

REGEN 2017

Inspiring new approaches in regenerative medicine

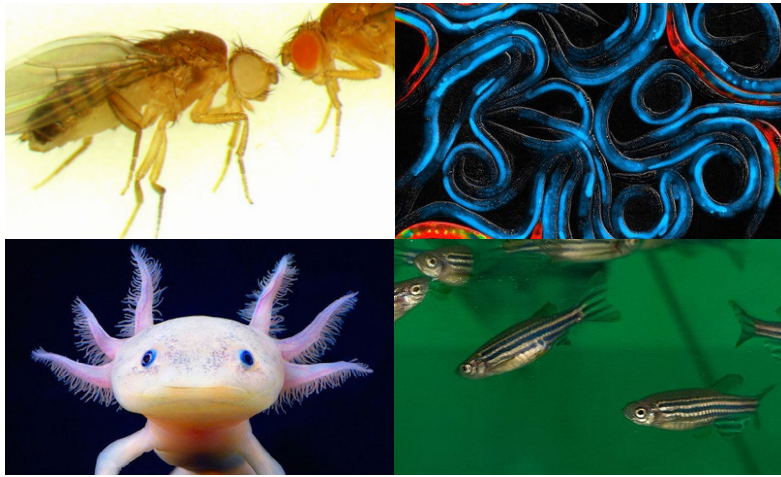


Learning from Nature: Comparative Biology of Tissue Regeneration and Aging

August 4-6, 2017
COBRE Symposium

MDI Biological Laboratory

COBRE



MDI Biological Laboratory Center of Biomedical Research Excellence in Regenerative Biology and Medicine

The Center of Biomedical Research Excellence (COBRE) grant was awarded to the MDI Biological Laboratory in September 2013 through funding from the National Institute of General Medical Sciences (NIGMS). The COBRE grant builds upon and expands the Institution's unique scientific expertise, providing support for junior scientists as they establish independent, extramurally funded research programs.

The Center focuses on defining the cellular and molecular mechanisms that make healing and regeneration possible and those that contribute to aging. Our work has the potential to improve the treatment of a broad range of injuries and diseases, including spinal cord injury, cancer, diabetes, and heart disease, as well as neurodegenerative diseases such as Alzheimer's, Parkinson's, and Huntington's diseases.

The ultimate goal of the research funded by the COBRE award is to develop therapies that will improve healing and regeneration in humans and slow the degenerative changes that occur with aging.

Keynote Speaker



Andrew Dillin, Ph.D University of California, Berkeley

Dr. Andrew Dillin is a Howard Hughes Medical Investigator and the Thomas and Stacey Siebel Distinguished Chair in Stem Cell Research at the Department of

Molecular and Cell Biology at Berkeley. He received his Bachelor of Science degree in Biochemistry from the University of Nevada, Reno, and then did his graduate thesis work with Professor Jasper Rine at UC Berkeley. After receiving his Ph.D., he was a Postdoctoral Fellow with Professor Cynthia Kenyon at UCSF, where he identified several of the determinants that regulate longevity in the nematode *C. elegans*. From there he spent several years at the Salk Institute for Biological Studies in La Jolla, California, as the Director of the Glenn Center for Aging Research, before a more recent move back to UC Berkeley.

Andrew's research focuses on the role of protein folding and maintenance in aging and neurodegenerative disease. He discovered that reduced insulin/IGF-1 signaling suppressed the toxic effects of human beta-amyloid—the protein linked to Alzheimer's disease—in worms. Even more intriguing, his lab found that the insulin/IGF-1 signaling pathways regulate two key proteins that influence how the cell handles toxic jumbles of proteins called aggregates: One protein breaks them up, while the other sequesters them in even bigger clumps. As organisms age, they lose the ability to manage the accumulation of toxic protein aggregates, so Andrew's lab is working to understand this process in hopes of finding clues that one day will help patients with neurodegenerative disease.

Schedule of Events

FRIDAY, AUGUST 4, 2017

3:00-5:00 PM

SYMPOSIUM REGISTRATION

Maren Conference Center

5:00-6:00 PM

DINNER

MDI Biological Laboratory Dining Hall

6:00 PM

KEYNOTE ADDRESS

Maren Auditorium

Introduction: Aric Rogers, Ph.D., MDI Biological Laboratory

Keynote: Andrew Dillin, Ph.D., University of California, Berkeley

7:00-9:00 PM

EVENING RECEPTION

Maren Conference Center

SATURDAY, AUGUST 5, 2017

7:00-8:15 AM

BREAKFAST

MDI Biological Laboratory Dining Hall

8:30 AM-12 PM

MORNING SESSION: REGENERATIVE GENE NETWORKS AND NEUROREGENERATION

Maren Conference Center

Session Chair: Jim Coffman, Ph.D., MDI Biological Laboratory

8:30

Aric Rogers, Ph.D., MDI Biological Laboratory
Opposing alterations in organismal growth, longevity, and resilience to stress in response to reduced mRNA translation in *C. elegans*

9:00

Eric Rottinger, Ph.D., Institute for Research on Cancer and Aging, Nice (IRCAN)
Regeneration is a partial redeployment of the embryonic gene regulatory network

Schedule of Events

SATURDAY, AUGUST 5, 2017

9:30

Nipam Patel, Ph.D., University of California, Berkeley
Germline regeneration in the crustacean, *Parhyale hawaiensis*

10:00

Sol Maria Pose Mendez, Ph.D., Technische Universität Braunschweig
Purkinje cell regeneration in the zebrafish cerebellum

10:15-10:45

BREAK

10:45

Xianmin Zeng, Ph.D., Buck Institute
Human iPSC-based disease modeling & drug screening for neurodegenerative disorders

11:15

Mitra Amiri Khabooshan, M.S., Australian Regenerative Medicine Institute
The role of roof and floor plate cells in zebrafish spinal cord regeneration

11:30

Ivanna Mayorenko, Ph.D. Student, Karolinska Institute
Inter-species comparison study of injury induced neurogenesis and tissue recovery after neuronal injury

11:45

Michael Orr, Ph.D. Student, University of Kentucky
Spiny mouse potential for spinal regeneration

12-1:30 PM

LUNCH

MDI Biological Laboratory Dining Hall

1:30-4:30 PM

AFTERNOON SESSION: STEM CELLS AND WOUND REPAIR

Maren Conference Center

Session Chair: Vicki Losick, Ph.D., MDI Biological Laboratory

Schedule of Events

SATURDAY, AUGUST 5, 2017

1:30

Voot Yin, Ph.D., MDI Biological Laboratory
The protein tyrosine phosphatase 1B inhibitor MSI-1436 stimulates regeneration of heart tissues in adult systems

2:00

Andrea Page-McCaw, Ph.D., Vanderbilt University
Multiple mechanisms drive calcium dynamics around laser-induced epithelial wounds

2:30

Jessica Sawyer, Ph.D., Duke University
Coordinating injury responses at an organ boundary

2:45

Pranidhi Sood, Ph.D., University of California, San Francisco
Transcriptional dynamics of single-cell regeneration in the ciliate *Stentor coeruleus*

3:00-3:15

BREAK

3:15

Heinrich Jasper, Ph.D., Genentech
Inflammation and immune modulation: tackling age-related stem cell dysfunction

3:45

William Jeffery, Ph.D., University of Maryland
Age related body and brain regeneration in Ascidian *Ciona intestinalis*

4:15

Susannah Helene Kassmer, Ph.D., University of California, Santa Barbara
Pou3-positive, blood borne cells are required for whole body regeneration in a basal chordate

4:30-7:30 PM

POSTER SESSION AND RECEPTION

Maren Conference Center
(Scan QR code for poster session abstracts)



7:30-9:00 PM

LOBSTER BANQUET

Dining Hall

Schedule of Events

SUNDAY, AUGUST 6, 2017

7:00-8:15 AM

BREAKFAST

MDI Biological Laboratory Dining Hall

8:30-11:15

MORNING SESSION: HEART AND LIMB REGENERATION

Maren Conference Center
Session Chair: James Godwin, Ph.D., MDI Biological Laboratory/Jackson Laboratory

8:30

Guo Huang, Ph.D., University of California, San Francisco

Molecular control of heart regeneration in development and evolution

9:00

Kenneth Poss, Ph.D., Duke University

9:30

Thomas Lisse, Ph.D., The Jackson Laboratory
GDNF: A trophic factor for cutaneous wound healing and hair follicle regeneration in mice

9:45-10:15

BREAK

10:15

Anna Huttenlocher, M.D., University of Wisconsin
Imaging wound repair in zebrafish

10:45

Jessica LaMae Whited, Ph.D., Harvard Medical School
Identifying roadblocks to regeneration by repeat deployment of the limb regeneration program

11:15

MEETING ADJOURNS

Opposing alterations in organismal growth, longevity and resilience to stress in response to reduced mRNA translation in *C. elegans*

Snow, S.¹, Howard, A.², Rollins, J.¹, Kapahi, P.³, Rogers, A.N.¹

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Different forms of physiological stress result in adaptive changes in gene expression in order to maintain homeostatic balance within cells and between tissues. Aging diminishes the ability to recover from perturbations in homeostasis and contributes to age-related disease. Inability to efficiently regulate protein homeostasis, or proteostasis, is considered a causative factor in protein conformational diseases like Alzheimer's and Parkinson's. An intervention with the potential to slow age-related decline involves adaptation to low nutrient availability, which alters gene expression, including the restriction and redirection of mRNA translation. Genetically attenuating translation ameliorates stress caused by unfolded proteins. We recently discovered that it also results in priming of the heat shock response in *C. elegans*, a molecular mechanism that enhances proteostatic maintenance through HSF-1 (Howard et al., Aging Cell, PMID: 27538368, 2016). However, the role of different tissues to low translation state in systemic health and organismal survival is not known. We found that lowering, but not abolishing, translation in either the hypodermis, germline, or neurons of nematodes increased lifespan, but had no effects or negative effects on lifespan when lowered in muscle or intestine. Furthermore, experiments in muscle-based proteotoxicity models indicated that lowering translation directly in muscle had only a small protective effect compared to lowering translation in tissues that increased lifespan. Interestingly, attenuating translation in muscle actually sped up development and increased fecundity. Results indicate that low translation state trade-offs between negative effects on development and positive effects on longevity can be uncoupled at the level of specific tissues. Furthermore, results suggest that organismal resources are redistributed upon lowering of translation through a coordinated but otherwise uncharacterized mechanism.

Regenerative Gene Networks + Neuroregeneration Abstracts

Regeneration is a partial redeployment of the embryonic gene regulatory network

Warner, J., Amiel, A., Röttinger, E.

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Regenerated body parts are essentially identical to the parts that were developed during embryogenesis. This observation is at the origin of a century old hypothesis proposing that regeneration utilizes developmental processes originally set aside for embryonic development. If this is true, the genetic interactions driving these two processes are predicted to be largely overlapping. In order to address this hypothesis, we used the sea anemone *Nematostella vectensis* that is perfectly suited to compare the gene regulatory networks underlying embryogenesis and regeneration. After characterizing in detail the regenerative capacity and the principal events underlying oral regeneration of *Nematostella*, we performed a high-resolution temporal RNAseq time-course spanning this process and compared it to embryonic RNAseq data. Combined with molecular analysis obtained from signaling pathway perturbation experiments, we show that regeneration is a partial and rewired re-deployment of the embryonic GRN rather than a complete recapitulation of the embryonic program.

Germline regeneration in the crustacean, *Parhyale hawaiensis*

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The amphipod crustacean, *Parhyale hawaiensis*, derives its primordial germ cells from a single precursor cell at the eight-cell stage. If this cell is ablated, the animal hatches without a detectable germline as assayed by morphology and the expression of *vasa* and *piwi*. Remarkably, however, these animals are fertile as adults, and in these ablated animals germline cells reappear about halfway through juvenile development. The progeny produced by germline replacement appear to be normal, at least in the lab environment. We have now used somatic transgenesis to determine the source of this replacement germline and find that it derives from mesoderm. We suggest that *Parhyale* possesses both a maternal mechanism involving cytoplasmic

Regenerative Gene Networks + Neuroregeneration Abstracts

localization to specify germline in the embryo, as well as a later inductive mechanism that acts as a backup system.

Purkinje cell regeneration in the zebrafish cerebellum

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Purkinje cells (PCs) are of special interest for regeneration studies, as they integrate all cerebellar information. With the help of a PC specific regulatory element we have established a cell ablation system mediated by tamoxifen-inducible apoptosis (PC-ATTACTM). Unlike localized wounding experiments, this conditional system has the advantage of not being counteracted by plasticity processes involving remaining PCs. Regenerative recovery of about half of the PC population occurs within about 10 days and can be monitored directly *in vivo* in the transparent zebrafish larvae. Immunohistochemistry revealed that during regeneration no rapid proliferation occurs, but rather a slight, long-lasting increase of cell proliferation, with sources of new cells being located in a median and lateral domain close to the newly arising PC pool.

This *in vivo* tracking is of special relevance to figure out how a single neuronal type can be restored, and reestablishes the circuitry of a whole brain compartment.

Funding: European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 703961.

Human iPSC-based disease modeling & drug screening for neurodegenerative disorders

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Human induced pluripotent stem cell (iPSC) technology offers the benefits of a cell line coupled with the advantage of using human primary cells. We have developed a large panel of iPSC lines for disease modeling and

Regenerative Gene Networks + Neuroregeneration Abstracts

drug discovery. These include: 1) normal and isogenic control lines, 2) patient-specific lines, 3) reporter lines. We have also established scalable protocols for generating differentiated neural cells in an assay ready format. I will discuss the utility of these lines for modeling Parkinson's disease and Alzheimer's disease as well as for neurotoxicity/neuroprotective assays.

The role of roof and floor plate cells in zebrafish spinal cord regeneration

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In mammals, spinal cord injury (SCI) results in permanent functional impairments due to axonal damage and neuronal loss. However, zebrafish have a robust ability to regenerate CNS tissue including spinal cord after injury. Ependymal cells lining the central canal of the zebrafish spinal cord are recruited after injury and they replenish lost tissue. Noteworthy, spinal cord and its cells are distinctly patterned through the dorsal-ventral (D-V) axis and it is not known how D-V identity of regenerated tissue is established after SCI. In this study, by using label retention assay we showed that ependymal cells located in the dorsal and ventral poles of the central canal of zebrafish spinal cord become quiet early in development and are maintained until adulthood. Proliferating Cell Nuclear Antigen (PCNA) immunostaining on the cross sections of zebrafish spinal cord showed that these label retaining cells can be occasionally proliferative, demonstrating that they are slow proliferating cells. Furthermore, we showed that these dorsal and ventral label retaining cells emerge from the roof and floor plate cell populations, respectively. Next we investigated the behaviour of the roof and floor plate cells in zebrafish spinal cord regeneration using an established larval spinal cord injury model. Our results suggested that the roof and floor plate cells regenerate their own population rather than originating other cell types. The unique dorsal and ventral positions of the label retaining ependymal cells and the fact that these cells are not multipotent progenitors, suggest that they may act as signalling centres to maintain the D-V identities of cells around the central canal during homeostasis or to re-establish this identity after injury. To determine the role of these cells in redefining of the D-V identity after injury, we are using laser and nitroreductase (NTR) genetic cell

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ablation of the roof and floor plate cells in our larval spinal cord injury model.

Inter-species comparison study of injury induced neurogenesis and tissue recovery after neuronal injury

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Functional regeneration of the central nervous system (CNS) after injury occurs in some vertebrates but not others. The sole presence of neural stem and progenitor cells within the tissue does not determine regenerative capacity, therefore both the tissue environment and the cellular response to the injury must also be considered. We investigated the process of neurogenesis and regenerative competence of closely related amphibians, as newts retain their ability to fully regenerate brain tissue as adults, while regeneration in frogs is lost after metamorphosis. We assess CNS regeneration mechanism in terms of the post-injury immune response and glial tissue environment using ischemic stroke and Parkinson's brain injury models. Through this study we expect to identify a path for modulating the activity of endogenous precursor cells in post-injury microenvironment for enhanced neurogenesis and regeneration.

Spiny mouse potential for spinal regeneration

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Following spinal cord injury (SCI) certain non-mammalian species and neonatal mammals are able to regenerate damaged neural pathways and regain functionality. Unfortunately, humans and rodents commonly used to experimentally model SCI do not exhibit central nervous system (CNS) regeneration. In order to identify key aspects of regeneration, researchers use comparative biology to identify differences between these regenerating and non-regenerating systems but large developmental and phylogenetic differences create a barrier to translation. Recently, spiny mice (genus *Acomys*) have been identified for their regenerative capabilities. Specifically, unique spiny mouse extracellular matrix and

Stem Cells and Wound Repair Abstracts

immune responses following injury result in reliable scar-free regeneration in peripheral organ systems, including substantial axon regrowth (Seifert et al., 2012) (Gawriluk et al., 2016). Both the extracellular matrix response and inflammation have been indicated as important barriers for regeneration after CNS injury, yet the CNS regenerative capabilities of spiny mice remain untested. We hypothesize that the unique spiny mouse injury response will lead to CNS regeneration following spinal cord injury. We will use immunohistology, confocal microscopy, and flow cytometry to identify the distribution, timing, and composition of the cellular and extracellular response to SCI. Additionally, we will determine the anatomical and functional recovery from injury using MRI, histology, and motor and sensory testing. Markers of cell proliferation and axonal track tracing will be used to determine the extent of CNS regeneration. Collectively, we will investigate the cellular and extracellular responses, anatomical and functional recovery, and axon regeneration in spiny mice following SCI, thereby identifying the potential for spiny mice as an adult mammalian model of CNS regeneration.

Multiple mechanisms drive calcium dynamics around laser-induced epithelial wounds

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When an epithelial sheet is wounded, a broad region of stationary cells become invasive and motile to close the wound. However, the mechanism that alerts these cells to a nearby wound is unclear. To investigate this mechanism we analyze the earliest response to an epithelial wound, namely a dramatic increase in cytosolic calcium that occurs in a broad region of cells. Our approach combines the highly tractable genetics of *Drosophila*, reproducible laser ablation, live imaging of a GCaMP calcium reporter, and a highly quantitative approach including computational analysis and modeling. We find that multiple mechanisms contribute to calcium dynamics of a single wound, including the entry of extracellular calcium through torn membranes proximal to the wound and a lysate-triggered signaling pathway in more distal cells. We hypothesize that

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these multiple mechanisms ensure that cells can detect and appropriately respond to a variety of wound types.

The protein tyrosine phosphatase 1B inhibitor MSI-1436 stimulates regeneration of heart tissues in adult systems

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Regenerative medicine holds substantial promise for repairing or replacing tissues and organs damaged by disease, injury and degeneration. Much of the field has focused on development of cell-, gene- and tissue engineering-based therapeutics. In contrast, development of small molecule regenerative medicine therapies is an emerging area. Using the adult zebrafish as a novel screening platform, we identified MSI-1436 as a first-in-class regenerative medicine drug candidate. MSI-1436 is a naturally occurring aminosterol that inhibits protein tyrosine phosphatase 1B. Treatment of adult zebrafish by intraperitoneal (IP) injection of MSI-1436 increased the rate of regeneration of the amputated caudal fin, which is comprised of bone, connective, skin, vascular and nervous tissues and also increased the rate of adult zebrafish heart regeneration. IP administration of MSI-1436 to adult mice for 4 weeks after induction of myocardial infarction increased survival, improved heart function, reduced infarct size, reduced ventricular wall thinning and increased cardiomyocyte proliferation. Satellite cell activation in injured mouse skeletal muscle was stimulated by MSI-1436. MSI-1436 was well tolerated by patients in Phase 1 and 1b obesity and type 2 diabetes clinical trials. Doses effective at stimulating regeneration are 5- to 50-times lower than the maximum well tolerated human dose. The demonstrated safety and well established pharmacological properties of MSI-1436 underscore the potential of this molecule as a novel treatment for heart attack and multiple other degenerative diseases.

Stem Cells and Wound Repair Abstracts

Coordinating injury responses at an organ boundary

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Injury at organ boundaries presents a challenge, as cells on either side of the boundary have distinct identities, and disruption of cell fate can lead to disease. We used the adult *Drosophila* midgut/hindgut boundary as a model to investigate coordination of injury repair between two organs with distinct developmental origins and tissue repair responses. We identified a specific population of adult midgut organ boundary intestinal stem cells (OB-ISCs) that is regulated by proximity to a specialized transition zone that shares molecular signatures of both midgut and hindgut organs, which we term the hybrid zone (HZ). During homeostasis, the HZ restrains OB-ISC proliferation. However, adult HZ/hindgut injury drives up-regulation of *upaired-3* cytokine and OB-ISC hyperplasia. If HZ disruption is severe, hyperplastic OB-ISCs expand across the inter-organ boundary. Our data suggest that inter-organ signaling is crucial in controlling OB-ISCs in homeostasis and injury repair.

Divergent effects of high fat diet on intrinsic and synaptic excitability in AgRP neurons

Transcriptional dynamics of single-cell regeneration in the ciliate

Stentor coeruleus

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Repair at the single cell scale is critical for an organism's wound healing response; however, little is known about regeneration at the level of an individual cell. We are developing a unicellular model, *Stentor coeruleus*, for studying regeneration. This giant ciliate has incredible regenerative abilities: almost any excised portion of the cell will give rise to a normally proportioned cell with intact subcellular organization. Early studies

Stem Cells and Wound Repair Abstracts

elucidated the morphology of regeneration (Morgan, 1901; Tartar, 1961), but little about the molecular basis of *Stentor's* regenerative abilities. We have recently published the genome of *Stentor* (Slabodnick et. al, 2017). Using RNA-seq to study the transcriptional dynamics underlying regeneration of a key highly ciliated organelle in the cell, we find highly conserved genes involved in centriole production. This detailed time course, in combination with RNAi manipulations, will help elucidate the fundamental principles of regeneration and healing at the scale of a single cell.

Inflammation and immune modulation: tackling age-related stem cell dysfunction

Heinrich Jasper

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In aging and diseased tissues, regeneration and regenerative therapies are limited by stem cell dysfunction and unfavorable tissue environments. Promising strategies to improve success include interventions that enhance stem cell function and that harness and boost endogenous tissue repair mechanisms. We study stem cells and tissue repair in barrier epithelia and the retina of *Drosophila* and mice to explore the causes and consequences of age-related regenerative dysfunction. These studies have led to the discovery of interventions targeting age-related inflammation, stem cell proliferation, stem cell metabolism, innate immune responses, and the commensal microbiota as strategies to enhance regeneration and extend lifespan. I will discuss these strategies and provide perspectives for the development of targeted interventions to improve tissue function in the elderly. I will highlight strategies to improve stem cell activity by targeting endogenous proliferation, differentiation and nutrient response pathways, and strategies to improve tissue repair by modulating innate immunity and host/commensal interactions. Combining such strategies is likely to significantly improve tissue homeostasis and regenerative therapies in the elderly, ultimately extending the healthy years of life.

Heart and Limb Regeneration Abstracts

Age Related Body and Brain Regeneration in the Ascidian *Ciona intestinalis*

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In the ascidian *Ciona intestinalis*, the body siphons and brain are capable of complete regeneration. Stem cell markers, cell proliferation indicators, and transplantation methods were used to study the location and behavior of stem cells involved in regeneration during the *Ciona* adult life cycle. The branchial sac, a pharyngeal organ, contain stem cell niches that supply proliferating progenitor cells to the regenerating siphons and brain following their extirpation. Wounding rather than removal of these organs did not induce stem cell activation, indicating that the stem cell niche shows specificity for regeneration. The rate and completeness of regeneration decreases with age, and regenerative capacity eventually disappears entirely in old age. The decline in regeneration capacity during the life cycle may be caused by the depletion and/or reduction of potency of stem cells in the branchial sac stem cell niche.

Pou3-positive, blood borne cells are required for whole body regeneration in a basal chordate

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Colonial ascidians such as *Botryllus schlosseri* are able to regenerate entire new bodies from nothing but fragments of vasculature. The specific cell types responsible for this regenerative growth are unknown. During the early stages of regeneration, cell proliferation occurs only in small, blood borne cells. Ablation of proliferating cells with Mitomycin C (MMC) leads to regeneration failure. In MMC treated animals, regeneration can be restored by injection of proliferating cells from an untreated animal. Small, proliferating cells in the blood express pou3 as well as vasa, and upon injury, appear to accumulate in distinct areas within the remodeling

Heart and Limb Regeneration Abstracts

vasculature. SiRNA mediated knockdown of pou3 leads to regeneration failure, indicating that pou3 expression in proliferating cells is required for their function during whole body regeneration. We hypothesize that pou3/vasa positive cells are blood borne stem cells, and are currently testing the contribution of pou3-positive stem cells to regenerating tissues.

Molecular control of heart regeneration in development and evolution

Guo Huang

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Robust tissue regenerative potential is widespread in many invertebrates, lower vertebrates, and even higher vertebrates of early developmental stages. It remains enigmatic why such a seemingly beneficial trait is lost in animal development and evolution. We use the heart as a model system to investigate the fundamental principle governing tissue regeneration. Our recent chemical screens in newborn mice and comparative analyses of cardiomyocytes across phylogeny converge on the importance of the endocrine system in triggering cardiomyocyte regenerative potential loss accompanied with thermogenesis increase in most mammals. Furthermore, our study suggests that long-lived mammalian species with low metabolism and body temperature may retain significant yet previously unknown cardiac regeneration capability. Altogether, our findings support a novel model of hormonal controlling cardiac regenerative capacity during the poikilotherms-to-homeotherms transition, and implicate the possible existence of remarkable regenerative capacities in some mammals of extreme longevity as an unappreciated anti-aging mechanism.

GDNF: A trophic factor for cutaneous wound healing and hair follicle regeneration in mice

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In mice and humans, postpartum wound repair in skin is associated

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with scar formation that prevents full tissue regeneration, including the regeneration of adnexal structures such as hair follicles. Recent findings have elaborated on the importance of hair follicles, which are regarded as key “purveyors” of scar-less skin regeneration after injury. Long-lived hair follicle bulge stem cells (BSCs) contribute to wound repair through neopidermal renewal, and factors derived from hair follicles themselves can promote the beneficial trans-differentiation of resident cells for proper tissue regeneration. Thus, the identification and understanding of factors that can promote wound-induced neogenic hair formation will be of clinical importance. Here we report on a previously unrecognized role of glial cell-derived neurotrophic factor (GDNF) toward hair and skin regeneration after injury in mice. We show that both recombinant GDNF and transgene overexpression of GDNF in the skin can promote wound closure, structural integrity of wound matrix and the formation of de novo hair follicles after 3-mm full thickness skin punctures. Furthermore, GDNF can initiate anagen (i.e. growth phase) and promote the regeneration of hair follicles after depilation. Given that members of the GDNF signaling apparatus, including co-receptors GFR 1 and RET tyrosine kinase, are expressed in keratin 15 (K15)-positive follicular BSCs, lineage-tracing and conditional knock-out studies were conducted utilizing K15+ cells. Using the K15-CrePR1:R26R-Confetti reporter mouse line in acute wound repair studies, we observed an increase in radial projections of multi-labeled progenitor cells from the hair bulge toward the neopidermis upon recombinant GDNF treatment. In conjunction with the findings that conditional ablation of Ret within K15+ BSCs (K15-CrePR1:Ret^{fllox/fllox}) led to impaired formation of anagen follicles following depilation, our results suggests that GDNF-RET signaling within BSCs is crucial for both hair and skin regeneration after injury. Besides the well-studied Wnt/ β -catenin signaling pathway, our findings show that the targeting of the GDNF signaling pathway within hair follicle stem cells may be considered for developing niche-based treatment options for both hair and skin regeneration following injury and disease.

Imaging wound repair in Zebrafish

Anna Huttenlocher
University of Wisconsin

Tissue injury can lead to scar formation or tissue regeneration. How regenerative animals sense initial tissue injury and transform wound signals into regenerative growth remains poorly understood. Previously,

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we found using zebrafish larval tail fins as a model, that wounding rapidly activated SFK downstream of early injury generated H₂O₂ in epithelia. The immediate reactive oxygen species and SFK in epithelia was important for late epimorphic regeneration of amputated fins. Using a new reporter of vimentin, we identify key upstream pathways that mediate vimentin expression along the wound edge. We also identify vimentin as an important pathway downstream of ROS and NF κ B signaling that mediates collagen expression at the wound and the formation of collagen projections during wound resolution of the zebrafish larval fin.

Identifying roadblocks to regeneration by repeat deployment of the limb regeneration program

Bryant, D.¹, Bryant, S.¹, Mariano, R.¹, Martinez Fernandez, J. 1, Payzin-Dogru, D.¹, Johnson, K.¹, and Whited, J.¹

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Axolotls are powerful models for understanding how regeneration of complex body parts can be achieved. We sought to determine if we could experimentally compromise the axolotl’s ability to regenerate limbs by repeated amputation and, if so, what molecular changes might underlie their inability to regenerate. Repeated limb amputation at the same site severely compromised the ability to regenerate. The data points to a defect in the initiation stage of regeneration. We compared global gene expression at three days post-amputation from compromised animals versus their sibling controls undergoing their first regenerative event. We identified transcripts aberrantly upregulated in compromised limbs as candidate antagonizers of regeneration. We demonstrated the sufficiency of one factor, *amphiregulin*, to antagonize normal regeneration in naive animals. We also discovered the inverse class of transcripts, which are more highly expressed in controls versus compromised animals. Future functional studies will leverage these genes in understanding the constraints on regeneration.

Poster Presenters

Alphabetical by presenting author

Utilization of the auxin-degradation system to eliminate P granules in *C. elegans*

Adkins, E., Sharp, C., Updike, D.

Axolotl tails as a model for regenerative angiogenesis

Montoro, R., Goss, C., Dickie, R.

Epicardium activation of *mmp13a* stimulates cardiomyocyte proliferation during adult zebrafish heart regeneration

Dykeman, C., Beauchemin, M. and Yin, V.P.

MiR-101 directs cross-talk between the epicardium and cardiomyocytes during zebrafish heart regeneration

FitzSimons, M., Beauchemin, M., Yin, V.

Elucidating the role of FGFR-4 in skeletal muscle homeostasis and regeneration

Galvis, L., Calhabeu, F., Marcelle, C.

Deciphering the wound repair strategy: cell growth vs division

Grendler, J. and Losick, V.

The loss of heparin sulphate editing enzyme Sulf1 reduces VEGF signaling and enhances endothelial glomerular injury and albuminuria

Masseli, A.K., Schroder, P.A., Beverly-Staggs, L.L., Schiffer, M., Müller-Deile, J., Haller, H.

Early life chronic stress programs the immune system via epigenetic and circadian regulation

Hartig, E., Gans, I., Zhu, S., Coffman, J.

Molecular mechanisms that generate muscle fibre type diversity during vertebrate evolution

Keenan, S. R., Ramialison, M., Currie, P. D.

Cardiac growth and coronary vessels development in a giant danio (*D. cf aequipinnatus*) heart

Lafontant, P.J.

Elucidating the effects of aging on muscle

Mason, E., Goody, M., Henry, C.

Epidermal damage as underlying cause of paclitaxel-induced peripheral neuropathy

Pellegrini, A., Bolduc, J., Dominy J., Rieger, S.

Girardia dorocephala transcriptome sequence, assembly, and validation through characterization of piwi homologs and stem cell progeny markers

Almazan, E.M.P., Lesko, S.L., Markey, M.P., Rouhana, L.

PQN-75 is secreted from the pharyngeal gland cells of *C. elegans* and is dispensable for germline development

Rochester, J., Tanner, P., Strange, K., Updike, D.

CRISPR-Based, Germline Specific Protein Overexpression and Visualization in *C. elegans*

Sharp, C., Updike D.

Let-7 regulation of TNF α activity stimulates heart tissue regeneration in adult zebrafish

Smith, AM., Dykeman, CA., Hohmann, A. and Yin, VP

Some cardiac and somatic parameters in zebrafish as tools for the evaluation of cardiovascular function

Vargas R., Vásquez I.



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2017 Regen Symposium

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