Prioritizing studies on regeneration in nontraditional model organisms

“Comparisons of equivalent stages of regeneration in multiple models offer the potential to identify conserved regulatory mechanisms that enable regeneration and contrast them with models that do not regenerate.”

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Increasingly more studies of nontraditional vertebrate model organisms with extraordinary regenerative capacities are providing valuable insight into the mechanisms of complex tissue regeneration. For example, the zebrafish (*Danio rerio*) can regenerate many tissues after injury including cardiac [1], fin appendages [2] and spinal cord [3]. Another ray-finned fish, the bichir (*Polypterus senegalus*) can also regenerate cardiac [4] and fin appendages [5,6]. Urodeles (salamanders and newts), such as the axolotl (*Ambystoma mexicanum*), can regenerate whole limbs [7]. Studies of models with robust regenerative capacities have advanced our understanding of regenerative mechanisms by identifying genes that are necessary and sufficient for regeneration *in vivo* (reviewed in [8]).

Regenerative biology has historically focused on defining the cellular and molecular mechanisms within individual species. Within the last 15 years, rapid advances in genome sequencing technology and gene editing strategies have advanced the understanding of the molecular and cellular processes that define tissue regeneration. Unfortunately, they have also unintentionally created silos that encase individual animal models and discourage examination of regenerative capacity in nontraditional model systems.

Tissue regeneration is a tightly regulated complex process that involves reprogramming of differentiated tissues into highly proliferative cells. Comparisons of equivalent

stages of regeneration in multiple models offer the potential to identify conserved regulatory mechanisms that enable regeneration and contrast them with models that do not regenerate. Small molecules can then be developed to reactivate these proregenerative mechanisms to potentially augment limited human regenerative capacity. Such comparative and subsequent therapeutic studies would be greatly enhanced by making critical investments to create the genetic and molecular tools to study these nontraditional models as we have done for major model organisms such as the mouse.

Remarkably, the same advances in genome sequencing technology and innovations in genome editing that stimulated single model system research are currently driving cross-species comparison in regenerative biology. Comparative studies of regeneration can be framed in a phylogenetic context where model organisms are selected to identify conserved gene regulatory mechanisms for regeneration. For jawed vertebrates (gnathostomes), particular taxa among ray-finned fishes (Actinopterygii) and lobe-finned fishes (Sarcopterygii) have been characterized to regenerate fin appendages and limbs among other tissues. Fin regeneration has been described in multiple subclasses of ray-finned fishes suggesting that it is an ancestral trait of all ray-finned fishes. Several teleosts, including zebrafish and killifish (*Fundulus heteroclitus*) [9], and the most basal extant

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ray-finned fish, the bichir (*Polypterus senegalus*) [5], all regenerate fin appendages. Among lobe-finned fishes, the axolotl [7], newt (*Notophthalmus viridescens*) [10] and *Xenopus* tadpoles [11] can regenerate appendages.

These limb regeneration traits are in stark contrast to mammals where it is limited to the very ends of digits in mice [12], rats [13], monkeys [14] and humans [15]. Given that these urodele taxa can regenerate limbs, it suggests that limb regeneration is an ancestral trait of urodeles. Furthermore, it is plausible that appendage regeneration is an ancestral trait of all jawed vertebrates as both ray-finned fish and urodele taxa can regenerate appendages. Alternatively, limb regeneration may be a derived trait. No reports of appendage regeneration have been published among cartilaginous fishes (chondrichthyes). The last common ancestor of jawed vertebrates appeared approximately 420 million years ago [16] providing for an opportunity to find common mechanisms for appendage regeneration.

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The ability to take advantage of nature's diversity by focusing on a wide range of animal models, particularly in a comparative context, can potentially offer insights into biological activities that are not available through the study of a single organism. A recent comparative study by King *et al.* identified a conserved gene regulatory circuit for appendage regeneration in zebrafish caudal fins, bichir pectoral fins and axolotl forelimbs [6]. Appendage regeneration in these models requires the formation of a highly proliferative tissue, called the blastema, following wound healing. Small noncoding RNAs, called miRNAs, are essential regulators of regeneration [17–19]. miRNAs negatively regulate target genes and are ideal candidate genes for comparative studies as they are highly conserved across animals [20]. Five miRNAs were commonly upregulated and five miRNAs were commonly downregulated. To understand the potential function of these miRNAs, a network of 1550 commonly differentially expressed mRNAs in all three models was built to determine functional relationships to 11 orthologous blastema-associated genes identified in previous studies. The discovery of a common genetic pathway was unexpected because these species last shared an ancestor more than 420 million years ago and because the tissue composition of each appendage was quite different – the axolotl forelimb, the zebrafish caudal fin and the bichir pectoral fin. The findings suggest that the instruction manual for regeneration is conserved during evolution.

With increasing knowledge of proregenerative mechanisms, the next challenge is to identify small molecules to enhance regeneration following injury in humans. A target-based strategy where compounds are identified to target particular genes, proteins or pathways is a complementary strategy. Proregenerative lead compounds could then be tested in nonregenerative models, such as the mouse, to determine whether they promote regeneration.

The demonstrated benefits of studying the genetic pathways for regeneration in highly regenerative species should motivate us to re-examine the allocation of research funds. Additional investment to create genetic and molecular resources to study nontraditional models, such as the zebrafish and axolotl, are needed to accelerate these comparative studies. The zebrafish represents a good start, its genome was characterized in 2003 and many genetic tools have been developed to work with it, which are already yielding fruit. The progress on therapies for heart regeneration, for instance, has been ‘spectacular’, according to Kenneth D Poss, PhD, director of the Regeneration Next Initiative at Duke University Medical Center in Durham, NC, USA, who discovered in 2002 that zebrafish can regenerate heart tissue after 20% of the ventricle has been removed [1].

But other model organisms are still unexploited, for instance, the bichir, the archaic-looking ‘walking fish’ that was a subject of a recent appendage regeneration study [6]. The bichir, which has lungs and pectoral fins that it uses to pull itself along on land, is of enormous interest to biologists because it represents the link between fish and early four-legged vertebrates. The discovery in 2012 that it can regenerate its fins suggests that appendage regeneration was a common property of vertebrates during the fin-to-limb evolutionary transition [5].

High levels of research funding using mouse models over several decades have built a vast repertoire of tools and resources for the mouse. Currently, over 70% of traditional research grants from the NIH involve mouse studies [21]. Increasing funding for studies that involve a broader set of model organisms, like the zebrafish and axolotl, across all biomedical fields would result in more tools and resources for these diverse models. In turn, these investments would provide the critical genetic and molecular tools and resources for nontraditional model organisms needed to accelerate comparative studies of regeneration.

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