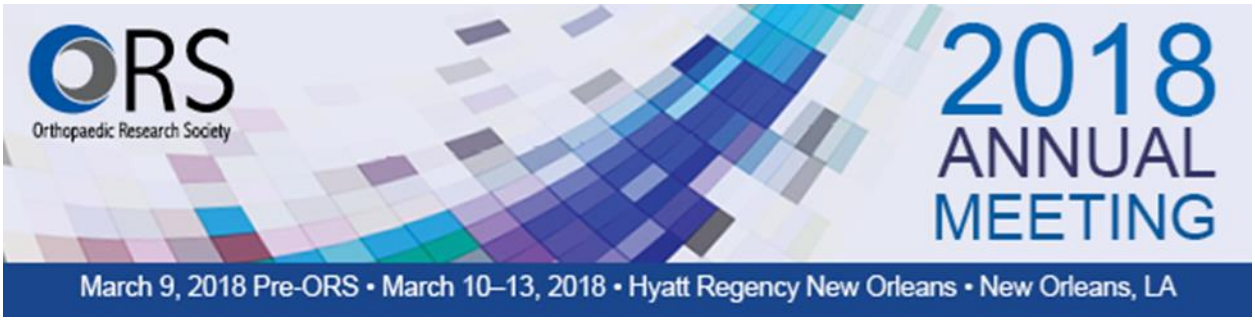

WORKSHOP
Limb Regeneration: What Can We Learn from Animal Models for Human Translation?

Organizers:
Jessica Lehoczky, PhD
Jessica Whited, PhD

Speakers:
Ken Muneoka, PhD
Thomas Lozito, PhD
S. Randal Voss, PhD

Limb Regeneration: What Can We Learn from Animal Models for Human Translation?

Nearly two million Americans currently live with limb amputations, and this number is rising due to increased prevalence of diseases that can require amputation (such as diabetes and peripheral artery disease). While prosthetic limbs are increasingly sophisticated, they are limited in key functions such as articulation and sensation. The ultimate goal of regenerative medicine in the context of orthopedics and limb loss is to stimulate limb regeneration in human patients. While humans have negligible innate composite tissue regeneration in limbs following injury, some non-human vertebrates have highly regenerative limbs/appendages. Basic research focused on regeneration in these species will lead to a molecular understanding of innate tissue renewal in vertebrates, and can ultimately be leveraged into translational research efforts. This workshop will introduce three model organisms currently used to gain a mechanistic understanding of limb/appendage regeneration in vertebrates. Axolotl salamanders will be highlighted as they spontaneously replace entire limbs following amputation. Cartilage regeneration during innate lizard tail regeneration will also be discussed. In addition, mouse digit tip regeneration will be presented, a form of mammalian appendage regeneration. Efforts to identify the molecular program behind these processes, as well as launching-off points for regenerative medicine, will be discussed.

Organizers:

Jessica Lehoczky, PhD
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Workshop Program:

8:00-8:10 --- Welcome and Introduction [Whited/Lehoczky]
8:10-8:30 --- "Blastema physiology and induced skeletal regeneration in mammals" [Muneoka]
3-5 minute Q&A
8:35-8:55 --- "Lizard tail regeneration as an instructive model of enhanced healing capabilities in an adult amniote" [Lozito]
3-5 minute Q&A
9:00-9:20 --- "Identifying transcriptional networks associated with appendage regeneration" [Voss]
5-10 minute Q&A and wrap-up

Invited Speakers:

Ken Muneoka, PhD
[Research Focus: Mouse digit tip regeneration]
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Blastema Physiology and Induced Skeletal Regeneration in Mammals

The mouse digit tip is the only part of the limb that displays regenerative ability, amputations at proximal level result in fibrotic healing and skeletal truncation. Blastema formation in digit tip regeneration is used as a model for regenerative competency, whereas non-regenerative digit amputation at the level of the sub-terminal phalanx (P2) has emerged as a test site for induced regeneration. BMP signaling is required for digit tip regeneration and BMP2 treatment stimulates endochondral ossification to regenerate the amputated portion of the P2 element (*Yu et al., BMP2 induces segment-specific skeletal regeneration from digit and limb amputations by establishing a new endochondral ossification center. Developmental Biology, 372 (2012) 263–273*). We now show that another BMP family member, BMP9, stimulates joint regeneration at the non-regenerative P2 amputation wound. This response involves regeneration of a distal skeletal element coupled with a cavity that forms an articulation with the P2 stump. Cells lining the regenerated cavity express *Prg4* identifying the cavity as synovial, and studies using the *Prg4* mutant demonstrates that *Prg4* is required for the response. The regeneration of the distal skeletal element initiates with a chondrogenic condensation that forms a nodule of cells expressing articular chondrocyte marker genes (*Col2a1*, *Fmod*, *Ucma*) and proteins (Acan and Dcx). The evidence indicates that BMP9 stimulates cells at a non-regenerative amputation wound to regenerate joint structures including a synovial cavity and a distal skeletal element lined with articular cartilage. Finally, we show that sequential treatment of the amputation wound with BMP2 and BMP9 stimulates the regeneration of stump bone that is contiguous with a synovial joint and a distal skeletal element. These findings demonstrate that a mammalian regeneration response can be stimulated and secondarily modified to control the polarity, continuity and complexity of the regenerated structures. Despite the non-regenerative nature of mammalian amputations, cells at the healing wound site retain the potential for regeneration of skeletal structures such as bone and joints when appropriately stimulated.

Supplemental Reading:

Dawson LA, et al., The periosteal requirement and temporal dynamics of BMP2-induced middle phalanx regeneration in the adult mouse. Regeneration, 4:140-150 (2017). [PMID: 28975034]

Dolan CP, et al., Digit tip regeneration: merging regeneration biology with regenerative medicine. Stem Cell Translational Medicine, Feb 5 (2018). [PMID: 29405625]

Thomas Lozito, PhD
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Lizard Tail Regeneration as an Instructive Model of Enhanced Healing Capabilities in an Adult Amniote

Lizards exhibit the remarkable ability to regenerate amputated tails, making them the closest relatives of mammals to display enhanced healing abilities as adults. Unlike salamanders, lizards are unable to regrow lost limbs, distinguishing lizards as the only vertebrates to combine regenerative tissues (i.e. tail) and non-regenerative tissues (i.e. limbs) in the same animal. These attributes specify lizards as extremely relevant model organisms for studying and manipulating adult regeneration. We have previously identified the lizard spinal cord as the critical source of signals responsible for patterning regenerated tails of Anolis lizards. In this study, we hypothesize that (1) lizard tail regeneration is induced by signals originating from the spinal cord, and (2) transplanting spinal cord pieces into amputated lizard limbs will improve healing. Towards investigating the regeneration-inductive activities of the lizard spinal cord, spinal cord pieces were removed and/or added to the stumps of amputated lizard tails. Spinal cord removal resulted in the complete loss of regeneration in amputated lizard tails, and transplantation of exogenous spinal cord pieces to tail stumps lacking endogenous spinal cords restored tail regeneration. Similarly, exogenous spinal cord pieces implanted as autografts within dorsal muscles of original tail portions induced normally structured ectopic regenerates at implantation sites, resulting in multi-pronged “forked” tails. Finally, exogenous spinal cord pieces implanted into the normally non-regenerative amputated hind limbs of lizards induced enhanced, yet tail-like, regenerates. Formation of ectopic tails was significantly diminished by co-treatment with Wnt and MMP inhibitors. In conclusion, this study has identified the lizard spinal cord as necessary and sufficient for inducing regeneration in a process involving hedgehog signaling. Furthermore, the signals produced by the lizard spinal cord are remarkably robust, capable of inducing regrowth in otherwise non-regenerative tissues. Future studies will test the abilities of lizard spinal cord-derived signals to enhance healing in mammals.

Supplemental Reading:

Lozito TP, et al., Lizard tail skeletal regeneration combines aspects of fracture healing and blastema-based regeneration. Development, 143:2946-57 (2016). [PMID: 27387871]

Londono R, et al., Cartilage and muscle cell fate and origins during lizard tail regeneration. Front Bioeng Biotechnol, 5:70 (2017). [PMID: 29164111]

S. Randal Voss, PhD
[Research Focus: Salamander limb regeneration]
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Identifying Transcriptional Networks Associated with Appendage Regeneration

Salamander regeneration has been studied for several hundred years, yet only recently has it become possible to dissect regenerative responses at a molecular level. In just the past 10 years, the primary salamander model, the Mexican axolotl (*Ambystoma mexicanum*), has seen expansion and enhancement of transcriptomic, genomic and transgenic resources, poising the axolotl to make new and significant contributions to stem cell biology and regenerative medicine. Still, there is need to pursue additional approaches that can rapidly lead to better understanding of signaling pathways that orchestrate regeneration. Here I show that axolotl embryos can be used as a chemical screening model to identify genes and signaling pathways that reveal novel and comparative insights about the molecular basis of tissue regeneration.

Supplemental Reading:

Ponomareva LV, et al., Using Ambystoma mexicanum (Mexican axolotl) embryos, chemical genetics, and microarray analysis to identify signaling pathways associated with tissue regeneration. Comp Biochem Physiol C Toxicol Pharmacol, 178:128-35 (2015). [PMID: 26092703]

Woodcock MR, et al., Identification of mutant genes and introgressed tiger salamander DNA in the laboratory axolotl, Ambystoma mexicanum. Scientific Reports, 7:6 (2017). [PMID: 28127056]