What Determines the Regenerative Capacity in Animals?

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The regenerative phenomenon is widespread, but regenerative capacity varies greatly across animals. Invertebrates and phylogenetically primitive vertebrates, such as salamanders and zebrafish, often possess a higher regenerative capacity than mammals have. Even in the same individual, different tissues or organs exhibit distinct regenerative capacity; for example, livers regenerate more readily than hearts in mammals. In addition, the younger animal is usually easier to regenerate than the older. Decades of research are beginning to yield explanations about why regenerative capacity differs markedly, based on cellular and molecular components and evolutionary ideas. Here, we discuss several reasons for differences in regenerative capacity, including the properties of stem cells, dedifferentiation and transdifferentiation potentials, expression of regeneration-associated genes, epigenetic regulators, and immune responses. Comprehensive analyses of these perspectives would provide new insights into how to promote regeneration in mammals.

Keywords: regeneration, stem cell, dedifferentiation, epigenetic, immune response

Regeneration occurs widely in the animal kingdom, although their regenerative capacity varies considerably. Invertebrates can regenerate the entire organisms (e.g., planarians and *Hydra*; Wittlieb et al. 2006, van Wolfswinkel et al. 2014). Phylogenetically primitive vertebrates, such as amphibians and fish, are capable of regenerating substantial parts of their body but not an entire organism. Urodele amphibians (salamanders) have a remarkable capability to regenerate a wide array of tissues and organs, including limbs, tails, jaws, spinal cords, and lenses. Similar to salamanders, teleost fish (zebrafish) can regrow hearts, fins, lenses, retinas, spinal cords, and so forth. By contrast, mammals have a very limited regenerative capacity. Severe damage to tissues or organs (e.g., hearts, limbs, or spinal cords) does not induce regenerative responses but rather a simple healing concomitant with fibrotic scarring. These collectively indicate that the capacity to regenerate generally decreases during evolutionary development. Moreover, regenerative capacity has a tendency to decline during ontogenic development or with age. Two classic examples are the transition from the fetal scarless wound healing to the typical adult scarring repair in mammals (Larson et al. 2010) and the gradual loss of limb regeneration from the larval stage to the adult in anuran amphibians (e.g., frogs; Mescher and Neff 2005). In the same individual, different tissues or organs display diverse degrees of regeneration: Livers regenerate more readily than hearts in mammals (Fausto et al. 2006, Porrello et al. 2011). Regenerative capacity is of great interest to scientists; why regenerative capacity differs across animals and tissues has been under continual investigation for several decades.

Given that cells are the foundation of regeneration, the availability of abundant cellular sources will inevitably determine the regenerative capacity. There are generally three mechanisms of yielding new cells *in vivo*, including the activation of stem or progenitor cells, the reversion of differentiated cells to their progenitors, and the conversion of one tissue cell into another (Jopling et al. 2011). The latter two are called *dedifferentiation* and *transdifferentiation*. Regenerative species either keep amounts of adult stem cells in their body or have a huge potential to undergo dedifferentiation and transdifferentiation in their adult cells. However, the three mechanisms are lacking in most adult mammals, which largely limits their regenerative capacity. The selected expression or silencing of regeneration-associated genes also affects regeneration. These genes might be possessed or expressed exclusively in regenerative species but not in nonregenerative species. In recent years, accumulating evidence strongly suggests that epigenetic regulators exert enormous influence on regeneration by modulating various aspects of regeneration processes (Cho et al. 2013, Gornikiewicz et al. 2013, Powell et al. 2013). Moreover, there has been a growing appreciation of contributions of the immune system to regeneration (Mescher and Neff 2005, Aurora and Olson 2014). The inflammatory microenvironment mediated by the immune response is essential for cell survival, growth, and function. Proper immune responses create a regeneration-permissive microenvironment, whereas

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aberrant immune responses cause a detrimental, inflammatory microenvironment that impedes regeneration.

Here, in light of recent literature, we first describe several well-studied regeneration models with emphasis on the cellular origins of regeneration. Then, we discuss the reasons for the differences in the regenerative capacity of animals at many levels, including the cellular mechanism, gene expression, epigenetic regulation, and immune response. We bring together common elements affecting regeneration and compare their contributions to regeneration. Our aim is to provide new insights into how to promote regeneration in mammals.

Regeneration models in invertebrates and the cellular basis

Planarians are tiny flatworms with the ability to regrow the entire organisms (figure 1a). This capacity depends on the abundant reserve of adult stem cells (throughout their bodies; neoblasts are small (5–8 μm in diameter), highly undifferentiated cells, and by morphology, they represent approximately 25%–30% of all planarian cells. In response to injury, neoblasts accumulate to form a regeneration blastema and then convert into any cell type required for regeneration. This pluripotency of neoblasts is similar to that of embryonic stem cells in mammals. Accordingly, neoblasts were long thought to be a homogeneous population of adult pluripotent stem cells. Consistently, Wagner and colleagues (2011) identified a subpopulation of neoblasts that can form large descendant-cell colonies and give birth to any cell type within the body. The subpopulation is described as clonogenic neoblasts *(cNeoblasts)*. Transplantation of a single cNeoblast could rescue the regeneration in irradiated planarians, suggesting cNeoblasts have the potential to regenerate a whole body. Based on multidimensional single-cell transcriptional profiling, however, a recent study has demonstrated that neoblasts are indeed heterogeneous, consisting of the pluripotent subpopulation (sigma-neoblasts) and the lineage-restricted progenitor subpopulation (zeta-neoblasts) (van Wolfswinkel et al. 2014). Zeta-neoblasts, as committed progenitor cells, can only yield postmitotic lineages, including epidermal cells, but they do not contribute to regeneration. Sigma-neoblasts are able to differentiate into any cell type (including zeta-neoblasts) and mainly responsible for regeneration. In addition, the cNeoblasts are likely contained in the sigma-neoblasts (van Wolfswinkel et al. 2014). Therefore, neoblasts are a mixed mass comprising pluripotent stem cells and lineage-restricted progenitor cells, although regeneration primarily depends on the pluripotent subpopulation.

Hydra is a member of the animal phylum Cnidaria, living in freshwater. The animal has a polarized, primary body axis and has two epithelial cell layers. Like planarians, *Hydra* exhibits strikingly high regeneration: When it is cut in half, the top half regenerates a foot, and the bottom half regenerates a head (figure 1b). Although *Hydra* is devoid of pluripotent stem cells, it has three stem cell types (ectodermal and endodermal epithelial stem cells and interstitial stem cells) throughout the body (figure 2b). The epithelial stem cells contribute to the regeneration of the epidermal layers (Wittlieb et al. 2006), and the interstitial stem cells contribute to the regeneration of the other tissues (Hemmrich et al. 2012). The interstitial stem cells have the multipotent potential to give rise to all other cells except epithelial cells, including neurons, nematocytes, secretory cells, and gametes. Therefore, three stem cell types together produce all cell types within the *Hydra* body, probably as the major reason for the unprecedented regenerative capacity.

Regeneration models in primitive vertebrates and the cellular basis

Urodele amphibians (salamanders) do not generate the entire body, but they can regrow substantial parts. Their limb regeneration is a well-characterized model for the regeneration of complex tissues. When amputated anywhere along the limb axis, salamanders completely regenerate the missing segments (figure 1c). Using lineage-tracing tools, Kragl and colleagues (2009) designed an elegant transplant experiment labeling limb tissue in axolotls (one kind of salamanders). They determined that the progenitors localized in the each tissue of limbs migrate to form the blastema (Kragl et al. 2009). In effect, blastema formation does not involve the conversion of one tissue cell to other tissue cells. This study has concluded that blastema is a heterogeneous pool of distinct lineage-restricted progenitor cells from its original limb tissue.

Are these lineage-restricted progenitor cells derived from the dedifferentiation of mature cells or from the activation of resident stem cells? Although the dominant view is that blastema originates from dedifferentiation, both mechanisms are actually implicated in blastema formation, depending on the species and tissues. For limb muscle regeneration in newts, Sandoval-Guzman and colleagues (2014) demonstrated that multinucleate myofibers fragment into proliferating, paired-box protein-7 (Pax7, a marker for muscle stem cells)-negative mononucleate cells in the blastema. Subsequently, these dedifferentiated cells generate limb muscles on the basis of genetic-fate mapping (Sandoval-Guzman et al. 2014). More recently, studies have shown that programmed cell death induces myofibers to dedifferentiate into muscle progenitor cells in newt limbs (Wang H et al. 2015). Do muscle stem cells contribute to blastema or not? The muscle stem cells, namely the Pax7-positive satellite cells, are indeed contained in the newt limb and activated to incorporate into the blastema after limb amputation (Morrison et al. 2006). Nevertheless, muscle stem cells seem to contribute rarely to muscle regeneration in newts, because Pax7-positive satellite cells are deficient in the blastema, except at the very early stage of blastema formation (Sandoval-Guzman et al. 2014). To the contrary, limb muscles in axolotls regenerate from activation of muscle satellite cells rather than from muscle dedifferentiation (Sandoval-Guzman et al. 2014). For that reason, an evolutionary diversity exists in the limb muscle

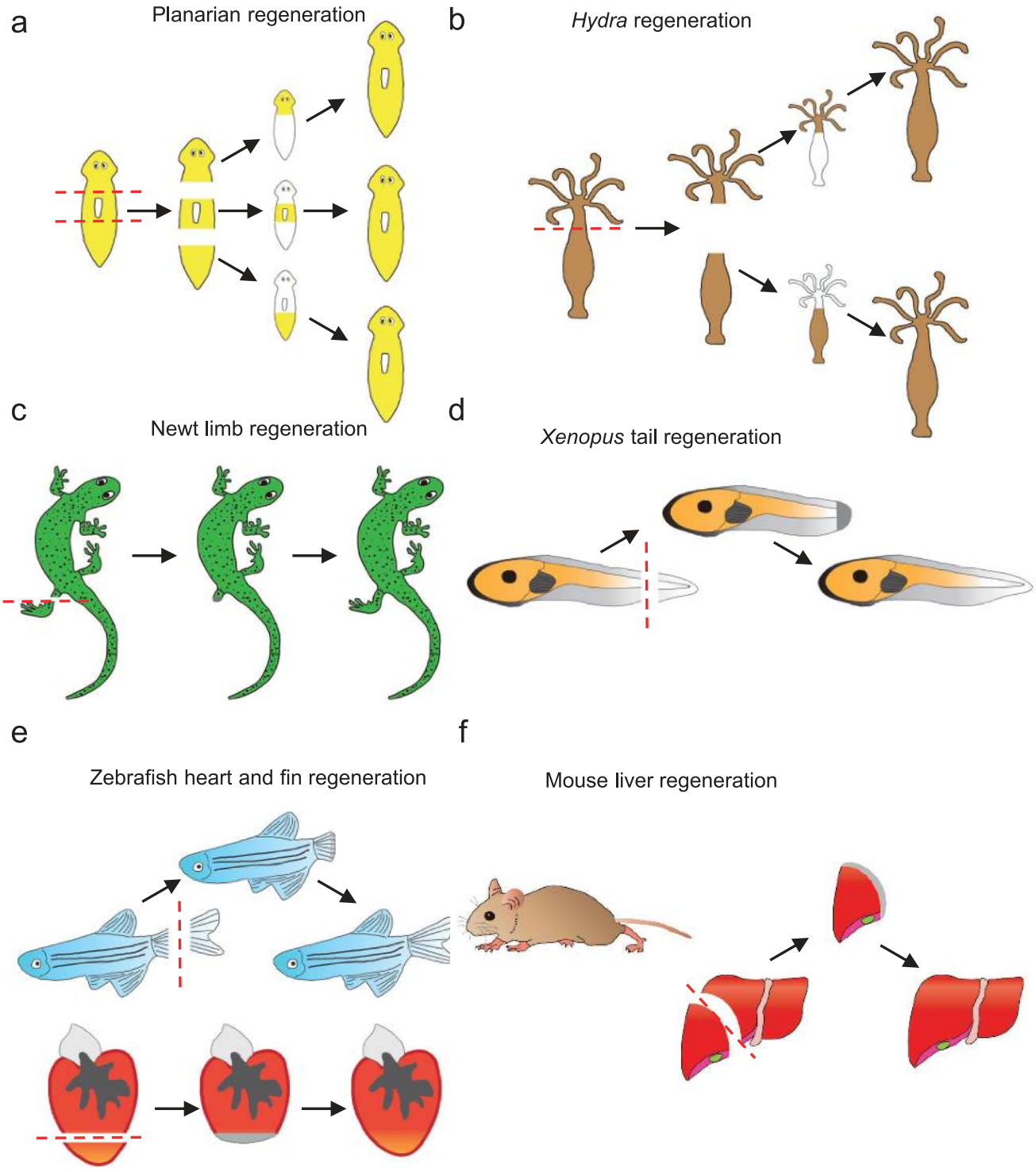

Figure 1. Schematics of animal regeneration models. (a and b) Planarians and* Hydra *have the highest regenerative capacity to regenerate the whole body. (c, d, and e) Lower or primitive vertebrates, such as newt,* Xenopus*, and zebrafish, can regrow lost parts, such as the limb, tail, fin, or heart. (f) Mouse regenerates liver. The red dashed line indicates amputation.

regeneration within the salamander species. As for other limb tissues, it is still not clear whether they each offer progenitor cells to the blastema through the dedifferentiation of stem cells, the activation of stem cells, or both during regeneration.

The anuran amphibian, *Xenopus laevis*, can regenerate its tail from the larval life to metamorphosis (figure 1d). After amputation, the injured tail regrows its lost part from the tail regeneration bud. The tail regeneration bud does not have the typical appearance of the limb blastema seen in

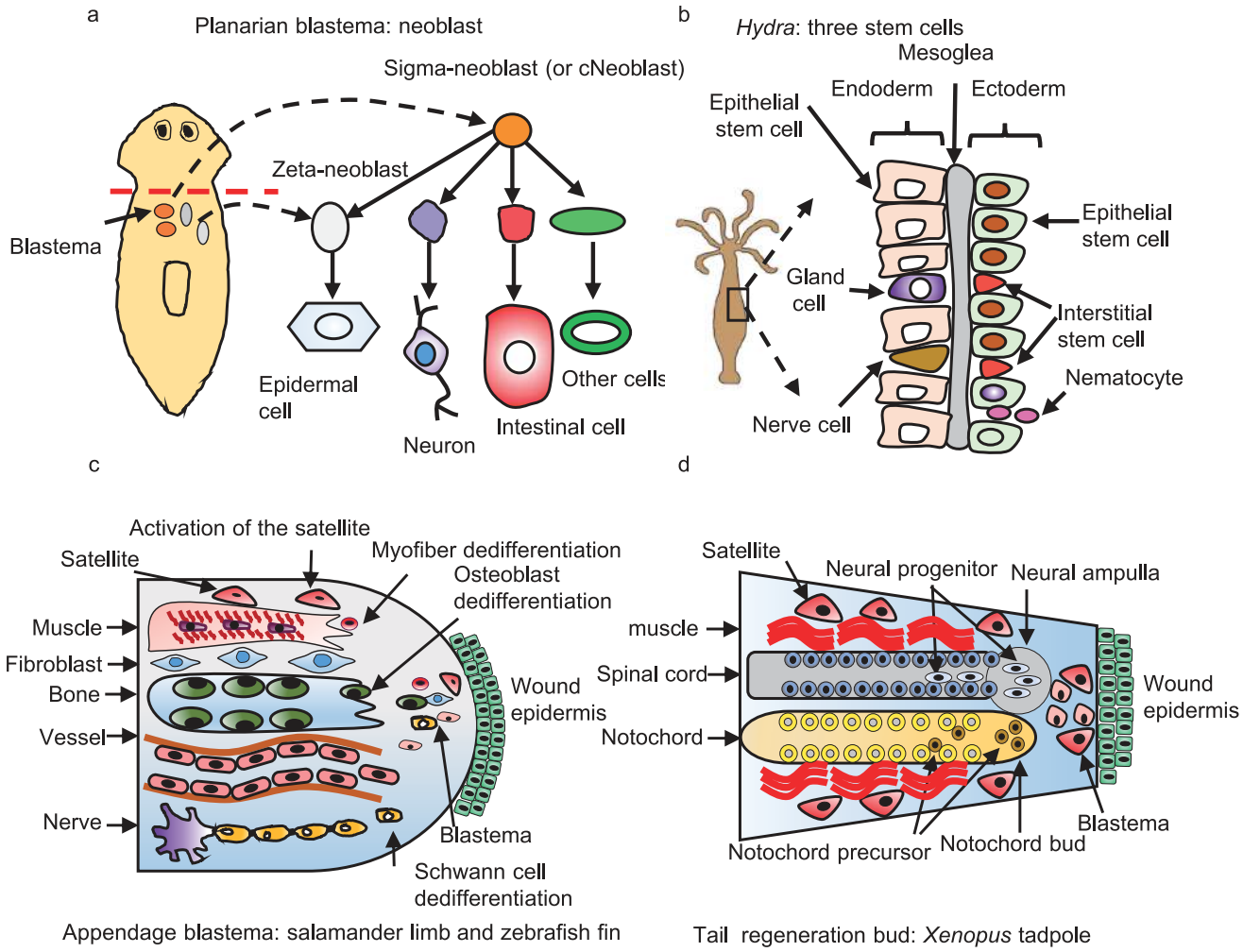

Figure 2. The cellular origins of regeneration in animal models. (a) Planarians' neoblasts consist of the pluripotent class (sigma-neoblast or cNeoblast) and the lineage-restricted progenitor class (zeta-neoblast). (b)* Hydra *regeneration involves three stem cells (endoderm and ectoderm epithelial cells as well as interstitial stem cells). (c) Vertebrate appendages, such as salamander limb and zebrafish fin, regrow similarly from the regeneration blastema. (d)* Xenopus *tadpole tail regrows from the regeneration bud containing neural ampulla, notochord bud, and blastema.

salamanders, including a notochord bud, neural ampulla, and blastema (figure 2d). Cell-lineage analyses have proven that lineage-restricted progenitor cells localized in the spinal cord, notochord, and muscle are activated and then migrate to form the three components of the regeneration bud, respectively (Gargioli and Slack 2004). These results are in line with the finding that there is no myofiber fragmentation during *Xenopus* tadpole tail regeneration (Rodrigues et al. 2012). In this regard, the activation of resident progenitor cells is the principal mechanism for tail regeneration in *Xenopus laevis*.

The teleost fish zebrafish is a versatile model system for studying regeneration because it can rebuild many tissues or organs, such as fins and hearts (figure 1e). Zebrafish fin is a complex appendage with bony fin rays, mesenchymal cells, nerve fibers, and vessels. Following amputation, zebrafish reconstructs its fin from the blastema (figure 2c). Cell-tracing studies have demonstrated that the lineage-restricted progenitor cells residing in each fin tissue migrate to form the blastema at the amputation plane (Tu and Johnson 2011), as has been seen in the salamander limb. Fin blastema formation seems to entail both dedifferentiation and activation of stem cells. For bone regeneration in the fin, differentiated osteoblasts undergo temporary dedifferentiation, migrate to the fin blastema, and then redifferentiate into osteoblasts (Knopf et al. 2011). With regard to the muscle component of the fin, however, it regrows from the activation of muscle stem cells, and no muscle dedifferentiation is observed after fin amputation (Rodrigues et al. 2012). Still, there is little knowledge of whether the other fin tissues provide lineage-restricted progenitor cells to the blastema by the dedifferentiation or activation of stem cells.

Zebrafish manifest a robust natural capacity for heart regeneration. After the surgical removal of approximately 20% of the ventricle, zebrafish heart regenerates completely without scarring (figure 1e; Jopling et al. 2010). Genetic-fate mapping experiments have consistently uncovered that dedifferentiation of pre-existing cardiomyocytes is the primary cellular mechanism for zebrafish heart regeneration (Jopling et al. 2010, Kikuchi et al. 2010). Following amputation, cardiomyocytes near the injury site display characteristics of dedifferentiation, such as reduced levels of sarcomeric contractile proteins and activation of the developmental transcription factor GATA-binding protein 4 (Gata4; Jopling et al. 2010, Kikuchi et al. 2010). In the heart regeneration model induced by genetic ablation of the ventricular cardiomyocytes, dedifferentiation of ventricular cardiomyocytes contributes to heart regeneration (Wang J et al. 2011). It is interesting to note that, in another ventricle-specific genetic ablation model, transdifferentiation of atrial cardiomyocytes to ventricular cardiomyocytes is involved in the regeneration of zebrafish ventricles (Zhang et al. 2013). It is likely that different types of injuries to the zebrafish heart might incite a diverse set of cellular regenerative responses.

Regeneration models in mammals and the cellular basis

Regenerative responses are quite limited in mammals. Although most of the mammalian tissues or organs rarely regenerate, some do display regeneration. The skin can continually renew and replace sloughed-off cells with basal layer cells during normal homeostasis or after injury (Plikus et al. 2012), and peripheral nerves can regrow the axon after injury (Scheib and Hoke 2013). In particular, the liver has a unique ability to regenerate—the prototype for mammalian organ regeneration. After removal of approximately 70% of the rodent liver, a partial hepatectomy (PHx) model, the remnant liver regenerates the entire liver within one week (figure 1f). The remnant liver does not essentially generate the lost lobes but simply increases in size until the liver has reached its original mass (i.e., "compensatory" regrowth). The hepatocyte proliferation has long been as the principal contributor to liver regeneration under this condition (Fausto et al. 2006). In addition to hepatocyte proliferation, one recent study demonstrates that hepatocyte hypertrophy makes equal contributions to liver regeneration after 70% PHx (Miyaoka et al. 2012). During the liver regeneration, most hepatocytes re-enter the cell cycle, but many of them do not divide and only enlarge their sizes. Quantitative data demonstrate that the number of hepatocytes increases by 1.6-fold during liver regeneration and that the total hepatocyte volume actually increases by approximately 1.5-fold because of hepatocyte enlargement. These results suggest that hepatocyte proliferation and hypertrophy equally contribute to liver regeneration following PHx.

Unlike PHx, which does not destroy the remnant liver, chronic liver diseases (e.g., chronic viral hepatitis, alcoholic liver disease, and nonalcoholic fatty liver disease) involve extensive hepatocyte death, inflammation, and fibrosis. Under these conditions, liver regeneration depends on the activation of liver progenitor cells (LPCs) rather than on the direct proliferation of mature hepatocytes (Itoh and Miyajima 2014). The disease-activated LPCs are "facultative" progenitor cells, which emerge only under damaged conditions, with a bilineage differentiation potential to generate hepatocytes and cholangiocytes. The canal of Hering is widely recognized as the origin for LPCs, although it is not formally proven (Itoh and Miyajima 2014). Several cell types have recently been proposed as possible candidates for the origins of LPCs. By using cholangiocyte-specific Cre driver strains, Espanol-Suner and colleagues (2012) demonstrated that cholangiocytes can produce LPCs after liver injury. In addition, mature hepatocytes are reported to turn into LPCs after certain liver injuries (Yanger et al. 2013), and hepatic stellate cells can act as LPCs to produce hepatocytes and contribute to liver regeneration (Kordes et al. 2014). Although distinct cellular origins of LPCs are proposed, their relative contributions to liver regeneration remain largely unknown. Moreover, it is unclear whether most of the cholangiocytes, mature hepatocytes, or hepatic stellate cells are competent to become LPCs or whether there is a special subpopulation of precursors for LPCs within them.

The underlying reasons for differences in regenerative capacity

The animal kingdom exhibits varying degrees of regeneration. Even in the same individual, some tissues manifest high regeneration, whereas other tissues manifest no regeneration. Often, younger tissues have higher regenerative capacity than older tissues have. What is accountable for such marked differences in regeneration capacity?

Stem or progenitor cells

The activation of stem/progenitor cells is the most popular way to generate new cells; it is reasonable to assume that the abundance of stem cells, to some extent, reflects the regenerative capacity. As we mentioned above, planarians, *Hydra*, and *Xenopus laevis* possess a large number of pluripotent, multipotent, or unipotent stem cells. Not surprisingly, they all have the high regenerative capacity. In adult mammals, a small number of tissue-specific stem cells are preferentially preserved in certain high-turnover tissues. For example, human skin and blood systems have the capacity to regenerate, which is largely because of the reserve of epidermal stem cells in the skin and hemopoietic stem cells in the bone marrow. Unfortunately, most of the adult mammalian tissues have few or no resident stem cells to support regeneration. This is likely one of the major limiting factors to regeneration. If, however, the small number of resident stem cells in the adult mammalian tissues could be stimulated and recruited, it is possible to promote regeneration. For instance, the mouse can achieve digit tip regeneration by stimulating the few distinct lineage-restricted progenitor

cells and forming blastema-like structure (Rinkevich et al. 2011). From this point, it is of great interest to illustrate the mechanisms by which stem or progenitor cells are activated *in vivo*. Moreover, many efforts should be done to decipher why invertebrates can sustain numerous stem cells for a lifetime and why mammals cannot. To summarize, animals or tissues with more stem cells generally possess higher regenerative capacity.

Dedifferentiation potential

Primitive vertebrates such as salamanders and zebrafish still regenerate substantial parts of their body, even without the presence of numerous stem cells. That is because they can produce new cells easily via dedifferentiation. Following injury, adult zebrafish cardiomyocytes rapidly dedifferentiate and re-enter the cell cycle to regenerate lost cardiomyocytes (Jopling et al. 2010, Kikuchi et al. 2010). Compared with zebrafish cardiomyocytes, adult human cardiomyocytes retain a limited ability to enter the cell cycle: A very low level (0.0006% to 1%) of constant cardiomyocyte turnover rate occurs throughout life (Senyo et al. 2013). It follows that the higher regeneration in zebrafish hearts is attributed to stronger dedifferentiation potentials in cardiomyocytes. Appendage regeneration is another example that stresses the importance of dedifferentiation. With fin amputation, zebrafish osteoblasts dedifferentiate, regain proliferative capacity, and regenerate bones (Knopf et al. 2011). Mammals fail to regenerate bones after the amputation of their bones, although internal bone defects can be healed below a critical size. In mammals, *de novo* osteoblasts deriving from mesenchymal stem cells contribute to the bone-healing process, without the occurrence of osteoblast dedifferentiation (Park et al. 2012). The scarcity of dedifferentiation in mammalian osteoblasts may be the underlying reason for low bone regeneration. Retina regeneration in zebrafish, chick, and mammals all depends on dedifferentiation of Müller glia, although mammalian retina has much lower regenerative capacity than that of zebrafish and chick. This is probably ascribed to lower dedifferentiation potentials in Müller glia in mammals than in zebrafish and chick (Goldman 2014). Although most tissue cells in mammals lose dedifferentiation potential, several tissue cells retain this ability. For instance, Schwann cells undergo dedifferentiation to engage in peripheral nerve regeneration (Scheib and Hoke 2013). Consistent with this idea, diminished dedifferentiation potential in aged Schwann cells impairs nerve regeneration in older bodies (Painter et al. 2014). Likewise, the dedifferentiation of renal proximal tubular epithelial cells contributes to kidney regeneration after acute kidney injury (Kusaba et al. 2014). Ischemic or toxic injury to kidney often results in the extensive death of proximal tubular epithelial cells, whereas the neighboring surviving cells dedifferentiate and proliferate. Consequently, dedifferentiated cells regenerate the lost cells and restore the integrity of nephrons. Thus, the potential to dedifferentiate will have a major impact on regeneration capability.

Although mammalian cells are hard to take natural dedifferentiation after injury, dedifferentiation can be induced *in vitro*. Mouse myotubes are induced to dedifferentiate and proliferate after treatment with extracts from regenerating limbs of newts (McGann et al. 2001) or after ectopic expression of the transcription factor, msh homeobox 1 (Msx1; Odelberg et al. 2000). These indicate that mammalian cells (like myotubes) remain the potential to dedifferentiate, although the potential needs to be stimulated.

Why do the cells of primitive vertebrates undergo dedifferentiation more easily than mammalian cells? Although the specific mechanisms are not clearly understood, cell-cycle regulators are found to play an essential role in controlling dedifferentiation. Terminally differentiated newt myotubes can dedifferentiate after injury because tumor suppressor retinoblastoma (Rb) proteins are phosphorylated, thereby allowing cells to re-enter the cell cycle (Tanaka et al. 1997). However, mammalian myotubes do not phosphorylate Rb proteins after injury and therefore fail to re-enter the cell cycle (Pajcini et al. 2010). This suggests that Rb phosphorylation may be a crucial barrier for muscle dedifferentiation in mammals. In support of this, transient inactivation of Rb and the alternative reading frame (ARF) tumor suppressor forces mammalian myotubes to re-enter the cell cycle and to lose differentiation properties (Pajcini et al. 2010). In addition, Rb and another Rb family member p130 can block cell-cycle genes and maintain the postmitotic state of mammalian adult cardiomyocytes; knockdown of Rb and p130 leads to the cell-cycle re-entry of adult cardiomyocytes (Sdek et al. 2011). As a cell-cycle inhibitor, the p53 tumor suppressor also hinders dedifferentiation. During salamander limb regeneration, an early down-regulation of p53 is a prerequisite for mesenchymal cell dedifferentiation and blastema formation (Yun et al. 2013). Because cell-cycle inhibitors block dedifferentiation in mammalian cells, targeted modification of these inhibitors is likely to promote dedifferentiation and regeneration. In addition to cell-cycle regulators, epigenetic regulators strictly control cellular differentiation and maintain the differentiated state, as potent barriers for dedifferentiation (Chen and Dent 2014). Targeting the epigenetic regulators has been applied to facilitate dedifferentiation. For example, the forced expression of transcription factors or treatment with small molecules changes the epigenetic regulators such as DNA methylation and histone modification, resulting in the complete dedifferentiation of somatic cells into pluripotent stem cells (Xu et al. 2015). Accordingly, the dedifferentiation potential may be enhanced artificially by targeting cell-cycle regulators or epigenetic regulators.

Transdifferentiation potential

Some animals and tissues regenerate highly by virtue of considerable transdifferentiation potentials. For example, newts and frogs can completely regenerate their lenses via cellular transdifferentiation. In newts, once the lens is removed, pigmented epithelial cells from the dorsal iris transdifferentiate into lens cells and regrow the entire lens

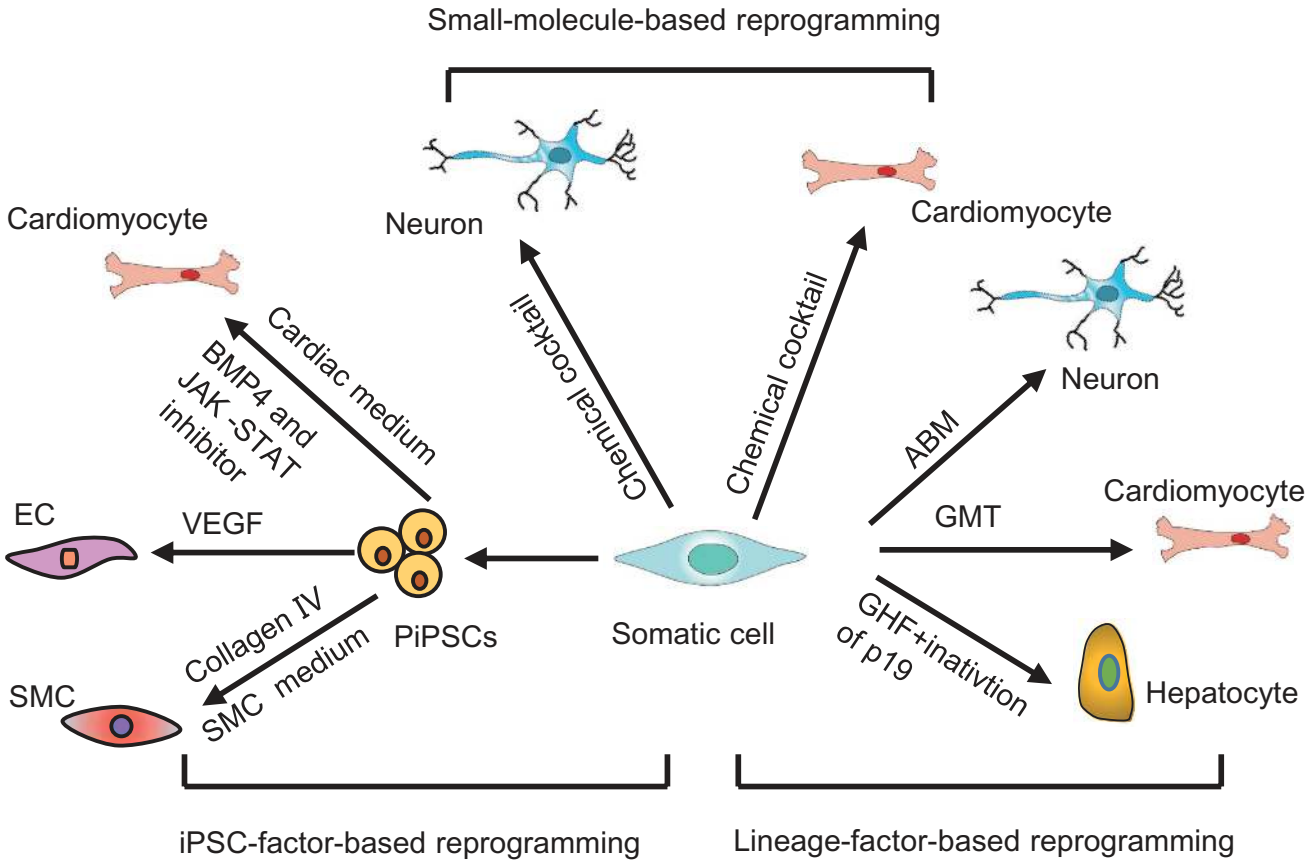

Figure 3. Direct reprogramming approaches. Somatic cells can be directly reprogrammed to another one by several reprogramming approaches (for a review, see Xu et al. 2015). The lineage factor–based reprogramming is mediated by the ectopic expression of lineage transcription factors, confirmed in the generation of neurons, cardiomyocytes, and hepatocytes. The iPSC factor–based reprogramming is to shortcut iPSC programming at the early stage and redirect cell fate by growth factors and chemical compounds, such as the generation of cardiomyocytes, endothelial cells (EC), and smooth muscle cells (SMC). Only a chemical cocktail of small-molecule compounds can reprogram somatic cells to neurons and cardiomyocytes, namely small molecule–based reprogramming. Abbreviations: Ascl1, Brn2, and Mytl1A, ABM; Gata4, Mef2C, and Tbx5, GMT; Gata4, Hnf1α, and Foxa3, GHF; partially reprogrammed iPSCs, PiPSCs; bone morphogenetic protein 4, BMP4; vascular endothelial growth factor, VEGF; Janus Kinase-signal transducer and activator of transcription, JAK-STAT.

(Barbosa-Sabanero et al. 2012). In the same manner, the frog lens can regenerate through the transdifferentiation of the corneal epithelium into lens cells during the larval stage (Barbosa-Sabanero et al. 2012). In contrast, the mammalian lens only has the ability to achieve incomplete regeneration from the lens's own epithelial cells (Gwon 2006), without transdifferentiation of other cells. Therefore, the loss of natural transdifferentiation in mammals appears to impede complete lens regeneration. Nonetheless, mammalian cells retain the transdifferentiation potential, which has to be incited by exogenous stimuli. The latent transdifferentiation in mammals is extensively confirmed by recent reprogramming strategies. Somatic cells, such as fibroblasts, can be induced into another lineage (e.g., neurons, cardiomyocytes, and hepatocytes) by several reprogramming approaches, including lineage factor–based reprogramming, induced pluripotent stem cell (iPSC) factor–based reprogramming, and small molecule–based reprogramming (figure 3; Xu et al. 2015). These reprogramming approaches (especially, small molecules–mediated reprogramming) will offer meaningful opportunities that allow deliberate transdifferentiation of one cell type to another cell type of interest *in vitro* and *in vivo*. Accordingly, artificially harnessing the transdifferentiation potential in mammals is a promising approach to promote regeneration.

Regeneration genes

Almost all the animals can heal wounds, but only some can regenerate. One hypothesis is that certain regeneration-specific genes are expressed exclusively in regenerative species and evolutionarily lost in nonregenerative species. One salamander-specific gene, Prod1, which encodes the

glycosylphosphatidylinositol-anchored protein, is found to support this hypothesis (Garza-Garcia et al. 2010). Prod1 is expressed in the blastema and essential for patterning and growth during the salamander limb regeneration. More importantly, no orthologue to Prod1 has been identified in *Xenopus*, zebrafish, and mammals (Garza-Garcia et al. 2010). Therefore, the regeneration gene is specifically possessed by salamanders. Another possibility is that nonregenerative and regenerative species both carry certain regeneration genes, but these genes promote regeneration only in regenerative species. This possibility is verified by the specific expression of the growth factor Fgf20a in zebrafish (Whitehead et al. 2005). Fgf20a is expressed early after fin amputation and initiates fin regeneration. By contrast, the orthologue of Fgf20a in mammals is not associated with regeneration. It is worth mentioning that selective expression of regeneration genes affects the regenerative capacity of different tissues even in the same animal. Newt lens can regenerate from pigmented epithelial cells of the dorsal iris but not from the ventral iris. One study showed that this situation is due to deficiency of one lens-specific regeneration gene, sine oculus-related homeobox 3 (Six3), in the ventral iris (Grogg et al. 2005). Six3 is required for lens development during embryogenesis but is only expressed in the dorsal iris after removal of newt lens, not in the ventral iris. However, when the ventral iris is transfected with Six3, it generates lens. Altogether, certain species and tissues with high regeneration have special regeneration genes, and these genes are induced upon injury.

Epigenetic regulators

The eukaryotic genome is packaged into chromatin consisting of DNA, histones, and nonhistone proteins. The chromatin structure has profound effects on gene expression, because it regulates the accessibility of transcription factors and transcriptional machinery to their target DNA. Chromatin can be remodeled as loose chromatin (euchromatin) or dense chromatin (heterochromatin) via epigenetic regulators, including DNA methylation, post-translational modifications of histones (e.g., acetylation and methylation), and ATP-dependent chromatin remodeling. Dynamic changes in chromatin states result in the increased or decreased expression of genes. In recent years, much progress has been made in the roles of DNA methylation and histone modifications in regulating regenerative capacity.

DNA methylation is the most studied epigenetic regulator, including cytosine methylation (5mC) and cytosine hydroxymethylation (5hmC). In general, high levels of DNA methylation repress gene expression and low levels of DNA methylation promote gene expression. The low DNA methylation pattern in the genome appears to closely associate with the regenerative capacity. In planarians, the levels of 5mC are undetectable in the genome and functional DNA methyltransferases are absent (Jaber-Hijazi et al. 2013). It is conceivable that global absence of DNA methylation may partly account for the pluripotency of planarian neoblasts and the remarkable regeneration ability of the taxon. Similarly, low DNA methylation is observed in the MRL/MpJ mouse model, which exhibits an enhanced regenerative response in a variety of organs, including livers, ears, and hair follicles (Gornikiewicz et al. 2013). The genome-wide DNA methylation profile of the adult MRL/MpJ mouse contains some features similar to that of its embryo or newborn. Moreover, some genes related to embryonic morphogenesis, such as EPH receptor A2 (Epha2), paired box gene 2 (Pax2), and GATA zinc finger domain containing 2A (Gatad2a), are hypomethylated and highly overexpressed in the adult MRL/MpJ mouse. Therefore, embryonic features of the genomic DNA methylation might be one important epigenetic mechanism underlying the enhanced regenerative capacity observed in the MRL/MpJ mouse. At the cellular level, DNA methylation status correlates with dedifferentiation potential. Zebrafish retina regeneration entails dedifferentiation of Müller glia into progenitor cells following a retinal injury. During the dedifferentiation process, DNA methylation pattern changes a lot, with a predominant early demethylation and a later *de novo* methylation. The early reduction of DNA methylation is required for Müller glia dedifferentiation (Powell et al. 2013). In addition, the promoters of pluripotency- and regeneration-associated genes are already hypomethylated in quiescent Müller glia before injury stimuli, and these genes are highly expressed at early stages after injury. The preexisting hypomethylation status in quiescent Müller glia suggests that pluripotency- and regeneration-associated genes are poised for activation in response to injury. Accordingly, low DNA methylation may contribute to the high dedifferentiation potential in zebrafish Müller glia. Similarly, the dedifferentiation of mature cells in the zebrafish fin into progenitor cells is accompanied by an early reduction of DNA methylation (Hirose et al. 2013). Moreover, differences in DNA methylation determine the expression of key regeneration-associated genes and largely affect regeneration. *Xenopus* tadpoles can regenerate a full limb after amputation, whereas *Xenopus* froglets (young frogs) can form only a simple cartilaginous spike structure after amputation. Although they both can form a blastema upon amputation, the froglet blastema fails to regenerate. The deficient expression of the regeneration-associated sonic hedgehog (Shh) gene in *Xenopus* froglets leads to loss of regeneration. The enhancer region of the Shh gene is highly methylated in the *Xenopus* froglet blastema and thereby silenced, but it is hypomethylated in the *Xenopus* tadpole blastema as well as in the salamander blastema (Yakushiji et al. 2007). This study points out that different DNA methylation levels surrounding regeneration-associated genes may affect regenerative capacity.

Histones (H2A, H2B, H3, and H4) can be modified by post-translational modifications, such as acetylation and methylation. Histone modifications have turned out to maintain stem cell pluripotency in planarian neoblasts and impact regeneration. The planarian homologs of the SET1/MLL family of H3K4me3 methyltransferases are expressed in pluripotent neoblasts. They promote the expression of genes

associated with the maintenance of stem cells by increasing transcriptional active H3K4me3 on the promoters (Hubert et al. 2013, Duncan et al. 2015). Planarian *Schmidtea mediterranea* histone deacetylase 1 (Smed-HDAC-1) that is specifically expressed in neoblasts also maintains the stem property of neoblasts (Eisenhoffer et al. 2008). Depletion of the SET/MLL or the Smed-HDAC-1 leads to the loss of planarian regeneration. Histone modifications have quite an impact on zebrafish fin regeneration. Normally, zebrafish fin developmental genes are silenced by bivalent H3K4me3/H3K27me3 histone marks in adult zebrafish; during the regeneration process, the repressive H3K27me3 mark is removed by H3K27me3 demethylases. As a result, the silent bivalent histone modifications convert to active states, derepressing those developmental genes (Stewart et al. 2009). In addition, histone deacetylase HDAC1 is detected in the fin blastema, and its knockdown impairs fin regeneration, possibly through reducing blastema proliferation and its later redifferentiation (Pfefferli et al. 2014).

Injured neurons in the peripheral nervous system (PNS) can successfully regenerate axons, whereas neurons within the central nervous system (CNS) typically fail to regenerate axons after injury. Adult dorsal root ganglion (DRG) neurons after the peripheral axotomy show increased active H4 acetylation surrounding the axon-regeneration genes, leading to their expression. However, DRG neurons after a central lesion fail to increase H4 acetylation, accompanied by no expression of those genes; when H4 acetylation is increased by administration of an HDAC inhibitor in the mouse model of spinal cord injury, axon regeneration is significantly improved (Finelli et al. 2013). Furthermore, peripheral nerve injury can trigger nuclear export of HDAC5 whereby HDAC5 levels are reduced in the nucleus. Reduced nuclear HDAC5 level, in turn, increases histone acetylation at the regeneration-promoting gene loci and activates their transcriptional expression. By contrast, HDAC5 nuclear export together with elevated histone acetylation does not occur in the injured neurons of CNS (Cho et al. 2013). This suggests that reduced HDAC5 and the resulting increased histone acetylation make great contributions to high regeneration in PNS compared with CNS. In the same manner, the histone acetyltransferase p300/CBP-associated factor (PCAF) complex elevates histone acetylation of the promoters of key regeneration-promoting genes after axonal injury in the PNS but not in the CNS (Puttagunta et al. 2014). All the studies demonstrate that different epigenetic responses to injury (such as histone acetylation) may lead to a discrepancy in regenerative capacity between PNS and CNS. The modulation of histone modifications, such as inhibiting the HDAC activity, has been emerging as a novel strategy to promote CNS regeneration. Histone modifications are also associated with aging-related loss of regenerative capacity. In livers of older mice, the CCAAT/enhancer-binding protein alpha (C/EBPα)–HDAC1 complex accumulates in the region of the E2F-dependent promoters of liver proliferation-associated genes, thereby suppressing these genes and reducing the regenerative capacity of older livers (Timchenko 2009). Changing the C/EBPα–HDAC1 complex can enhance liver regeneration and even make mice fail to stop liver regeneration when regenerating liver reaches its original size (Jin et al. 2015). Taken together, histone modifications have profound effects on regenerative capacity.

Immune responses

The immune system is implicated in tissue homeostasis and wound repair. Meanwhile, the inflammatory interactions of immune cells and fibroblasts often bring about scarring or fibrosis. Comparative analyses of animal regeneration display an inverse relationship between the evolution of the immune system and the regenerative capacity (Mescher and Neff 2005, Aurora and Olson 2014). The more phylogenetically primitive urodele amphibians (salamanders) appear to have weaker cellular and humoral immune responses in terms of the specificity, speed of onset, and memory compared with adult anuran amphibians (frogs). On the contrary, salamanders can regenerate limbs completely, whereas frogs fail to regenerate limbs, indicating that regenerative capacity declines as the immune system advances. In addition, zebrafish have a higher CNS regeneration than mammals have, which is associated with a much weaker and shorter inflammatory response to CNS injury in zebrafish than in mammals (Kyritsis et al. 2014). By comparing the degrees of regeneration in different stages of life, many studies have revealed that the age-dependent decline in regeneration may relate to the gradual maturation of the immune system. For instance, the regenerative capacity in tails or limbs progressively decreases in the frog as it transits from the larval stage to the postmetamorphic stage. This transition is closely linked to the maturation of the adaptive immunity (Mescher and Neff 2005). The immune response in the larval stage is relatively ancestral and much less well developed than that in the postmetamorphic stage, which has a highly evolved immune system resembling that of mammals. In mammals, the transition from the fetal scarless wound healing to the adult typical scarring is accompanied by a gradual increase in the level of inflammation, immune cells, and pro-inflammatory cytokines (Mescher and Neff 2005, Larson et al. 2010). These studies seem to point out that loss of regeneration in animals relates to the development of the immune system.

The immune response does not always hinder regeneration. Successful regeneration, in effect, demands proper immune responses. For example, immune responses are indispensable to both salamander limb regeneration and neonatal mouse heart regeneration (Godwin et al. 2013, Han et al. 2015). Common functions of the immune response in regeneration include scavenging cellular debris, activating progenitor cells, and promoting angiogenesis (Aurora and Olson 2014). Many immune cells, cytokines, and complements are engaged in the processes. Among them, macrophage responses play an important part in regeneration. After the amputation of axolotl limbs, macrophages are

recruited early into the regeneration blastema, whereas the systemic depletion of macrophages leads to the failure of full limb regeneration as well as extensive fibrosis (Godwin et al. 2013). Macrophage recruitment similarly participates in the regeneration of neonatal mouse hearts; when macrophages are depleted, hearts fail to regenerate and form fibrotic scarring (Aurora et al. 2014). Although macrophage responses are essential for regeneration, distinct macrophage responses result in differences in regeneration. Two types of macrophages, which function differently, have been characterized: M1 macrophages are pro-inflammatory and secrete soluble factors to stimulate fibrosis and scar formation, whereas M2 macrophages are anti-inflammatory and reparative. The polarization of M2 macrophages to M1 macrophages is closely connected with changes in regenerative capacity in mouse hearts (Aurora et al. 2014). Fetal hearts can regenerate within the first week after birth, but afterward, hearts lose the ability to regenerate, instead forming fibrotic scarring (Porrello et al. 2011). Comparison of the immune responses to myocardial infarction in mice at postnatal day 1 (P1, regenerative period) and P14 (nonregenerative period) reveals prominent M2 macrophages in the P1 and M1 macrophages in the P14 (Aurora et al. 2014). Consistent with this result, embryo-derived cardiac macrophages (M2 macrophages) decrease in amount with age and are progressively replaced by monocyte-derived macrophages (M1 macrophages) in adults (Lavine et al. 2014). Moreover, the shift or polarization of M2 macrophages to M1 macrophages has been reported to result in the loss of regeneration in other tissues or organs, such as skeletal muscles, brains, livers, and kidneys (Aurora and Olson 2014, Forbes and Rosenthal 2014). Other immune components pertaining to regeneration capacity are comprehensively discussed in several reviews (Mescher and Neff 2005, Forbes and Rosenthal 2014). In this regard, modulating the immune response at the right time (e.g., the polarization of M1 and M2 macrophages) may be a novel strategy to promote regeneration.

Conclusions

Tremendous strides have been made in delineating the regeneration processes and the cellular and molecular mechanisms of regeneration in various animal models. After comparing many aspects of regeneration among animals, we suggest several possible reasons why regenerative capacity differs. Those reasons may account for the low regeneration observed in mammals and provide a novel avenue for promoting regeneration in mammals. Because adult mammals have insufficient stem cells, the induction of dedifferentiation and transdifferentiation is crucial to obtaining cellular sources of regeneration. The high stability of adult mammalian cells prevents them from changing their cell states, which considerably restrains their dedifferentiation and transdifferentiation potentials. To address the issue, it is crucial to decipher how the differentiated states of mature cells are maintained. Cell-cycle inhibitors and epigenetic regulators appear to maintain the differentiated state (Holmberg and Perlmannpus 2012), both of which are easily eliminated during regeneration in regenerative species but not in nonregenerative species. Comparative analyses of regenerative and non-regenerative species will help unravel the fundamental mechanisms of removing the cell-cycle and epigenetic barriers to dedifferentiation and transdifferentiation. When the differentiated state is disrupted, somatic cells go into unstable or plastic states at which cell fates can be deliberately directed by exogenous stimuli. To direct the cell fate, it is a key to have a good knowledge of how the cell fate is determined. Accumulating evidence has shown that master transcription factors, epigenetic regulators, and signaling pathways play a pivotal role in determining cell fate (Xu et al. 2015). Correspondingly, the ectopic expression of master transcription factors and/or the modulation of epigenetic regulators and signaling pathways with small molecules have successfully converted somatic cells to stem cells or to directly another lineage (Xu et al. 2015). However, these strategies are largely restricted to experiments *in vitro*; therefore, future work should strive to improve these strategies and apply them to generate those wanted cells for regeneration *in vivo*. Because some genes responsible for regeneration are evolutionarily and developmentally silenced or lost, the reactivation or reintroduction of these genes or the addition of their proteins may enhance regeneration. Just as importantly, we need to dissect the causes of their loss or silencing (e.g., epigenetic silencing), which will allow us to design strategies to increase their expression.

Even with appropriate cell sources for regeneration, a proper local microenvironment is essential for better cell survival, growth, and function. Current cell-based therapies show a low efficacy resulting from the low survival and integration rate of transplanted cells in the inflammatory microenvironment. Therefore, creating a regeneration-permissive microenvironment is vital for regeneration. Because complete suppression of immune responses and inflammation compromises regeneration (Forbes and Rosenthal 2014), careful scrutiny of the immune responses in regenerative models and mammals after injury may allow researchers to distinguish the good immune responses from the bad. Then, we will be able to block the negative effects of the immune response at the right time, which might enhance mammalian regeneration.

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