoreceptor damage and regeneration, larval albino zebrafish (14-20 days post-fertilization) were exposed to intense light based on the modification of an existing protocol, but reduced in size to accommodate the larval fish. Fish were light treated for up to 4 days and tissue was harvested and processed for immunohistochemical markers of photoreceptors and proliferation. In a separate experiment aimed at determining whether larval animals regenerated new photoreceptors, BrdU incorporation was used to tag proliferating Muller glia and their progenitors, and then the fish were allowed to grow for either 15 or 60 days. Finally, larval fish were exposed to either 5-FU or control media starting at 1 dpL and analyzed for proliferation at 3 dpL. In all cases, quantification of immunolabeled cells was performed on captured images and statistical differences between groups was determined using a student’s T-test.

Results: The modified light treatment protocol resulted in damage to both rod and cone photoreceptors and a robust proliferation response that increased from 1 to 4 dpL. BrdU incorporation confirmed that progenitor cells differentiated into photoreceptors by 15 dpL. Finally, we showed that 5-FU inhibited the regeneration response in larval fish.

Conclusions: Collectively, these findings have laid the foundation for future experiments to screen for molecules that overcome the inhibition of 5-FU.
Understanding urodele limb regeneration in the context of tetrapod limb development

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Purpose: Pattern formation during embryonic development is an intriguing phenomenon wherein undifferentiated cells assemble into higher order tissue assemblages within a spatiotemporal framework. While the tetrapod limb has been an important model for understanding the cellular and molecular basis for pattern formation during development, the control of pattern formation during limb regeneration is poorly understood. The urodele *Ambystoma mexicanum* depicts close to perfect limb regeneration via epimorphosis and pattern formation is a key aspect of this process. Interestingly, gene expression patterns suggest that limb development in urodeles may differ substantially compared to other tetrapods. Additionally, urodele limb development appears to follow an alternate route compared to other tetrapods in terms of ossification pattern, presence of an ectodermal signaling center and some gene expression topology. This begs the question as to whether similar developmental mechanisms are operating in urodeles and other tetrapods during limb development and whether these differences can partly explain regenerative ability in salamanders and newts?

Methods: Using developing and regenerating *Ambystoma mexicanum* limbs, this study examines the spatiotemporal expression pattern of key genes known to control patterning and growth during mouse and chick limb development. Whole mount *in-situ* hybridizations (WISH) for *Fgf8*, *En-1*, *Rfng*, *Bmp4*, *Wnta5a* and *Lmx1* were done on regenerating forelimbs at early blastema, late blastema, cone/palette, early digit condensation and late digit condensation stages. We used real time PCR to substantiate (using paired t-test) and quantify the observed spatial expression patterns in regenerating limbs. Cell migration pattern during regeneration was analyzed partially using posterior GFP transplants.

Results: Gene expression patterns in axolotls during limb regeneration stand apart from the general vertebrate limb development in most of the cases presented here. Classical ectodermal markers like *En-1*, *Fgf8* and *Rfng* show mesenchymal expression during axolotl limb regeneration. Spatial restrictions like ventral expression of *En-1* and apical ectodermal ridge expression of *Fgf8* is not seen during regeneration. *Bmp4*, *Wnt5a* and *Lmx1* show interdigital expressions. Real time validations for *En-1* and *Fgf8* do not show spatial restriction either. Cell tracking during limb regeneration using posterior GFP half cuffs show posterior restriction of GFP positive cells.

Conclusions: Different models for limb regeneration talk about the regional restriction of cellular clusters, which when disturbed leads to malformations or no regeneration. This study looks into specific regional gene expression patterns and cellular clustering during axolotl limb regeneration. Robust epidermal and mesenchymal expression of some of these genes during the very early stages of regeneration reveals the widespread outburst of active gene transcription. There is a burst of overlapping gene expression and then disharmony with tetrapod limb development for a number of genes. Unlike most tetrapods, axolotls show an anterior-posterior and dorsal-ventral balance in terms of few gene expression patterns. Cellular clustering sets in at a very early stage (10 dpa according to the present study). GFP labeled posterior cells do not cross the anterior-posterior boundary and maintain regional loyalty.
Sensory hair cells that detect sound require numerous hair-like protrusions known as stereocilia. It is crucial that the stereocilia length is maintained for the entire life of an organism to sustain hearing ability. The length of a stereocilium depends on the regulation of its actin core, which is a bundle of exceptionally stable parallel actin filaments. The actin filaments are heavily crosslinked by several proteins including fascin-2, an abundant actin crosslinking protein that is critical for stereocilia length maintenance. To better understand stereocilia homeostasis, we sought to measure the dynamics of fascin-2 using a novel transgenic mouse where GFP-fascin-2 expression is induced by cre recombinase. Overexpression of fascin-2 caused a slight increase in stereocilia length, but did not otherwise perturb stereocilia morphology or auditory performance. We assessed GFP-fascin-2 dynamics in stereocilia of hair cells from explants of early postnatal transgenic mice by measuring fluorescence recovery after photobleaching (FRAP). GFP-fascin-2 fluorescence recovered over a timecourse of a few minutes by uniformly incorporating along the entire length of stereocilia. In addition, fluorescence recovery was faster in the stereocilia of outer hair cells (OHCs) than that of inner hair cells (IHCs), perhaps because the former possess stereocilia of shorter length and smaller diameter compared to the latter. These results contrast with photobleaching of GFP-actin, which did not recover fluorescence along the stereocilia length but instead recovered only at the tips over hours long timecourse. Therefore, actin and the actin crosslinker fascin-2 have markedly different behavior in stereocilia with fascin-2 being turned over much more rapidly compared to actin filaments.
Characterization of miR-133 during zebrafish photoreceptor development, maintenance, and regeneration
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Purpose: MicroRNAs (miRNA) are small RNA molecules that lower gene expression by binding to target sequences in the 3' UTR of certain mRNAs. miRNAs are predicted to be involved in the expression of thousands of genes, but their role in tissue regeneration has not been thoroughly characterized. Previous reports demonstrated a role for miR-133 in the regeneration of the adult zebrafish caudal fin and cardiac muscle, suggesting a potential universal role for miR-133 in tissue regeneration. Therefore, this study aimed at determining the function of miR-133 during adult photoreceptor regeneration in zebrafish. Its role in photoreceptor development and maintenance was also examined.

Methods: Two transgenic lines were utilized to induce the over- and under-expression of miR-133 under the heat-shock promoter hsp70. Wild-type (AB) animals were used as controls. Tg(hsp70:miR-133sp) animals possess a series of miR-133 binding sites that act as a "sponge" to reduce existing miR-133 levels. In contrast, Tg(hsp70:miR-133pre) allows for the over-expression of miR-133. We utilized these two lines to reveal the role of miR-133 during photoreceptor development, maintenance, and regeneration in zebrafish. In each case, retinal tissue was harvested at various time points and processed for immunohistochemical localization of specific cell markers.

Results: Our preliminary results indicated that loss of miR-133 during retinal development did not effect rod or cone cell differentiation. However, loss of miR-133 immediately prior to phototoxic light damage to adult retinal photoreceptors resulted in an early onset of photoreceptor apoptosis. In turn, this initiated a significantly increased regeneration response earlier than control retinas. In contrast, sustained high levels of miR-133 expression resulted in a delay in photoreceptor cell death and a decreased regeneration response. These data raise the possibility that sustained high levels of miR-133 are neuroprotective to photoreceptors. We therefore tested whether miR-133 was required for long-term photoreceptor maintenance, but found no differences in photoreceptor number following 60 days of sustained over- and under-expression of miR-133.

Conclusion: Together, these data indicate that miR-133 is not required for photoreceptor development or maintenance, but plays an early neuroprotective role following photoreceptor damage.
Bardet-Biedl Syndrome (BBS) is a rare autosomal recessive genetic disorder caused by the mutations in any of the 20 currently known disease associated genes. BBS patients present with several clinical features including hyperphagia associated obesity, type II diabetes, and high blood pressure. Data from several model systems indicate a role for the BBS proteins in primary cilia signaling. Cilia are microtubule based cellular appendages known to serve as signaling centers in development and adult tissue homeostasis. One signaling pathway which requires a functioning cilium in mammals is the hedgehog pathway. Cilia mediated hedgehog signaling is critically important for several developmental processes such as neural tube patterning and limb development. However, little is known about the roles for cilia mediated hedgehog signaling in the adult brain. Here we use a transgenic allele to assess hedgehog pathway activity in the developing and adult hypothalamus of control and BBS mutant mice. Preliminary results suggest that in preobese BBS mutant mice there is an increased amount of reporter activity in the ventromedial (VMH) region of the hypothalamus. Future studies will determine the levels of hedgehog reporter activity in development and upon adult onset obesity to determine if altered cilia mediated hedgehog signaling plays an impact on adult feeding behaviors.