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Regeneration enhancers: Starting a journey to unravel regulatory events in tissue regeneration

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Abstract

Regeneration, an ability to replace lost body parts, is widespread across animal species. While mammals poorly regenerate most tissues, teleost fish and urodele amphibians possess remarkable regenerative capacity. Earlier work demonstrated that genes driving regeneration are evolutionarily conserved, indicating that a key factor in diverse tissue regeneration is not the presence or absence of regeneration-driving genes but the mechanisms controlling activation of these genes after injury. Thus, understanding the regulatory events of tissue regeneration could provide the means for unlocking latent capacities for tissue regeneration. After injury, cells undergo extensive epigenetic changes to establish new transcriptional programs for tissue regeneration. Gene transcription in eukaryotes is a complicated process that requires specific interactions between *trans*-acting regulators and *cis*-regulatory DNA elements. Among *cis*-regulatory elements, enhancers are essential to control precise gene expression. Recently, multiple regeneration/injury-associated enhancers have been identified in several model organisms. In this review, we highlight these recently discovered regeneration/injury enhancers and their specific characteristics. We also discuss how abnormal regulation of regeneration enhancers influences animal development and physiology. Investigation of regeneration enhancers potentially allows us to begin understanding the fundamental biology of tissue regeneration and inspires new solutions for manipulating regenerative ability.

1. Introduction

Regeneration, an ability to replace lost body parts, is widespread across animal species, but regenerative capacity is unevenly distributed among tissues, organs, and species. For instance, planaria possess remarkable regenerative capacity in that they can regrow an entire body from a small fragment or even from a single stem cell [1, 2]. Among vertebrates, teleost fish and urodele amphibians are capable of regenerating damaged tissues, including appendages (fins or limbs), brains and hearts [3]. In contrast, adult mammals, including mice and humans, have limited ability to regenerate multiple tissues, such as those in the limbs, brain and heart [3].

Organisms have evolved multiple cellular mechanisms to retain regenerative capacity. First, resident stem (or progenitor) cells play crucial roles in replenishing lost cells. Stem cells are

activated by injury to produce the same type of stem cells (self-renewal) and differentiated cells [4]. Self-renewing stem cells do not differentiate but maintain an undifferentiated status in order to preserve an adequate pool of stem cells. The other daughter cells undergo differentiation to regenerate lost cells. Second, new cells are generated through dedifferentiation [4]. The preexisting differentiated cells lose their mature status and acquire progenitor cell properties to produce additional cells. The dedifferentiated cells proliferate and eventually redifferentiate into the original cell types. In general, dedifferentiation occurs within the same lineage, and different cell lineages do not contribute to the generation of cell types in other lineages. For example, during appendage regeneration, muscle does not give rise to epidermis, and epidermis does not give rise to muscle [5]. Lastly, differentiated cells change their identities following injury either to generate transient cell states, such as repair cells, or to convert into other cell types (referred to as transdifferentiation or adaptive cellular reprogramming) [6, 7]. When nerves are severed, the differentiated Schwann cells become specialized repair Schwann cells, which form regeneration tracks to facilitate nerve regeneration [8]. After massive loss of pancreatic insulin-producing β -cells, glucagon-producing α -cells are transdifferentiated into β -cells to replace lost β -cells [9]. Collectively, the diverse cellular mechanisms of regeneration are adapted in various circumstances to repair injured tissues.

Regardless of the cellular mode of regeneration, cells must undergo extensive epigenetic changes to establish new transcriptional programs for tissue regeneration [10]. Gene transcription in eukaryotes is a complicated process that requires specific interactions between transcription factors and *cis*-regulatory DNA elements. Among *cis*-regulatory elements, enhancers are essential to controlling precise gene expression. Since the first enhancer of the polyomavirus simian virus 40 (SV40) virus was discovered [11], numerous enhancers controlling development and diseases have been identified [12, 13]. Such findings raise the possibility that tissue injuries may activate regeneration-associated enhancers to establish genetic programs, by which cells attain new attributes for tissue regeneration. The identification of regeneration-associated enhancers and their binding factors could potentially allow us to better understand the fundamental biology of tissue regeneration and inspire new solutions for manipulating regenerative events.

Only in past few years have the regeneration/injury-associated enhancers been identified in several model organisms (Table 1) [14–36]. In this review, we highlight recently discovered regeneration/injury enhancers and their specific characteristics. We also discuss how abnormal regulation of regeneration enhancers influences animal development and physiology.

2. Enhancers as key *cis*-regulatory DNA elements controlling gene transcription

Enhancers are key *cis*-acting DNA regulatory elements that govern various biological events, including development and physiology. Promoters and enhancers are major *cis*-regulatory elements, but the major difference between them lies in location: promoters act proximally to their target genes, while enhancers control gene transcription regardless of the distance

from the target genes. Active enhancers directly interact with their target promoters through physical enhancer-promoter loops that allow distal enhancers to relay regulatory information over long distances [37]. A single gene is typically regulated by multiple enhancers, each of which contains a distinct combination of sequence-specific transcription factor binding sites. Cooperative interactions among binding factors enable the orchestration of complex and precise gene transcription, determining tissue- or cell-type-specific regulation in response to internal and external signals [38]. Recent studies to identify disease-associated regulatory elements demonstrated that substantial numbers of disease-associated alleles are found in noncoding regions, particularly enhancers, and a number of studies are beginning to document that mutations in enhancers lead to developmental disorders and diseases [39–41]. Thus, studies of mechanisms by which enhancer activity is precisely regulated in a given context are currently an area of great interest.

3. Enhancers activated by both developmental and injury cues

Development and regeneration present obvious similarities, and the molecular bases for each event clearly resemble each other. For example, limb development and regeneration form analogous limb structures using common key factors, including retinoic acid (RA), fibroblast growth factor 8 (FGF8), and sonic hedgehog (SHH) [42]. These similarities raise the question of whether the same enhancer could be activated by developmental cues as well as injury to direct identical target gene expression.

The anterior-posterior axis of the developing limb bud is specified by signals from the zone of polarizing activity (ZPA), which is governed by the expression level of *Shh*. Limb-specific *Shh* expression is regulated by a well-characterized developmental enhancer: the ZPA regulatory sequence (ZRS) [43]. The ZRS is highly conserved across limbed vertebrates, and a transgenic reporter assay with ZRS showed limb bud activity *in vivo* [44]. Multiple limb-less snake species have a deletion in the ZRS, which leads to loss of limb development [45, 46], implying the developmental roles of the ZRS in limb formation. Recently, Suzuki et al. applied CRISPR/Cas9 genome editing to mutate the ZRS sequence in the newt *Pleurodeles waltl*, an animal with the ability to regenerate amputated limbs [21]. Notably, disruption of the ZRS in *P. waltl* significantly decreased *Shh* expression, thereby impairing digit reformation during limb regeneration. These studies suggest that developmental enhancers are reactivated by injury to restore the developmental factors for tissue regeneration.

Epicardial enhancer studies also provided additional evidence for the presence of enhancers activated by both development and injury [23, 24]. A comparative genomic study of DNA regions surrounding epicardial factors, which play crucial roles in the epicardium during heart development in vertebrates, defined multiple evolutionarily conserved sequences. Among these conserved sequences, two regions identified as epicardial enhancers can direct gene expression in the epicardium of developing mouse hearts. While these enhancers exhibit no activity in uninjured hearts of adult mice, they are reactivated by cardiac injury to drive epicardial gene expression. Further analysis also revealed that the CCAAT/enhancer binding protein (C/EBP) transcription factor and the swItch/sucrose non-fermentable (SWI/SNF) complex mediate enhancer activation during heart development and injury [23, 24].

Thus, these works provide evidence that the same enhancers facilitate active gene transcription during development and regeneration (Fig. 1A).

4. Enhancers restricted to injury or regeneration

The growing body of research on tissue regeneration suggests that regeneration is distinct from development. Regeneration includes regeneration-specific processes, such as wound healing, dedifferentiation, transdifferentiation, robust proliferation within a short period, and nerve-dependence [47]. Therefore, although identical gene sets are used for development and regeneration, the enhancers responsible for development and regeneration might be distinct.

Bone morphogenetic protein 5 (Bmp5) is a crucial signaling molecule required for bone and cartilage development and regeneration [48]. Developmental expression of mouse *Bmp5* is controlled by anatomy-specific enhancers, each of which drives *Bmp5* expression in discrete domains of bone and cartilage [49]. Recently, Guether et al. mapped out additional *Bmp5* regulatory elements that appear to contain injury-induced enhancers in multiple tissues [25]. Interestingly, this injury response element is not activated during development, suggesting that development and regeneration programs target the same gene via distinct regulatory sequences (Fig. 1B).

Regeneration-associated genes in zebrafish are induced by injury, and their expression remains sufficient to promote regeneration. This observation suggests that the expression of regeneration-associated genes in zebrafish is directed by enhancers preferential or specific to regeneration. *leptin b (lepb)* is robustly expressed in response to fin and cardiac injuries, and the short sequences upstream of *lepb* are enriched by acetylation of histone H3 lysine 27 (H3K27ac), a typical active enhancer marker, in regenerating tissue [18]. A transgenic reporter assay confirmed that this regulatory element, coupled with various minimal promoters, is sufficient to direct expression in fin and heart during regeneration, but not development, implying that this short noncoding sequence is a *lepb*-linked regeneration-specific enhancer (*LEN*). As regeneration completes, the elevated *LEN* activity is attenuated, revealing that regeneration enhancer activity is transient (Fig. 2). Interestingly, the regeneration-specific attribute of *LEN* can be applied to the enhancer engineering approach, in which anti- or pro-regenerative factors, such as the dominant negative form of *fibroblast growth factor receptor (fgfr)* and mitogens, are coupled with *LEN* to drive regeneration-restricted expression without developmental induction. These approaches enable the modulation of regenerative ability without altering normal zebrafish development, indicating the lack of *LEN* activity during development [18]. Recent studies in the imaginal disc of *Drosophila* and the nephric tubule of *Xenopus* also revealed new regeneration enhancers controlling injury-induced gene expression [15, 17, 22]. Notably, the *wnt*-linked *Drosophila* regeneration enhancer is epigenetically silenced at the late larval stage, resulting in regenerative failure [15]. These results suggest that epigenetic regulation of regeneration enhancers governs their activities and subsequent regenerative ability (for more reviews, see [50]).

A recent report has revealed that targeted deletion of a regeneration enhancer disrupts the regeneration program and subsequently impairs regeneration [31]. An intron of *sterile alpha motif (SAM) domain containing protein 14 (Samd14)* harbors an E-box-GATA element containing an enhancer, which is occupied by GATA-2 in mouse erythroid cells. A severe deficiency in red blood cells (anemia) increases the accessibility of this Samd14-Enh in erythroid progenitor cells, which undergo erythropoiesis to regenerate lost red blood cells. The function of Samd14-Enh was examined by generating genetically modified mice, in which the Samd14-Enh sequence was precisely deleted by genome editing. Samd14-Enh $-/-$ mice exhibited reduced *Samd14* transcription under homeostatic conditions but normal steady-state hematopoiesis. While *Samd14* expression increases in response to acute anemia, Samd14-Enh deletion significantly abrogates this anemia-induced Samd14 elevation and abolishes the activation of the stem cell factor/c-Kit regenerative program. As a result, Samd14-Enh $-/-$ mice poorly regenerate red blood cells and are more susceptible to severe anemia. Notably, several other Samd14-Enh-like loci also display features similar to Samd14-Enh, including increased chromatin accessibility and target gene expression in severe anemia and evolutionary conservation, providing evidence that a group of regeneration enhancers with similar attributes is widespread throughout the genome to control transcription of regeneration genes in the same cluster [31].

Pathological enhancer mutations in human patients can also impact regeneration but not development. Transcription factor GATA-2 function is required for hematopoietic stem cell (HSC) genesis during embryogenesis as well as hematopoiesis in adults [51]. GATA-2 expression is regulated by multiple enhancers, and one crucial GATA-2 enhancer harbors conserved E-box, GATA, and Ets binding motifs [32]. Mice carrying mutations in both E-Box and Ets motifs fail to establish HSCs, indicating that these two motifs are required for HSC genesis. One GATA-2 related disease is characterized by a single-nucleotide mutation in an Ets binding site. Strikingly, mice harboring the same single-nucleotide Ets mutation develop HSC establishment during embryogenesis but exhibit severely impaired hematopoietic regeneration after bone marrow injury. These studies highlight the power of microdissecting enhancers and the importance of regeneration enhancers for translational research.

5. Silenced regeneration/injury enhancers in uninjured tissues

As mentioned above, regeneration enhancers display dynamic activity; they are activated by injury and then return to a naïve state when regeneration is complete (Fig. 2). The intriguing questions are how their activity is precisely regulated, and what are the phenotypic alterations caused by misregulated regeneration enhancers? In this section, we discuss how epigenetic regulators influence the activity of regeneration/injury-associated enhancers and subsequent phenotypic outcomes.

After peripheral nerve injury, the axons distal to the injury site degenerate. In response to axon degeneration, Schwann cells, which enwrap peripheral nerve axons, rapidly activate *c-Jun* to reprogram differentiated Schwann cells into repair Schwann cells, transient cells that play a crucial role in axonal regeneration and nerve repair [52]. These highly specialized repair Schwann cells exhibit unique features. Repair Schwann cells appear to be

dedifferentiated Schwann cells, as they downregulate genes required in differentiated states, such as major myelin genes including the key myelin transcription factor *Egr2/Krox20* and myelin basic protein (*Mbp*), and reactivate genes expressed in immature Schwann cells, such as *neural cell adhesion molecule (Ncam)* and *glial fibrillary acidic protein (Gfap)* [8]. In addition, repair Schwann cells gain novel features associated with neither mature nor immature Schwann cells. At the molecular level, several genes, such as *Shh*, *Glial cell-derived neurotrophic factor (Gdnf)*, or *Artemin*, are exclusively expressed in repair Schwann cells [53]. Functionally, repair Schwann cells assist regeneration by triggering the expression of injury-related genes, including trophic factors and cytokines, to promote axon growth and to attract macrophages for removing debris, respectively [8]. Therefore, Schwann cells are another interesting model for studying injury-induced transcriptional programs and underlying regeneration/injury-associated enhancers.

To identify regulatory elements and their dynamics in Schwann cells upon peripheral nerve injury, Hung and colleagues performed H3K27ac chromatin immunoprecipitation followed by sequencing (ChIP-seq) with sham and injured rat sciatic nerve, a large portion of which is composed of Schwann cells [27]. This epigenetic study identified a substantial number of H3K27ac-enriched regulatory elements in Schwann cells of uninjured and injured nerve tissues (referred to as control and repair Schwann cells). H3K27ac enrichment in control and repair Schwann cells displays a strong correlation with the expression level of genes close to H3K27ac peaks; H3K27ac-enriched peaks in controls are found near the major myelin genes whose expression is reduced upon injury, and DNA regions surrounding injury-induced genes are highly marked by H3K27ac in repair Schwann cells. Motif analysis reveals that enriched motifs of putative enhancers in control samples are binding sites for *Egr2* and *Sox10*, key transcription factors for Schwann cell differentiation [54, 55], while injury-induced enhancer candidates are enriched with cognate sites for *c-Jun* and *Runx2*, major injury-associated transcription factors in Schwann cells [52]. In mice, another rodent model, some analogous injury-induced enhancers are likely hypomethylated by nerve injury, suggesting that injury-induced enhancers may be evolutionarily conserved [29].

To address the impact of epigenetic modulators in Schwann cells, Ma et al. generated a conditional knock-out (cKO) mouse model of *Eed* [28], an essential subunit of polycomb repressive complex2 (PRC2) that mediates trimethylation of H3K27 [56]. Mice with *Eed* deletion in Schwann cells develop myelin normally in the early postnatal stage. However, adult mice with *Eed* cKO in Schwann cells display myelin abnormalities, including progressive hypermyelination and morphological changes [28]. Hypermyelination raises the possibility of upregulation of myelin-related genes, but gene expression analysis determined that *Eed* deletion did not highly induce the expression of myelin-related genes. Interestingly, a substantial portion of nerve injury-activated genes in repair Schwann cells was significantly upregulated in uninjured *Eed*-deficient sciatic nerve [28], indicating that *Eed* inactivation interferes with the suppression of injury-activated genes in uninjured tissues (Fig. 3). These results suggest that regeneration/injury enhancers are silenced in uninjured tissue by unknown mechanisms to repress genes that respond to injury, implying the presence of negative regulators for regeneration/injury enhancers in uninjured states [57].

6. Wound-healing enhancers hijacked by cancer

The skin in mice harbors two distinct lineages, hair follicle (HF) and epidermis (Epd), each of which contains their own resident stem cells [58]. Under homeostatic conditions, each lineage replenishes its population through the activation of its own stem cells (referred to as homeostatic regeneration). When quiescent stem cells are activated under homeostatic conditions, stem cells generate additional stem cells (self-renewal) and short-lived progenitors called transit-amplifying cells (TACs) [59]. TACs divide rapidly multiple times and then undergo differentiation to restore HF cells. In addition to homeostatic regeneration, following wound injury, HF and Epd stem cells are capable of regenerating damaged skin. Intriguingly, in response to injury, both HF and Epd stem cells near the wound site gain plasticity so that they are able to exit their niche to participate in wound healing, even though the damaged skin is not their own lineage [58]. An interesting question is how transcriptional programs are rapidly changed under various conditions for different skin stem cell lineages.

Using epigenome profiles of multiple transcription factors and specific histone modification markers in HF stem cells and their TACs, Adam et al. identified that superenhancers (SEs), a cluster of enhancers greatly enriched with mediator (Med1) [60], emerge near genes specifically expressed in HF stem cells [33]. A substantial portion of SEs in stem cells are inhibited in their daughter TACs, but TACs gain new SE signatures in the regions close to the genes selectively transcribed in TACs. These results indicate that enhancers in these two populations are significantly remodeled during lineage progression. In this regard, transgenes containing densely clustered sequences (epicenters) within HF stem cell SEs can direct gene expression in stem cells but not in daughter TACs. The investigators also evaluated the activity of SEs in an in vitro culture dish, a context similar to the wounded skin, and found that the majority of stem cell SEs lose their activity in cultured stem cells. Intriguingly, a small group of stem cell SEs remains active in vitro by shifting their epicenters to the regions enriched with motifs for wound-related factors, such as Activator protein 1 (AP-1), Krüppel-like factor (KLF), and Forkhead (FOX) families. In vivo reporter assays confirmed that these epicenters induced in vitro are truly activated by injury in stem cell progenies of mouse skin [33]. These findings prove that enhancers are dynamic between homeostatic and injury-induced regenerative conditions.

Regeneration and tumorigenesis similarly achieve plasticity to expand their cell numbers. Whereas regeneration programs accurately control the balance between cell proliferation and differentiation to ultimately repair wounded tissue without overgrowth, proliferation is persistent in tumor cells. These similarities and discrepancies between regeneration and tumorigenesis raise the questions of whether tumor cells hijack regulatory events associated with regeneration to constantly proliferate and, if this happens, what makes tumor cells infinitely divide. Recently, Ge and colleagues explored this “hijack hypothesis” by comparing global open chromatin landscapes of mouse skin stem cells under homeostatic, wound-induced and tumorigenic conditions [34].

Comparative analysis of global open chromatin landscapes, which could be considered enhancers, in HF, Epd, and wound-induced skin stem cell populations have revealed that

wound injury rapidly collapses enhancers specific to either HF or Epd stem cells but establishes new injury-specific enhancers. When the wound heals, injury-specific enhancers are resolved, and each stem cell population regains their own characteristics. These results indicate that stem cells, regardless of origin, switch from lineage-specific to common injury-specific enhancer signatures upon injury (Fig. 4). In addition, wound-induced stem cells simultaneously express *Klf5* and *Sox9*, which are lineage-associated transcription factors exclusively expressed in HF or Epd stem cells, respectively. Dual *Klf5* and *Sox9* expression is essential for repair but transient in wound-induced stem cells, as the mutually exclusive expression of *Klf5* and *Sox9* in HF and Epd stem cells, respectively, is restored after regeneration completes [34]. These results suggest that wound-induced stem cells achieve plasticity by converting lineage-specific features to injury-specific transcriptional features upon injury and subsequently revert to a certain lineage when regeneration terminates.

Interestingly, analysis of open chromatin landscape and transcriptional programs in tumor cells revealed that tumor stem cells significantly share differentially expressed genes and accessible chromatin regions with wound-induced stem cells (Fig. 4). Additionally, tumor cells exhibit the dual expression of *Klf5* and *Sox9*, similar to wound-induced stem cells. These data provide evidence that tumor cells hijack regulatory events associated with tissue repair. However, in contrast to wound-induced stem cells, tumor cells lock tumorigenic features by exposing new cancer-specific enhancers (Fig. 4) and by maintaining the dual expression of *Klf5* and *Sox9* [34]. Thus, epigenetic plasticity plays crucial roles in tissue repair, but misregulation of wound-induced regulatory events can lead to cancer.

7. Memorized injury-induced enhancers

Most injury-induced enhancers are transient because they return to normal levels after completion of regeneration (Fig. 2). However, several studies have highlighted that a subset of injury-induced enhancers maintain an open status after injury is healed (Fig. 5), influencing the potential of a cell to elicit future injury responses or cause diseases [30, 35].

Some patients with central nervous system (CNS) injury develop neuropathic pain, in which somatosensory nerves transmit disordered signals to the brain. One major question is why neuropathic pain persists long after the CNS injury is repaired [61]. CNS injury activates microglial cells, the resident myeloid cells of the CNS, and the activated microglial cells produce cytokines, such as tumor necrosis factor alpha (TNF α) and interleukin-1 beta (IL-1 β). The secreted cytokines mediate immune and inflammatory responses and play an essential role in the pathogenesis of chronic pain [62]. Denk and colleagues performed a genome-wide analysis of monomethylation of histone H3 lysine 4 (H3K4me1), a typical active enhancer marker, in microglial cells to define how nerve injury alters enhancers in microglial cells and subsequently influences neuropathic pain [30]. Some injury-induced enhancers in microglial cells appear to be latent enhancers, in which target gene expression returns to normal but enhancers are persistently enriched by H3K4me1, implying that a subset of injury-induced enhancers in microglial cells are epigenetically memorized. These memorized enhancers are associated with genes controlling immune and inflammatory responses. These results suggest that abnormal activation of these memorized enhancers in microglial cells may induce inflammatory reactions, resulting in chronic pain (Fig. 5).

The epigenetic memory of injury-induced enhancers in nonimmune cells has recently been reported in mouse skin epidermis [35]. After the skin is injured, the epidermis undergoes inflammatory reactions, which are required for wound healing. Interestingly, Naik et al. discovered that skin with previous inflammatory injury or inflammation-promoting chemical treatment heals wounds much faster than naïve skin. Notably, the enhanced regenerative ability occurs at 180 days after the first inflammation exposure, indicating that the inflammation response is memorized in the skin. An assay for transposase-accessible chromatin followed by sequencing (ATAC-seq) analysis with skin epithelial stem cells showed that several regions retain their open status at day 180, long after inflammation resolves (Fig 5). Moreover, a transgenic reporter assay confirmed that some of these regions drive reporter gene expression much faster in inflammation-experienced skin, implying that a subset of inflammation-induced enhancers are memorized to accelerate reactivation of a secondary assault. The authors also identified *Aim2* as a mediator of “inflammation memory”. *Aim2* null mice are not capable of achieving faster wound healing postinflammation, whereas persistent *Aim2* expression in the epidermis without preinflammation reaction is sufficient to enhance wound healing [35]. These studies indicate that injury-induced enhancers may be primed to improve regenerative ability for a secondary injury.

8. Conclusion

Although regeneration has been studied for a long time, regulatory elements controlling regeneration, such as regeneration enhancers, have been recently documented in diverse species (Table 1). In the near future, advances in sequencing and epigenetics along with *in vivo* enhancer analysis will identify more regeneration enhancers. The next important questions are how regeneration enhancers have evolved in animal species and how these changes influence regenerative abilities positively or negatively. Other critical steps are to elucidate essential motifs, their binding factors and their upstream signaling pathways. These studies will illuminate the *cis*-regulatory logic underlying tissue regeneration, such as how injury signals are transformed to trigger regeneration programs in a tissue-specific manner, and what are the master regulators of tissue regeneration. Although limitations remain in defining regulators, recently developed new proteomic approaches will enable researchers to find proteins associated with regeneration in *in vivo* models [63–66]. After that, such knowledge will lead to the discovery of new targets and innovative strategies to enhance tissue repair in humans.

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Fig. 1. Diverse classes of regeneration/injury enhancers

(A) Enhancers shared between development and regeneration/injury. The ZPA regulatory sequence (ZRS) and *Raldh2* and *Wt1* epicardial enhancers are activated by developmental and injury cues. (B) Regeneration/injury-restricted enhancers. Distinct enhancers are activated by development and regeneration. *Bmp5* injury responsible elements, *lepb*-linked regeneration enhancer (*LEN*), *Drosophila wnt*-associated damage-induced enhancer (BRV118), and Samd14-Enh are responsible for regeneration or injury, but not development.

Fig. 2. Dynamic activity of regeneration/injury-associated enhancers.

(A) Regeneration/injury enhancer activity. Regeneration/injury enhancers are not active in uninjured tissues. Upon injuries, regeneration/injury enhancers are activated to drive target gene expression and their activity is maintained during regeneration. When regeneration or injury is resolved, the activity of regeneration/injury enhancers is returned to the level of the uninjured tissues. (B) Example of dynamic regeneration enhancer activity. Zebrafish *LEN* is not active in uninjured fin (0 day post-amputation (dpa)). Fin amputation activate *LEN* to direct reporter gene expression (12 hours post-amputation (hpa)). *LEN* activity is maintained during regeneration (2 and 4 dpa). When fin regeneration completes (30 dpa), *LEN* activity is decommissioned and thus reporter gene expression is not detectable. (B) is adapted from [18].

Fig. 3. Silenced regeneration/injury enhancer in uninjured tissues.

After peripheral nerve injury, injury-induced enhancers emerge in Schwann cells. However, the loss of PRC2 activity results in aberrant activation of injury-induced enhancers in uninjured cells, leading to upregulation of myelin-regulated genes and subsequent hypermyelination and morphological change.

Fig. 5. Epigenetically memorized injury-induced enhancers.

Each track represents ATAC-seq peaks of uninjured tissues (A), early (B) and late phase (C) of regenerating tissues, and completely regenerated tissues (D). A subset of injury-induced enhancers is not completely resolved long after completion of regeneration. These epigenetically memorized enhancers may mediate aberrant activation of immune cells in the central nervous system (CNS), resulting in chronic neuropathic pain. In mouse skin, the memorized enhancers contribute to faster healing of secondary wound.

Table 1.
Injury or regeneration-associated regulatory elements

Tissues, Species	Description	Identification	Functional assay	Ref.
Entire body, Acoel worm	Whole body regeneration-responsive elements	ATAC-seq	N.D.	[14]
Imaginal disc, <i>Drosophila</i>	Injury-induced expression of <i>wg</i> and <i>wnt6</i> (BRV118)	Serial transgenic assays	Tg, deletion	[15, 16]
Imaginal disc, <i>Drosophila</i>	Injury-induced regulatory elements	ATAC-seq, H3K4me1 and H3K27ac ChIP-seq	Tg	[17]
Fin and heart, Zebrafish	<i>lepb</i> -linked regeneration-specific enhancer (<i>LEN</i>)	H3K27ac ChIP-seq	Tg, enhancer engineering	[18]
Heart, Zebrafish	Cardiac tissue regeneration enhancers	H3.3 profiling	Tg	[19]
Fin and heart, Zebrafish	<i>ctgfa</i> regulatory elements (<i>careg</i>)	Targeted reporter line	Tg	[20]
Limb, Newts	ZRS (evolutionarily conserved limb developmental enhancer)	Evolutionarily Conserved sequences	Mutation	[21]
Nephric tubules, <i>Xenopus</i>	Regeneration signal-response enhancers (RSREs)	Evolutionarily Conserved sequences	Tg	[22]
Heart, Mouse	Injury-induced epicardial enhancers	Evolutionarily Conserved sequences	Tg	[23, 24]
Skeletal system, Mouse	Injury-induced expression of <i>Bmp5</i>	Serial BAC transgenic assays	Tg	[25]
Adult skeletal muscle, Mouse	Muscle cell regeneration-associated genes	H3K27ac, H3K4me1, H3K4me3 ChIP-seq	N.D.	[26]
Schwann cells, Rat	Nerve injury-induced enhancers	H3K27ac ChIP-seq	N.D.	[27, 28]
Schwann cells, Mouse	Differentially methylated DNA regions after nerve injury	WGSB-seq	N.D.	[29]
Microglial cells, Mouse	Injury-induced microglial enhancers	H3K4me1 ChIP-seq	N.D.	[30]
Red blood cells, Mouse	<i>Samd14</i> enhancers activated in severe anemia	GATA-2 ChIP-seq and FAIRE-seq	Deletion	[31]
Hematopoietic stem cells, Mouse	<i>Gata2</i> +9.5 enhancer	Single-nucleotide human disease mutation	Point mutation	[32]
Skin, Mouse	Wound-induced super-enhancers	Various ChIP-seqs	Tg	[33]
Skin stem cells, Mouse	Wound-induced enhancers	ATAC-seq	Tg	[34]
Skin stem cells, Mouse	Wound-induced enhancers, Some are inflammatory memory enhancers	ATAC-seq	Tg	[35]
Engrafted human epidermis to mice, Human	Wound-induced enhancers, One targets <i>TSLP</i>	FAIRE-seq	N.D.	[36]

wg: wingless

ZRS: Zone of polarizing activity (ZPA) regulatory sequence

TSLP: thymic stromal lymphopoietin

H3K27ac: Acetylation of histone H3 lysine 27

H3K4me1: Mono-methylation of histone H3 lysine 4

H3K4me3: Tri-methylation of histone H3 lysine 4

ChIP-seq: Chromatin immunoprecipitation followed by sequencing

WGSB-seq: Whole-genome shotgun bisulfite sequencing

FAIRE-seq: Formaldehyde-assisted isolation of regulatory elements sequencing

ATAC-seq: Assay for transposase-accessible chromatin followed by sequencing

H3.3: A histone H3 variant is selectively enriched at transcriptionally active genes, promoters, insulators, and enhancers

BAC: Bacterial artificial chromosome

Tg: Transgenic reporter assay in animal

N.D. Not Determined.