Inducing Vertebrate Limb Regeneration: A Review of Past Advances and Future Outlook

Devon Davidian[1] and Michael Levin[1,2]

[1]Allen Discovery Center at Tufts University, Medford, Massachusetts 02155, USA

[2]Wyss Institute for Biologically Inspired Engineering, Harvard University, Boston, Massachusetts 02115, USA

Correspondence: michael.levin@tufts.edu

Cold Spring Harbor Perspectives in Biology
www.cshperspectives.org

Limb loss due to traumatic injury or amputation is a major biomedical burden. Many vertebrates exhibit the ability to form and pattern normal limbs during embryogenesis from amorphous clusters of precursor cells, hinting that this process could perhaps be activated later in life to rebuild missing or damaged limbs. Indeed, some animals, such as salamanders, are proficient regenerators of limbs throughout their life span. Thus, research over the last century has sought to stimulate regeneration in species that do not normally regenerate their appendages. Importantly, these efforts are not only a vital aspect of regenerative medicine, but also have fundamental implications for understanding evolution and the cellular control of growth and form throughout the body. Here we review major recent advances in augmenting limb regeneration, summarizing the degree of success that has been achieved to date in frog and mammalian models using genetic, biochemical, and bioelectrical interventions. While the degree of whole limb repair in rodent models has been modest to date, a number of new technologies and approaches comprise an exciting near-term road map for basic and clinical progress in regeneration.

Patients who lose limbs to injury, cancer, or degenerative diseases must readjust nearly every aspect of their lives. The most recent study aiming to quantify limb loss in the United States found that, as of 2005, there are 1.6 million individuals living with limb loss, with the number of amputees increasing by 185,000 each year to a projected 3.5 million by 2050 (Ziegler-Graham et al. 2008). Besides the unquantifiable suffering it imposes, limb loss presents life-long challenges, some of which currently have no adequate solution. Although inorganic prosthetics are improving (Windrich et al. 2016; Trent et al. 2020), organic restoration of a lost appendage would be the definitive solution. While great progress has been made in methods to treat superficial wounds resulting in rapid, nearly scarless healing (Moore et al. 2018; Monavarian et al. 2019), complete renewal of vertebrate limbs remains a fundamental challenge to overcome. Because most vertebrates create perfect limbs during embryogenesis, it is clear that their genomes and cellular pathways possess the information needed to create the needed anatomical structures. Could this information be reaccessed during adulthood in cases of traumatic injury or disease-induced amputation?

Appendage regeneration is not only a profound unmet biomedical need. It is also a paradigm case of a more fundamental problem of

Editors: Kenneth D. Poss and Donald T. Fox
Additional Perspectives on Regeneration available at www.cshperspectives.org

 doi: 10.1101/cshperspect.a040782
Cite this article as *Cold Spring Harb Perspect Biol* 2022;14:a040782

evolutionary, developmental, and synthetic biology: how do cells cooperate to build specific complex organs and stop precisely when the correct anatomical structure is complete (Pezzulo and Levin 2016)? How are cellular collectives driven to build one structure rather than another from exactly the same cellular building blocks (e.g., hand vs. foot), and could those same information-processing mechanisms be exploited to create needed anatomical structures in regenerative or even synthetic bioengineering settings?

As nature would have it, large-scale regeneration is not restricted to science fiction. Many invertebrates, such as hydra (Holstein et al. 2003), planaria (Elliott and Sánchez Alvarado 2013), and various arthropods (Maruzzo and Bortolin 2013), display perfect regeneration from injuries that would otherwise be fatal for many other organisms (Birnbaum and Alvarado 2008). Importantly, there are also several vertebrate models that serve as proof-of-principle for complex organ regeneration. For example, in salamanders, this capacity is retained well into adulthood (Brockes 1997), enabling regeneration of eyes, jaws, limbs, heart, ovaries, and spinal cord (Maden 2008; McCusker and Gardiner 2011). Other amphibians, such as frogs, retain the ability to regenerate up to a specific developmental stage (typically metamorphosis [Dent 1962]) but experience decline of regenerative capacity as they mature (Dent 1962).

Remarkably, instances of regeneration are also observed in non-amphibian vertebrates, from fish (Poss et al. 2003; Zupanc 2008; Sîrbulescu and Zupanc 2011) to mammals (Muneoka and Dawson 2020). Such instances of regeneration are typically restricted to specialized structures such as the lizard tail (Jacyniak et al. 2017; Lozito and Tuan 2017), mouse digit tip (Lehoczky 2017; Dawson et al. 2018; Seifert and Muneoka 2018), and deer antler (Kierdorf et al. 2007; Li et al. 2014), while wound healing in other tissues within these animals typically follows a general fibrotic response (Gurtner et al. 2008). Examples of regeneration exist even in humans; the human digit tip has been observed to regenerate in children (Illingworth 1974), although this regenerative capacity is diminished in adulthood (Shieh and Cheng 2015; Jafari et al. 2017). The routine, reliable, and very rapid growth of bone, vasculature, and innervation in adult deer antler (up to ~1 cm per day) suggests that there is nothing fundamental preventing complex appendages from regenerating in mammals (Price et al. 2005; Kierdorf et al. 2007; Li 2012).

Harnessing the power of the body's healing processes to stimulate the creation of a complex anatomical structure is a fundamentally daunting scientific prospect with tantalizing potential for addressing biomedical needs from birth defects to cancer to aging. Much work has been done over the last century to attempt to trigger this process in species that normally do not regenerate. While endogenous mechanisms in regeneration-competent species have been amply reviewed (Lehoczky 2017; Londono et al. 2018; Seifert and Muneoka 2018), the development of future biomedical therapies in this field depends on a thorough synthesis of prior results in nonregenerative models. Thus, here we provide an overview of what has been achieved by various interventions in five major categories: (1) surgical/engineered interventions, (2) biochemical pathway targeting, (3) murine transgenic lines with enhanced regenerative capacity, (4) targeted manipulations of cellular membrane potential, and (5) applied bioelectric interventions. We end with a perspective on promising future directions in this exciting, multidisciplinary field.

EPIMORPHIC REGENERATION: A BRIEF OVERVIEW

Naturally occurring vertebrate limb regeneration uses a regenerative process known as epimorphic regeneration to produce and pattern new tissues from the wound edge. Epimorphic regeneration begins with epithelial wound closure and the accumulation of a mass of undifferentiated cells; the resulting structure—the blastema (Allen et al. 2016; Seifert and Muneoka 2018; Muneoka and Dawson 2020)—is the source of new tissues. Rapid re-epithelialization of the wound bed and blastemal cell accumulation is followed by a thickening of the wound

Cold Spring Harbor Perspectives in Biology
www.cshperspectives.org

epidermis, transforming it to a central blastemal signaling center known as the apical epithelial (or ectodermal) cap (AEC) (Han et al. 2005; Seifert and Muneoka 2018). The most highly regenerative vertebrates, salamanders, achieve this re-epithelialization in under 12 h postamputation (Repesh and Oberpriller 1978; Carlson et al. 1998), while other regenerative vertebrates, such as developing frogs, accomplish this in 24 h (Pearl et al. 2008; Beck et al. 2009). The regenerative AEC is crucial to proper epimorphic regeneration, as replacing the AEC with mature skin completely inhibits regeneration (Gidge and Rose 1944; Mescher 1976). In contrast, poorly regenerative vertebrates take 2 to 3 times longer to re-epithelialize, and fail to establish a functional AEC (Han et al. 2005; Londono et al. 2018; Muneoka and Dawson 2020).

It has been suggested that regeneration arises from a reactivation of the developmental mechanisms that drive limb outgrowth during development. One common family of genetic regulators implicated in vertebrate limb regeneration are fibroblast growth factor (FGF) proteins, having functional roles in limb outgrowth and morphogenesis with signaling functions in the apical ectodermal ridge (AER) of the developing limb bud (Martin 1998). In chick embryos, FGF8 and FGF10 have been shown to be endogenous inducers of limb bud formation (an apical ectodermal factor; Mahmood et al. 1995; Ohuchi et al. 1997), while ectopic treatment with FGF1, FGF2, or FGF4 similarly induces the formation of supernumerary limbs during development (Cohn et al. 1995). Interestingly, during limb regeneration in developing frogs (i.e., limb amputation prior to metamorphosis), FGF8 is promptly reexpressed in the newly formed AEC (Christen and Slack 1997). Likewise, salamander limb development and regeneration both use the expression of FGF8 in the AER/AEC, while other FGF proteins are exclusive to either limb development (FGF4, FGF10) or regeneration (FGF1, FGF2) (flanks, Christensen et al. 2002; and blastemas of *Ambystoma*, Dungan et al. 2002; Sun et al. 2002; Giampaoli et al. 2003). Conserved signals between development and regeneration also include bone morphogenic proteins (BMPs) (Pajni-Underwood et al. 2007; Yu et al. 2010, 2012; Makanae et al. 2014; Norrie et al. 2014; Vieira et al. 2019), Msh homeobox proteins (Msx) (Davidson et al. 1991; Kostakopoulou et al. 1996; Koshiba et al. 1998; Han et al. 2003; Johnson et al. 2020), homeobox proteins (Hox) (Gardiner et al. 1995; Kostakopoulou et al. 1996; Nelson et al. 1996), and Sonic hedgehog (Shh) (Riddle et al. 1993; Chiang et al. 2001; Yakushiji et al. 2009). However, it remains an open question as to whether the high-level mechanisms that drive self-limiting morphogenesis toward a specific anatomical outcome are the same as those functioning in regeneration. Many efforts over the last 100 years have targeted diverse aspects of the process, in attempts to induce a limb-repair cascade in species that normally do not activate it after injury.

SURGICAL AND ENGINEERED INTERVENTIONS TO PROMOTE VERTEBRATE LIMB REGENERATION

Researchers have used various surgical approaches to improve regeneration, including the transfer of regenerative blastemal tissue and implantation of extracellular matrix (ECM) protein-based grafts. Early blastemal transplantation studies in Urodeles suggested that blastemal tissue was quite plastic and could adopt new regenerative fate if grafted onto heterotopic amputation sites (Weiss 1927; Schaxel 1934). However, it was later realized that, at a certain regenerative stage, the Urodele blastema becomes functionally independent of the host environment and will reliably regenerate all of the appropriate donor structures irrespective of where it is placed (Trampusch 1966; Stocum and Dearlove 1972; Pescitelli and Stocum 1980; Pecorino et al. 1996a,b).

In mouse digit tips, the nail bed is a known regulator and driving force behind successfully regenerating digit tips (Takeo et al. 2013; Leung et al. 2014). Interestingly, if the digit is amputated at the intermediate phalange, P2 (which is nonregenerative), grafting of a segment of P3 nail/nail bed epithelium induces minor bone growth directed upward toward the implanted nail epithelium (Mohammad et al. 1999), sug-

gesting this epithelium is coordinating new bone growth. In contrast, grafting of regenerative fibroblasts derived from P3 blastemas into a host P2 amputation wound bed fails to induce any regenerative effect (Wu et al. 2013). Alternatively, if P2 fibroblasts are grafted into an amputated blastema forming P3, they will actively contribute toward regeneration of the P3 segment and restore P3 connective tissues. These findings suggest that the primary driver of epimorphic regeneration is a wound microenvironment conducive for regeneration, and that grafting of regenerative tissues alone is insufficient to drive full-scale regeneration. Additionally, these studies demonstrate that properly established regenerative environments have the capacity to reprogram nonregenerative cells (Wu et al. 2013).

Regenerative fibroblasts appear to have the capacity to participate in regeneration even if derived from nonregenerative origin tissue. However, regenerative success may be driven partly by the tissue microenvironment in which they are inserted. This is supported by studies showing that growth medium, conditioned by regenerative stem cells (i.e., containing bioactive compounds secreted by highly regenerative cells such as those responsible for deer antler regeneration [Shabbir et al. 2015; Li et al. 2017; Rong et al. 2019]), can be used to accelerate wound healing and enhance overall regeneration in cutaneous injuries when administered as a cell-free media (Li et al. 2017; Menéndez-Menéndez et al. 2017; Rong et al. 2019). This finding, taken together with the results demonstrating the modular nature of fibroblasts, suggests that a proregenerative tissue microenvironment can have great influence over the activity of the native cells and failure to establish a proper microenvironment may significantly impact the regenerative capability of tissue.

Surgical manipulation of developing limbs can shed light on potential drivers for limb regeneration. For example, if the hand of a developing mouse or frog embryo is modified surgically to bring together dissimilar tissue along an axis (e.g., anterior adjacent to posterior tissue), the resultant hand develops supernumerary digits (Wanek et al. 1989; Yokoyama et al. 1998)—a phenomenon that has been interpreted as an intercalation rule that triggers growth at regions of nonadjacent positional information (Bryant and Iten 1977; Mittenthal and Trevarrow 1984; Crawford and Stocum 1988; Beck et al. 1999). This resembles the induction of supernumerary limbs in adult axolotls through the grafting of contralateral epidermis containing dermal cells of contrary axis identity (Endo et al. 2004). Ectopic growth also occurs upon grafting mouse embryonic limb buds onto amputated neonatal limbs. Grafting of the embryonic limb bud to an amputated forelimb of a neonatal mouse promotes significant bone and cartilage regeneration with the resulting regenerate complexity related to the developmental stage of the grafted limb bud (Fig. 1; Masaki and Ide 2007). Interestingly, the resulting regenerate outcome was not dependent on the native structure of the grafted limb bud, as grafts using cell limb bud homogenates formed equally complex regenerate structures (Masaki and Ide 2007). This suggests that a key determinant of limb outgrowth and morphogenesis during regeneration is the complement of cells and tissues in the regenerate's microenvironment.

An important driver and promoter of the blastemal microenvironment is the AEC (Shimokawa et al. 2012). In nonregenerative systems, the wound bed of an amputated limb or digit does not form a blastema but rather is slowly re-epithelialized with mature non-AEC epithelia through the migration of keratinocytes, fibroblasts, and myofibroblast-driven wound contraction (Martin 1997; Han et al. 2005; Gonzalez et al. 2016). This action of wound closure with mature epithelia typifies general fibrosis and this inclination toward fibrosis may be acting against potential regeneration. For example, in highly regenerative salamanders, covering of the wound with mature skin is known to inhibit regeneration (Mescher 1976). Interestingly, regenerative responses have been reported in nonregenerative vertebrates following the enforced inhibition of normal wound response re-epithelialization. For example, the formation of blastemal-like tissue was observed in P2 digit amputations using the super healing mouse strain, MRL-mice (Gourevitch et al. 2009),

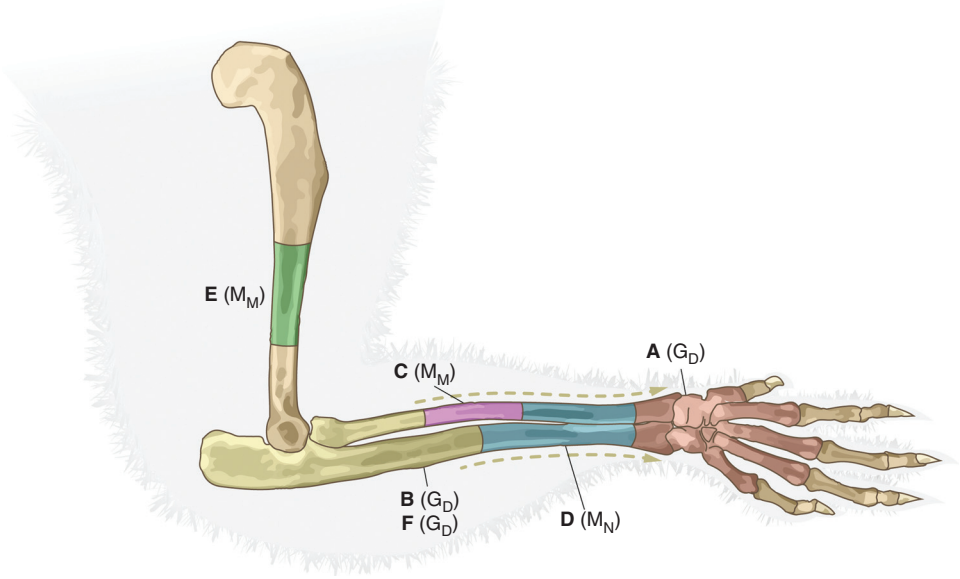

Figure 1. A schematic representation of bone growth in the context of experiments using targeted biochemical pathway treatments in mouse and rooster model(s). Colors are used to distinguish specific publications from one another and represent their reported growth. Data from Yu et al. (2012) (green) represents a medial amputation to the tibial bone transecting the fibula; this was concatenated to a forelimb representation over a humerus to conserve space. It is important to note that, anatomically, the tibia is proximal of the hind paw and distal to the femur. Colored dotted lines represent the entire growth area for a specific citation; these are only shown if other citation(s) overlap. (*A*) Based on Taylor et al. (1994), (*B*) based on Kostakopoulou et al. (1996), (*C*) based on Suckow et al. (1999), (*D*) based on Masaki and Ide (2007), (*E*) based on Yu et al. (2012), and (*F*) based on Makanae and Satoh (2018). (G_D) Developing *Gallus gallus* model, (M_N) neonatal murine model, (M_M) mature murine model.

which reportedly re-epithelialize the amputation at a rate 72 h slower than typical C57bl/6 strain mice (Turner et al. 2010a). This blastemal-like tissue experienced waves of significant cell proliferation, but it also underwent waves of significant apoptosis that ultimately led to its destruction followed by standard wound closure and fibrotic healing (Gourevitch et al. 2009; Turner et al. 2010a). In similar fashion, blastemal formation was reported in P2 digit amputations when the epithelial cell migratory blocker dibutyryl (db)-cAMP was administered to the wound bed (McCormack 1983). In addition, repeated mechanical removal of the wound epithelium along with repeated treatment with NaCl solution promoted blastemal formation in P2 mouse digit amputations (Neufeld 1980). Limb regeneration was also induced in nonregenerative frogs through repeated epithelial trauma to the amputated limb stump along with NaCl treatment (Poležajew 1936; Rose 1945; Polezhayev 1946). These reports suggest that the mode by which nonregenerative systems cover the wound, using mature epithelium, is highly inhibitory to regeneration. This result may be attributed to the inability of mature skin to generate a proper regenerative microenvironment because it lacks the regenerative AECs. Indeed, the physiological properties of the overlying ectoderm (specifically, its ability to conduct ion flows across the epithelial barrier) have been suggested to be a driver for both the determination of the embryonic limb field and for regenerative capacity (Borgens 1984).

In addition to the importance of the wound covering, the deposition of underlying connective tissue is starkly different in regenerative versus nonregenerative wound healing. Fibrotic

(scar forming) wound healing is characterized by significant deposition by tissue fibroblasts of connective tissue ECM, such as collagen I, in place of normal parenchymal tissue (Gurtner et al. 2008). Researchers studying wound healing in lower back cutaneous injuries of fetal mice discovered a lineage-restricted group of fibroblast cells, known as engrailed-1-lineage (En1) fibroblasts, responsible for the transition from scarless healing to scar-forming fibrosis (Jiang et al. 2018). Specifically, fibroblasts that expressed En1 during an embryonic state (EPFs, for En1-past expression fibroblasts) were shown to directly contribute toward fibrosis while En1-naive fibroblasts (ENFs) do not (Rinkevich et al. 2015). Further analysis showed that grafting of EPFs in lower back wound beds promoted heavy deposition of collagen I and fibrosis while grafting of ENFs in the same injuries promoted scarless wound healing, a moderate and porous reticular ECM lattice (contrary to what is seen in dense cross-hatch fibrotic lattice), and increased blood vessel infiltration of the regenerate tissues (Jiang et al. 2018). Interestingly, an ENF-generated ECM lattice alone was sufficient to drive tissue wound healing toward a scarless fate (Jiang et al. 2018), showing that the underlying ECM is a significant regulator of regenerative outcome. The porous nature of the resulting ECM lattice deposited by these ENF cells is reminiscent of blastemal tissue, which is known to highly express matrix metalloproteinases (Santosh et al. 2011; Yates et al. 2012; Johnson et al. 2020) that degrade ECM components. Likewise, increasing collagen enrichment in the blastema directly inhibits regeneration in salamanders (Satoh et al. 2012).

The regenerative AEC and ECM dually promote regenerative healing, creating the appropriate microenvironment to stimulate epimorphic regeneration. In addition to AEC and ECM grafting, scaffolding materials typically composed of naturally occurring ECM components, or other organic compounds such as silk, can be manufactured and implanted into injured tissue fissures and act as a framework for the regenerate structure (Sundelacruz and Kaplan 2009; Benders et al. 2013; Caudwell et al. 2015). These scaffolds can be loaded with regenerative stem cells, bioactive compounds, or drugs to promote regeneration (Pritchard and Kaplan 2011; Yucel et al. 2014; Golding et al. 2016). In contrast to a regenerating limb, grafting treatments typically aim for targeted regeneration and restoration of specific tissues within a given organ structure, such as a fractured radial bone (Suckow et al. 1999) or a damaged heart valve (D'Onofrio et al. 2011). These engineered scaffolds, whether derived from synthetic or biological material, act as a foundation for natural growth of resident cells and tissues in which the scaffold is implanted. Several scaffolding techniques may have significant implications in the context of limb regeneration. Significant bone regeneration of a rat radial bone, which had been resected 11 mm at its center, was reported after grafting with decellularized porcine small intestinal submucosa (pSIS) scaffolds (Suckow et al. 1999). Specifically, rats provided with pSIS grafts showed about an 80% restitution of resected bone at 6 mo postsurgery with evidence of bone marrow formation (Fig. 1; Suckow et al. 1999). This same pSIS scaffolding technique was used to reconstitute canine Achilles musculotendinous junction following excision of the distal third of gastrocnemius muscle and proximal half of the associated Achilles tendon bundle (Turner et al. 2010b). After 6 mo, canines with pSIS scaffolds showed complete tissue regeneration, restoration of contractile force to ~50%, significant reinnervation with new neuromuscular junctions and significant muscular vascularization (Turner et al. 2010b). Similar success was observed in the restoration of skeletal muscle in mice using fibrin scaffolds (Page et al. 2011) or pSIS scaffolds (Valentin et al. 2010). More recently, decellularized human nail bed (dcNB) was used to construct an engineered scaffold (eNB) that differed from the dcNB, as it contained seeded bone mesenchymal stem cells (BMSCs) (Yu et al. 2019b). eNB scaffolds, when implanted into the back of nude mice, promoted the restoration of the nail plate and nail epithelium. Furthermore, expression of Msx1, an important component of regeneration and limb outgrowth (Kostakopoulou et al. 1996; Han et al. 2003; Johnson et al. 2020), was shown to be significantly expressed in eNB implants

compared to dcNB alone, BMSC alone, or sham control (Yu et al. 2019b). These engineering-based interventions show significant promise for regenerative interventions in vertebrates (for a more comprehensive review of scaffolding approaches, see Chen 2014 and Darnell et al. 2017).

These surgical and engineering-based interventions reveal key lessons about regenerative tissues and how they can be used to augment limb wound healing. A blastema, if provided time to reach a "critical mass," can autonomously build its preordained missing tissues without needing positional information from its surroundings. However, blastemal cells are insufficient to alone drive regeneration if grafted into a nonregenerative environment. In addition to blastemal tissue, developing limb bud tissue, when grafted to amputated limbs of the same identity, can enhance regeneration but ultimately falls short of producing anything resembling the lost limb. Grafting of biologic composite material such as ECM scaffolds can indeed stimulate a regenerative response; in its current state, scaffolding approaches can be used to effectively modulate the regrowth of a specific tissue. However, in the case of limbs or digits, the complexity of the multitissue system has yet to be met with a sufficiently complex engineering solution. A perfusion decellularization approach was recently applied to an entire human cadaver forearm, preserving all ECM complexity and structure (Gerli et al. 2018). This decellularized matrix was posited to supply cells with perfectly organized, natural ECM to infiltrate as a means to regenerate the missing limb. However, cell-seeding technologies and challenges with nutrient supply deep within the developing tissue must be solved to successfully use this decellularized limb matrix.

BIOCHEMICAL PATHWAY TARGET INTERVENTION FOR INDUCING VERTEBRATE REGENERATION

Researchers have had success with techniques using limb developmental bioactive molecules to initiate a regenerative response in poorly or nonregenerative tissues. One family of signaling molecules showing success in various nonregenerative models is the FGF proteins. FGF proteins are expressed in the apical epidermis in developing limb buds and are known to reexpress in these same regions following limb amputation in regenerative vertebrates (Christen and Slack 1997; Han et al. 2001, 2005; Giampaoli et al. 2003). In nonregenerative systems, this reexpression of FGF proteins is absent (Han et al. 2005). In addition, inhibition of FGF signaling within regenerative systems effectively blocks the formation of blastema and subsequent regeneration (Poss et al. 2000; Lin and Slack 2008). FGF proteins are such potent stimulators of limb growth that forced expression can rescue limb development following AER removal (Niswander et al. 1993; Fallon et al. 1994; Mahmood et al. 1995), or can even lead to the formation of ectopic limbs during development (Crossley et al. 1996; Ohuchi et al. 1997, an apical ectodermal factor). The importance of FGF signaling is underscored by regenerative neuronal dependence.

Early studies denervating regenerating Urodele limb stumps inhibited subsequent limb regeneration while grafting neuronal tissue to denervated limb stumps could rescue regeneration (Singer 1952; Thornton 1970). These experiments established the basis for neural dependence in Urodele regeneration that has been confirmed in other amphibious vertebrate regenerators (Simpson 1961; Endo et al. 2004; Mondia et al. 2011; Mitogawa et al. 2018). It was postulated that neurons are responsible for supplying the regenerating tissues with pro-regenerative bioactive molecules through neuronal secretion. This hypothesis is further supported by evidence showing that blastemal expression of FGF proteins is directly linked to innervation of the regenerating limb stump (Christensen et al. 2001; Satoh et al. 2017). Neurons in *Ambystoma mexicanum* (axolotl) synthesize FGF8 and BMP7 and transport these bioactive compounds to the regenerating limb stump through the axon (Satoh et al. 2016). In newts, nerve-dependent regeneration has been linked back to FGF2 expression in the AEC and inhibition of limb regeneration by denervation can be rescued by ectopic application of FGF2 at

the limb amputation site (Mullen et al. 1996). Remarkably, ectopic limb growth can be stimulated in axolotl simply by deviating the innervating limb axon to an alternative location containing an epidermal lesion (Endo et al. 2004). Nerve dependency of ectopic limb formation in axolotl can be mimicked by implantation of beads soaked in BMP2, -7/FGF8, -2 in the proximal limb (Han et al. 2005; Makanae et al. 2014; Satoh et al. 2018; Vieira et al. 2019).

The regenerative potential of these neuronally associated bioactive molecules translates to other vertebrate systems as well. Ectopic application of FGF2 or FGF4 to amputated wing buds in developing embryonic *Gallus gallus* (red junglefowl) induced a robust regenerative response that often resulted in the complete reconstitution of the amputated limb (Taylor et al. 1994; Kostakopoulou et al. 1996). Specifically, treating developing limbs with FGF2 following distal zeugopod amputations resulted in the regeneration of missing zeugopod structures as well as the formation of some carpal and digit structures (Taylor et al. 1994). Similarly, amputated developing limb bud outgrowths of chickens, treated with FGF4, were able to develop complete humeral and zeugopod structures, and in some cases, incomplete autopod structures (Fig. 1; Kostakopoulou et al. 1996). Furthermore, in developing frogs and chickens, ectopic application of FGF2 and FGF8 on developing limbs with resected lower limb parts (presumptive zeugopods) resulted in the regeneration of the zeugopod (Fig. 1; Makanae and Satoh 2018; Satoh et al. 2018).

Innervation of a regenerating stump in Urodele is directly linked to the secretion of an additional neurotrophic factor, newt anterior gradient protein (nAG). nAG was found to be initially expressed in the neuronal sheath and subsequently in glands within the AEC (Kumar et al. 2007). Forced AEC expression of nAG was sufficient to rescue denervated limb buds leading to complete regeneration of aneurogenic limbs (Kumar et al. 2007).

Denervation of developing *Ambystoma maculatum* (spotted salamander) limb buds results in the formation of complete aneurogenic limbs (Popiela 1976) that, surprisingly, retain full regenerative capacity (Yntema 1959; Steen and Thornton 1963). Kumar found that these aneurogenic limbs are capable of expressing the *A. maculatum* homolog to nAG (mAG) in their limb stump AEC despite being aneurogenic (Kumar et al. 2011), and reestablishment of neuronal dependence in these limbs inhibits the expression of mAG upon subsequent neural denervation (Kumar et al. 2011). This group also identified nAG homologs in mice, rats, frogs, and humans, which have yet to be characterized in the context of regeneration (Kumar et al. 2011).

In mature *Xenopus laevis*, increasing the neuronal innervation in a regenerating limb stump (hyperinnervation) drastically improves the resulting morphology of a limb regenerate, shifting regenerate outcome from a typically produced hypomorphic spike to an elongated regenerate with underlying structures more closely resembling a mature limb (Fig. 2; Mitogawa et al. 2018). Limb stump hyperinnervation increases blastemal expression of important growth and patterning genes: FGF2/-8, BMP7, hoxa11/-13, and Shh (Mitogawa et al. 2018). In the absence of hyperinnervation, forced blastemal expression of BMP7, FGF2/-8, and Shh via DNA electroporation leads to improved regenerate outcomes: increased length, enhanced cartilage structures and joint-like structures, similar to what is seen in hyperinnervated limbs (Mitogawa et al. 2018). Furthermore, induced expression of Shh in nonregenerative froglet limb amputations results in increased regenerative length and more complex patterning of regenerate bone (Yakushiji et al. 2009), similar to yet more moderate than what is seen with combinatorial approach of BMP7, FGF2/8, and Shh treatment. It should be noted that innervated stumps regenerated better overall, with more complex morphology, than those with supplemental gene expression alone (Mitogawa et al. 2018).

Likewise, the neurosteroid progesterone, which has been established as a potent promoter of wound healing and regeneration of both neural (Koenig et al. 2000; Chávez-Delgado et al. 2003; Rosales-Cortes et al. 2003) and nonneural injuries (McEwen 1991; Routley and Ashcroft 2009; including bone remodeling, Hennighausen and Robinson 2001), was shown to dramat-

Cold Spring Harbor Perspectives in Biology
www.cshperspectives.org

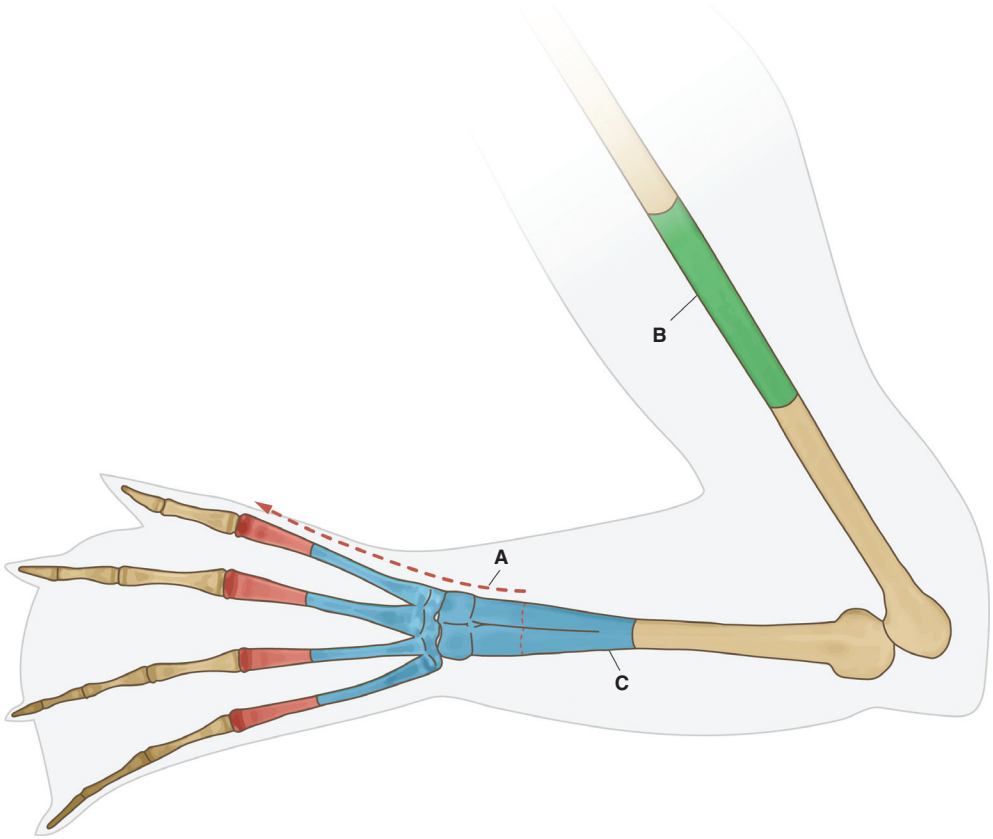

Figure 2. A schematic representation of bone growth in the context of experiments using targeted biochemical pathway treatments in mature Anura. Colors are used to distinguish specific publications from one another and represent their reported growth. It is important to note that Herrera-Rincon et al. (2018) amputations were performed at the midfemur of the hindlimb and are depicted here in the humerus to conserve space. Colored dotted lines represent the entire growth area for a specific citation; only shown if other shown citation(s) overlap. (*A*) Based on Lin et al. (2013), (*B*) based on Herrera-Rincon et al. (2018), and (*C*) based on Mitogawa et al. (2018). All studies used adult *Xenopus laevis*.

ically improve the regenerate outcome of adult *X. laevis* hindlimb amputees (Herrera-Rincon et al. 2018). Brief 24 h application of progesterone, delivered through a wearable silk-based hydrogel biodome device (Golding et al. 2016), resulted in a dramatic regenerative response lasting nearly 10 mo (Herrera-Rincon et al. 2018). Moreover, these partially regenerated hindlimbs developed a paddle-like morphology with highly vascularized and innervated tissues as well as significant femoral bone growth into the paddle structure (Fig. 2; Herrera-Rincon et al. 2018). Similarly, fibrin-based scaffolds seeded with Shh + FGF10 + thymosin β4 (Tβ4) induced the near complete regeneration of forelimb wrist-level amputees in adult *X. laevis* (Lin et al. 2013). The regenerate hand contained intermediate skeletal elements with metacarpal bone, phalange epiphysial plates, and joint structures along with protruding digits (Fig. 2). This significant improvement in regeneration was underscored by the expression of known regenerative (and developmental) regulators Shh, Wnt3a, Wnt5a, FGF8, FGF10, and FGF20 (Lin et al. 2013). These outcomes, although significant improvements over hypomorphic spikes of

controls, were not cosmetically similar to a normal forelimb, suggesting that important triggers of the limb regeneration cascade remain to be discovered.

Nonamphibious regenerative vertebrate models display contrasting dependence on nerve supply and regeneration. Early work in lizards shows that augmenting nerve supply, while not leading to the complete regeneration of a limb, can induce a moderate regenerative response (Simpson 1961; Singer 1961). In the murine digit regeneration model, there is strong evidence showing that neural tissue is involved in proregenerative paracrine signaling and denervation via sciatic nerve resection of the limb significantly reduces the ability of the digit tip to mount subsequent regenerative responses (Johnston et al. 2016). Furthermore, transplantation of Schwan cell precursor cells (SCPs), or local deposition of SCP paracrine factors platelet-derived growth factor AA or oncostatin M, can rescue digit denervation (Johnston et al. 2016). In contrast, a normally regenerated murine digit tip is significantly less innervated than its nonamputated counterpart, having 7.5-fold fewer axons in the connective tissue branches and 5.6-fold fewer in the bone marrow branch; but despite being significantly less innervated, this digit can fully regenerate with successive amputation (Dolan et al. 2019). This suggests neural dependence may be less of a driver for regeneration of these tissues than originally thought (Dolan et al. 2019). Can the regenerative capability of the digit tip be linked back to quantitative amounts of neural tissue, as it has been in amphibians (Singer 1952, 1954; Thornton 1970), and could mice continually mount a regenerative response in the digit tip with persistent amputations? This has yet to be tried in mice but evidence in axolotls suggests that even highly regenerative systems have their limitations when faced with repetitive assault (Bryant et al. 2017a,b).

While no treatments have led to complete regenerative rescue in proximal digit amputations to date, there are several targeted biochemical treatments that have shown promise in initiating a partial regenerative response. Mouse digit tips exposed to various developmental growth factors demonstrate significant increases in regenerative capacity. For example, if FGF2 expression is induced through β-catenin stabilization, proximal P3 mouse digit tip amputations exhibit partial regeneration leading to an increased nail bed regenerate (Fig. 3; Takeo et al. 2013). Combinatorial treatment pairing mouse-induced pluripotent stem cells (iPSCs) with beads loaded with a cocktail of bioactive compounds, BMP2/FGF8/Tβ4/Wnt3a on amputated mid-P2 digits, leads to significant regeneration of the lost P2 bone (Chen et al. 2017). This regenerative response is characterized by significant bone growth, increased length, and the formation of bone marrow structures in the newly formed bone (Fig. 3; Chen et al. 2017). Singular treatment with BMP2 in mouse digit P2 amputations leads to significant net bone outgrowth of over 60% (compared to sham control) in only 30 d posttreatment; with some mice even developing epiphyseal plate-like distal structures at 160 d postamputation (Fig. 3; Yu et al. 2012; Dawson et al. 2017). Interestingly, this response requires the periosteum layer of the bone (Dawson et al. 2017) and its removal abolishes the regenerative effects of BMP2 treatment (Dawson et al. 2017). This data links the known chondrogenic response of injured periosteal tissue (Colnot 2009; Dawson et al. 2016) as a proponent to this BMP2-mediated bone regeneration. Building upon these findings, researchers performed sequential treatment using BMP2 and BMP9 in mice following P2 digit amputation, where BMP2 was administered first to drive digit bone outgrowth, while subsequent treatment with BMP9 successfully stimulated the formation of a joint and epiphyseal plate (Yu et al. 2019a). The effectiveness of BMP2-mediated bone regeneration is not restricted to the digit, as midshaft hindlimb amputations treated with BMP2 also exhibit significant bone growth (Yu et al. 2012). Remarkably, BMP2-induced bone regeneration in these midshaft limb amputees successfully fused their tibia and fibula bones 8 wk postamputation in a morphologically accurate way (Fig. 1; Yu et al. 2012). Similarly, neonatal mice with mid-radial-ulnar forelimb amputations experienced significant bone re-

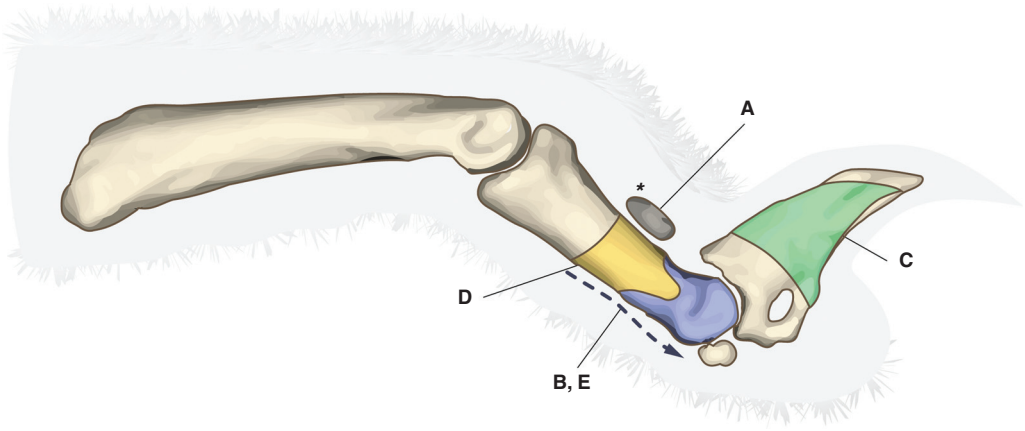

Figure 3. A schematic representation of bone growth in the context of experiments using targeted biochemical pathway treatments in mouse digit models. Colors are used to distinguish specific publications from one another and represent their reported growth. Colored dotted lines represent the entire growth area for a specific citation; only shown if other shown citation(s) overlap. Asterisk indicates formation of adjacent endochondral ossification centers lacking proper positioning with relation to cut boundary. (*A*) Based on Agrawal et al. (2011), (*B*) based on Yu et al. (2012), (*C*) based on Takeo et al. (2013), (*D*) based on Chen et al. (2017), and (*E*) based on Dawson et al. (2017). All studies used adult mice.

Cold Spring Harbor Perspectives in Biology
www.cshperspectives.org

generation when gelatin beads containing BMP7 were implanted at the amputation site (Fig. 1; Masaki and Ide 2007).

A quite different approach was taken by one group of researchers who noted the importance of ECM breakdown during successful regenerative events. During regeneration-induced ECM breakdown, a small cryptic peptide is released into the local tissue (Agrawal et al. 2011). After isolating this cryptic peptide, they found that treatment of mid-P2 phalange amputations with this cryptic peptide-induced small ossification centers just adjacent to the amputation site (Fig. 3). Although this peptide could induce the formation of new bone (Agrawal et al. 2011), the new bone formation did not follow proper morphogenesis, suggesting that simply providing the cell-type building blocks of complex organs is not sufficient—patterning must be regulated or induced.

MURINE TRANSGENIC LINES WITH ENHANCED REGENERATIVE CAPACITY FOLLOWING DIGIT AMPUTATION

Establishment of transgenic inbred lines is commonplace in model-based research. With new transgenic lines developed rapidly for various applications, some become well known for their proficiency to perform other tasks outside of their canonical purpose. In this section, we will discuss the remarkable discovery of enhanced regeneration found in two murine transgenic lines originally purposed for the study of non-wound healing systems.

In the early 1990s, researchers selectively bred murine B6, C3H, AKR, and large strains to develop the Murphy Roths large/lymphoproliferative (lpr) mouse strain (MRL/MpJ) (Watson et al. 1992). The capacity of these mice to perform scarless wound healing in multiple tissues, including dermal lesions (Beare et al. 2006; Davis et al. 2007; Buckley et al. 2011) and ear hole punch injuries (Clark et al. 1998; Fitzgerald et al. 2008), led them to be named "super super-healing" MRL mouse strain (Heydemann 2012). Classical regeneration observed in MRL mice is characterized by rapid wound re-epithelialization and formation of a proper regenerative blastema (Clark et al. 1998). When MRL mice were tested for their ability to mount a regenerative response following standard P3 digit amputation, it was found that MRL mice regrew their digits at an accel-

erated rate compared to outbreed strains C57BL/6 and DBA/2 (Chadwick et al. 2007). In addition to accelerated re-epithelialization, a number of transcriptional differences were observed differentially expressed in regenerating P3 MRL mice, including a number of transcripts involved in Wnt signaling (LRP6) (Tamai et al. 2000), limb bud polarization factor (Formin-1) (Zeller et al. 1999), and growth factor regulators (SPARC) (Chadwick et al. 2007). When MRL mice were tested for more severe digit regeneration (amputations midway through the P2 phalange), they showed a unique propensity to form blastemal-like tissues despite being unable to mount a formal regenerative response (Gourevitch et al. 2009). Still, MRL mice re-epithelialized their injuries more rapidly than the C57BL/6 or Swiss Webster strains against which they were tested. These reports of the wound re-epithelialization speed of MRL mice during P2 digit amputation are contrasted with other researchers' reports of significantly delayed wound re-epithelialization of MRL mice compared with C57BL/6 mice following mid-P2 phalange amputations (Turner et al. 2010a). Ultimately, reports of digit amputations in MRL mice demonstrate that, despite initial steps toward digit regeneration, MRL mice cannot sustain the blastema; cells undergo apoptosis, the blastema collapses, and regeneration is aborted and replaced with standard fibrotic wound healing (Gourevitch et al. 2009; Turner et al. 2010a).

In addition to this rather serendipitous finding, overexpression of Msx1 and Msx2 in vertebrates has proven effective in inducing regeneration in vertebrates. Msx proteins are readily expressed during the formation of a blastema in Urodele (Koshiba et al. 1998), *Xenopus* (Endo et al. 2000), and *Murinae* (Reginelli et al. 1995; Han et al. 2003; Lehoczky et al. 2011; Johnson et al. 2020), and have been shown to be required for successful regeneration (Han et al. 2003). Using the nonregenerative refractory period of the *Xenopus* model, researchers induced expression of Msx1 using heat shock protein 70 promotor and showed that, although full regeneration was not achieved, Msx1 regenerates showed improved characteristics such as increased blastema accumulation, increased blastemal outgrowth, thickened AEC, and increased number of toes forming as a result of treatment (Barker and Beck 2009). Following this study, researchers used a combinatorial approach of overexpression of both Msx1 and Msx2 in proximal nonregenerating P3 digit amputations in mice with high efficacy (Taghiyar et al. 2017). Specifically, bone-derived mesenchymal stem cells were modified to overexpress Msx1 and Msx2 and subsequently embedded at the amputation site 4 d postamputation. This treatment led to complete regeneration of the distal P3 bone 6 wk postamputation (Taghiyar et al. 2017). Overexpression of Msx1 and Msx2 brings critical gene-derived patterning information to the wound site, as it was shown that treated mice demonstrated increased expression of known patterning genes BMP4 and FGF8 (Taghiyar et al. 2017). Besides inducing these patterning genes, Msx1 and Msx2 have well-known roles in tissue patterning during development and regeneration (Reginelli et al. 1995; Bensoussan-Trigano et al. 2011; Lehoczky et al. 2011; Vieux-Rochas et al. 2013; Johnson et al. 2020) that likely contribute to the reported result (Taghiyar et al. 2017). The efficacy of this approach in supplying the wound site with patterning information is thought to be the key behind its success.

The RNA-binding protein Lin28 was eventually used to create another strain of mice with significantly improved healing responses, iLin28a transgenic mouse or "iLin28a Tg" (Shyh-Chang et al. 2013). Lin28 was first discovered in *C. elegans* where it was shown to be a regulator of developmental transition from a larval state into adulthood by controlling cell progenitor fate and renewal (Ambros and Horvitz 1984; Moss et al. 1997). Two Lin28 paralogs, Lin28a and Lin28b (Viswanathan et al. 2008), are highly expressed in embryonic stem cells (Rybak et al. 2008) but are progressively lost as fetal development progresses toward adulthood (Shyh-Chang and Daley 2013). At the tissue level, Lin28 is highly expressed in the embryonic epidermis and developing limb buds and is undetectable after birth (Yang and Moss 2003).

Because embryonic tissues are known to be highly regenerative compared to their adult counterparts (Deuchar 1976; Young et al. 1983; Alvarado and Tsonis 2006), researchers sought to use the delaying powers of Lin28 to force adult tissues into an embryonic-like state by overexpression of Lin28 in mice (Shyh-Chang and Daley 2013). The iLin28a Tg mice exhibited regenerative responses to hair follicle removal, ear hole punch injuries, and even neonatal proximal digit amputation (including the complete recapitulation of lost second phalangeal bone and soft tissue) (Shyh-Chang et al. 2013). Researchers found that regenerating bone of iLin28a Tg mice highly expressed Lin28, while that of wild-type mice lacked Lin28 expression (Shyh-Chang et al. 2013). Yet Lin28 expression failed to provide any regenerative potential in adult digit amputees (Shyh-Chang et al. 2013). Lin28 is most known for its function as a repressor of microRNA lethal-7 (let-7), although researchers analyzing Lin28-induced regeneration clearly demonstrate that let-7 repression, although necessary, is not alone sufficient to account for the observed regenerative responses (Shyh-Chang et al. 2013). In addition to its role in regulating let-7, Lin28 is a known regulator of translation for the metabolic enzymes Pfkp, Pdha1, Idh3b, Sdha, Ndufb3, and Ndufb8, which have been shown through metabolic profiling to enhance oxidative metabolism (Shyh-Chang et al. 2013). The authors tested the contribution of Lin28 and its enhancement of oxidative metabolism by testing pharmacological compounds (3BP or 2-deoxy-D-glucose) targeting oxidative phosphorylation and their propensity to influence tissue repair. Pharmacological treatment with these compounds led to significant increases in tissue repair comparable to Lin28 induction groups (Shyh-Chang et al. 2013). In addition to its role as a repressor for let-7, Lin28 acts as a potent stimulator of regeneration through the regulation of cellular metabolic processes that ultimately led to a cellular microenvironment simulating a more embryonic state, thus enhancing the regenerative capacity of tissues (Reddien 2013; Shyh-Chang et al. 2013).

TARGETED MANIPULATIONS OF CELL MEMBRANE POTENTIAL (V_{mem}) FOR REGENERATION

All cells, not just neurons, communicate via bioelectric signaling (Levin et al. 2017). A key aspect of bioelectric networks in tissues are the spatial patterns of standing cell membrane resting potentials, V_{mem}, which regulate downstream cell behaviors and morphogenesis at the organ level (Levin and Martyniuk 2018). Targeting V_{mem} for the purpose of influencing wound healing and regeneration has grown in popularity as researchers begin to unravel all the cellular processes in which V_{mem}, and more broadly bioelectric signaling, regulate (Zhao 2009; Tseng and Levin 2013; Zhao et al. 2020) complementing classical work focused on transepithelial electric fields (Borgens 1982, 1983).

Bioelectric signaling has been shown to influence (and in many cases control) cellular migration (Stump and Robinson 1983; Nishimura et al. 1996), proliferation (Vodovnik et al. 1992; Sundelacruz et al. 2009; Barghouth et al. 2015), differentiation (Zhao et al. 2015; Liu et al. 2017; Yan et al. 2017), and neuronal growth (Patel and Poo 1982; Stump and Robinson 1983; McCaig and Rajnicek 1991), all processes vital for proper tissue regeneration and wound healing. Furthermore, a natural consequence of tissue injury is the generation of an electric current, known as an injury current, surrounding the wound site. In organisms with elevated regenerated capacity, injury currents are tightly regulated and efficiently drive migratory galvanotactic cell types such as neurons and immune cells toward the wound (Becker 1967; Adams et al. 2007; Reid et al. 2009).

In the context of wound healing and regeneration, bioelectric responses occur when the ionic microenvironment changes in response to tissue injury. These changes are induced by the destruction of the endogenous transepithelial potential (TEP) generated by conjoined epithelial cells that line all tissues (Zhao 2009). Injury of TEP-producing epithelia generates a region of depolarization at the injury site, which, when coupled with adjacent active TEP-producing epithelia, creates an electric di-

pole. This generates an endogenous electric current (injury current) that travels through the resistive tissue matrix, creating an electric field (Nuccitelli 2003; Zhao 2009). Endogenous currents, and the fields they generate, act directly on membranes of cells within the field, enabling them to orient to and migrate along electric field lines leading directly to the wound (Gross et al. 1986; McCaig et al. 2009; Kucerova et al. 2011; Yao et al. 2011; Cao et al. 2014). Manipulating these currents in injured tissues can precisely alter subsequent wound healing responses. For example, treating the wound bed of murine corneal injuries with aminophylline (an enhancer of chloride flux leading to an increased TEP within adjacent healthy epithelium) nearly doubles the rate of wound healing (Song 2004). In addition to accelerated wound healing, neuronal invasion and directedness in the corneal tissue was increased as a result (Song 2004). Manipulation of transepithelial electric fields is currently a very promising target for a number of tissue-level repair contexts (Zhao et al. 2006, 2020; Reid et al. 2011).

Tadpole tails, which contain a complex pattern of spinal cord, muscles, nerves, and vasculature, are regenerative from the moment of initial tail growth up until the tail is lost during development (Beck et al. 2009). However, there is an intermediate period during which the tail cannot regenerate; but after this refractory period (Beck et al. 2003; Ivanova et al. 2018), regenerative capacity is regained until metamorphosis (Beck et al. 2003). Amputated tadpole tails exhibit temporal changes in V_{mem} at the amputation site coincident with the state of their regenerative capability (Adams et al. 2007). In regenerating tails, V_{mem} at the amputation site undergoes an initial depolarization at 6 h postamputation followed by a repolarization of the tissue 24 h postamputation (Adams et al. 2007). During the refractory period, the initial depolarized state at the amputation site persists and is indicative of an inability to mount a regenerative response (Adams et al. 2007). These underlying bioelectric signatures of regeneration in the tadpole tail are supported by measurements of currents in amputated tadpole tails. Proper tadpole tail regeneration was marked by an initial outward current that later reversed to an inward current (known as a rectifying current) between 12 and 24 h postamputation (Reid et al. 2009). Later, it was discovered that O_2 flux, reactive oxygen species production, and hypoxia-inducible factor 1α (HIF-1α), a master regulator of O_2 homeostasis, mediate tadpole tail regeneration (Ferreira et al. 2018). Interestingly, HIF-1α stabilization can be used to promote tail regeneration during the refractory period and was shown to regulate endogenous electric current reversal occurring in normally regenerating tail buds (Ferreira et al. 2018). These temporal bioelectric dynamic changes within the tadpole tail are required for proper regeneration; this is supported by work showing that inducing these known shifts in bioelectric state during the refractory period can rescue regeneration (Adams et al. 2007; Reid et al. 2009).

During the tadpole tail regeneration, regenerative potential is eliminated through the inhibition of proton pump (H^+ V-ATPase) caused by the failure of tissue to repolarize within 24 h following amputation; this repolarization is required for up-regulation of Msx1, Notch, and other key regenerative genes (Adams et al. 2007; Tseng et al. 2010). The importance of this bioelectric state was further illustrated by research showing that the forced expression of a yeast H^+ pump (PMA1.2) during refractory period tail amputations rescued regeneration of refractory tails and tails in which endogenous V-ATPase activity was abrogated (Adams et al. 2007). Because frog H^+ V-ATPAse has no sequence or structural homology to the PMA1.2 that can substitute for it, these data show that the necessary and sufficient signal for tail regeneration is not a specific gene product, but a physiological state.

Later, it was found that regenerating tadpole *X. laevis* tails activate H^+ V-ATPase activity 6 h postamputation, which leads to a downstream activation of Na^+ currents through voltage-gated sodium channels (Na_V). This local Na_V activation leads to downstream activation of a key transcriptional regulator, sodium-induced kinase, which acts to regulate the transcription of known regenerative modulators Msx1 and Notch, thus leading to proper tail regeneration

(Tseng et al. 2010). Furthermore, to elicit more precise control over proton current, researchers showed that introduction of a light-gated Archaerhodopsin-based H^+-ATPase rescued tail regeneration during normal tadpole tail refractory periods, while allowing for precise optogenetic channel activation via exposure to 463 nm light source (Adams et al. 2013). These studies clearly demonstrate the potential for bioelectric mechanisms to govern regeneration and demonstrate the ability to exploit these measures as tools to induce regeneration in otherwise nonregenerative tissues. Importantly, the sodium flux can be induced by an ionophore—a small molecule signal that does not require transgenes and is therefore more suitable for application in human patients (Tseng et al. 2010).

APPLIED BIOELECTRIC INTERVENTIONS STIMULATING VERTEBRATE REGENERATION

While molecular bioelectrics targets native ion channels or V_{mem}, a more classical approach uses electrodes to deliver external electric fields to modulate tissue bioelectric state. One example used a bimetallic electrode (a fusion of platinum and silver wire) with a measured electric field of 66.7 mV/mm in Ringers solution (Smith 1967). Implantation of this electrode at the bed of an amputated adult frog forelimb (*Rana pipiens*) led to a significant regenerative response resulting in several millimeters of growth, wound closure by a thickened epithelium, formation of a blastema-like structure, and regeneration of loose poorly differentiated mesenchymal tissue, all within a 3 mo period (Fig. 4; Smith 1967). Later experiments using the same nonregenerative adult *R. pipiens* forelimb model, with a slightly modified battery-powered implantation device, reinforced the potential for applied current to stimulate regeneration and revealed that anatomical positioning of electrodes had a significant impact on the regenerative potential of this treatment (Smith 1974). It was discovered that dorsal-postaxial placement of the anode, delivering 1.0×10^{-7} A over 1 mo, resulted in significant forelimb regeneration measured 4-mo postamputation. Moderate regeneration (increased bone and tissue growth) was observed in 16 of 21 cases while remarkable regeneration (characterized by the formation of moveable digits) was shown in five of 21 cases. Most notably, one of these five animals regenerated a complete forelimb indistinguishable from the original, with all digits and internal structures properly formed and oriented (Fig. 4; Smith 1974). In the instance of full limb regeneration, researchers allowed this frog 1 yr to regenerate as the organism displayed a continued regenerative response (Fig. 5A–D; Smith 1974). Borgens later revisited these experiments in 1977 using the same model organism and electrode implant techniques, including electrode placement and dipole orientation. However, the magnitude of current applied in these experiments was approximately double to that used by Smith (0.2 μA vs. 0.1 μA). Likewise, Borgens et al. (1977) reported noteworthy regeneration in frogs treated with applied currents, observing the formation of new bone and bone-like cartilage structures, new muscle and nervous tissue, despite no formation of new digits or complete forelimbs (Fig. 4). These findings also corroborated the importance of electrode placement as well as dipole orientation underlying this regenerative response (Borgens et al. 1977).

Remarkably, electrode implants have also proven effective in murine limb regeneration. Becker assessed the potential for bimetallic electrode implantation to induce regeneration in Sprague–Dawley rats, whose forelimbs were amputated at the lower 2/3 of the distal humerus. Implantation of these devices resulted in blastema formation followed by significant bone and tissue regeneration (Becker 1972). In contrast to Smith's bimetallic electrode, Becker's electrode was modified by a 10 MΩ resistor to yield delivered currents ranging from 3 to 6 nA, which was reported to lead to the regeneration of the lost humeral bone, in some cases including the formation of a joint. In these experiments, 21 animals were used, with 10 analyzed at or before 7–11 d postamputation (Becker 1972). Much of the remarkable regeneration was seen in animals given between 12 and 28 d to regenerate. In this limited group of 11 animals, two formed an initial blastema that eventually was lost to fibrosis;

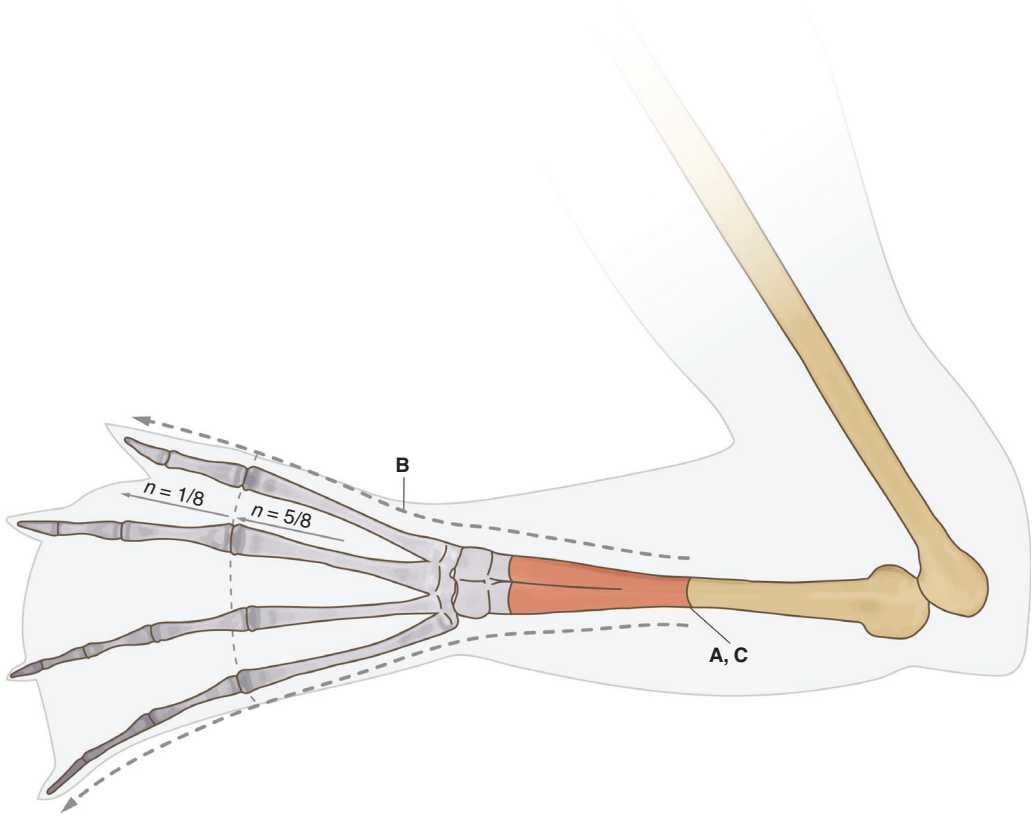

Figure 4. A schematic representation of bone growth in the context of experiments using applied electric currents in Anura. Colors are used to distinguish specific publications from one another and represent their reported growth. Colored dotted lines represent the entire growth area for a specific citation; only shown if other shown citation(s) overlap. (*A*) Based on Smith (1967), (*B*) based on Smith (1974), and (*C*) based on Borgens et al. (1977). All cited examples used a mature *Rana pipiens* model.

three showed significant blastemal response with bone, cartilage, and muscle regrowth; and six displayed even more impressive regeneration, having near complete distal humeral restitution with two epiphyseal centers. In one case (within the group of six), Becker observed the formation of a complete humeral epiphysial plate along with a complimentary joint (Fig. 6). Another surprising finding was one instance of the formation of a supernumerary humerus, preceded by a larger than normal blastema (Becker 1972). Unfortunately, the actual current of the devices used was not reported. Nevertheless, this influence of electric current on bone formation is complemented by prior work showing that endogenous electric currents arise from naturally occurring formation of biological PN (positive and negative) junction diodes through collagen and apatite networks that translate mechanical forces to electrical currents (Becker 1967). As bone is introduced to load and compression forces, it will deposit new bone on the concave/compressed side in correspondence with the endogenously formed cathodal surface generated by these collagen/apatite junctions (Becker 1967).

These findings of electrically induced rat limb regeneration would be later corroborated by Smith in 1981. Smith performed mid-antebrachium amputations in Sprague–Dawley rats and implanted the same bimetallic electrode that he used in frog forelimb amputations in

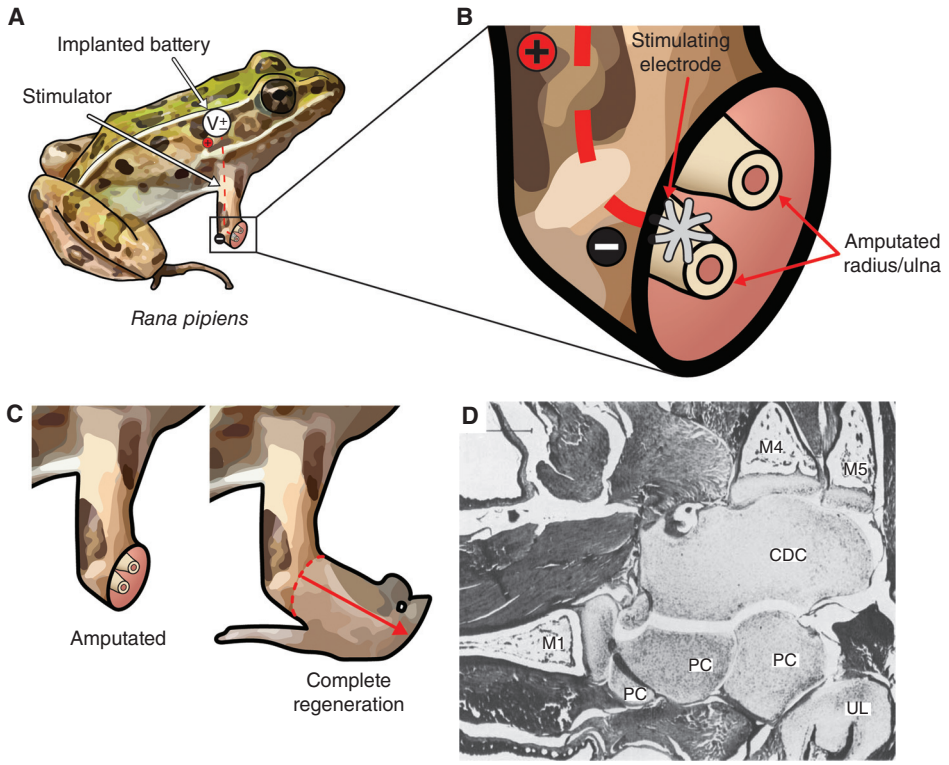

Figure 5. Summary schematic showing a specific example of regeneration induced by applied electric current in the adult frog, *Rana pipiens*. (*A*) Illustration of adult *R. pipiens* frog showing the amputation plane at the first 1/3 of the forearm and the placement of the embedded stimulating wire connected to a battery delivering 0.1 μA of current to the wound site. (*B*) Enhanced illustration showing the wound site and placement of the negative stimulating electrode at the dorsal-postaxial region of the amputation plane. (*C*) Illustration showing the resultant regeneration of an individual frog, a complete regrowth of the lost tissue, which was reported as the best regenerate of the study (note that one digit grows at an angle, as is normal for this species). (*D*) Excerpt from Smith (1974) showing a cross section of the palm of the regenerated arm with labeled segments. (M1, M4, M5) Bases of metacarpals, (UL) ulnar, (PC) proximal carpal, (CDC) common distal carpal. (Panel *D* from Smith 1974; reprinted, with permission, from John Wiley and Sons © 1974.)

1974 (Smith 1974), with cathodal placement in the dorsal-postaxial region above the ulna at the amputation surface (Smith 1981). Similar to his work in frogs, Smith observed significant bone, skeletal muscle, and cartilage regeneration, and in some cases the early stages of joint formation (Fig. 6; Smith 1981). It should be noted that the polarity of the electric field induced by Smith's electrode placement, and magnitude of current (1 μA; ~1000X greater than Becker's), within the forelimb is contrary to that done by Becker in humeral amputations. Nevertheless, Smith's observations confirm the ability of electric currents to stimulate significant regeneration in murine limbs. Despite these compelling reports, not much work has been done recently to decipher the significant regenerative capacities induced through bioelectric intervention, with most attention being paid to biochemical and genetic control mechanisms. This trend parallels the history of bioelectrics in developmental biology, although the development of modern molecular tools and the integration of bioelectric signaling with transcriptional cascades has begun to shed increasing attention on the importance of ion current- and voltage-based

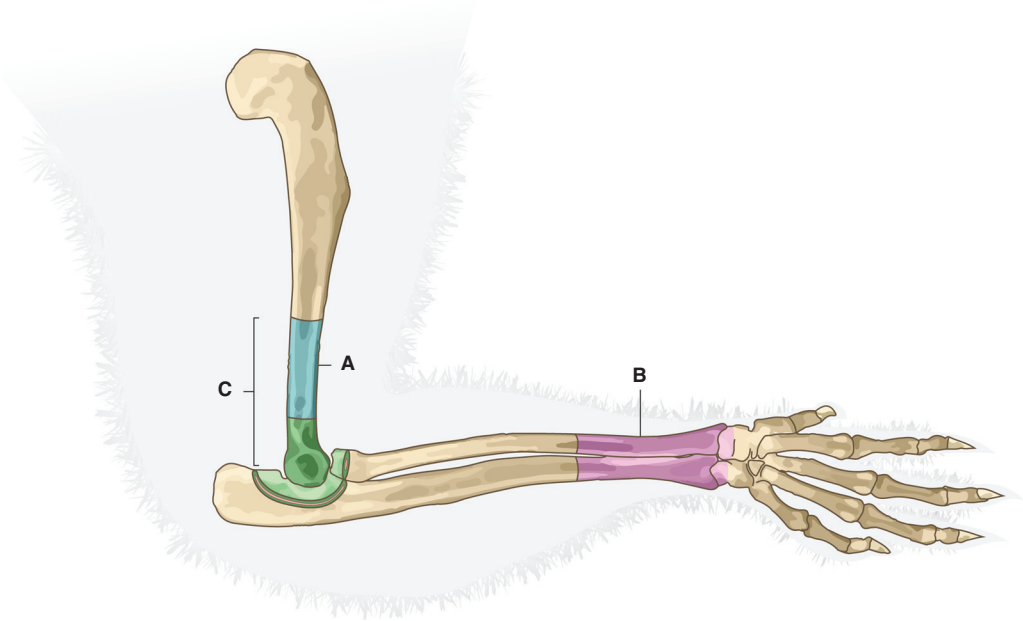

Figure 6. A schematic representation of bone growth in the context of experiments using applied electric currents in Muridae. Colors are used to distinguish specific publications from one another and represent their reported growth. (*A*) Based on work in Libbin et al. (1979), (*B*) based on work in Smith (1981), and (*C*) based on work in Leppik et al. (2016). Both examples were performed in the Sprague–Dawley rat model.

mechanisms (Nuccitelli et al. 1986; Bates 2015; Levin 2020, 2021).

Leppik et al. recently revitalized this approach (Becker 1972; Libbin et al. 1979), demonstrating the capacity for applied electric currents to stimulate regeneration in nonregenerating rat limbs. Using the bimetallic electrode implant, Leppik observed that, within a 28 d regeneration period, electrically stimulated rat limbs showed significant regeneration with substantial bone growth, increased vascularization of regenerate tissue, and decreased neuromas (Fig. 6; Leppik et al. 2016). Although both Leppik and Libbin described a reduced effect compared to that reported by Becker (Fig. 6), two significant electrically driven results are noteworthy. First, neuroma formation is characteristic of fibrotic healing postinjury (Watson et al. 2010), and electric stimulation completely inhibited neuroma formation (Leppik et al. 2016). Second, normal fibrotic healing of an amputated limb (or proximal digit) results in rapid capping of the bone marrow cavity (Turner et al. 2010a). Electrically stimulated limbs showed a prolonged opening of the marrow cavity that remained open for as long as 7 d postamputation compared to control animals whose marrow was completely capped by 7 d postamputation (Leppik et al. 2016). Bone marrow is known to be rich in mesenchymal stem cells that play a significant role during bone repair (Colnot 2009) and have been heavily investigated for their capacity to rejuvenate numerous tissue types (Penfornis and Pochampally 2011). Thus, marrow access into the regenerating tissues may have contributed to the regeneration observed in electrically stimulated limbs.

In 2010, a group of researchers approached the use of electrical stimulation in a novel way by creating a bioreactor coined "BioDome" (Hechavarria et al. 2010). The BioDome is designed to maintain a hydrated environment at the wound interface while delivering bioactive compounds and electrical stimulation to the amputation site. The key component of this bioreactor is its implemented hydrogel. This hydrogel pro-

vides the hydrated environment, osmotic control over the wound, and acts as a precise delivery mechanism for embedded bioactive compounds whose release kinetics are regulated by the properties of the hydrogel, which can be carefully manipulated (Pritchard and Kaplan 2011). Integral to this design is the capability not only to deliver a drug or growth factor of choice to the wound, but also to simultaneously treat the wound with electrical stimulation to modulate its bioelectric state. Control over the wound's bioelectric state alone has been shown to have the capacity to induce regeneration in nonregenerative vertebrates (Jenkins et al. 1996; Tseng et al. 2010; Adams et al. 2013; Leppik et al. 2016). Coupling this with bioactive compound delivery may prove to be a highly effective therapeutic.

CONCLUSION: MECHANISMS CONTRIBUTING TO REPORTED REGENERATIVE IMPROVEMENT

Appendage regeneration is in a unique position to contribute therapies that significantly improve quality of life. Accelerating research in regenerative fields has already borne fruit with studies developing techniques to regenerate significant amounts of lost tissue (Suckow et al. 1999; Kumar et al. 2007; Yakushiji et al. 2009; Turner et al. 2010b; Yu et al. 2012; Lin et al. 2013; Herrera-Rincon et al. 2018) and even features such as joints that closely resemble healthy structures (Yu et al. 2019a). Five general approaches have been used to achieve varying degrees of regeneration in poorly regenerative vertebrate systems: surgical intervention, biochemical pathway targeted interventions, selective breeding, pharmacological V_{mem} augmentation, and exogenously applied electric fields. Targeted manipulation of biochemical pathways via systemic or local treatment with pharmacological substances has proven to be the most effective and reproducible means to induce vertebrate limb regeneration in nonregenerative systems. Taken together, the body of work on regenerative induction suggests a few common features (summarized in Table 1).

Targeting native biochemical pathways to induce regeneration is promising because it uses cells' native capacity to grow tissue, predicated by embryonic development but perhaps suppressed through evolutionary time. These therapies are designed to rejuvenate cellular mechanisms that emulate those used during development and repurpose them for adult limb regeneration. Of the various biochemical stimulators discussed, those employing BMP and FGF proteins are the most effective and consistent stimulators of limb outgrowth across nonregenerative adult vertebrate systems (Taylor et al. 1994; Kostakopoulou et al. 1996; Mullen et al. 1996; Christen and Slack 1997; Yu et al. 2010, 2012, 2019a; Lin et al. 2013; Takeo et al. 2013; Vieira et al. 2019). Treatments with BMP2 (Yu et al. 2012, 2019a) and BMP9 (Yu et al. 2019a) in mice were highly effective at partial regeneration of amputated limbs as these treatments significantly enhanced underlying bone and joint regeneration. Bone outgrowth is a known determinant of successfully regeneration in nonregenerative vertebrates (Sensiate and Marques-Souza 2019). The effectiveness of BMP and FGF in stimulating limb regeneration can be seen from amphibious (Makanae et al. 2014; Satoh et al. 2016; Vieira et al. 2019) to mammalian vertebrates (Han et al. 2003; Yu et al. 2012, 2019a), and ongoing research may yet discover how BMP's may be used to successfully and completely regenerate lost limbs.

Interestingly, the only ever reported instance of inducing complete vertebrate appendage regeneration occurring in a nonregenerative system was achieved through the manipulation of bioelectrics (Smith 1974). This outcome has yet to be reproduced with modern tools and molecular characterization, but the recent successes of modulation of resting potential to manipulate human (Sundelacruz et al. 2008, 2013, 2016) and rodent (Feng et al. 2017; Mobini et al. 2017) stem cells and amphibian tail and limb regeneration (Fig. 7A–F; Adams et al. 2007, 2013; Tseng et al. 2010) suggest that bioelectric interventions are likely to be a key modality (Herrera-Rincon et al. 2017). Bioelectric controls of cell behaviors (Blackiston et al. 2009) and tissue-level morphogenetic processes (Reid et al. 2009; Lois et al. 2010; Perathoner et al. 2014; Bates 2015; Ferreira et al. 2016, 2018; Dahal et al. 2017; Daane et al. 2018; Lanni

Table 1. Scored results from reported observations across the various bodies of work investigated in this review

References	Model	Age	Amputation	Treatment	Time	B/C	M	N
Smith et al. 1967	*Rana pipens*	Adult	Radial/ulnar	0.67 V/cm (Ringers)	Until sac.	++	++	NR
Becker 1972	*Rattus*	Adult	Humeral	$3–5 \times 10^{-9}$ Å (Ringers)	Until sac.	+	+	NR
Smith et al. 1974	*R. pipens*	Adult	Radial/ulnar	1.03×10^{-7} Å (tissue)	30 d	+++*	+++*	NR
Borgens et al. 1977	*R. pipens*	Adult	Radial/ulnar	2.0×10^{-7} Å (tissue)	21–30 d	+	+	+
Libbin et al. 1979	*Rattus*	Adult	Humeral	$3–10 \times 10^{-9}$ Å (NR)	4–14 d	+	-	NR
Smith et al. 1981	*Mus musculus*	Adult	Radial/ulnar	1.04×10^{-6} Å (NR)	Until sac.	++	++	NR
Taylor et al. 1994	*Gallus gallus*	Embryonic	Radial/ulnar	FGF2	Until sac.	+++	NR	NR
Kostakopoulou et al. 1996	*G. gallus*	Embryonic	Radial/ulnar	FGF4	Until sac.	+++	NR	NR
Suckow et al. 1999	*M. musculus*	Adult	Radial	Porcine small intestinal mucosa	Until sac.	+++	NR	NR
Masaki and Ide 2007	*M. musculus*	Neonatal	Radial/ulnar	Gelatin + BMP7	Until sac.	++	NR	NR
Turner et al. 2010b	*Canis lupus familiaris*	Adult	Achilles musculotendinous junction	Porcine small intestinal mucosa	Until sac.	NR	++++	+++
Agrawal et al. 2011	*M. musculus*	Adult	P2 digit	Cryptic peptide	Until sac.	+	NR	NR
Yu et al. 2012	*M. musculus*	Adult	P2 digit	BMP2	Until sac.	+++	NR	NR
Yu et al. 2012	*M. musculus*	Adult	Tibia/fibula	BMP2	Until sac.	++	NR	NR
Lin et al. 2013	*Xenopus laevis*	Adult	Wrist	β-Catenin limb bud cells + (Shh, FGF10, Tβ4)	Until sac.	+++	+++	+++
Takeo et al. 2013	*M. musculus*	Adult	Proximal P3 digit	Stabilized β-catenin	Until sac.	++++	NR	NR
Leppik et al. 2016	*Rattus*	Adult	Humeral	Applied current (not measured)	Until sac.	+	-	-
Chen et al. 2017	*M. musculus*	Adult	P2 digit	iPSC + (BMP2, FGF8, Tβ4, WNT3a)	Until sac.	++	NR	NR
Dawson et al. 2017	*M. musculus*	Adult	P2 digit	BMP2	Until sac.	+++	NR	NR
Makanae and Satoh et al. 2018	*G. gallus*	Embryonic	Radial/ulnar	FGF2, FGF8	Until sac.	++++	NR	NR
Herrera-Rincon et al. 2018	*X. laevis*	Adult	Femur	Progesterone	24 h	++	+++	+++
Mitogawa et al. 2018	*X. laevis*	Adult	Radioulna	Hyperinnervation	Until sac.	+++	+++	NR
Mitogawa et al. 2018	*X. laevis*	Adult	Radioulna	BMP7, FGF2/-8, Shh	Until sac.	++	NR	NR

Regeneration was scored from + = little regeneration to ++++ = complete regeneration (+++* = significant regeneration with 1 instance of complete regeneration). Although no score of ++++ was given to any report, various works did exemplify high levels of regeneration (+++). When reporting values of applied current, parentheses are used to describe the medium in which the current was measured, as this holds significance in comparing across experimental reports. Time refers to the amount of time a given treatment was applied (not the amount of time the animals were given to regenerate). Until sac. = treatment was applied until animals were sacrificed.

(B/C) Bone and/or cartilage, (M) muscle, (N) nerve(s), (NR) not reported.

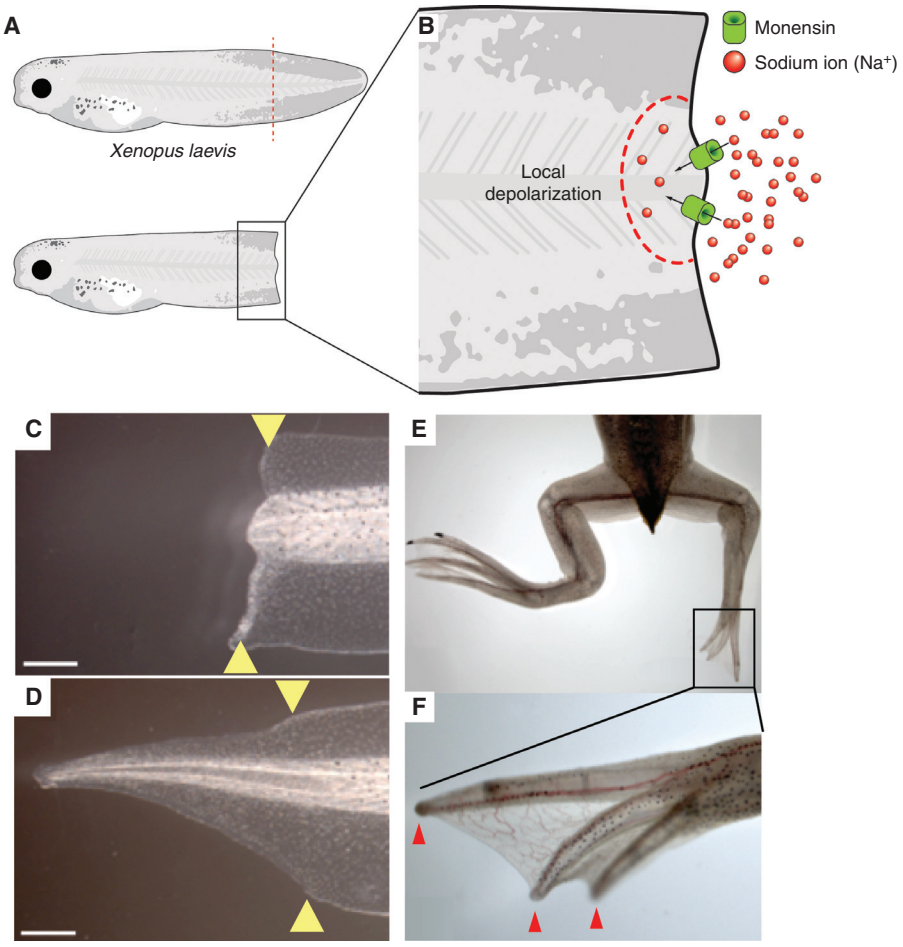

Figure 7. Summary schematic showing the effects of monensin treatment used to rescue regeneration in *Xenopus laevis* appendages. (*A*) Illustration of *X. laevis* tadpole at developmental stage ~45 during what is known as the refractory period (nonregenerative), showing intact and amputated specimens. (*B*) Enhanced illustration showing the mechanism by which monensin (a sodium ionophore) locally depolarizes the tail amputation site. (*C*) Morphology of a tail 8 d after being amputated during the tadpole refractory period, showing no growth. (*D*) Morphology of a regenerated tail that was also amputated during the refractory period and treated with a 1-h monensin soak, demonstrating its ability to rescue regeneration. (Panels *C* and *D* from Tseng et al. 2010, reprinted under a Creative Commons Attribution-Noncommercial-Share Alike 3.0 Unported License [CC-BY-NC-SA].) (*E*) *Xenopus* froglets, at the nonregenerative stage, exhibit regeneration of hindlimbs when treated for 24 h with a monensin soak (Tseng and Levin 2013). (*F*) Close-up of distal regenerate showing fairly normal resultant structure and morphology. Yellow arrowheads in panels *C* and *D* indicate amputation plane. Red arrowheads indicate new digits. Scale bars, 500 µm. (Panels *C* and *D* used with permission from Tseng et al. 2010; panels *E* and *F* kindly provided by AiSun Tseng [Levin laboratory].)

et al. 2019; Levin 2021) can act during multiple steps thought to be part of the regenerative control cascade (Fig. 8). Overall, it is likely that successful outcomes will be achieved by approaches with two components: wearable bioreactors to provide a regeneration-permissive, supportive environment, and a combination of bioelectric, biochemical, and haptic signals that trigger growth and initiate self-limiting, locale-appropriate morphogenetic cascades. By perfecting these aspects for limb regeneration, it is likely that the progress will lead not only to applied

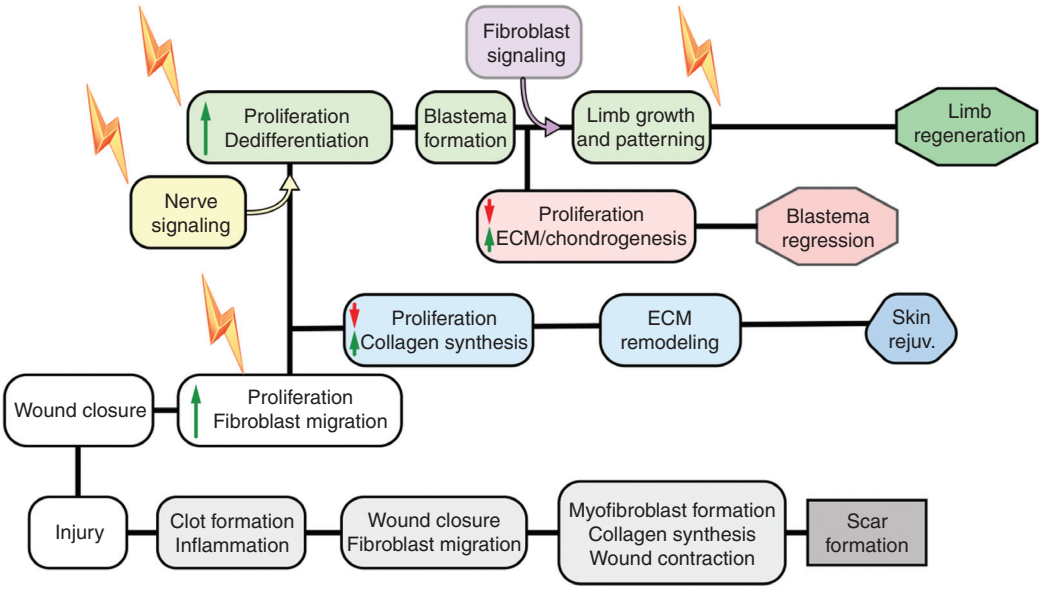

Figure 8. Flowchart summarizing the various processes and outcomes following injury in the context of wound healing and regeneration. This diagram was constructed after Figure 2 of Gardiner (2005) as a foundation; yellow lightning symbols indicate processes where bioelectric signaling can work as a regulatory trigger (including effects on cell proliferation, guidance of innervation, control of differentiation and immune cell migration, and tissue-level morphogenetic decisions). (ECM) Extracellular matrix. (Permission for modification of Figure 2 by Gardiner 2005 granted by Mary Ann Liebert © 2005.)

biomedical outcomes but also to a much deeper basic understanding of the endogenous controls of growth and form and the plasticity with which they can be controlled.

ACKNOWLEDGMENTS

We gratefully acknowledge support of the Allen Discovery Center program through The Paul G. Allen Frontiers Group (12171).

REFERENCES

Adams DS, Masi A, Levin M. 2007. H^+ pump-dependent changes in membrane voltage are an early mechanism necessary and sufficient to induce *Xenopus* tail regeneration. *Development* **134:** 1323–1335. doi:10.1242/dev.02812

Adams DS, Tseng A-S, Levin M. 2013. Light-activation of the Archaerhodopsin H^+-pump reverses age-dependent loss of vertebrate regeneration: sparking system-level controls in vivo. *Biol Open* **2:** 306–313. doi:10.1242/bio.20133665

Agrawal V, Kelly J, Tottey S, Daly KA, Johnson SA, Siu BF, Reing J, Badylak SF. 2011. An isolated cryptic peptide influences osteogenesis and bone remodeling in an adult mammalian model of digit amputation. *Tissue Eng Part A* **17:** 3033–3044. doi:10.1089/ten.tea.2011.0257

Allen JM, Ross KG, Zayas RM. 2016. Regeneration in invertebrates: model systems. In *eLS*, pp. 1–9. Wiley, Chichester, UK.

Alvarado AS, Tsonis PA. 2006. Bridging the regeneration gap: genetic insights from diverse animal models. *Nat Rev Genet* **7:** 873–884. doi:10.1038/nrg1923

Ambros V, Horvitz H. 1984. Heterochronic mutants of the nematode *Caenorhabditis elegans*. *Science* **226:** 409–416. doi:10.1126/science.6494891

Barghouth PG, Thiruvalluvan M, Oviedo NJ. 2015. Bioelectrical regulation of cell cycle and the planarian model system. *Biochim Biophys Acta* **1848:** 2629–2637. doi:10.1016/j.bbamem.2015.02.024

Barker DM, Beck CW. 2009. Overexpression of the transcription factor Msx1 is insufficient to drive complete regeneration of refractory stage *Xenopus laevis* hindlimbs. *Dev Dyn* **238:** 1366–1378. doi:10.1002/dvdy.21923

Bates E. 2015. Ion channels in development and cancer. *Annu Rev Cell Dev Biol* **31:** 231–247. doi:10.1146/annurev-cellbio-100814-125338

Beare AHM, Metcalfe AD, Ferguson MWJ. 2006. Location of injury influences the mechanisms of both regeneration and repair within the MRL/MpJ mouse. *J Anat* **209:** 547–559. doi:10.1111/j.1469-7580.2006.00641.x

Beck F, Chawengsaksophak K, Waring P, Playford RJ, Furness JB. 1999. Reprogramming of intestinal differentiation and intercalary regeneration in *Cdx2* mutant mice. *Proc Natl Acad Sci* **96:** 7318–7323. doi:10.1073/pnas.96.13.7318

Beck CW, Christen B, Slack JMW. 2003. Molecular pathways needed for regeneration of spinal cord and muscle in a vertebrate. *Dev Cell* **5:** 429–439. doi:10.1016/S1534-5807(03)00233-8

Beck CW, Izpisúa Belmonte JC, Christen B. 2009. Beyond early development: *Xenopus* as an emerging model for the study of regenerative mechanisms. *Dev Dyn* **238:** 1226–1248. doi:10.1002/dvdy.21890

Becker RO. 1967. The electrical control of growth processes. *Med Times* **95:** 657–669.

Becker RO. 1972. Stimulation of partial limb regeneration in rats. *Nature* **235:** 109–111. doi:10.1038/235109a0

Benders KEM, van Weeren PR, Badylak SF, Saris DBF, Dhert WJA, Malda J. 2013. Extracellular matrix scaffolds for cartilage and bone regeneration. *Trends Biotechnol* **31:** 169–176. doi:10.1016/j.tibtech.2012.12.004

Bensoussan-Trigano V, Lallemand Y, Saint Cloment C, Robert B. 2011. Msx1 and Msx2 in limb mesenchyme modulate digit number and identity. *Dev Dyn* **240:** 1190–1202. doi:10.1002/dvdy.22619

Birnbaum KD, Alvarado AS. 2008. Slicing across kingdoms: regeneration in plants and animals. *Cell* **132:** 697–710. doi:10.1016/j.cell.2008.01.040

Blackiston DJ, McLaughlin KA, Levin M. 2009. Bioelectric controls of cell proliferation: ion channels, membrane voltage and the cell cycle. *Cell Cycle* **8:** 3527–3536. doi:10.4161/cc.8.21.9888

Borgens RB. 1982. What is the role of naturally produced electric current in vertebrate regeneration and healing. *Int Rev Cytol* **76:** 245–298. doi:10.1016/S0074-7696(08)61793-3

Borgens RB. 1983. The role of ionic current in the regeneration and development of the amphibian limb. *Prog Clin Biol Res* **110:** 597–608.

Borgens RB. 1984. Are limb development and limb regeneration both initiated by an integumentary wounding? A hypothesis. *Differentiation* **28:** 87–93. doi:10.1111/j.1432-0436.1984.tb00270.x

Borgens RB, Vanable JW, Jaffe LF. 1977. Bioelectricity and regeneration. I: Initiation of frog limb regeneration by minute currents. *J Exp Zool* **200:** 403–416. doi:10.1002/jez.1402000310

Brockes JP. 1997. Amphibian limb regeneration: rebuilding a complex structure. *Science* **276:** 81–87. doi:10.1126/science.276.5309.81

Bryant SV, Iten LE. 1977. Intercalary and supernumerary regeneration in regenerating and mature limbs of *Notophthalmus viridescens*. *J Exp Zool* **202:** 1–15. doi:10.1002/jez.1402020102

Bryant DM, Sousounis K, Farkas JE, Bryant S, Thao N, Guzikowski AR, Monaghan JR, Levin M, Whited JL. 2017a. Repeated removal of developing limb buds permanently reduces appendage size in the highly-regenerative axolotl. *Dev Biol* **424:** 1–9. doi:10.1016/j.ydbio.2017.02.013

Bryant DM, Sousounis K, Payzin-Dogru D, Bryant S, Sandoval AGW, Martinez Fernandez J, Mariano R, Oshiro R, Wong AY, Leigh ND, et al. 2017b. Identification of regenerative roadblocks via repeat deployment of limb regeneration in axolotls. *NPJ Regen Med* **2:** 30. doi:10.1038/s41536-017-0034-z

Buckley G, Metcalfe AD, Ferguson MWJ. 2011. Peripheral nerve regeneration in the MRL/MpJ ear wound model. *J Anat* **218:** 163–172. doi:10.1111/j.1469-7580.2010.01313.x

Cao L, McCaig CD, Scott RH, Zhao S, Milne G, Clevers H, Zhao M, Pu J. 2014. Polarizing intestinal epithelial cells electrically through Ror2. *J Cell Sci* **127:** 3233–3239.

Carlson MR, Bryant SV, Gardiner DM. 1998. Expression of Msx-2 during development, regeneration, and wound healing in axolotl limbs. *J Exp Zool* **282:** 715–723. doi:10.1002/(SICI)1097-010X(19981215)282:6<715::AID-JEZ7>3.0.CO;2-F

Caudwell M, Crowley C, Khan WS, Wong JML. 2015. Systematic review of preclinical and clinical studies on scaffold use in knee ligament regeneration. *Curr Stem Cell Res Ther* **10:** 11–18. doi:10.2174/1574888X09666140710101435

Chadwick RB, Bu L, Yu H, Hu Y, Wergedal JE, Mohan S, Baylink DJ. 2007. Digit tip regrowth and differential gene expression in MRL/Mpj, DBA/2, and C57BL/6 mice. *Wound Repair Regen* **15:** 275–284. doi:10.1111/j.1524-475X.2007.00216.x

Chávez-Delgado ME, Mora-Galindo J, Gómez-Pinedo U, Feria-Velasco A, Castro-Castañeda S, López-Dellamary Toral FA, Luquin-De Anda S, García-Segura LM, García-Estrada J. 2003. Facial nerve regeneration through progesterone-loaded chitosan prosthesis. A preliminary report. *J Biomed Mater Res Part B Appl Biomater* **67B:** 702–711. doi:10.1002/jbm.b.10059

Chen TH. 2014. Tissue regeneration: from synthetic scaffolds to self-organizing morphogenesis. *Curr Stem Cell Res Ther* **9:** 432–443. doi:10.2174/1574888X09666140507123401

Chen Y, Xu H, Lin G. 2017. Generation of iPSC-derived limb progenitor-like cells for stimulating phalange regeneration in the adult mouse. *Cell Discov* **3:** 17046. doi:10.1038/celldisc.2017.46

Chiang C, Litingtung Y, Harris MP, Simandl BK, Li Y, Beachy PA, Fallon JF. 2001. Manifestation of the limb prepattern: limb development in the absence of sonic hedgehog function. *Dev Biol* **236:** 421–435. doi:10.1006/dbio.2001.0346

Christen B, Slack JM. 1997. FGF-8 is associated with anteroposterior patterning and limb regeneration in *Xenopus*. *Dev Biol* **192:** 455–466. doi:10.1006/dbio.1997.8732

Christensen RN, Weinstein M, Tassava RA. 2001. Fibroblast growth factors in regenerating limbs of *Ambystoma*: cloning and semi-quantitative RT-PCR expression studies. *J Exp Zool* **290:** 529–540. doi:10.1002/jez.1097

Christensen RN, Weinstein M, Tassava RA. 2002. Expression of fibroblast growth factors 4, 8, and 10 in limbs, flanks, and blastemas of *Ambystoma*. *Dev Dyn* **223:** 193–203. doi:10.1002/dvdy.10049

Clark LD, Clark RK, Heber-Katz E. 1998. A new murine model for mammalian wound repair and regeneration. *Clin Immunol Immunopathol* **88:** 35–45. doi:10.1006/clin.1998.4519

Cohn MJ, Izpisúa-Belmonte JC, Abud H, Heath JK, Tickle C. 1995. Fibroblast growth factors induce additional limb development from the flank of chick embryos. *Cell* **80:** 739–746. doi:10.1016/0092-8674(95)90352-6

Colnot C. 2009. Skeletal cell fate decisions within periosteum and bone marrow during bone regeneration. *J Bone Miner Res* **24:** 274–282. doi:10.1359/jbmr.081003

Crawford K, Stocum DL. 1988. Retinoic acid proximalizes level-specific properties responsible for intercalary regeneration in axolotl limbs. *Development* **104:** 703–712. doi:10.1242/dev.104.4.703

Crossley PH, Minowada G, MacArthur CA, Martin GR. 1996. Roles for FGF8 in the induction, initiation, and maintenance of chick limb development. *Cell* **84:** 127–136. doi:10.1016/S0092-8674(00)80999-X

Daane JM, Lanni J, Rothenberg I, Seebohm G, Higdon CW, Johnson SL, Harris MP. 2018. Bioelectric-calcineurin signaling module regulates allometric growth and size of the zebrafish fin. *Sci Rep* **8:** 10391. doi:10.1038/s41598-018-28450-6

Dahal GR, Pradhan SJ, Bates EA. 2017. Inwardly rectifying potassium channels influence *Drosophila* wing morphogenesis by regulating Dpp release. *Development* **144:** 2771–2783.

Darnell M, Young S, Gu L, Shah N, Lippens E, Weaver J, Duda G, Mooney D. 2017. Substrate stress-relaxation regulates scaffold remodeling and bone formation in vivo. *Adv Healthcare Mater* **6:** 1601185. doi:10.1002/adhm.201601185

Davidson DR, Crawley A, Hill RE, Tickle C. 1991. Position-dependent expression of two related homeobox genes in developing vertebrate limbs. *Nature* **352:** 429–431. doi:10.1038/352429a0

Davis TA, Amare M, Naik S, Kovalchuk AL, Tadaki D. 2007. Differential cutaneous wound healing in thermally injured MRL/MPJ mice. *Wound Repair Regen* **15:** 577–588. doi:10.1111/j.1524-475X.2007.00266.x

Dawson LA, Simkin J, Sauque M, Pela M, Palkowski T, Muneoka K. 2016. Analogous cellular contribution and healing mechanisms following digit amputation and phalangeal fracture in mice. *Regeneration (Oxf)* **3:** 39–51. doi:10.1002/reg2.51

Dawson LA, Yu L, Yan M, Marrero L, Schanes PP, Dolan C, Pela M, Petersen B, Han M, Muneoka K. 2017. The periosteal requirement and temporal dynamics of BMP2-induced middle phalanx regeneration in the adult mouse. *Regeneration* **4:** 140–150. doi:10.1002/reg2.81

Dawson LA, Schanes PP, Kim P, Imholt FM, Qureshi O, Dolan CP, Yu L, Yan M, Zimmel KN, Falck AR, et al. 2018. Blastema formation and periosteal ossification in the regenerating adult mouse digit. *Wound Repair Regen* **26:** 263–273. doi:10.1111/wrr.12666

Dent JN. 1962. Limb regeneration in larvae and metamorphosing individuals of the South African clawed toad. *J Morphol* **110:** 61–77. doi:10.1002/jmor.1051100105

Deuchar EM. 1976. Regeneration of amputated limb-buds in early rat embryos. *J Embryol Exp Morphol* **35:** 345.

Dolan CP, Yan M, Zimmel K, Yang TJ, Leininger E, Dawson LA, Muneoka K. 2019. Axonal regrowth is impaired during digit tip regeneration in mice. *Dev Biol* **445:** 237–244. doi:10.1016/j.ydbio.2018.11.010

D'Onofrio A, Cresce GD, Bolgan I, Magagna P, Piccin C, Auriemma S, Fabbri A. 2011. Clinical and hemodynamic outcomes after aortic valve replacement with stented and stentless pericardial xenografts: a propensity-matched analysis. *J Heart Valve Dis* **20:** 319–325; discussion 326.

Dungan KM, Wei TY, Nace JD, Poulin ML, Chiu IM, Lang JC, Tassava RA. 2002. Expression and biological effect of urodele fibroblast growth factor 1: relationship to limb regeneration. *J Exp Zool* **292:** 540–554. doi:10.1002/jez.10077

Elliott SA, Sánchez Alvarado A. 2013. The history and enduring contributions of planarians to the study of animal regeneration. *WIREs Dev Biol* **2:** 301–326. doi:10.1002/wdev.82

Endo T, Tamura K, Ide H. 2000. Analysis of gene expressions during *Xenopus* forelimb regeneration. *Dev Biol* **220:** 296–306. doi:10.1006/dbio.2000.9641

Endo T, Bryant SV, Gardiner DM. 2004. A stepwise model system for limb regeneration. *Dev Biol* **270:** 135–145. doi:10.1016/j.ydbio.2004.02.016

Fallon JF, López A, Ros MA, Savage MP, Olwin BB, Simandl BK. 1994. FGF-2: apical ectodermal ridge growth signal for chick limb development. *Science* **264:** 104–107. doi:10.1126/science.7908145

Feng JF, Liu J, Zhang L, Jiang JY, Russell M, Lyeth BG, Nolta JA, Zhao M. 2017. Electrical guidance of human stem cells in the rat brain. *Stem Cell Reports* **9:** 177–189. doi:10.1016/j.stemcr.2017.05.035

Ferreira F, Luxardi G, Reid B, Zhao M. 2016. Early bioelectric activities mediate redox-modulated regeneration. *Development* **143:** 4582–4594. doi:10.1242/dev.142034

Ferreira F, Raghunathan V, Luxardi G, Zhu K, Zhao M. 2018. Early redox activities modulate *Xenopus* tail regeneration. *Nat Commun* **9:** 4296. doi:10.1038/s41467-018-06614-2

Fitzgerald J, Rich C, Burkhardt D, Allen J, Herzka AS, Little CB. 2008. Evidence for articular cartilage regeneration in MRL/MpJ mice. *Osteoarthr Cartil* **16:** 1319–1326. doi:10.1016/j.joca.2008.03.014

Gardiner DM. 2005. Ontogenetic decline of regenerative ability and the stimulation of human regeneration. *Rejuvenation Res* **8:** 141–153. doi:10.1089/rej.2005.8.141

Gardiner DM, Blumberg B, Komine Y, Bryant SV. 1995. Regulation of HoxA expression in developing and regenerating axolotl limbs. *Development* **121:** 1731–1741. doi:10.1242/dev.121.6.1731

Gerli MFM, Guyette JP, Evangelista-Leite D, Ghoshhajra BB, Ott HC. 2018. Perfusion decellularization of a human limb: a novel platform for composite tissue engineering and reconstructive surgery. *PLoS ONE* **13:** e0191497. doi:10.1371/journal.pone.0191497

Giampaoli S, Bucci S, Ragghianti M, Mancino G, Zhang F, Ferretti P. 2003. Expression of FGF2 in the limb blastema of two Salamandridae correlates with their regenerative capability. *Proc Biol Sci* **270:** 2197–2205. doi:10.1098/rspb.2003.2439

Gidge NM, Rose SM. 1944. The role of larval skin in promoting limb regeneration in adult Anura. *J Exp Zool* **97:** 71–93. doi:10.1002/jez.1400970105

Golding A, Guay JA, Herrera-Rincon C, Levin M, Kaplan DL. 2016. A tunable silk hydrogel device for studying limb

regeneration in adult *Xenopus laevis*. *PLoS ONE* **11:** e0155618. doi:10.1371/journal.pone.0155618

Gonzalez ACDO, Costa TF, Andrade ZDA, Medrado ARAP. 2016. Wound healing—a literature review. *An Bras Dermatol* **91:** 614–620. doi:10.1590/abd1806-4841.20164741

Gourevitch DL, Clark L, Bedelbaeva K, Leferovich J, Heber-Katz E. 2009. Dynamic changes after murine digit amputation: the MRL mouse digit shows waves of tissue remodeling, growth, and apoptosis. *Wound Repair Regen* **17:** 447–455. doi:10.1111/j.1524-475X.2009.00492.x

Gross D, Loew LM, Webb WW. 1986. Optical imaging of cell membrane potential changes induced by applied electric fields. *Biophys J* **50:** 339–348. doi:10.1016/S0006-3495(86)83467-1

Gurtner GC, Werner S, Barrandon Y, Longaker MT. 2008. Wound repair and regeneration. *Nature* **453:** 314–321. doi:10.1038/nature07039

Han MJ, An JY, Kim WS. 2001. Expression patterns of Fgf-8 during development and limb regeneration of the axolotl. *Dev Dyn* **220:** 40–48. doi:10.1002/1097-0177(2000)9999:9999<::AID-DVDY1085>3.0.CO;2-8

Han M, Yang X, Farrington JE, Muneoka K. 2003. Digit regeneration is regulated by *Msx1* and *BMP4* in fetal mice. *Development* **130:** 5123–5132. doi:10.1242/dev.00710

Han M, Yang X, Taylor G, Burdsal CA, Anderson RA, Muneoka K. 2005. Limb regeneration in higher vertebrates: developing a roadmap. *Anat Rec B New Anat* **287B:** 14–24. doi:10.1002/ar.b.20082

Hechavarria D, Dewilde A, Braunhut S, Levin M, Kaplan DL. 2010. Biodome regenerative sleeve for biochemical and biophysical stimulation of tissue regeneration. *Med Eng Phys* **32:** 1065–1073. doi:10.1016/j.medengphy.2010.07.010

Hennighausen L, Robinson GW. 2001. Signaling pathways in mammary gland development. *Dev Cell* **1:** 467–475. doi:10.1016/S1534-5807(01)00064-8

Herrera-Rincon C, Guay J, Levin M. 2017. Bioelectrical coordination of cell activity toward anatomical target states. In *Regenerative engineering and developmental biology* (ed. Gardiner DM), pp. 55–112. CRC, Boca Raton, FL.

Herrera-Rincon C, Golding AS, Moran KM, Harrison C, Martyniuk CJ, Guay JA, Zaltsman J, Carabello H, Kaplan DL, Levin M. 2018. Brief local application of progesterone via a wearable bioreactor induces long-term regenerative response in adult *Xenopus* hindlimb. *Cell Rep* **25:** 1593–1609.e7. doi:10.1016/j.celrep.2018.10.010

Heydemann A. 2012. The super super-healing MRL mouse strain. *Front Biol* **7:** 522–538. doi:10.1007/s11515-012-1192-4

Holstein TW, Hobmayer E, Technau U. 2003. Cnidarians: an evolutionarily conserved model system for regeneration? *Dev Dyn* **226:** 257–267. doi:10.1002/dvdy.10227

Illingworth CM. 1974. Trapped fingers and amputated finger tips in children. *J Pediatr Surg* **9:** 853–858. doi:10.1016/S0022-3468(74)80220-4

Ivanova AS, Ermakova GV, Zaraisky AG, Tereshina MB. 2018. Patterns of mitosis and activation of the map-kinase cascade during tadpole tail regeneration in the refractory period of *Xenopus laevis* development. *Russ J Dev Biol* **49:** 260–263. doi:10.1134/S1062360418050028

Jacyniak K, McDonald RP, Vickaryous MK. 2017. Tail regeneration and other phenomena of wound healing and tissue restoration in lizards. *J Exp Biol* **220:** 2858–2869. doi:10.1242/jeb.126862

Jafari P, Muller C, Grognuz A, Applegate LA, Raffoul W, di Summa PG, Durand S. 2017. First insights into human fingertip regeneration by echo-Doppler imaging and wound microenvironment assessment. *Int J Mol Sci* **18:** 1054. doi:10.3390/ijms18051054

Jenkins LS, Duerstock BS, Borgens RB. 1996. Reduction of the current of injury leaving the amputation inhibits limb regeneration in the red spotted newt. *Dev Biol* **178:** 251–262. doi:10.1006/dbio.1996.0216

Jiang D, Correa-Gallegos D, Christ S, Stefanska A, Liu J, Ramesh P, Rajendran V, De Santis MM, Wagner DE, Rinkevich Y. 2018. Two succeeding fibroblastic lineages drive dermal development and the transition from regeneration to scarring. *Nat Cell Biol* **20:** 422–431. doi:10.1038/s41556-018-0073-8

Johnson GL, Masias EJ, Lehoczky JA. 2020. Cellular heterogeneity and lineage restriction during mouse digit tip regeneration at single-cell resolution. *Dev Cell* **52:** 525–540.e5. doi:10.1016/j.devcel.2020.01.026

Johnston APW, Yuzwa SA, Carr MJ, Mahmud N, Storer MA, Krause MP, Jones K, Paul S, Kaplan DR, Miller FD. 2016. Dedifferentiated Schwann cell precursors secreting paracrine factors are required for regeneration of the mammalian digit tip. *Cell Stem Cell* **19:** 433–448. doi:10.1016/j.stem.2016.06.002

Kierdorf U, Kierdorf H, Szuwart T. 2007. Deer antler regeneration: cells, concepts, and controversies. *J Morphol* **268:** 726–738. doi:10.1002/jmor.10546

Koenig HL, Gong WH, Pelissier P. 2000. Role of progesterone in peripheral nerve repair. *Rev Reprod* **5:** 189–199. doi:10.1530/ror.0.0050189

Koshiba K, Kuroiwa A, Yamamoto H, Tamura K, Ide H. 1998. Expression of *Msx* genes in regenerating and developing limbs of axolotl. *J Exp Zool* **282:** 703–714. doi:10.1002/(SICI)1097-010X(19981215)282:6<703::AID-JEZ6>3.0.CO;2-P

Kostakopoulou K, Vogel A, Brickell P, Tickle C. 1996. "Regeneration" of wing bud stumps of chick embryos and reactivation of Msx-1 and Shh expression in response to FGF-4 and ridge signals. *Mech Devt* **55:** 119–131. doi:10.1016/0925-4773(95)00492-0

Kucerova R, Walczysko P, Reid B, Ou J, Leiper LJ, Rajnicek AM, McCaig CD, Zhao M, Collinson JM. 2011. The role of electrical signals in murine corneal wound re-epithelialization. *J Cell Physiol* **226:** 1544–1553. doi:10.1002/jcp.22488

Kumar A, Godwin JW, Gates PB, Garza-Garcia AA, Brockes JP. 2007. Molecular basis for the nerve dependence of limb regeneration in an adult vertebrate. *Science* **318:** 772–777. doi:10.1126/science.1147710

Kumar A, Delgado JP, Gates PB, Neville G, Forge A, Brockes JP. 2011. The aneurogenic limb identifies developmental cell interactions underlying vertebrate limb regeneration. *Proc Natl Acad Sci* **108:** 13588–13593. doi:10.1073/pnas.1108472108

Lanni JS, Peal D, Ekstrom L, Chen H, Stanclift C, Bowen ME, Mercado A, Gamba G, Kahle KT, Harris MP. 2019. Integrated K^+ channel and K^+Cl^- cotransporter functions are

required for the coordination of size and proportion during development. *Dev Biol* **456:** 164–178. doi:10.1016/j.ydbio.2019.08.016

Lehoczky JA. 2017. Are fingernails a key to unlocking the puzzle of mammalian limb regeneration? *Exp Dermatol* **26:** 478–482. doi:10.1111/exd.13246

Lehoczky JA, Robert B, Tabin CJ. 2011. Mouse digit tip regeneration is mediated by fate-restricted progenitor cells. *Proc Natl Acad Sci* **108:** 20609–20614. doi:10.1073/pnas.1118017108

Leppik LP, Froemel D, Slavici A, Ovadia ZN, Hudak L, Henrich D, Marzi I, Barker JH. 2016. Effects of electrical stimulation on rat limb regeneration, a new look at an old model. *Sci Rep* **5:** 18353. doi:10.1038/srep18353

Leung Y, Kandyba E, Chen YB, Ruffins S, Chuong CM, Kobielak K. 2014. Bifunctional ectodermal stem cells around the nail display dual fate homeostasis and adaptive wounding response toward nail regeneration. *Proc Natl Acad Sci* **111:** 15114–15119. doi:10.1073/pnas.1318848111

Levin M. 2020. Revisiting Burr and Northrop's "The electrodynamic theory of life" (1935). *Biol Theory* **15:** 83–90. doi:10.1007/s13752-020-00341-y

Levin M. 2021. Bioelectric signaling: reprogrammable circuits underlying embryogenesis, regeneration, and cancer. *Cell* **184:** 1971–1989. doi:10.1016/j.cell.2021.02.034

Levin M, Martyniuk CJ. 2018. The bioelectric code: an ancient computational medium for dynamic control of growth and form. *BioSystems* **164:** 76–93. doi:10.1016/j.biosystems.2017.08.009

Levin M, Pezzulo G, Finkelstein JM. 2017. Endogenous bioelectric signaling networks: exploiting voltage gradients for control of growth and form. *Annu Rev Biomed Eng* **19:** 353–387. doi:10.1146/annurev-bioeng-071114-040647

Li C. 2012. Deer antler regeneration: a stem cell-based epimorphic process. *Birth Defects Res C Embryo Today* **96:** 51–62. doi:10.1002/bdrc.21000

Li C, Zhao H, Liu Z, McMahon C. 2014. Deer antler—a novel model for studying organ regeneration in mammals. *Int J Biochem Cell Biol* **56:** 111–122. doi:10.1016/j.biocel.2014.07.007

Li M, Luan F, Zhao Y, Hao H, Liu J, Dong L, Fu X, Han W. 2017. Mesenchymal stem cell-conditioned medium accelerates wound healing with fewer scars. *Int Wound J* **14:** 64–73. doi:10.1111/iwj.12551

Libbin RM, Person P, Papierman S, Shah D, Nevid D, Grob H. 1979. Partial regeneration of the above-elbow amputated rat forelimb. II: Electrical and mechanical facilitation. *J Morphol* **159:** 439–451. doi:10.1002/jmor.1051590309

Lin G, Slack JMW. 2008. Requirement for Wnt and FGF signaling in *Xenopus* tadpole tail regeneration. *Dev Biol* **316:** 323–335. doi:10.1016/j.ydbio.2008.01.032

Lin G, Chen Y, Slack JMW. 2013. Imparting regenerative capacity to limbs by progenitor cell transplantation. *Dev Cell* **24:** 41–51. doi:10.1016/j.devcel.2012.11.017

Liu J, Yan XL, Zheng XL, Mei L, Wang S, Han J, Yan H. 2017. Electric field exposure promotes epithelial-mesenchymal transition in human lens epithelial cells via integrin β1-FAK signaling. *Mol Med Rep* **16:** 4008–4014. doi:10.3892/mmr.2017.7086

Lois N, Reid B, Song B, Zhao M, Forrester J, McCaig C. 2010. Electric currents and lens regeneration in the rat. *Exp Eye Res* **90:** 316–323. doi:10.1016/j.exer.2009.11.007

Londono R, Sun AX, Tuan RS, Lozito TP. 2018. Tissue repair and epimorphic regeneration: an overview. *Curr Pathobiol Rep* **6:** 61–69. doi:10.1007/s40139-018-0161-2

Lozito TP, Tuan RS. 2017. Lizard tail regeneration as an instructive model of enhanced healing capabilities in an adult amniote. *Connect Tissue Res* **58:** 145–154. doi:10.1080/03008207.2016.1215444

Maden M. 2008. Axolotl/newt. In *Molecular embryology* (ed. Sharpe PT, Mason I), pp. 467–480. Humana, Totowa, NJ.

Mahmood R, Bresnick J, Hornbruch A, Mahony C, Morton N, Colquhoun K, Martin P, Lumsden A, Dickson C, Mason I. 1995. A role for FGF-8 in the initiation and maintenance of vertebrate limb bud outgrowth. *Curr Biol* **5:** 797–806. doi:10.1016/S0960-9822(95)00157-6

Makanae A, Satoh A. 2018. Ectopic Fgf signaling induces the intercalary response in developing chicken limb buds. *Zoological Lett* **4:** 8. doi:10.1186/s40851-018-0090-2

Makanae A, Mitogawa K, Satoh A. 2014. Co-operative Bmp- and Fgf-signaling inputs convert skin wound healing to limb formation in urodele amphibians. *Dev Biol* **396:** 57–66. doi:10.1016/j.ydbio.2014.09.021

Martin P. 1997. Wound healing—aiming for perfect skin regeneration. *Science* **276:** 75–81. doi:10.1126/science.276.5309.75

Martin GR. 1998. The roles of FGFs in the early development of vertebrate limbs. *Genes Dev* **12:** 1571–1586. doi:10.1101/gad.12.11.1571

Maruzzo D, Bortolin F. 2013. Arthropod regeneration. In *Arthropod biology and evolution* (ed. Minelli A, Boxshall G, Fusco G), pp. 149–169. Springer, Berlin.

Masaki H, Ide H. 2007. Regeneration potency of mouse limbs. *Dev Growth Differ* **49:** 89–98. doi:10.1111/j.1440-169X.2007.00909.x

McCaig CD, Rajnicek AM. 1991. Electrical fields, nerve growth and nerve regeneration. *Exp Physiol* **76:** 473–494. doi:10.1113/expphysiol.1991.sp003514

McCaig CD, Song B, Rajnicek AM. 2009. Electrical dimensions in cell science. *J Cell Sci* **122:** 4267–4276. doi:10.1242/jcs.023564

McCormack F. 1983. "Post amputation partial blastema formation in adult mice." Undergraduate thesis, Life and Environmental Sciences, Carroll College, Helena, MT.

McCusker C, Gardiner DM. 2011. The axolotl model for regeneration and aging research: a mini-review. *Gerontology* **57:** 565–571. doi:10.1159/000323761

McEwen BS. 1991. Non-genomic and genomic effects of steroids on neural activity. *Trends Pharmacol Sci* **12:** 141–147. doi:10.1016/0165-6147(91)90531-V

Menéndez-Menéndez Y, Otero-Hernández J, Vega JA, Pérez-Basterrechea M, Pérez-López S, Álvarez-Viejo M, Ferrero-Gutiérrez A. 2017. The role of bone marrow mononuclear cell-conditioned medium in the proliferation and migration of human dermal fibroblasts. *Cell Mol Biol Lett* **22:** 29. doi:10.1186/s11658-017-0055-z

Mescher AL. 1976. Effects on adult newt limb regeneration of partial and complete skin flaps over the amputation surface. *J Exp Zool* **195:** 117–127. doi:10.1002/jez.1401950111

Mitogawa K, Makanae A, Satoh A. 2018. Hyperinnervation improves *Xenopus laevis* limb regeneration. *Devl Biol* **433:** 276–286. doi:10.1016/j.ydbio.2017.10.007

Mittenthal JE, Trevarrow WW. 1984. Intercalary regeneration in legs of crayfish: proximal segments. *Dev Biol* **101:** 40–50. doi:10.1016/0012-1606(84)90115-5

Mobini S, Leppik L, Thottakkattumana Parameswaran V, Barker JH. 2017. In vitro effect of direct current electrical stimulation on rat mesenchymal stem cells. *PeerJ* **5:** e2821. doi:10.7717/peerj.2821

Mohammad KS, Day FA, Neufeld DA. 1999. Bone growth is induced by nail transplantation in amputated proximal phalanges. *Calcif Tissue Int* **65:** 408–410. doi:10.1007/s002239900722

Monavarian M, Kader S, Moeinzadeh S, Jabbari E. 2019. Regenerative scar-free skin wound healing. *Tissue Eng Part B Rev* **25:** 294–311. doi:10.1089/ten.teb.2018.0350

Mondia JP, Levin M, Omenetto FG, Orendorff RD, Branch MR, Adams DS. 2011. Long-distance signals are required for morphogenesis of the regenerating *Xenopus* tadpole tail, as shown by femtosecond-laser ablation. *PLoS ONE* **6:** e24953. doi:10.1371/journal.pone.0024953

Moore AL, Marshall CD, Barnes LA, Murphy MP, Ransom RC, Longaker MT. 2018. Scarless wound healing: transitioning from fetal research to regenerative healing. *Wiley Interdiscip Rev Dev Biol* **7**. doi:10.1002/wdev.309

Moss EG, Lee RC, Ambros V. 1997. The cold shock domain protein LIN-28 controls developmental timing in *C. elegans* and is regulated by the lin-4 RNA. *Cell* **88:** 637–646. doi:10.1016/S0092-8674(00)81906-6

Mullen LM, Bryant SV, Torok MA, Blumberg B, Gardiner DM. 1996. Nerve dependency of regeneration: the role of distal-less and FGF signaling in amphibian limb regeneration. *Development* **122:** 3487–3497. doi:10.1242/dev.122.11.3487

Muneoka K, Dawson LA. 2020. Evolution of epimorphosis in mammals. *J Exp Zool B Mol Dev Evol* **336:** 165–179.

Nelson CE, Morgan BA, Burke AC, Laufer E, DiMambro E, Murtaugh LC, Gonzales E, Tessarollo L, Parada LF, Tabin C. 1996. Analysis of *Hox* gene expression in the chick limb bud. *Development* **122:** 1449–1466. doi:10.1242/dev.122.5.1449

Neufeld DA. 1980. Partial blastema formation after amputation in adult mice. *J Exp Zool* **212:** 31–36. doi:10.1002/jez.1402120105

Nishimura KY, Isseroff RR, Nuccitelli R. 1996. Human keratinocytes migrate to the negative pole in direct current electric fields comparable to those measured in mammalian wounds. *J Cell Sci* **109:** 199–207. doi:10.1242/jcs.109.1.199

Niswander L, Tickle C, Vogel A, Booth I, Martin GR. 1993. FGF-4 replaces the apical ectodermal ridge and directs outgrowth and patterning of the limb. *Cell* **75:** 579–587. doi:10.1016/0092-8674(93)90391-3

Norrie JL, Lewandowski JP, Bouldin CM, Amarnath S, Li Q, Vokes MS, Ehrlich LIR, Harfe BD, Vokes SA. 2014. Dynamics of BMP signaling in limb bud mesenchyme and polydactyly. *Dev Biol* **393:** 270–281. doi:10.1016/j.ydbio.2014.07.003

Nuccitelli R. 2003. A role for endogenous electric fields in wound healing. *Curr Top Dev Biol* **58:** 1–26.

Nuccitelli R, Robinson K, Jaffe L. 1986. On electrical currents in development. *Bioessays* **5:** 292–294. doi:10.1002/bies.950050616

Ohuchi H, Nakagawa T, Yamamoto A, Araga A, Ohata T, Ishimaru Y, Yoshioka H, Kuwana T, Nohno T, Yamasaki M, et al. 1997. The mesenchymal factor, FGF10, initiates and maintains the outgrowth of the chick limb bud through interaction with FGF8, an apical ectodermal factor. *Development* **124:** 2235–2244. doi:10.1242/dev.124.11.2235

Page RL, Malcuit C, Vilner L, Vojtic I, Shaw S, Hedblom E, Hu J, Pins GD, Rolle MW, Dominko T. 2011. Restoration of skeletal muscle defects with adult human cells delivered on fibrin microthreads. *Tissue Eng Part A* **17:** 2629–2640. doi:10.1089/ten.tea.2011.0024

Pajni-Underwood S, Wilson CP, Elder C, Mishina Y, Lewandoski M. 2007. BMP signals control limb bud interdigital programmed cell death by regulating FGF signaling. *Development* **134:** 2359–2368. doi:10.1242/dev.001677

Patel N, Poo MM. 1982. Orientation of neurite growth by extracellular electric fields. *J Neurosci* **2:** 483–496. doi:10.1523/JNEUROSCI.02-04-00483.1982

Pearl EJ, Barker D, Day RC, Beck CW. 2008. Identification of genes associated with regenerative success of *Xenopus laevis* hindlimbs. *BMC Dev Biol* **8:** 66. doi:10.1186/1471-213X-8-66

Pecorino LT, Brockes JP, Entwistle A. 1996a. Semi-automated positional analysis using laser scanning microscopy of cells transfected in a regenerating newt limb. *J Histochem Cytochem* **44:** 559–569. doi:10.1177/44.6.8666741

Pecorino LT, Entwistle A, Brockes JP. 1996b. Activation of a single retinoic acid receptor isoform mediates proximodistal respecification. *Curr Biol* **6:** 563–569. doi:10.1016/S0960-9822(02)00542-0

Penfornis P, Pochampally R. 2011. Isolation and expansion of mesenchymal stem cells/multipotential stromal cells from human bone marrow. *Methods Mol Biol* **698:** 11–21. doi:10.1007/978-1-60761-999-4_2

Perathoner S, Daane JM, Henrion U, Seebohm G, Higdon CW, Johnson SL, Nusslein-Volhard C, Harris MP. 2014. Bioelectric signaling regulates size in zebrafish fins. *PLoS Genet* **10:** e1004080. doi:10.1371/journal.pgen.1004080

Pescitelli MJ, Stocum DL. 1980. The origin of skeletal structures during intercalary regeneration of larval *Ambystoma* limbs. *Dev Biol* **79:** 255–275. doi:10.1016/0012-1606(80)90115-3

Pezzulo G, Levin M. 2016. Top-down models in biology: explanation and control of complex living systems above the molecular level. *J R Soc Interface* **13:** 20160555. doi:10.1098/rsif.2016.0555

Poležajew LW. 1936. Sur la restauration de la capacité régénérative chez les anoures. *Arch d'Anat Microsc* **32:** 439–463.

Polezhayev LW. 1946. The loss and restoration of regenerative capacity in the limbs of tailless Amphibia. *Biol Rev Camb Philos Soc* **21:** 141–147. doi:10.1111/j.1469-185X.1946.tb00319.x

Popiela H. 1976. In vivo limb tissue development in the absence of nerves: a quantitative study. *Exp Neurol* **53:** 214–226. doi:10.1016/0014-4886(76)90293-4

Cold Spring Harbor Perspectives in Biology
www.cshperspectives.org

Poss KD, Shen J, Nechiporuk A, McMahon G, Thisse B, Thisse C, Keating MT. 2000. Roles for Fgf signaling during zebrafish fin regeneration. *Dev Biol* **222:** 347–358. doi:10.1006/dbio.2000.9722

Poss KD, Keating MT, Nechiporuk A. 2003. Tales of regeneration in zebrafish. *Dev Dyn* **226:** 202–210. doi:10.1002/dvdy.10220

Price J, Faucheux C, Allen S. 2005. Deer antlers as a model of mammalian regeneration. *Curr Top Dev Biol* **67:** 1–48. doi:10.1016/S0070-2153(05)67001-9

Pritchard EM, Kaplan DL. 2011. Silk fibroin biomaterials for controlled release drug delivery. *Expert Opin Drug Deliv* **8:** 797–811. doi:10.1517/17425247.2011.568936

Reddien Peter W. 2013. Lin28: time for tissue repair. *Cell* **155:** 738–739. doi:10.1016/j.cell.2013.10.025

Reginelli AD, Wang YQ, Sassoon D, Muneoka K. 1995. Digit tip regeneration correlates with regions of Msx1 (Hox 7) expression in fetal and newborn mice. *Development* **121:** 1065–1076. doi:10.1242/dev.121.4.1065

Reid B, Song B, Zhao M. 2009. Electric currents in *Xenopus* tadpole tail regeneration. *Dev Biol* **335:** 198–207. doi:10.1016/j.ydbio.2009.08.028

Reid B, Graue-Hernandez EO, Mannis MJ, Zhao M. 2011. Modulating endogenous electric currents in human corneal wounds—a novel approach of bioelectric stimulation without electrodes. *Cornea* **30:** 338–343. doi:10.1097/ICO.0b013e3181f7f2de

Repesh LA, Oberpriller JC. 1978. Scanning electron microscopy of epidermal cell migration in wound healing during limb regeneration in the adult newt, *Notophthalmus viridescens*. *Am J Anat* **151:** 539–555. doi:10.1002/aja.1001510408

Riddle RD, Johnson RL, Laufer E, Tabin C. 1993. Sonic hedgehog mediates the polarizing activity of the ZPA. *Cell* **75:** 1401–1416. doi:10.1016/0092-8674(93)90626-2

Rinkevich Y, Walmsley GG, Hu MS, Maan ZN, Newman AM, Drukker M, Januszyk M, Krampitz GW, Gurtner GC, Lorenz HP, et al. 2015. Skin fibrosis. Identification and isolation of a dermal lineage with intrinsic fibrogenic potential. *Science* **348:** aaa2151. doi:10.1126/science.aaa2151

Rong X, Chu W, Zhang H, Wang Y, Qi X, Zhang G, Wang Y, Li C. 2019. Antler stem cell-conditioned medium stimulates regenerative wound healing in rats. *Stem Cell Res Ther* **10:** 326. doi:10.1186/s13287-019-1457-9

Rosales-Cortes M, Peregrina-Sandoval J, Bañuelos-Pineda J, Castellanos-Martínez EE, Gómez-Pinedo UA, Albarrán-Rodríguez E. 2003. Regeneration of the axotomised sciatic nerve in dogs using the tubulisation technique with Chitosan biomaterial preloaded with progesterone. *Rev Neurol* **36:** 1137–1141.

Rose SM. 1945. The effect of NaCl in stimulating regeneration of limbs of frogs. *J Morphol* **77:** 119–139. doi:10.1002/jmor.1050770202

Routley CE, Ashcroft GS. 2009. Effect of estrogen and progesterone on macrophage activation during wound healing. *Wound Repair Regen* **17:** 42–50. doi:10.1111/j.1524-475X.2008.00440.x

Rybak A, Fuchs H, Smirnova L, Brandt C, Pohl EE, Nitsch R, Wulczyn FG. 2008. A feedback loop comprising lin-28 and let-7 controls pre-let-7 maturation during neural stem-cell commitment. *Nat Cell Biol* **10:** 987–993. doi:10.1038/ncb1759

Santosh N, Windsor LJ, Mahmoudi BS, Li B, Zhang W, Chernoff EA, Rao N, Stocum DL, Song F. 2011. Matrix metalloproteinase expression during blastema formation in regeneration-competent versus regeneration-deficient amphibian limbs. *Dev Dyn* **240:** 1127–1141. doi:10.1002/dvdy.22503

Satoh A, Hirata A, Makanae A. 2012. Collagen reconstitution is inversely correlated with induction of limb regeneration in *Ambystoma mexicanum*. *Zoolog Sci* **29:** 191–197. doi:10.2108/zsj.29.191

Satoh A, Makanae A, Nishimoto Y, Mitogawa K. 2016. FGF and BMP derived from dorsal root ganglia regulate blastema induction in limb regeneration in *Ambystoma mexicanum*. *Dev Biol* **417:** 114–125. doi:10.1016/j.ydbio.2016.07.005

Satoh A, Mitogawa K, Saito N, Suzuki M, Suzuki K-iT, Ochi H, Makanae A. 2017. Reactivation of larval keratin gene (krt62.L) in blastema epithelium during *Xenopus* froglet limb regeneration. *Dev Biol* **432:** 265–272. doi:10.1016/j.ydbio.2017.10.015

Satoh A, Mitogawa K, Makanae A. 2018. Nerve roles in blastema induction and pattern formation in limb regeneration. *Int J Dev Biol* **62:** 605–612. doi:10.1387/ijdb.180118as

Schaxel J. 1934. Zur determination der regeneration der axolotl-extremität. *Dokl Akad Nauk SSSR* **4:** 246–248.

Seifert AW, Muneoka K. 2018. The blastema and epimorphic regeneration in mammals. *Dev Biol* **433:** 190–199. doi:10.1016/j.ydbio.2017.08.007

Sensiate LA, Marques-Souza H. 2019. Bone growth as the main determinant of mouse digit tip regeneration after amputation. *Sci Rep* **9:** 9720. doi:10.1038/s41598-019-45521-4

Shabbir A, Cox A, Rodriguez-Menocal L, Salgado M, Badiavas EV. 2015. Mesenchymal stem cell exosomes induce proliferation and migration of normal and chronic wound fibroblasts, and enhance angiogenesis in vitro. *Stem Cells Dev* **24:** 1635–1647. doi:10.1089/scd.2014.0316

Shieh SJ, Cheng TC. 2015. Regeneration and repair of human digits and limbs: fact and fiction. *Regeneration (Oxf)* **2:** 149–168. doi:10.1002/reg2.41

Shimokawa T, Yasutaka S, Kominami R, Shinohara H. 2012. Wound epithelium function in axolotl limb regeneration. *Okajimas Folia Anat Jpn* **89:** 75–81. doi:10.2535/ofaj.89.75

Shyh-Chang N, Daley George Q. 2013. Lin28: primal regulator of growth and metabolism in stem cells. *Cell Stem Cell* **12:** 395–406. doi:10.1016/j.stem.2013.03.005

Shyh-Chang N, Zhu H, de Soysa TY, Shinoda G, Seligson MT, Tsanov Kaloyan M, Nguyen L, Asara JM, Cantley LC, Daley GQ. 2013. Lin28 enhances tissue repair by reprogramming cellular metabolism. *Cell* **155:** 778–792. doi:10.1016/j.cell.2013.09.059

Simpson SB. 1961. Induction of limb regeneration in the lizard, *Lygosoma laterale*, by augmentation of the nerve supply. *Exp Biol Med* **107:** 108–111. doi:10.3181/00379727-107-26550

Singer M. 1952. The influence of the nerve in regeneration of the amphibian extremity. *Q Rev Biol* **27:** 169–200. doi:10.1086/398873

Singer M. 1954. Induction of regeneration of the forelimb of the postmetamorphic frog by augmentation of the nerve supply. *J Exp Zool* **126:** 419–471. doi:10.1002/jez.1401260304

Singer M. 1961. Induction of regeneration of body parts in the lizard, Anolis. *Exp Biol Med* **107:** 106–108. doi:10.3181/00379727-107-26549

Sîrbulescu RF, Zupanc GK. 2011. Spinal cord repair in regeneration-competent vertebrates: adult teleost fish as a model system. *Brain Res Rev* **67:** 73–93. doi:10.1016/j.brainresrev.2010.11.001

Smith SD. 1967. Induction of partial limb regeneration in *Rana pipiens* by galvanic stimulation. *Anat Rec* **158:** 89–97. doi:10.1002/ar.1091580110

Smith SD. 1974. Effects of electrode placement on stimulation of adult frog limb regeneration. *Ann NY Acad Sci* **238:** 500–507. doi:10.1111/j.1749-6632.1974.tb26816.x

Smith SD. 1981. The role of electrode position in the electrical induction of limb regeneration in subadult rats. *J Electroanal Chem Interfacial Electrochem* **128:** 661–670. doi:10.1016/S0022-0728(81)80265-3

Song B. 2004. Nerve regeneration and wound healing are stimulated and directed by an endogenous electrical field in vivo. *J Cell Sci* **117:** 4681–4690. doi:10.1242/jcs.01341

Steen TP, Thornton CS. 1963. Tissue interaction in amputated aneurogenic limbs of *Ambystoma* larvae. *J Exp Zool* **154:** 207–221. doi:10.1002/jez.1401540208

Stocum DL, Dearlove GE. 1972. Epidermal-mesodermal interaction during morphogenesis of the limb regeneration blastema in larval salamanders. *J Exp Zool* **181:** 49–61. doi:10.1002/jez.1401810106

Stump RF, Robinson KR. 1983. *Xenopus* neural crest cell migration in an applied electrical field. *J Cell Biol* **97:** 1226–1233. doi:10.1083/jcb.97.4.1226

Suckow MA, Voytik-Harbin SL, Terril LA, Badylak SF. 1999. Enhanced bone regeneration using porcine small intestinal submucosa. *J Invest Surg* **12:** 277–287. doi:10.1080/089419399272395

Sun X, Mariani FV, Martin GR. 2002. Functions of FGF signalling from the apical ectodermal ridge in limb development. *Nature* **418:** 501–508. doi:10.1038/nature00902

Sundelacruz S, Kaplan DL. 2009. Stem cell- and scaffold-based tissue engineering approaches to osteochondral regenerative medicine. *Semin Cell Dev Biol* **20:** 646–655. doi:10.1016/j.semcdb.2009.03.017

Sundelacruz S, Levin M, Kaplan DL. 2008. Membrane potential controls adipogenic and osteogenic differentiation of mesenchymal stem cells. *PLoS ONE* **3:** e3737. doi:10.1371/journal.pone.0003737

Sundelacruz S, Levin M, Kaplan DL. 2009. Role of membrane potential in the regulation of cell proliferation and differentiation. *Stem Cell Rev* **5:** 231–246. doi:10.1007/s12015-009-9080-2

Sundelacruz S, Levin M, Kaplan DL. 2013. Depolarization alters phenotype, maintains plasticity of predifferentiated mesenchymal stem cells. *Tissue Eng Part A* **19:** 1889–1908. doi:10.1089/ten.tea.2012.0425.rev

Sundelacruz S, Levin M, Kaplan DL. 2016. Comparison of the depolarization response of human mesenchymal stem cells from different donors. *Sci Rep* **5:** 18279. doi:10.1038/srep18279

Taghiyar L, Hesaraki M, Sayahpour FA, Satarian L, Hosseini S, Aghdami N, Baghaban Eslaminejad M. 2017. Msh homeobox 1 (Msx1)- and Msx2-overexpressing bone marrow-derived mesenchymal stem cells resemble blastema cells and enhance regeneration in mice. *J Biol Chem* **292:** 10520–10533. doi:10.1074/jbc.M116.774265

Takeo M, Chou WC, Sun Q, Lee W, Rabbani P, Loomis C, Taketo MM, Ito M. 2013. Wnt activation in nail epithelium couples nail growth to digit regeneration. *Nature* **499:** 228–232. doi:10.1038/nature12214

Tamai K, Semenov M, Kato Y, Spokony R, Liu C, Katsuyama Y, Hess F, Saint-Jeannet JP, He X. 2000. LDL-receptor-related proteins in Wnt signal transduction. *Nature* **407:** 530–535. doi:10.1038/35035117

Taylor GP, Anderson R, Reginelli AD, Muneoka K. 1994. FGF-2 induces regeneration of the chick limb bud. *Dev Biol* **163:** 282–284. doi:10.1006/dbio.1994.1144

Thornton CS. 1970. Amphibian limb regeneration and its relation to nerves. *Am Zool* **10:** 113–118. doi:10.1093/icb/10.2.113

Trampusch HAL. 1966. Regeneration from inter-ocular grafts. *Arch Zool Ital* **51:** 787–825.

Trent L, Intintoli M, Prigge P, Bollinger C, Walters LS, Conyers D, Miguelez J, Ryan T. 2020. A narrative review: current upper limb prosthetic options and design. *Disabil Rehabil Assist Technol* **15:** 604–613. doi:10.1080/17483107.2019.1594403

Tseng A, Levin M. 2013. Cracking the bioelectric code: probing endogenous ionic controls of pattern formation. *Commun Integr Biol* **6:** e22595. doi:10.4161/cib.22595

Tseng AS, Beane WS, Lemire JM, Masi A, Levin M. 2010. Induction of vertebrate regeneration by a transient sodium current. *J Neurosci* **30:** 13192–13200. doi:10.1523/JNEUROSCI.3315-10.2010

Turner NJ, Johnson SA, Badylak SF. 2010a. A histomorphologic study of the normal healing response following digit amputation in C57bl/6 and MRL/MpJ mice. *Arch Histol Cytol* **73:** 103–111. doi:10.1679/aohc.73.103

Turner NJ, Yates AJ, Weber DJ, Qureshi IR, Stolz DB, Gilbert TW, Badylak SF. 2010b. Xenogeneic extracellular matrix as an inductive scaffold for regeneration of a functioning musculotendinous junction. *Tissue Eng Part A* **16:** 3309–3317. doi:10.1089/ten.tea.2010.0169

Valentin JE, Turner NJ, Gilbert TW, Badylak SF. 2010. Functional skeletal muscle formation with a biologic scaffold. *Biomaterials* **31:** 7475–7484. doi:10.1016/j.biomaterials.2010.06.039

Vieira WA, Wells KM, Raymond MJ, De Souza L, Garcia E, McCusker CD. 2019. FGF, BMP, and RA signaling are sufficient for the induction of complete limb regeneration from non-regenerating wounds on *Ambystoma mexicanum* limbs. *Dev Biol* **451:** 146–157. doi:10.1016/j.ydbio.2019.04.008

Vieux-Rochas M, Bouhali K, Mantero S, Garaffo G, Provero P, Astigiano S, Barbieri O, Caratozzolo MF, Tullo A, Guerrini L, et al. 2013. BMP-mediated functional cooperation between Dlx5;Dlx6 and Msx1;Msx2 during mam-

Cold Spring Harbor Perspectives in Biology
www.cshperspectives.org

malian limb development. *PLoS ONE* **8:** e51700. doi:10.1371/journal.pone.0051700

Viswanathan SR, Daley GQ, Gregory RI. 2008. Selective blockade of MicroRNA processing by Lin28. *Science* **320:** 97–100. doi:10.1126/science.1154040

Vodovnik L, Miklavcic D, Sersa G. 1992. Modified cell proliferation due to electrical currents. *Med Biol Eng Comput* **30:** CE21–CE28.

Wanek N, Muneoka K, Bryant SV. 1989. Evidence for regulation following amputation and tissue grafting in the developing mouse limb. *J Exp Zool* **249:** 55–61. doi:10.1002/jez.1402490111

Watson ML, Rao JK, Gilkeson GS, Ruiz P, Eicher EM, Pisetsky DS, Matsuzawa A, Rochelle JM, Seldin MF. 1992. Genetic analysis of MRL-lpr mice: relationship of the Fas apoptosis gene to disease manifestations and renal disease-modifying loci. *J Exp Med* **176:** 1645–1656. doi:10.1084/jem.176.6.1645

Watson J, Gonzalez M, Romero A, Kerns J. 2010. Neuromas of the hand and upper extremity. *J Hand Surg Am* **35:** 499–510. doi:10.1016/j.jhsa.2009.12.019

Weiss P. 1927. Potenzprüfung am regenerationsblastem. I: Extremitäten-bildung aus schwanzblastern im extremitätenfeld bei triton. *Wilhelm Roux Arch Entwickl Mech Org* **111:** 317–340. doi:10.1007/BF02080952

Windrich M, Grimmer M, Christ O, Rinderknecht S, Beckerle P. 2016. Active lower limb prosthetics: a systematic review of design issues and solutions. *Biomed Eng Online* **15:** 140. doi:10.1186/s12938-016-0284-9

Wu Y, Wang K, Karapetyan A, Fernando WA, Simkin J, Han M, Rugg EL, Muneoka K. 2013. Connective tissue fibroblast properties are position-dependent during mouse digit tip regeneration. *PLoS ONE* **8:** e54764. doi:10.1371/journal.pone.0054764

Yakushiji N, Suzuki M, Satoh A, Ide H, Tamura K. 2009. Effects of activation of hedgehog signaling on patterning, growth, and differentiation in *Xenopus* froglet limb regeneration. *Dev Dyn* **238:** 1887–1896. doi:10.1002/dvdy.22011

Yan T, Jiang X, Guo X, Chen W, Tang D, Zhang J, Zhang X, Zhang D, Zhang Q, Jia J, et al. 2017. Electric field-induced suppression of PTEN drives epithelial-to-mesenchymal transition via mTORC1 activation. *J Dermatol Sci* **85:** 96–105. doi:10.1016/j.jdermsci.2016.11.007

Yang DH, Moss EG. 2003. Temporally regulated expression of Lin-28 in diverse tissues of the developing mouse. *Gene Expr Patterns* **3:** 719–726. doi:10.1016/S1567-133X(03)00140-6

Yao L, Pandit A, Yao S, McCaig CD. 2011. Electric field-guided neuron migration: a novel approach in neurogenesis. *Tissue Eng Part B* **17:** 143–153. doi:10.1089/ten.teb.2010.0561

Yates CC, Hebda P, Wells A. 2012. Skin wound healing and scarring: fetal wounds and regenerative restitution. *Birth Defects Res C Embryo Today* **96:** 325–333. doi:10.1002/bdrc.21024

Yntema CL. 1959. Regeneration in sparsely innervated and aneurogenic forelimbs of Amblystoma larvae. *J Exp Zool* **140:** 101–123. doi:10.1002/jez.1401400106

Yokoyama H, Endo T, Tamura K, Yajima H, Ide H. 1998. Multiple digit formation in *Xenopus* limb bud recombinants. *Dev Biol* **196:** 1–10. doi:10.1006/dbio.1998.8856

Young HE, Bailey CF, Dalley BK. 1983. Gross morphological analysis of limb regeneration in postmetamorphic adult *Ambystoma*. *Anat Rec* **206:** 295–306. doi:10.1002/ar.1092060308

Yu L, Han M, Yan M, Lee EC, Lee J, Muneoka K. 2010. BMP signaling induces digit regeneration in neonatal mice. *Development* **137:** 551–559. doi:10.1242/dev.042424

Yu L, Han M, Yan M, Lee J, Muneoka K. 2012. BMP2 induces segment-specific skeletal regeneration from digit and limb amputations by establishing a new endochondral ossification center. *Dev Biol* **372:** 263–273. doi:10.1016/j.ydbio.2012.09.021

Yu L, Dawson LA, Yan M, Zimmel K, Lin YL, Dolan CP, Han M, Muneoka K. 2019a. BMP9 stimulates joint regeneration at digit amputation wounds in mice. *Nat Commun* **10:** 424. doi:10.1038/s41467-018-08278-4

Yu Y, Cui H, Zhang D, Liang B, Chai Y, Wen G. 2019b. Human nail bed-derived decellularized scaffold regulates mesenchymal stem cells for nail plate regeneration. *J Tissue Eng Regen Med* **13:** 1770–1778. doi:10.1002/term.2927

Yucel T, Lovett ML, Kaplan DL. 2014. Silk-based biomaterials for sustained drug delivery. *J Control Release* **190:** 381–397. doi:10.1016/j.jconrel.2014.05.059

Zeller R, Haramis AG, Zuniga A, McGuigan C, Dono R, Davidson G, Chabanis S, Gibson T. 1999. Formin defines a large family of morphoregulatory genes and functions in establishment of the polarising region. *Cell Tissue Res* **296:** 85–93. doi:10.1007/s004410051269

Zhao M. 2009. Electrical fields in wound healing—an overriding signal that directs cell migration. *Semin Cell Dev Biol* **20:** 674–682. doi:10.1016/j.semcdb.2008.12.009

Zhao M, Song B, Pu J, Wada T, Reid B, Tai G, Wang F, Guo A, Walczysko P, Gu Y, et al. 2006. Electrical signals control wound healing through phosphatidylinositol-3-OH kinase-γ and PTEN. *Nature* **442:** 457–460. doi:10.1038/nature04925

Zhao H, Steiger A, Nohner M, Ye H. 2015. Specific intensity direct current (DC) electric field improves neural stem cell migration and enhances differentiation towards βIII-tubulin+ neurons. *PLoS ONE* **10:** e0129625. doi:10.1371/journal.pone.0129625

Zhao S, Mehta AS, Zhao M. 2020. Biomedical applications of electrical stimulation. *Cell Mol Life Sci* **77:** 2681–2699. doi:10.1007/s00018-019-03446-1

Ziegler-Graham K, MacKenzie EJ, Ephraim PL, Travison TG, Brookmeyer R. 2008. Estimating the prevalence of limb loss in the United States: 2005 to 2050. *Arch Phys Med Rehabil* **89:** 422–429. doi:10.1016/j.apmr.2007.11.005

Zupanc GK. 2008. Adult neurogenesis and neuronal regeneration in the brain of teleost fish. *J Physiol Paris* **102:** 357–373. doi:10.1016/j.jphysparis.2008.10.007

Cold Spring Harbor Perspectives in Biology

Inducing Vertebrate Limb Regeneration: A Review of Past Advances and Future Outlook

Devon Davidian and Michael Levin

Cold Spring Harb Perspect Biol 2022; doi: 10.1101/cshperspect.a040782 originally published online August 16, 2021