Recent advancements in understanding fin regeneration in zebrafish

Ivonne M. Sehring | Gilbert Weidinger

Institute of Biochemistry and Molecular Biology, Ulm University, Ulm, Germany

Correspondence
Gilbert Weidinger, Institute of Biochemistry and Molecular Biology, Ulm University, Albert-Einstein-Allee 11, 89081 Ulm, Germany. Email: gilbert.weidinger@uni-ulm.de

Funding information
Deutsche Forschungsgemeinschaft, Grant/Award Numbers: 251293561, 316249678, 414077062; European Union, Grant/Award Number: 01KL1704

Abstract
Zebrafish have the remarkable ability to fully regenerate a lost appendage, faithfully restoring its size, shape and tissue patterning. Studies over the past decades have identified mechanisms underlying the formation, spatial organization, and regenerative growth of the blastema, a pool of proliferative progenitor cells. The patterning of newly forming tissue is tightly regulated to ensure proper rebuilding of anatomy. Precise niche regulation of retinoic acid and sonic hedgehog signaling ensures adherence to ray—interray boundaries. The molecular underpinnings of systems underlying re-establishment of pre-amputation size and shape (positional information) are also slowly starting to emerge. Osteoblasts play an important role as a cellular source of regenerating skeletal elements, and in zebrafish both osteoblast dedifferentiation as well as de novo osteoblast formation occurs. Both dedifferentiation and proliferation are tightly controlled, which makes it interesting to compare it to tumorigenesis, and to identify potential players involved in these processes.

This article is categorized under:
Adult Stem Cells, Tissue Renewal, and Regeneration > Regeneration

KEYWORDS
bone, dedifferentiation, positional information, regeneration, zebrafish

1 | INTRODUCTION

Across the animal kingdom, the extent of regenerative capacity varies tremendously not only between species, but also between organs (Ricci & Srivastava, 2018). Adult mammals can efficiently repair damage to the skin, the liver, and to epithelia of the lung, kidney and gut (Barker, 2014; Kotton & Morrisey, 2014; Yang, Liu, & Fogo, 2014), but their ability to restore other organs is highly limited. In contrast, several non-mammalian vertebrates possess an astonishing capacity to regenerate complex structures even after a severe tissue loss, an ability that is most dramatically apparent in limb and fin regeneration in salamanders and teleost fish. While appendage amputation results in formation of a collagen-rich scar and failure to replace lost tissue in mammals—with the exception of the digit tips (Simkin, Han, Yu, Yan, & Muneoka, 2013), appendage regeneration in salamanders and fish involves full restoration of all cell-types (i.e., nerves, bone, vasculature, connective tissue, etc.), of tissue architecture and appendage pattern along all three axes, and of appendage function. The urodele appendage can be divided into three morphologically distinct domains, the stylopod...
(upper limb), zeugopod (lower limb), and autopod (hand or foot), which contain endochondral bones (endoskeleton). In fish dorsal and anal fins, the endoskeletal elements, the radials (or hypurals in the caudal fin), occupy only a small portion of the entire fin structure, and the majority of the fin skeleton is composed of exoskeletal fin rays (see below).

The zebrafish *Danio rerio* is a powerful vertebrate model to study regeneration, as it can restore organs that poorly regenerate in mammals, including the heart, brain, spinal cord, and the appendages/fins (Gemberling, Bailey, Hyde, & Poss, 2013). Studying appendage regeneration in zebrafish has the advantage that fins are easily accessible, their partial amputation has no major detrimental effect on the animals in lab conditions, and regeneration can be easily followed in live fish. After amputation of the exoskeletal part of the zebrafish caudal fin, it reliably regenerates within 2–3 weeks, restoring not only its exact pre-amputation size, but also its patterning and tissue organization. Individual fish can repeatedly regenerate the caudal fin throughout their life span (Azevedo, Grotek, Jacinto, Weidinger, & Saúde, 2011). Since fins are thin and quite transparent (with the exception of pigment cells), regeneration of several tissues, including bone, can be monitored by live imaging *in vivo* (Xu, Volkery, & Siekmann, 2015). Additionally, the zebrafish genome has been sequenced and annotated, a plethora of mutant strains and transgenic lines have been established, and multiple tools for genetic manipulations are available.

### 2 | THE ZEBRAFISH CAUDAL FIN MODEL

As a member of the teleost class of vertebrates, zebrafish possess two sets of paired fins, the pelvic and pectoral fins, and the unpaired caudal, anal and dorsal fins. All these fin types regenerate, and although there are studies using the pectoral fins as model, the caudal fin is mainly used for regeneration studies.

The zebrafish caudal fin consists of several endochondral bony elements at its base, which are muscularized, while the visible part of the fin that extends from the body is formed by fin rays, and does not contain muscle. Fin rays (lepidotrichia) are exoskeletal elements of dermal origin, that is they are directly formed from osteoblasts without a cartilage template (Géraudie & Landis, 1982; Landis & Géraudie, 1990) (Figure 1a). Each ray consists of a succession of repetitive bony units, the segments, which are connected to each other by collagenous ligaments, the joints. The proximal-most segment is connected to the endochondral bones (the fan-like hypurals, hemal spine of preural 2, and parhypural) and striated muscles of the fin base, which control fin movement during swimming. At the distal tip, non-mineralized skeletal elements, the so-called actinotrichia, which are a fish-specific feature, top each ray (König, Page, Chassot, & Jaźwińska, 2018). All but the most lateral rays are bifurcated at a distal position (Figure 1c). Each lepidotrichium is composed of two opposed concave hemirays (Figure 1b,d). The hemirays are lined by a single layer of flattened osteoblasts on the inner and outer surface, which deposit the bone matrix (Johnson & Bennett, 1998) (Figure 1b). In contrast to zebrafish endochondral bones, the lepidotrichia do not contain osteocytes, that is bone-forming cells that become entrapped in bone matrix (Apschner, Schulte-M merker, & Witten, 2011). Cells positive for the osteoclast marker tartrate-resistant acid phosphatase (TRAP) are absent in the uninjured fin; however, after amputation they are not only detected in the regenerate but also proximal to the amputation plane (Blum & Begemann, 2015a).

Yet, potential functions of osteoclasts during fin regeneration have so far not been described. The core of space between the two hemirays, the intrarays, contains nerves, arterial capillaries and connective tissue (fibroblasts). Interray tissue is formed by soft connective tissue (fibroblasts) covered by epidermis and contains venous capillaries (Becerra, Montes, Bexiga, & Junqueira, 1983).

### 3 | CAUDAL FIN REGENERATION

After partial fin amputation, a precise sequence of events proceeds to ensure the successful regeneration of the lost tissue.

In addition to the recruitment of neutrophils and macrophages, one of the earliest responses to the injury is the migration of epithelial cells, which form an epithelium covering the wound (Figure 2a) (Poleo, Brown, Laforest, & Akimenko, 2001; Shibata, Ando, Murase, & Kawakami, 2018). Within the next hours to days, the epithelium becomes a multilayered structure, termed the wound or regeneration epidermis (Chen et al., 2015; Shibata, Ando, et al., 2018). In contrast to mammalian limb injury, which is associated with local tissue degradation (Simkin et al., 2015), in zebrafish the regeneration process starts immediately after wound closure. The next crucial step, which separates regeneration from mere wound healing, is the formation of a blastema (Figure 2a,b). Generally speaking, a blastema is an
accumulation of undifferentiated, proliferative cells which are thought to contain the majority of the cells necessary to regenerate the missing structure (Pfefferli & Jazwinska, 2015; Poss, Keating, & Nechiporuk, 2003). Historically, regeneration of a particular organ/structure has been described to involve formation of a blastema if a population of small cells lacking the morphology of differentiated cells can be identified simply by light microscopy using non-specific histological stainings. Typical examples include regenerating salamander limbs, and the regeneration of planarian heads or tails. Yet in other regenerating systems, for example the zebrafish heart, blastemas fulfilling the above definition cannot be distinguished, although a zone of proliferating cardiomyocytes can be detected using molecular markers at the wound border of regenerating hearts (Poss, Wilson, & Keating, 2002; Schnabel, Wu, Kurth, & Weidinger, 2011). Thus, whether blastemas (as an accumulation of easily identifiable undifferentiated cells) form or do not form during regeneration likely does not reflect fundamental differences in the underlying cellular mechanisms, but might rather be related to the scale at which proliferative cells accumulate at the wound site. In the zebrafish fin, accumulation of small undifferentiated cells occurs mainly in the regions distal to the bony rays, and much less so in interray areas, thus each fin ray appears to form its own blastema (Figure 2b). This conclusion is also supported by the expression pattern of genes like the Msx homeobox family member msxb, a marker for undifferentiated, progenitor-like cells, which is strongest distally to the bony rays (Figure 2b). Similarly, the activity of several signaling pathways that are required for fin regeneration is largely confined to the regenerate distally to the rays. For example, bmp4 is exclusively expressed in the regenerate tissue atop of each ray (Murciano et al., 2002), and components of the Wnt/β-catenin pathway are restricted there as well (Wehner et al., 2014). Overall, it thus appears that blastemas sensu stricto form only distally to bony rays.

Blastemas are initially formed by distally migrating and proliferating fibroblasts, followed by dedifferentiated osteoblasts. Subsequently, the blastema is partitioned into several regions with different proliferation profiles. In particular, a small group of cells at the distal tip of the blastema hardly proliferates and was suggested to barely participate in building the regenerate but to rather serve as a signaling center that organizes proliferation and patterning of other regions of the blastema (Nechiporuk & Keating, 2002; Wehner et al., 2014). The progenitor cells in the proximal blastema...
differentiate to restore the lost tissue; in the fin this is best described for osteoblasts, which are located in lateral regions of the blastema. These undergo a succession of specification and differentiation steps along the proximodistal axis, with runx2-positive osteoblast progenitors being located distally in the proximal blastema, succeeded by sp7 (osterix)-positive committed osteoblasts and bglap (osteocalcin)-positive differentiated cells further proximally (Brown, Fisher, & Iovine, 2009).

A large number of signaling pathways have been shown to be involved in wound healing, blastema formation and regenerative growth. Since these have been reviewed elsewhere, we will concentrate on other aspects of fin regeneration here (Gemberling et al., 2013; Sehring, Jahn, & Weidinger, 2016; Wehner & Weidinger, 2015).

### 4 | FIN AND BODY SCALING

When a fin is amputated, it regenerates only the missing parts, and thus grows to pre-amputation length with high precision. Fin size is also tightly regulated during regular, physiological growth of the zebrafish, since the ratio of fin to body length remains constant. The molecular mechanisms ensuring fin/body scaling and those mediating regeneration to pre-amputation length are very incompletely understood; interestingly, it is also unclear to which extent these two scaling processes share molecular mechanisms.

The isometric, physiological growth of the fins (proportional to body size) is controlled through mechanisms regulating segment length and segment number (Iovine & Johnson, 2000). Most of the limited knowledge we have about molecular mechanisms regulating isometric growth comes from the analysis of mutants that display disturbed scaling of fin size relative to body size. In short fin (sofb123) mutants, where shorter fins result from short bony ray segments, the gap junction protein connexin43 (cx43) is mutated (Iovine, Higgins, Hindes, Coblitz, & Johnson, 2005; Sims, Eble, &...
Iovine, 2009). Concurrently, in the another long fin (alf<sup>αldc86</sup>) mutant, which possesses elongated segments, increased cx43 expression was observed (Sims et al., 2009). While these genetic data strongly suggest that communication between cells via gap junctions is essential for proper scaling of segments, the molecules that presumably need to travel through the tissue via gap junctions have not been identified. Causative for the increased growth of fins in alf mutants are gain-of-function mutations in a potassium (K+) channel, knck5b, which increase K+ conductance of the channel (Perathoner et al., 2014). This intriguingly indicates that isometric fin growth is controlled by bioelectrical signals. Segment length and fin size is also increased when the protein phosphatase calcineurin is inhibited (Daane et al., 2018; Kujawski et al., 2014). Surprisingly, calcineurin inhibition has no effect on growth in knck5b (alf) mutants, and calcineurin was suggested to directly modify conductance of the channel (Daane et al., 2018). How bioelectrical signals are induced and regulated and how knck5b/calcineurin signals regulate fin growth, will be interesting avenues for future research. While fin growth is isometric to body growth under physiological conditions, during regeneration of amputated fins, a fast growth rate is achieved by switching to allometric growth. When the fin is restored to its original size, the fin returns to isometric growth, preventing excessive outgrowth. This switch between isometric and allometric growth might, at least in part, be regulated via calcineurin, since calcineurin activity is reduced during regenerative growth (Kujawski et al., 2014).

5 | POSITIONAL INFORMATION

When the fin is amputated at a distal position, only the missing distal part will be regenerated. After amputation close to the fin base, the whole fin will be restored (White, Boffa, Jones, & Petkovich, 1994). In addition, the shape of the fin along the dorsoventral axis will faithfully regenerate, that is lateral fin rays regrow to a bigger length than medially located rays. Faithful regeneration of only the missing anatomy is also observed in salamander limbs. These are highly patterned along the proximodistal axis, that is an upper arm is anatomically very different from a lower arm or hand, and this pattern is faithfully restored during regeneration. Thus, cells at the amputation plane in salamanders are known to contain information about their proximodistal location, and this positional memory ensures regeneration of only the appropriate missing structures distal to the amputation plane (Nacu & Tanaka, 2011). In zebrafish, all bony segments of a fin ray exhibit a uniform anatomy, except for the segment where bifurcation occurs, and except for the fact that proximal segments are thicker than distal segments (see also below). Due to this rather uniform anatomy of the fin rays along the proximodistal axis, it is difficult to apply the same concept of positional memory that has been developed in salamanders to zebrafish fins. Yet, a system that measures how much of a ray is missing must also exist in zebrafish fins, since each ray regrows until its pre-amputation size is achieved. One remarkable feature of this system is that after a proximal amputation, the growth rate is faster than after a distal amputation, and a higher number of proliferating cells can be detected (Lee, Grill, Sanchez, Murphy-Ryan, & Poss, 2005). This ensures that the regeneration process of the longer lateral rays is completed simultaneously with that of the shorter medial rays (Figure 3a).

6 | PROPERTIES OF THE POSITIONAL INFORMATION SYSTEM

An important question is whether positional information is autonomous to individual fin rays, or rather only established in the context of an entire fin. Shibata et al. tested this question using elegant transplantation experiments of rays along the dorsoventral (medial-lateral) axis of the fin (Shibata, Liu, Kawasaki, Sakai, & Kawakami, 2018). When long rays located in lateral positions are grafted into the center of the fin, which contains shorter rays, the grafted rays regenerate to a greater length than the adjacent central rays. Likewise, central rays transplanted to the lateral side regenerate to shorter lengths than their neighbors. Interestingly, these rays exhibit an intermediate length, between the length appropriate for the original-position and the one of the new adjacent rays. Thus, it appears that the positional information for the dorsoventral axis is retained within the cells of individual fin rays, yet some communication between adjacent rays exists. Moreover, Shibata et al. could show that this ray-autonomous positional information is quite long-lived, as it was kept even after a second amputation. It would be interesting to test if, with further repeated amputations of a transplanted ray, the ray-intrinsic information would persist, or if the positional information from the surrounding region would eventually prevail.

During axolotl limb regeneration, it was shown that blastemas transplanted between different proximodistal amputation planes induce regeneration of all tissue distal to their original position, independent of their current location.
Thus, blastemas appear to autonomously contain positional information. In contrast, zebrafish fin distal blastemas transplanted to a proximal amputation site did not induce less outgrowth of the regenerate, as one might assume if they retained a memory of their origin (Shibata, Liu, et al., 2018). However, the absence of obvious anatomical heterogeneity along the proximodistal axis in zebrafish fin rays makes it more difficult to reveal differences in proximal vs. distal regenerates, and thus these transplantation experiments might lack the sensitivity necessary to reveal blastema-autonomous positional memory. Nevertheless, differences between the positional information system between salamanders and zebrafish were also indicated by lineage tracing of fibroblasts. Tryptophan hydroxylase 1b (tph1b) is upregulated in the zebrafish blastema after amputation, and lineage tracing of tph1b+ blastemal cells using a tph1b:CreERT2 transgenic line revealed that these cells give rise to fibroblasts in the regenerate (Tornini et al., 2016). Under conditions of mosaic recombination, the authors could observe an organization of the blastema which they term “pre-patterning,” where in most cases the spatial localization of a clone within the blastema is retained in its spatial contribution to the regenerate; for example, proximal blastemal cells have a higher likelihood to contribute to proximal clones in the regenerated fin. Interestingly, this proximodistal identity of a clone is not conserved through multiple amputations; re-amputation of fins with proximal clones resulted in a heterogeneous distribution of the progeny along the proximodistal axis of the second regenerate. These observations suggest that in zebrafish fibroblasts do not carry positional information. This is in contrast to salamanders, where cells containing positional information are assumed to be localized within the connective tissue (Bryant, French, & Bryant, 1981; Nacu et al., 2013). Whether other cell types within the zebrafish blastema possess autonomous memory of their location along the proximodistal axis remains to be tested.

In summary, transplantation of entire fin rays has indicated that they contain autonomous information about their pre-amputation length, which differs along the dorsoventral axis (medial-lateral axis) of the fin. Thus, rays appear to
translates this information into differential growth rates of at least two components: One allows cells to assess their position along the proximodistal axis of the ray and another one measures the different degree of anatomical heterogeneity along the proximodistal axis in salamander limbs and fish fin rays, where regeneration of different anatomy dependent on the amputation plane is only necessary in salamanders. Yet, a system ensuring proper scaling of the fin along the proximodistal and dorsoventral axis during regeneration exists, and several studies have attempted to shed light on its molecular nature.

### 7 | POSITIONAL INFORMATION VIA GRADED EXPRESSION OF MOLECULES

Most studies have assumed that a molecular system ensuring fin regeneration to pre-amputation length would consist of at least two components: One allows cells to assess their position along the proximodistal axis of the ray and another translates this information into differential growth rates—proximal high, distal low. The first system might consist of molecules that are expressed in a graded fashion along the proximodistal axis (Adell, Cebrià, & Saló, 2010; Wolpert, 2016). These gradients might be established by the diffusion of molecules, and a mathematical model invoking such gradients was found to be able to explain the growth patterns of regenerating zebrafish fins (Rolland-Lagan, Paquette, Tweedle, & Akimenko, 2012). Yet, it is difficult to imagine that stable gradients could be established by diffusion in a structure that is up to 4 mm in length and heavily vascularized, and indeed no evidence for the existence of gradients of diffusible molecules along the proximodistal axis of fin rays exists. Accordingly, better candidates for graded expression might be molecules tethered to the extracellular matrix or to the plasma membrane of cells. Prod1 is a GPI-linked protein that was shown to be expressed in a gradient in salamander limbs, and in gain-of-function experiments it can instruct distally located cells to adopt more proximal identity in salamanders (Da Silva, Gates, & Brockes, 2002; Kumar, Gates, & Brockes, 2007). While it thus is the best candidate mediating positional memory in any vertebrate regeneration model, loss-of-function data confirming its role are missing, and no homologs of Prod1 have been identified outside salamanders (Garza-Garcia, Harris, Esposito, Gates, & Driscoll, 2009).

To identify molecules that are present in a graded pattern in nonamputated zebrafish fins, Rabinowitz et al. compared transcriptomic, proteomic and metabolomic profiles between different regions along the proximodistal axis of the caudal fin (Rabinowitz et al., 2017). Their comprehensive study identified over a thousand transcripts that are differentially expressed between proximal and distal regions. Comparison of transcriptomic and proteomic data revealed a surprisingly small number of these transcripts to have a comparable graded protein expression, suggesting that extensive posttranscriptional regulation takes place, which reduces the differences in protein expression along the axis of the fin. The authors propose a high confidence list of 32 candidate molecules which exhibit a significant difference on both RNA and protein level between proximal and distal regions. Here, proximally as well as distally enriched molecules exist. In-depth analysis of two candidates, the proximally enriched aldehyde dehydrogenase Aldh1l1 and the distally enriched carbonic anhydrase Ca2, revealed their conserved differential expression pattern across different fins (caudal, dorsal and pectoral fins). Graded expression of these molecules also scaled with the shorter and longer fin length seen in short fin (sof) and long fin (lof) mutant lines. Thus, these enzymes might indeed be involved in the establishment of positional memory during regeneration. The data obtained in this study are a valuable starting point for further work aimed at identifying molecular players involved in positional memory.

One caveat with the interpretation of such expression data is that fin ray bone displays different levels of maturity and thickness along the proximodistal axis (Blum & Begemann, 2015a). Fins grow by sequential addition of new segments distally, and fin growth continues also in adult fish. While newly added segments are relatively thin, the bone matrix broadens and thickens over time, and the curvature of the hemirays increases. Thus, a gradient of bone amount and maturity exists along the proximodistal axis of the fin, which could be causative for the differential expression of molecules along this axis of the fin. Thus, it will be important to test whether molecules expressed in a gradient indeed mediate positional memory. On the other hand, it is conceivable that fins actually use this gradient of bone amount and maturity to assess the proximodistal position of amputation planes.
In the pelvic fin, fin rays are of different length along the anteroposterior axis. Sequenced-based profiling has revealed a graded expression of many genes along this axis (Nachtrab, Kikuchi, Tornini, & Poss, 2013). This study as well as the caudal fin study (Rabinowitz et al., 2017) showed a differential expression for genes involved in developmental processes, including many transcription factors. These findings suggest that transcription factors that are active during development retain a differential expression in adult tissues, where they are important for tissue size regulation and positional memory.

## 8 | Interpretation of Positional Information: Regulation of Differential Growth Rates

More progress has been made in the identification of molecules that are involved in regulating differential growth rates at proximal vs. distal amputation planes (Figure 3b). Different regeneration rates might be obtained by variation in the expression strength of amputation-induced genes. The vacuolar-type proton transporter H+ -ATPase (v-ATPase) displays such a dynamic expression: it is earlier and more strongly upregulated in proximal than in distal blastemas, resulting in a similarly patterned difference in H+ efflux (Monteiro et al., 2014). Inhibition of v-ATPase function reduces proliferation and subsequently regenerative growth. Regulation of proliferation by v-ATPase was also shown during Xenopus tail regeneration (Adams, Masi, & Levin, 2007). A potential link between v-ATPase function and cell proliferation is the Fibroblast growth factor (Fgf) signaling pathway, which controls cell proliferation during blastema formation (Poss et al., 2000). Higher Fgf signaling activity in proximal than distal regenerates has also been shown to be causative for their faster growth and the Fgf signaling target gene mkp3 is more strongly expressed in proximal than distal regenerates (Lee et al., 2005). V-ATPase knockdown inhibits mkp3 expression, suggesting a potential requirement of v-ATPase activity for Fgf signaling activity (Monteiro et al., 2014). Retinoic acid (RA) signaling is required for blastema proliferation (Blum, Begemann, Rauch, Geisler, & Ingham, 2012). Aldh1a2 is more strongly expressed in proximal than in distal stumps (Monteiro et al., 2014), indicating that patterned RA signaling might also be modulated by v-ATPase activity and might be involved in regulating the differential growth rates of proximal vs. distal blastemas. V-ATPase might also act upstream of mTORC1 activation by regulating lysosomal acidification (Takayama, Muto, & Kikuchi, 2018). Lysosomal acidification and mTORC1 activation are stronger in proximal than in distal regions, constituting further candidates for systems mediating differential regeneration rates. Amino acids, especially leucin, are important activators for mTORC1 (Zheng et al., 2016), which is a central regulator of cell growth. Interestingly, the study by Rabinowitz et al., which looked for molecules differentially expressed along the proximodistal axis (discussed in detail above), identified leucin to be proximally enriched in unamputated fins (Rabinowitz et al., 2017). Whether v-ATPase activity regulates Fgf and RA signaling directly remains to be determined. v-ATPase might regulate the pathways via modulating endocytosis and vesicle recycling events, ultimately affecting the expression of downstream genes, as it was shown for Notch, Wnt and VEGF signaling (Cruciat et al., 2010; Rath, Liebl, Fürst, Vollmar, & Zahler, 2014; Vaccari, Duchi, Cortese, Tacchetti, & Bilder, 2010). At any rate, additional studies, particularly genetic loss- and gain-of-function analyses, are necessary to further validate these findings.

## 9 | Ray—Interray Boundaries

After amputation of the caudal fin, not only its shape and size are restored, but also the complex pattern of lepidotrichia bone, blood vessels, nerves, connective tissue (fibroblasts), pigment cells and their relative arrangement to one another. Fin rays are separated by interray tissue, which has a similar architecture as skin, consisting of multi-layered epidermis covering mesenchymal connective tissue (fibroblasts) (Figure 1c,d). Regeneration of the rays is driven by blastemas, which form atop of each fin ray, and new bone forms as a direct straight extension of the existing amputated bone of each ray. Interray skin likewise regenerates, and under normal conditions, no thickening or spreading of bony tissue in the regenerate can be observed, rather the ray-interray organization is faithfully restored (Stewart & Stankunas, 2012). This indicates that there are mechanisms in place which control ray—interray patterning. One study proposed the involvement of retinoic acid (RA) signaling in the establishment of the ray—interray organization (Blum & Begemann, 2015b). Aldh1a2, the rate-limiting enzyme for the synthesis of RA, is highly expressed in the blastema (Mathew et al., 2009), as is the RA receptor rarga (White et al., 1994). Blum & Begemann proposed that the RA-degrading enzyme
cyp26a1 establishes an RA-free niche in the proximal basal epidermal layer of the regenerate, which is responsible for the strict adherence of regenerating osteoblasts to the ray (Figure 4a). Abolition of this zoning by either RA administration or inhibition of cyp26a1 activity results in ectopic bone formation in interray locations (Figure 4b), indicating that an initial arrangement of pre-osteoblasts in the nascent blastema is necessary for correct organization of ray—interray tissues. This is supported by the observation that blood vessels in the blastema also ignore ray—interray boundaries after RA treatment. The authors could further show that RA degradation is required for sonic hedgehog a (shha) expression. During zebrafish fin regeneration, shha is expressed in basal epidermal cells on both sides of the distal stump and regenerate, and its expression domain splits into two discrete domains preceding ray bifurcation (Lee et al., 2009; Quint et al., 2002). Ectopic expression of shha and bmp2b between the blastemas of two sister rays induces ectopic bone formation (Quint et al., 2002), and laser ablation of shha-expressing basal epidermal cells results in a delay in branching (Zhang, Jeradi, Strähle, & Akimenko, 2012). Altogether, these data indicate that during fin regeneration, shha signaling is involved in the regulation of lepidotrichia branching by mediating epithelial-mesenchymal interactions. This potential role is challenged by a study which showed a permanent expression of shha in two separate domains in the basal layer of the epidermis, also in regions of regenerating fin where bifurcations do not occur (Figure 4b), thus arguing that shha expression in two separate domains is not sufficient to trigger the formation of bifurcations (Azevedo, Sousa, Jacinto, & Saúde, 2012). Consistently, Shha/Smo signaling inhibition blocks ray bifurcation, although the two distinct epidermal shha positive domains are retained (Armstrong, Henner, Stewart, & Stankunas, 2017). The authors thus propose a model in which shha-driven Hh/Smo signaling promotes ray bifurcation by directing the migration of osteoblast progenitors into two spatially separated pools, while the splitting of shha-expressing epidermis itself is not Hh/Smo signaling dependent.

10 | THE FATE OF OSTEOBLASTS

An important aspect in understanding fin regeneration is to determine the cellular source(s) of regenerating skeletal elements. Recently, work in several systems, including the zebrafish fin, has revealed that regeneration can involve intriguing cases of cellular plasticity (Tata & Rajagopal, 2016). Potential sources of regenerating tissue can be stem/progenitor cells, or differentiated cells. However, differentiated cells are usually postmitotic. Thus, when differentiated cells are recruited as source cells, they would have to undergo a process of dedifferentiation, that is they have to lose morphological, functional and/or molecular characteristics of the differentiated state and gain characteristics of a (developmental) progenitor state. Another interesting question is whether cells can alter or expand their potency during regeneration. That is, can stem/progenitor cells that are committed to give rise to one cell type during homeostasis gain the potency to form also other cells types during regeneration, or can differentiated cells even transdifferentiate into another cell type, either directly or via dedifferentiation into a multipotent progenitor? From several studies examining the source of osteoblasts during fin regeneration, it appears that there is not just one single source, but that bone regeneration is achieved by cellular mechanisms displaying a high degree of plasticity and redundancy. Using genetic fate mapping and transgenic reporters of the osteoblast differentiation status, it has been shown that differentiated osteoblasts close to the amputation plane dedifferentiate, that is, they lose the expression of the differentiation marker bglap.
(osteocalcin), gain the progenitor marker runx2, proliferate, and migrate toward the amputation plane and beyond into the forming blastema (Knopf et al., 2011; Sousa et al., 2011; Stewart & Stankunas, 2012). These cells stay lineage restricted and only give rise to osteoblasts in the regenerate (Knopf et al., 2011; Tu & Johnson, 2011). Similarly, eight more cell types, including epidermis, dermal fibroblasts and endothelium, have been proposed to remain lineage restricted during fin regeneration, using tracing of randomly induced clones of cells expressing GFP (Stewart & Stankunas, 2012; Tu & Johnson, 2011).

While it is thus well established that bone can regenerate from differentiated osteoblasts in the zebrafish fin (Figure 5a), in a study combining genetic ablation and fate mapping approaches, Singh et al. found that osteoblasts can also regenerate through de novo differentiation from cells that do not have osteoblast character (Singh, Holdway, & Poss, 2012). Genetic ablation of osteoblasts in the zebrafish fin was achieved by treating fish expressing nitroreductase under control of osterix (sp7) regulatory elements with metrodinazole. The osterix transcription factor is critical for the differentiation of osteoblast precursor cells and is considered a specific marker of committed osteoblasts in the fin (DeLaurier et al., 2010; Li, Felber, Elks, Croucher, & Roehl, 2009; Nakashima et al., 2002). Interestingly, after ablation of seemingly all osterix + osteoblasts, bone can nevertheless regenerate (Singh et al., 2012). This indicates that a non-osteoblast population of cells can differentiate into osteoblasts and can drive complete bone regeneration (Figure 5b). From this observation, two important questions arise. First, what is the identity and normal fate of these non-osteoblastic cells that are able to regenerate bone, and second, are these cells always deployed to contribute to bone regeneration, or do they only get activated when the potential standard source of regenerating osteoblasts is not available? Such an activation of a dormant regenerative mechanism can be observed in several other systems. For example, mammalian pancreatic β-cells regenerate by self-replication (Dor, Brown, Martinez, & Melton, 2004), yet after β-cell ablation, they can be generated by transdifferentiation of α-cells (Thorel et al., 2010). Likewise, biliary cells can transdifferentiate to hepatocytes when the default pathway of liver regeneration by hepatocyte proliferation is blocked (Choi, Ninov, Stainier, & Shin, 2014; Schaub et al., 2018) The potential identity of cells contributing to osteoblast replenishment was recently addressed in a study by Ando et al. (Ando, Shibata, Hans, Brand, & Kawakami, 2017). Using genetic lineage tracing, they found that cells expressing matrix metalloproteinase 9 (mmp9) function as osteoblast progenitor cells (OPC) not only during fin regeneration, but also during fin bone maintenance. Mmp9 might seem like an unlikely marker for osteoblasts, as it is assumed to be expressed in osteoclasts in mammals (De Vrieze, Sharif, Metz, Flik, & Richardson, 2011; Sharif, de Bakker, & Richardson, 2014). However, the authors could show that mmp9 and the osteoclast marker TRAP are expressed in different cell populations in the fin. In uninjured fins, mmp9 positive cells are located in the joints between lepidotrichia segments. Intriguingly, newly regenerated joint cells initially express the osteoblast progenitor marker runx2, but do not become positive for osterix, indicating that they represent immature osteoblasts. However after amputation, lineage tracing of mmp9+ cells using a mmp9:CreERT2 transgenic line showed that progeny of these cells migrate into the regenerate and become positive for osterix, suggesting that they serve as OPCs (Figure 5c). At presumptive new joint sites, they remain osterix negative. Presumptive joint cells sequentially upregulate hoxa13a, evx1 and pthlha and maintain expression of these joint-specific genes, indicative for a commitment to a new cell fate (McMillan et al., 2018). Treatment with RA results in a loss of these markers, upregulation of the differentiation marker bglap and ectopic bone deposition in the joints. These observations are in good agreement with the RA data discussed above, strengthening the model of tightly controlled spatially diverse RA levels steering osteoblast positioning and bone formation.

In the future it will be very interesting to determine, by quantitative lineage tracing, the relative importance of osteoblast dedifferentiation versus de novo osteoblast formation from mmp9+ or other yet unidentified cells for bone regeneration in the zebrafish fin. It is likely that the availability of both differentiated and progenitor cells for bone formation makes bone regeneration more robust.

### REGENERATION VERSUS TUMORIGENESIS

During regeneration, cell proliferation and dedifferentiation must be precisely regulated and executed to restore the organization of tissues. In contrast to regeneration, tumor formation represents a tissue formation process in adults that has gone awry. Tumor suppressor genes, which are inactivated in mammalian tumors, regulate a wide array of cellular activities, including growth, proliferation, and differentiation (Sherr, 2004). The relationship between tumor formation and regeneration has been controversially debated. In particular, it has remained unclear whether tumor suppressors play a beneficial or debilitating role in regeneration (Pomerantz & Blau, 2013). By restricting proliferation, they might
**FIGURE 5** Sources for osteoblasts during regeneration. Schematic longitudinal view of a single ray. (a) Mature osteoblasts in the stump dedifferentiate, migrate into the blastema and re-differentiate. (b) After osteoblast ablation, osteoblasts form *de novo* in the regenerate. (c) Osteoblast progenitor cells (OPC) in the joints migrate into the blastema and differentiate to osteoblasts.
impair regeneration and thus might have to be inactivated for successful regenerative growth. Conversely, their function in controlling organ size might be utilized to orchestrate proper tissue formation during regeneration. For the tumor suppressor protein Arf, which is formed from an alternative transcript of the cdkn2 gene, which also codes for p16, no ortholog can be found in zebrafish (Hesse, Kouklis, Ahituv, & Pomerantz, 2015). Interestingly, a transgene containing the regulatory elements of the human Arf gene is not expressed in zebrafish, except after fin amputation in the blastema (Hesse et al., 2015). However, expression of the Arf coding sequence blocks fin regeneration. This might imply that the regenerative context resembles tumor formation and that successful regeneration requires relaxed tumor suppressing mechanisms. This hypothesis was addressed in a systematic gene expression study where normal fin tissue was compared to blastema tissue, and genes upregulated in the blastema were cross-referenced to the Oncomine cancer database (Hagedorn et al., 2016). This study found that 40% of the blastema-upregulated genes are overexpressed in melanoma, indicating that expression of these genes plays a role both in tissue regeneration and in melanoma progression. This group of genes included several members of the FK506-binding protein family (FKBP proteins). It was previously shown that FK506 induces excessive fin outgrowth during regeneration (Kujawski et al., 2014). Of note, in uninjured fins FK506 treatments led to increased cell proliferation at the distal tip of the fins only, resulting in allometric fin outgrowth but not dysmorphic growth. The FK506-FKBP complex inhibits the protein phosphatase calcineurin (Liu et al., 1991), and calcineurin activity in the blastema is at a minimum during maximal regenerative growth (Kujawski et al., 2014). Altogether, these data suggest that during regeneration, pathways controlling growth are modified to allow fast regeneration, and these pathways might also be disturbed during tumorigenesis (Figure 6).

### CONCLUSIONS

Zebrafish regenerate an amputated caudal fin to its previous size within 2–3 weeks. The fin is not only restored in both its size and shape, but also in its patterning and tissue organization. Multicellular mechanisms are organized through local interactions, which are influenced by global spatial patterning. The findings discussed here provide the beginnings of a comprehensive understanding of the molecular control of positional memory and patterning during fin regeneration. However, to date, many studies into the mechanisms of fin regeneration are based on pharmacological approaches or global genetic interventions, thus genetic analyses and cell-type-specific manipulations will be necessary to provide further insight into the mechanisms and to understand how they are dynamically remodeled during regeneration.

### CONFLICT OF INTEREST

The authors have declared no conflicts of interest for this article.

### AUTHOR CONTRIBUTIONS

Ivonne Sehring: Conceptualization; writing-original draft, review, and editing. Gilbert Weidinger: Conceptualization; Writing-original draft, review, and editing.
REFERENCES

Adams, M. S., Ko, D. S., & Levin, M. (2007). H+ pump-dependent changes in membrane voltage are an early mechanism necessary and sufficient to induce Xenopus tail regeneration. Development, 134(7), 1323–1335. https://doi.org/10.1242/dev.02812

Adell, T., Cebrià, F., & Saló, L. (2010). Gradients in planarian regeneration and homeostasis. Cold Spring Harbor Perspectives in Biology, 2(1), a000505. https://doi.org/10.1101/cshperspect.a000505

Ando, K., Shibata, E., Hans, S., Brand, M., & Kawakami, A. (2017). Osteoblast production by reserved progenitor cells in zebrafish bone regeneration and maintenance. Developmental Cell, 43(5), 643–650.e3. https://doi.org/10.1016/j.devcel.2017.10.015

Apschner, A., Schulte-Merker, S., & Witten, P. E. (2011). Not all bones are created equal – Using zebrafish and other teleost species in osteogenesis research. Methods in Cell Biology, 105, 239–255. https://doi.org/10.1016/B978-0-12-381320-6.00010-2

Armstrong, B. E., Henner, A., Stewart, S., & Stankunas, K. (2017). Shh promotes direct interactions between epidermal cells and osteoblast progenitors to shape regenerated zebrafish bone. Development (Cambridge, England), 144(7), 1165–1176. https://doi.org/10.1242/dev.143792

Azevedo, A. S., Grotek, B., Jacinto, A., Weidinger, G., & Saúde, L. (2011). The regenerative capacity of the zebrafish caudal fin is not affected by repeated amputations. PLoS One, 6(7), e22820. https://doi.org/10.1371/journal.pone.0022820

Azevedo, A. S., Sousa, J., Jacinto, A., & Saúde, L. (2012). An amputation resets positional information to a proximal identity in the regenerating zebrafish caudal fin. BMC Developmental Biology, 12, 24. https://doi.org/10.1186/1471-213X-12-24

Barker, N. (2014). Adult intestinal stem cells: Critical drivers of epithelial homeostasis and regeneration. Nature Reviews Molecular Cell Biology, 15(1), 19–33. https://doi.org/10.1038/nrm3721

Becerra, J., Montes, G. S., Bexiga, S. R., & Junqueira, L. C. (1983). Structure of the tail fin in teleosts. Cell and Tissue Research, 230(1), 127–137. https://doi.org/10.1007/bf00216033

Blum, N., & Begemann, G. (2015a). Osteoblast de- and redifferentiation are controlled by a dynamic response to retinoic acid during zebrafish fin regeneration. Development, 142(17), 2894–2903. https://doi.org/10.1242/dev.120204

Blum, N., & Begemann, G. (2015b). Retinoic acid signaling spatially restricts osteoblasts and controls ray-interray organization during zebrafish fin regeneration. Development, 142(17), 2888–2893. https://doi.org/10.1242/dev.120212

Blum, N., Begemann, G., Rauch, G. J., Geisler, R., & Ingham, P. W. (2012). Retinoic acid signaling controls the formation, proliferation and survival of the blastema during adult zebrafish fin regeneration. Development (Cambridge, England), 139(1), 107–116. https://doi.org/10.1242/dev.065391

Brown, A. M., Fisher, S., & Lovine, M. K. (2009). Osteoblast maturation occurs in overlapping proximal-distal compartments during fin regeneration in zebrafish. Developmental Dynamics, 238(11), 2922–2928. https://doi.org/10.1002/dvdy.22114

Bryant, S. V., French, V., & Bryant, P. J. (1981). Distal regeneration and symmetry. Science, 212(4498), 993–1002. https://doi.org/10.1126/science.212.4498.993

Chen, C. H., Merriman, A. F., Savage, J., Willer, J., Wahlig, T., Katsanis, N., ... Poss, K. D. (2015). Transient laminin beta 1a induction defines the wound epidermis during zebrafish fin regeneration. PLoS Genetics, 11(8), e105437. https://doi.org/10.1371/journal.pgen.1005437

Choi, T.-Y., Ninov, N., Stainier, D. Y. R., & Shin, D. (2014). Extensive conversion of hepatic biliary epithelial cells to hepatocytes after near total loss of hepatocytes in zebrafish. Gastroenterology, 146(3), 776–788. https://doi.org/10.1053/j.gastro.2013.10.019

Cruciat, C.-M., Okhawara, B., Acebron, S. P., Karaulhanov, E., Reinhard, C., Ingelfinger, D., ... Niehrs, C. (2010). Requirement of prorenin receptor and vacuolar H+–ATPase-mediated acidification for Wnt signaling. Science (New York, N.Y.), 327(5964), 459–463. https://doi.org/10.1126/science.1179802

Da Silva, S. M., Gates, P. B., & Brockes, J. P. (2002). The new ortholog of CD59 is implicated in proximodistal identity during amphibian limb regeneration. Developmental Cell, 3(4), 547–555. https://doi.org/10.1016/S1534-5807(02)00288-5

Daane, J. M., Lanni, J., Rothenberg, I., Seebohm, G., Higdon, C. W., Johnson, S. L., & Harris, M. P. (2018). Bioelectric-calcineurin signaling module regulates allometric growth and size of the zebrafish fin. Scientific Reports, 8(1), 10391. https://doi.org/10.1038/s41598-018-28450-6

De Vrieze, E., Sharif, F., Metz, J. R., Flik, G., & Richardson, M. K. (2011). Matrix metalloproteinases in osteoclasts of ontogenetic and regenerating zebrafish scales. Bone, 48(4), 704–712. https://doi.org/10.1016/j.bone.2010.12.017

DeLaurier, A., Eames, B. F., Blanco-Sánchez, B., Peng, G., He, X., Swartz, M. E., ... Kimmel, C. B. (2010). Zebrafish sp7:EGFP: A transgenic for studying otic vesicle formation, skeletogenesis, and bone regeneration. Genesis, 48(8), 505–511. https://doi.org/10.1002/dvg.20639

Dor, Y., Brown, J., Martinez, O. I., & Melton, D. A. (2004). Adult pancreatic β-cells are formed by self-duplication rather than stem-cell differentiation. Nature, 429(6987), 41–46. https://doi.org/10.1038/nature02520
Echeverri, K., & Tanaka, E. M. (2005). Proximodistal patterning during limb regeneration. Developmental Biology, 279(2), 391–401. https://doi.org/10.1016/j.ydbio.2004.12.029

Garza-Garcia, A., Harris, R., Espósito, D., Gates, P. B., & Driscoll, P. C. (2009). Solution structure and phylogenetics of Prod1, a member of the three-finger protein superfamily implicated in salamander limb regeneration. PLoS One, 4(9), e7123. https://doi.org/10.1371/journal.pone.0007123

Gemberling, M., Bailey, T. J., Hyde, D. R., & Poss, K. D. (2013). The zebrafish as a model for complex tissue regeneration. Trends in Genetics, 29(11), 611–620. https://doi.org/10.1016/j.tig.2013.07.003

Géraudie, J., & Landis, W. J. (1982). The fine structure of the developing pelvic fin dermal skeleton in the trout Salmo gairdneri. The Anatomical Record, 206(2), 141–156. https://doi.org/10.1002/1099-1004(198210)206:2<141::AID-ARV3202060217>3.0.CO;2-K

Hagedorn, M., Siegfried, G., Hooks, K. B., Khatib, A.-M., Hagedorn, M., Siegfried, G., ... & Poss, K. D. (2013). Transcriptional components of anteroposterior positional information during zebrafish fin regeneration genes with expression data of human tumors in silico uncovers potential novel melanoma markers. Oncotarget, 7(44), 71567–71579. https://doi.org/10.18632/oncotarget.12257

Hesse, R. G., Kouklis, G. K., Ahituv, N., & Pomerantz, J. H. (2015). The human ARF tumor suppressor senses blastema activity and suppresses epimorphic tissue regeneration. eLife, 4, e07702. https://doi.org/10.7554/eLife.07702

Iovine, M. K., Higgins, E. P., Hindes, A., Coblitz, B., & Johnson, S. L. (2005). Mutations in connexin43 (GJA1) perturb bone growth in zebrafish fins. Developmental Biology, 278(1), 2008–2219. https://doi.org/10.1016/j.ydbio.2004.11.005

Iovine, M. K., & Johnson, S. L. (2000). Genetic analysis of isometric growth control mechanisms in the zebrafish caudal fin. Genetics, 155(3), 1321–1329.

Johnson, S. L., & Bennett, P. (1998). Chapter 16 growth control in the ontogenetic and regenerating zebrafish fin. Methods in Cell Biology, 59, 201–311. https://doi.org/10.1016/S0091-679X(08)61831-2

Knopf, F., Hammond, C., Chekuru, A., Kurth, T., Hans, S., Weber, C. W., ... & Weidinger, G. (2011). Bone regenerates via dedifferentiation of osteoblasts in the zebrafish fin. Developmental Cell, 20(5), 713–724. https://doi.org/10.1016/j.devcel.2011.04.014

König, D., Page, L., Chassot, B., & Jazwinka, A. (2018). Dynamics of actinotrichia regeneration in the adult zebrafish fin. Developmental Biology, 433(2), 416–432. https://doi.org/10.1016/j.ydbio.2017.07.024

Kotton, D. N., & Morrisey, E. E. (2014). Lung regeneration: Mechanisms, applications and emerging stem cell populations. Nature Medicine, 20(8), 822–832. https://doi.org/10.1038/nm.3642

Kujawski, S., Lin, W., Kitte, F., Bömkel, M., Fuchs, S., Arulmozhiwarman, G., ... & Antos, C. L. (2014). Calcineurin regulates coordinated outgrowth of zebrafish regenerating fins. Developmental Cell, 28(5), 573–587. https://doi.org/10.1016/j.devcel.2014.01.019

Kumar, A., Gates, P. B., & Brookes, J. P. (2007). Positional identity of adult stem cells in salamander limb regeneration. Comptes Rendus – Biologies, 330(6–7), 485–490. https://doi.org/10.1016/j.crvi.2007.01.006

Landis, W. J., & Géraudie, J. (1990). Organization and development of the mineral phase during early ontogenesis of the bony fin rays of the trout Oncorhynchus mykiss. The Anatomical Record, 228(4), 383–391. https://doi.org/10.1002/ar.1092280404

Lee, Y., Grill, S., Sanchez, A., Murphy-Ryan, M., & Poss, K. D. (2005). Fgf signaling instructs position-dependent growth rate during zebrafish fin regeneration. Development (Cambridge, England), 132(23), 5173–5183. https://doi.org/10.1242/dev.02101

Lee, Y., Hami, D., De Val, S., Kagermeier-Schenk, B., Wills, A. A., Black, B. L., ... & Poss, K. D. (2009). Maintenance of blastema proliferation by functionally diverse epidermis in regenerating zebrafish fins. Developmental Biology, 331(2), 270–280. https://doi.org/10.1016/J.YDBIO.2009.05.545

Li, N., Felber, K., Elks, P., Croucher, P., & Roehl, H. H. (2009). Tracking gene expression during zebrafish osteoblast differentiation. Developmental Dynamics, 238(2), 459–466. https://doi.org/10.1002/dvdy.21838

Liu, J., Farmer, J. D., Lane, W. S., Friedman, J., Weissman, I., & Schreiber, S. L. (1991). Calcineurin is a common target of cyclophilin-cyclosporin A and FKBP-FK506 complexes. Cell, 66(4), 807–815. https://doi.org/10.1016/0092-8674(91)90124-H

Mathew, L. K., Sengupta, S., Franzosa, J. A., Perry, J., La Du, J., Andreassen, E. A., & Tanguay, R. L. (2009). Comparative expression profiling reveals an essential role for raldh2 in epimorphic regeneration. The Journal of Biological Chemistry, 284(48), 33642–33653. https://doi.org/10.1074/jbc.M109.011668

McMillan, S. C., Zhang, J., Phan, H.-E., Jeradi, S., Probst, L., Hammerschmidt, M., & Akimenko, M.-A. (2018). A regulatory pathway involving retinoic acid and calcineurin demarcates and maintains joint cells and osteoblasts in the fin regenerate. Development (Cambridge, England), 145, dev.161158. https://doi.org/10.1242/dev.161158

Monteiro, J., Aires, R., Becker, J. D., Jacinto, A., Certal, A. C., & Rodrigo-León, J. (2014). V-ATPase proton pumping activity is required for adult zebrafish appendage regeneration. PLoS One, 9(3), e92594. https://doi.org/10.1371/journal.pone.0092594

Murciano, C., Fernández, T. D., Durán, I., Maseda, D., Ruiz-Sánchez, J., Becerra, J., ... & Mari-Beffa, M. (2002). Ray–Interray interactions during fin regeneration of Danio rerio. Developmental Biology, 252(2), 214–224. https://doi.org/10.1006/dbio.2002.0848

Nachtrab, G., Kikuchi, K., Tornini, V. A., & Poss, K. D. (2013). Transcriptional components of anteroposterior positional information during zebrafish fin regeneration. Development, 140(18), 3754–3764. https://doi.org/10.1242/dev.098798

Nacu, E., Glausch, M., Le, H. Q., Damanik, F. F. R., Schuez, M., Knapp, D., ... & Tanaka, E. M. (2013). Connective tissue cells, but not muscle cells, are involved in establishing the proximo-distal outcome of limb regeneration in the axolotl. Development, 140(3), 513–518. https://doi.org/10.1242/dev.081752

Nacu, E., & Tanaka, E. M. (2011). Limb regeneration: A new development? Annual Review of Cell and Developmental Biology, 27(1), 409–440. https://doi.org/10.1146/annurev-cellbio-092910-154115
Nakashima, K., Zhou, X., Kunkel, G., Zhang, Z., Deng, J. M., Behringer, R. R., & de Crombrugghe, B. (2002). The novel zinc-finger-containing transcription factor osterix is required for osteoblast differentiation and bone formation. *Cell*, 108(1), 17–29. https://doi.org/10.1016/S0092-8674(01)00622-5

Nechiporuk, A., & Keating, M. T. (2002). A proliferation gradient between proximal and mxb-expressing distal blastema directs zebrafish fin regeneration. *Development (Cambridge, England)*, 129(11), 2607–2617.

Perathoner, S., Daane, J. M., Henrion, U., Seebohm, G., Hidgon, C. W., Johnson, S. L., ... Harris, M. P. (2014). Bioelectric signaling regulates size in zebrafish fins. *PLoS Genetics*, 10(1), e1004080. https://doi.org/10.1371/journal.pgen.1004080

Pfefferli, C., & Jazwinska, A. (2015). The art of fin regeneration in zebrafish. *Regeneration*, 2(2), 72–83. https://doi.org/10.1002/reg.2.33

Poleo, G., Brown, C. W., Laforest, L., & Akimenko, M. A. (2001). Cell proliferation and movement during early fin regeneration in zebrafish. *Developmental Dynamics*, 222(4), 380–390. https://doi.org/10.1002/dvdy.1152

Pomerantz, J. H., & Blau, H. M. (2013). Tumor suppressors: Enhancers or suppressors of regeneration? *Development (Cambridge, England)*, 140, 2502–2512, 12. https://doi.org/10.1242/dev.1084210

Poss, K. D., Keating, M. T., & Nechiporuk, A. (2003). Tales of regeneration in zebrafish. *Developmental Dynamics*, 222(2), 347–358. https://doi.org/10.1002/dfdy.10220

Poss, K. D., Wilson, L. G., & Keating, M. T. (2002). Heart regeneration in zebrafish. *Proceedings of the National Academy of Sciences of the United States of America*, 99(13), 8713–8718. https://doi.org/10.1073/pnas.122571799

Rabinowitz, J. S., Rohitaille, A. M., Wang, Y., Ray, C. A., Thummel, R., Gu, H., ... Moon, R. T. (2017). Transcriptomic, proteomic, and metabolomic landscape of positional memory in the caudal fin of zebrafish. *Proceedings of the National Academy of Sciences of the United States of America*, 114(5), E717–E726. https://doi.org/10.1073/pnas.1620755114

Rath, S., Liebl, J., Fürst, R., Vollmar, A. M., & Zahler, S. (2014). Regulation of endothelial signaling and migration by v-ATPase. *Angiogenesis*, 17(3), 587–601. https://doi.org/10.1007/s10456-013-9408-z

Ricci, L., & Srivastava, M. (2018). Wound-induced cell proliferation during animal regeneration. *Wiley Interdisciplinary Reviews: Developmental Biology*, 7(5), e321. https://doi.org/10.1002/wdev.321

Rolland-Lagan, A.-G., Paquette, M., Tweedle, V., & Akimenko, M.-A. (2012). Morphogen-based simulation model of ray growth and joint patterning during fin development and regeneration. *Development*, 139(6), 1188–1197. https://doi.org/10.1242/dev.073452

Schaub, J. R., Huppert, K. A., Kurial, S. N. T., Hsu, B. Y., Cast, A. E., Donnelly, B., ... Willenbring, H. (2018). De novo formation of the biliary system by TGF-β-mediated hepatocyte transdifferentiation. *Nature*, 557(7704), 247–251. https://doi.org/10.1038/s41586-018-0075-5

Schnabel, K., Wu, C.-C., Kurth, T., & Weidinger, G. (2011). Regeneration of cryoinjury induced necrotic heart lesions in zebrafish is associated with epicardial activation and cardiomyocyte proliferation. *PLoS ONE*, 6(4), e18503. https://doi.org/10.1371/journal.pone.0018503

Sehring, I. M., Jahn, C., & Weidinger, G. (2016). Zebrafish fin and heart: What's special about regeneration? *Current Opinion in Genetics & Development*, 40, 48–56. https://doi.org/10.1016/j.gde.2016.05.011

Sharif, F., de Bakker, M. A. G., & Richardson, M. K. (2014). Osteoclast-like cells in early zebrafish embryos. *Cell Journal*, 16(2), 211–224.

Sherr, C. J. (2004). Principles of tumor suppression. *Cell*, 116(2), 235–246. https://doi.org/10.1016/S0092-8674(03)01075-4

Shibata, E., Liu, Z., Kawasaki, T., Sakai, N., & Kawakami, A. (2016). Heterogeneous fates and dynamic rearrangement of regenerative epidermis-derived cells during zebrafish fin regeneration. *Development (Cambridge, England)*, 145(8), dev162016. https://doi.org/10.1242/dev.162016

Shibata, E., Liu, Z., Kawasaki, T., Sakai, N., & Kawakami, A. (2018). Robust and local positional information within a fin ray directs fin length during zebrafish regeneration. *Development, Growth & Differentiation*, 60(6), 354–364. https://doi.org/10.1111/dgd.12558

Simkin, J., Han, M., Yu, L., Yan, M., & Muneoka, K. (2013). The mouse digit tip: From wound healing to regeneration (pp. 419–435). Totowa, NJ: Humana Press. https://doi.org/10.1007/978-1-62703-505-7_24

Simkin, J., Sammarco, L., Dawson, L., Schanes, P., Yu, L., & Muneoka, K. (2015). The mammalian blastema: Regeneration at our fingertips. *Regeneration*, 2(3), 93–105. https://doi.org/10.1002/reg.2.36

Sims, K., Eble, D. M., & Iovine, M. K. (2009). Connexin43 regulates joint location in zebrafish fins. *Developmental Biology*, 327(2), 410–418. https://doi.org/10.1016/j.ydbio.2008.12.027

Singh, S. P., Holdway, J. E., & Poss, K. D. (2012). Regeneration of amputated zebrafish fin rays from de novo osteoblasts. *Developmental Cell*, 22(4), 879–886. https://doi.org/10.1016/J.DECYDEV.2012.03.006

Sousa, A., Fonseca, M., Simões, M., Leon, J., ... Nissen, R. (2011). Differentiated skeletal cells contribute to blastema formation during zebrafish fin regeneration. *Development (Cambridge, England)*, 138(18), 3897–3905. https://doi.org/10.1242/dev.064717

Stewart, S., & Stankunas, K. (2012). Limited dedifferentiation provides replacement tissue during zebrafish fin regeneration. *Developmental Biology*, 365(2), 339–349. https://doi.org/10.1016/J.YDBIO.2012.02.031

Takayama, K., Muto, A., & Kilukuchi, Y. (2018). Leucine/glutamine and α-ATPase/lysosomal acidification via mTORC1 activation are required for position-dependent regeneration. *Scientific Reports*, 8(1), 8278. https://doi.org/10.1038/s41598-018-26664-2
Tata, P. R., & Rajagopal, J. (2016). Cellular plasticity: 1712 to the present day. *Current Opinion in Cell Biology*, 43, 46–54. https://doi.org/10.1016/J.CEB.2016.07.005

Thorel, F., Népote, V., Avril, I., Kohno, K., Desgraz, R., Chera, S., & Herrera, P. L. (2010). Conversion of adult pancreatic α-cells to β-cells after extreme β-cell loss. *Nature*, 464(7292), 1149–1154. https://doi.org/10.1038/nature08894

Tornini, V. A., Puliafito, A., Slota, L. A., Thompson, J. D., Nachtrab, G., Kaushik, A.-L., ... Poss, K. D. (2016). Live monitoring of blastemal cell contributions during appendage regeneration. *Current Biology*, 26(22), 2981–2991. https://doi.org/10.1016/J.CUB.2016.08.072

Tu, S., & Johnson, S. L. (2011). Fate restriction in the growing and regenerating zebrafish fin. *Developmental Cell*, 20(5), 725–732. https://doi.org/10.1016/J.DEVCEL.2011.04.013

Vaccari, T., Duchi, S., Cortese, K., Tacchetti, C., & Bilder, D. (2010). The vacuolar ATPase is required for physiological as well as pathological activation of the notch receptor. *Development (Cambridge, England)*, 137(11), 1825–1832. https://doi.org/10.1242/dev.045484

Wehner, D., Cizelsky, W., Vasudevaro, M. D., Özhan, G., Haase, C., Kagermeier-Schenk, B., ... Weidinger, G. (2014). Wnt/β-catenin signaling defines organizing centers that orchestrate growth and differentiation of the regenerating zebrafish caudal fin. *Cell Reports*, 6(3), 467–481. https://doi.org/10.1016/j.celrep.2013.12.036

Wehner, D., & Weidinger, G. (2015). Signaling networks organizing regenerative growth of the zebrafish fin. *Trends in Genetics: TIG*, 31(6), 336–343. https://doi.org/10.1016/j.tig.2015.03.012

White, J. A., Boffa, M. B., Jones, B., & Petkovich, M. (1994). A zebrafish retinoic acid receptor expressed in the regenerating caudal fin. *Development*, 120(7), 1861–1872.

Wolpert, L. (2016). *Positional information* (pp. 1–5). Chichester, England: John Wiley & Sons, Ltd. https://doi.org/10.1002/9780470015902.a0020914.pub2

Xu, C., Volkery, S., & Siekmann, A. F. (2015). Intubation-based anesthesia for long-term time-lapse imaging of adult zebrafish. *Nature Protocols*, 10(12), 2064–2073. https://doi.org/10.1038/nprot.2015.130

Yang, H.-C., Liu, S.-J., & Fogo, A. B. (2014). Kidney regeneration in mammals. *Nephron. Experimental Nephrology*, 126(2), 50. https://doi.org/10.1159/000360661

Zhang, J., Jeradi, S., Strähle, U., & Akimenko, M.-A. (2012). Laser ablation of the sonic hedgehog-a-expressing cells during fin regeneration affects ray branching morphogenesis. *Developmental Biology*, 365(2), 424–433. https://doi.org/10.1016/J.YDBIO.2012.03.008

Zheng, L., Zhang, W., Zhou, Y., Li, F., Wei, H., Peng, J., ... Peng, J. (2016). Recent advances in understanding amino acid sensing mechanisms that regulate mTORC1. *International Journal of Molecular Sciences*, 17(10), 1636. https://doi.org/10.3390/ijms17101636

---

**How to cite this article:** Sehring IM, Weidinger G. Recent advancements in understanding fin regeneration in zebrafish. *WIREs Dev Biol*. 2020;9:e367. https://doi.org/10.1002/wdev.367