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Special Issue: Studying mechanisms of regeneration in amphibian and reptilian vertebrate models

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Regeneration, or the *de novo* formation of new organs, is observed in many different classes of vertebrates (reviewed in Alibardi, 2010). In teleost fishes, regeneration of heart muscle as well as the cartilage, nerves, and skin of tail fins have been characterized (reviewed in Poss, 2010; Tanaka and Ferretti, 2009). Anurans and urodele amphibians have both been the subject of classic studies of regeneration. The salamander is capable of regeneration of both limbs and tail following amputation, and the *Xenopus laevis* frog can regrow these structures at the tadpole stage (reviewed in Tanaka and Ferretti, 2009; Han et al., 2005; Slack et al., 2008). Among amniote vertebrates, lizards retain substantial capacity for regeneration of the skin, musculoskeletal and nervous tissues after loss of the tail (reviewed in Alibardi, 2010). This issue features studies of regeneration in amphibian and reptilian vertebrate models.

Historically, studies of regeneration have focused on a specific model system, and only recently have comparative studies of regeneration been undertaken across species. This effort has been advanced by genome sequencing efforts that have allowed for the rapid development of molecular reagents for comparison of orthologous genes and pathways among the vertebrates. A central question as these comparative studies advance is whether the mechanisms regulating regeneration represent variation on a monophyletic trait, or whether regenerative mechanisms have evolved multiple times in vertebrates. This question directly impacts the applicability of regenerative studies in these vertebrate species for potential clinical therapeutic approaches. Genomic sequencing of tetrapod vertebrate models, including the chicken, the lizard *Anolis carolinensis*, and the frog *Xenopus tropicalis*, highlight the degree to which gene homologues are conserved in evolution (International Chicken Genome Sequencing Consortium, 2004; Hellsten et al., 2010; Alföldi et al., 2011). The capacity for regeneration is substantially reduced in mammals, except during immediate post-natal periods as was demonstrated in cardiac muscle regrowth (Porrello et al., 2011). However, if the genes and pathways regulating regeneration were conserved among vertebrates, this would open up future approaches to harness these mechanisms in mammals, including humans.

Analyses of regeneration in the tadpole stage of the African clawed frog, *Xenopus laevis*, have leveraged the molecular reagents developed for this classic developmental model (Sive et al., 2000). In particular, the generation of transgenic *Xenopus* tadpoles is a major advance in molecular genetic analysis of the regenerative process. Overexpression of key regulatory genes can be achieved, and the use of fluorescent reporter genes allows for lineage tracing and studies of cell autonomy. In this issue, Slack and colleagues (Lin et al., 2012)

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demonstrate the power of these transgenic tools in dissecting the regulation of regeneration of specific cellular populations. They report generation of both temperature sensitive and doxycycline inducible transgenes and use them to study whether Wnt and FGF signaling are required during regeneration. Tissues carrying transgenes for Dickkopf1 (*dkk1*), an antagonist of Wnt signaling, or the dominant negative form of the FGF receptor XFD were transplanted into wild-type tadpoles (Lin & Slack, 2008). Using a combination of modern and classical techniques, they were able to demonstrate essential roles for both during regeneration.

While tadpoles are capable of tail regeneration at earlier stages, later stages lack regrowth after amputation, making *Xenopus* a model of regenerative capacity that has temporal windows, as seen in humans (Illingworth, 1974; Muller et al., 1999). Interestingly, specific regulation of bioelectrical signaling can induce tadpole tail regeneration in later stages (Levin, 2009). A longstanding question has been how biophysical changes, such as those established by ion concentration or transmembrane voltage gradients, direct the regulation of transcriptional activation in the regenerative process. In this issue, Tseng & Levin (2012) explore the role of transmembrane potentials and its effects on chromatin-mediated gene regulation. By modulating a chloride channel in *X. laevis*, a voltage gradient was generated within the tadpole tail. Depolarization during regenerative stages led to decrease in growth response post-amputation, while the opposite was observed at later refractory stages. Tseng and Levin (2012) hypothesize that the link between the voltage gradient and chromatin modification may be through the Na+-coupled monocarboxylate transporter, SLC5A8/SMCT1, through its action of transporting the histone deacetylase (HDAC) inhibitor, butyrate.

Mescher and colleagues (King et al., 2012) utilize the *Xenopus* model to explore the relationship between inflammation and regeneration. Modulation of an inflammatory response is a critical first step prior to the regenerative process. Mescher and colleagues report that treatment of the amputated *Xenopus* hindlimb with certain anti-inflammatory agents inhibits the regenerative process, while therapy with others promote it. They also demonstrate that pro-inflammatory agents can regulate the levels of pro-inflammatory, dedifferentiation, and limb-patterning genes. These findings indicate that regeneration requires a balance between factors that promote local inflammation versus those that promote blastema formation.

Standard methods for analyzing the regenerating limb in *Xenopus* rely on tissue treatments, such as decalcifying agents, which make the tissues incompatible with most immunohistochemical analyses. In order to advance molecular studies of *Xenopus* tadpole limb regeneration, a new protocol using the contrast-agent Hexabrix in microcomputer tomography (microCT) is presented Slack and colleagues (Chen et al., 2012). Since the regenerating endoskeleton is primarily cartilaginous, this tissue is difficult to detect using standard microCT techniques. Optimized concentrations of Hexabrix and imaging parameters yielded high-resolution images of cartilaginous tissue. This novel imaging protocol, utilizing a clinically available, low toxicity contrast agent, provides a valuable tool for regeneration research.

Urodele amphibians such as the axolotl (*Ambystoma mexicanum*) are the classic model of limb and tail regeneration. In this issue, Makanae and Satoh (2012) demonstrate the utility of the accessory limb model (ALM), which can be induced in axolotl by a skin wound, deviation of a nerve, and subsequent contralateral skin graft. The ALM can be utilized to study nerve function and apical ectodermal cap induction and to compare wound healing with blastema formation. In particular, the authors utilize the ALM to demonstrate that FAK/Src signaling plays a role in cell migration as in amniote vertebrates.

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Among amniotes, squamate reptiles are able to regenerate spinal cord, hyaline cartilage, axial muscle groups, and skin after tail loss. Lizards have evolved unique fracture planes in their caudal vertebrae to facilitate the process of autotomy, or self-amputation. Vickaryous and colleagues (Delorme et al., 2012) analyze the processes of wound healing and regeneration following autotomy at a fracture plane versus involuntary amputation elsewhere in the leopard gecko, *Eublepharis macularius*. Their findings indicate that regeneration is an intrinsic property of the tail, regardless of location (e.g., proximity to a fracture plane) or method of amputation. The authors also provide evidence of the activation of wound healing genes and isolated expression of a marker of apoptosis in the tail stump and regenerating tail.

The recent sequencing of the first non-avian reptile, the green anole lizard, *Anolis carolinensis*, promises to greatly advance molecular studies of lizard tail regeneration (Alföldi et al., 2011).The green anole has been a favorite model for studies of evolutionary genetics, neuroendocrinology, and behavioral ecology. There are also a number of studies describing the cellular and histological features of the regenerative process in this lizard species (reviewed in Alibardi, 2010). However, there are conflicting interpretations of these studies, many of which were carried out before stem cell and developmental concepts had been refined with the availability of molecular genetic approaches. In this issue, Wilson-Rawls and colleagues (Fisher et al., 2012) take advantage of these advances to describe features of the regenerated *A. carolinensis* tail not previously appreciated. They identify irregularly spaced foramina that transmit the vasculature but not nerves in the regenerated cartilage tube. In addition, they present a detailed analysis of regenerated muscle bundles and show that they are quite different from those in the original tail. These muscles display unique tendinous attachments and a distribution of connective tissue not found in the original tail.

To complement the histological analysis of the regenerated tail of *A. carolinensis* Fisher and colleagues (Ritzman et al., 2012) analyze the gross anatomy of the original and regenerated tail from a comparative and functional perspective. In the original tail, the extrinsic tail muscles, mm. caudofemoralis longus and brevis, are more restricted than other *Anolis* species, reflecting possible differences in locomotor performance. Muscle origins and insertions are also described and illustrated for the original and regenerated intrinsic musculature. The regenerated tail musculature is strikingly different, consisting of radially-organized longitudinal myomeres of variable size, compared to the regularly spaced, interdigitating muscle segments and intramuscular septa of the original tail. The functional anatomy of the regenerated tail suggests an appendage that is less capable of coordinated, fine-scale movements.

The studies in this special issue employ diverse methods and levels of analysis to investigate regeneration in amphibians and reptiles. In particular, the availability of molecular sequences and reagents has led to advances in studies of regeneration in non-mammalian vertebrate species. With the rapidly decreasing costs of technologies such as RNA-Seq, which does not involve the high up-front investments required for microarrays, gene expression studies can be carried out on any of a number of species where genomic sequence is available. These studies represent the first step towards carrying out comparative mechanistic studies between vertebrate models of regeneration, to identify both conserved and species-specific mechanisms.

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