Review

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The zebrafish as a model for complex tissue regeneration

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For centuries, philosophers and scientists have been fascinated by the principles and implications of regeneration in lower vertebrate species. Two features have made zebrafish an informative model system for determining mechanisms of regenerative events. First, they are highly regenerative, able to regrow amputated fins, as well as a lesioned brain, retina, spinal cord, heart, and other tissues. Second, they are amenable to both forward and reverse genetic approaches, with a research toolset regularly updated by an expanding community of zebrafish researchers. Zebrafish studies have helped identify new mechanistic underpinnings of regeneration in multiple tissues and, in some cases, have served as a guide for contemplating regenerative strategies in mammals. Here, we review the recent history of zebrafish as a genetic model system for understanding how and why tissue regeneration occurs.

A versatile model system

Zebrafish are native to river basins in and surrounding East India and were established as a laboratory model system first by Streisinger and colleagues during the 1970s, as a potential means to apply genetic analysis to vertebrate development [1,2]. Over the decades that have followed, zebrafish have become a valuable tool to dissect embryogenesis. Experimental advantages of zebrafish for this use include large clutches, rapid external development, amenability to mutagenesis, a relatively small genome, and a reasonably short generation time. By utilizing these advantages, researchers have uncovered key factors in myriad developmental events, from early germ layer patterning to how tissues derived from these layers acquire form and function [3,4]. Recently, zebrafish have been used increasingly to investigate additional aspects of biology, including behavior, stem cells, and disease [5–9].

In this review, we provide an overview of how, over the past decade, zebrafish have become a primary model system for vertebrate tissue regeneration (see Glossary). We have focused on their remarkable regeneration of fins, heart, and central nervous system structures, although they also regenerate jaw, hair cells (lateral line), pancreas, liver, and kidney [10–20]. We summarize what is known about mechanisms of regeneration in different tissues and contexts, and describe how new discoveries and approaches in zebrafish are impacting the field of tissue regeneration.

Zebrafish fin regeneration

Zebrafish fins are complex appendages that quickly and reliably regenerate after amputation, restoring both size and shape. The key regenerative units are their many rays of dermal bone, which are segmented and lined by osteoblasts. Rays are cylindrical and hollowed, with two concave hemirays surrounding an inner mesenchymal tissue that is innervated, vascularized, and comprised primarily of fibroblasts. An amputated fin ray is covered within the first several hours by epidermis, and within 1–2 days, a regeneration blastema forms. The blastema is a proliferative mass of morphologically similar cells, formed through disorganization and distal migration of fibroblasts and osteoblasts (or scleroblasts) proximal to the amputation plane. As with blastemas in other classical regenerating

Glossary

Blastema: a proliferative mass of morphologically similar cells that accumulates in certain tissues after trauma and develops into the lost structures.
CRISPR-Cas: the Cas9 protein can be targeted through a CRISPR guide RNA to induce site-specific double-stranded DNA breaks for targeting genome editing.
Dedifferentiation: process by which a differentiated cell reverts to a less differentiated state to enable proliferation or differentiation.
Epicardium: mesothelial cell type that covers the periphery of the heart and can act as progenitor tissue for fibroblasts, vascular support cells, and possibly other cells.
Fate mapping: permanent labeling of a cell type to determine the contribution of these cells and their progeny during developmental and regenerative events.
Genetic ablation: selective killing of a specific cell type by the expression of a toxin, pro-apoptotic factor, or pro-drug converting enzyme.
Müller glia: specialized glial cells found in the retina that act as neuronal support cells and resident stem cells after injury.
Myocardial infarction (MI): massive cardiac muscle cell death and a leading cause of morbidity and mortality in humans, typically caused by coronary artery occlusion and ischemia.
Osteoblasts: bone-depositing cells.
Positional memory: the process by which spared adult cells retain positional information to recover only those structures lost by injury, of correct size and pattern.
Radial glial cell: glial cells in the brain and spinal cord that act as neuronal progenitors during development and after injury.
Regeneration: events by which lost or damaged tissue is replaced through endogenous mechanisms, restoring organ form and function.
Telencephalon: the most rostral of two subdivisions of the developing forebrain, the caudal subdivision being the diencephalon.
Transdifferentiation: conversion from one differentiated cell type to another.
Transection: a precise transverse cut into the tissue that leaves much of the surrounding tissue undisturbed.

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systems, such as the salamander limb and planarian head, the fin ray blastema is the major source of new structures.

The ability of teleost fish to regenerate amputated fins was first reported in 1786, in pectoral fins of goldfish, by Broussonet [21]. Although Thomas Hunt Morgan was fascinated by fin regeneration at the turn of the 20th century [22], it took nearly an additional century for fin regeneration to reach the genetic era. In 1995, Johnson and Weston described a screen for mutations that disrupt regeneration of tailfins in adult zebrafish [23], arguably the first experiments to demonstrate a technical advantage of studying regeneration in zebrafish. This screen was novel not only in its application of genetics to vertebrate regeneration, but also in its use of temperature-sensitive (TS) mutations in zebrafish. Given that regeneration is expected in most cases to re-employ genes used during early development, a TS screen enables identification of mutations in adults that would be lethal during early development. Over the next decade, genetic screening uncovered a handful of mutations that inhibited fin regeneration and could be localized to specific molecular defects by positional cloning [24–27]. These discoveries have contributed to the molecular models described below; yet, there has been a large time gap since the most recent identification of a regeneration gene by mutagenesis. New advances in high-throughput genome, exome, and transcriptome sequencing are likely to reboot forward genetic approaches to studying regeneration [28–33].

To best understand regeneration in any system, one must conclusively know the sources of the different cell types that are restored after injury. Only recently have modern genetic fate-mapping approaches been applied to address this question, including Cre recombinase-based technology used routinely in mice for lineage analysis. Multiple recent studies used transgenic Cre lines to focus on bone-forming osteoblasts. Their results indicated that differentiated osteoblasts transiently downregulate the osteogenic program, or dedifferentiate, as they contribute to the blastema. After this, resident osteoblasts contribute only osteoblasts to new regenerated structures [34–37]. This idea of lineage restriction was extended to other cell types, such as endothelium, epidermis, and fibroblasts, by other studies [38]. These findings agree with similar lineage restriction observed in axolotl (Mexican salamander) limbs and mouse digit tips [39–41]. However, these studies could not exclude rare transdifferentiation events; neither do most of them address possible ancillary mechanisms under different injury contexts. For instance, osteoblasts form and bone regenerates efficiently even when resident osteoblasts are potently ablated, indicating that other cell types are capable of differentiating into osteoblasts and supporting bone regeneration [35].

Many groups have examined the molecular mechanisms underlying the formation and proliferation of the blastema. In response to injury, increased expression of key signaling components of the Wnt/β-catenin and Activin-βA pathways are detectable by 3-h post amputation (hpa) [42,43], followed by upregulation of retinoic acid (RA), insulin-like growth factor (Igf), and fibroblast-like growth factor (Fgf) signaling pathway components by 6 hpa [24,44,45]. Although more complete functional testing is needed, one model for blastema formation is that increases in RA synthesis in response to injury induce expression of *igf2b* and *wnt10b*. These ligands then signal through canonical Wnt and Igf pathways to induce expression of *fgf20a*, a marker and critical regulator of blastema formation [43,44]. Independent of this signaling cascade, *activin-βA* is upregulated in the inter-ray region and is involved in reorganization of the underlying mesenchyme during blastema formation [42]. Blockade of these signaling pathways results in improper wound healing and blastema formation, implicating them in initiation of the blastema.

Blastema formation is only one step in zebrafish fin regeneration, and fins must then grow to the appropriate size. Regenerative outgrowth occurs by two processes: maintenance of a proliferative compartment at the distal end of the regenerate, and differentiation of more proximal cells. The proliferative compartment is maintained by signaling interactions between the mesenchyme and basal epidermis [46]. In addition to regulating blastema formation, RA, Fgf, and canonical Wnt signaling positively regulate blastemal proliferation and outgrowth, whereas noncanonical Wnt signaling inhibits these events [43,45,47]. Inhibition of Igf receptors or the Tgf-β receptor *alk4* also blocks blastemal proliferation during outgrowth, further indicating continued requirements for these pathways [42,44]. Interestingly, inhibition or ectopic activation of the Notch signaling pathway results in a regenerative block, leading authors to propose models in which Notch signaling, through an unknown mechanism, enhances blastemal proliferation while suppressing osteoblast differentiation during regeneration [48,49].

In addition to Notch signaling, other pathways have been examined for their ability to influence differentiation within the blastema. Bmp and Hedgehog signaling induce bone formation in the regenerate when ectopically activated, suggesting that the normal function of these molecules is to drive redifferentiation of osteoblasts in the proximal blastema [50,51].

Finally, fins provide a potentially useful model for considering the mechanisms by which an appendage regains its original shape and size after amputation. This phenomenon of positional memory, in which adult cells in the stump somehow retain and recall the correct developmental coordinates and instructions, remains a mystery in many ways. Regeneration occurs at different rates depending on the proximodistal amputation plane, regulation that involves position-dependent control of amounts of Fgf signaling [47]. Signals responsible for this, and factors that retain coordinates in adult fins and enact precise recovery, remain to be found and are likely to be broadly relevant to regeneration in other systems.

Heart regeneration

There is no significant regeneration of adult mammalian cardiac muscle after experimental injury paradigms. This deficiency is highly relevant to human disease, given that ischemic myocardial infarction (MI) and scarring is a primary cause of morbidity and mortality. Zebrafish have a high natural ability for heart regeneration and, thus, can inform as to how this process occurs or might be induced [52]. There are currently several injury models that