Tissue regeneration enhancer elements: A way to unlock endogenous healing power.

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Abstract

Regenerative capacity is widespread throughout almost all animal phyla. However, the distribution pattern remains incompletely understood. Various examples show that very closely related species display different regenerative capacities. Why and how have diverse regenerative capacities evolved across species? One prevailing thought in the field of regeneration is that most regeneration-associated factors are evolutionarily conserved, suggesting the existence of an innate tissue regeneration ability in all species. However, its regulation is differentially controlled in distinct species, resulting in heterogeneous regenerative capabilities. In this review, we discuss regeneration-associated enhancers, the key *cis*-regulatory elements controlling gene expression, their underlying molecular mechanisms, and their influence on regenerative capacity. Understanding the regulatory mechanisms of regeneration enhancers can provide fundamental insights into tissue regeneration and further help us develop therapeutic strategies to unlock latent healing powers in humans.

Introduction

Regeneration can be considered as an adaptive trait for evolution and survival, but incongruously, regenerative capacity is unevenly distributed and the distribution seems to be random or inexplicable even within species (Sanchez Alvarado, 2000; Brockes et al., 2001; Maginnis, 2006). For example, while planarians can, in general, regenerate the whole body from a very tiny fragment, some planarian species show limited regenerative capacity (Newmark and Sanchez Alvarado, 2002; Liu et al., 2013; Sikes and Newmark, 2013; Umesono et al., 2013). In teleost fish species, zebrafish and medaka display different regenerative capacities of the heart (Poss et al., 2002; Ito et al., 2014; Lai et al., 2017). Likewise, while many urodele species (e.g., *Notophthalmus viridescens* (newt) and *Ambystoma mexicanum* (axolotl)) can regenerate amputated limbs, some urodele species (e.g., *Necturus maculosus, Ambystoma tigrinum*) show little or no such ability (Scadding, 1977; Maginnis, 2006). Similarly, unlike normal laboratory mice, African spiny mice can completely regrow skin, bone, or ear tissues after massive losses (Seifert et al., 2012; Matias Santos et al., 2016; Simkin et al., 2017).

The presence of highly regenerative species seems to suggest the existence of species-specific regeneration genes. However, few species-specific genes have been described; *Prod1* is one such gene that has been reported to exist solely in the highly regenerative salamander (da Silva et al., 2002). In fact, the prevailing thought is that crucial regeneration-driving genes are present in most animal genomes, implying the existence of an innate ability to regenerate lost tissues in most animals. Thus, a key

aspect of diverse regenerative capacities is not the presence or absence of regeneration genes, but in the mechanisms controlling the activation of these genes after injury.

The poor regeneration displayed by most higher vertebrates might be indicative of the absence of regulatory events driving regeneration gene expression upon injury. Defining how and why these organisms lose these regulatory events might provide mechanistic insights into tissue regeneration. By contrast, highly regenerative organisms, such as zebrafish and salamander, can be used to elucidate the regulatory mechanisms controlling regeneration gene expression. Recent advances in sequencing and epigenetic techniques combined with traditional transgenic assays have discovered numerous enhancers, key *cis*-regulatory DNA elements controlling spatiotemporal gene expression, including regeneration-associated enhancers (Kang et al., 2016; Goldman et al., 2017). In this review, we explore the current understanding of genes and epigenetic regulatory mechanisms that contribute to the varying regenerative responses among and within species. We first describe a species-specific regeneration gene and the importance of differentially expressed conserved genes in tissue regeneration. We then review recent discoveries of regulatory elements controlling the expression of regeneration genes: regeneration enhancers. Finally, we discuss how regenerative abilities may be altered during evolution.

Species-specific limb regeneration gene

Species-specific genes may contribute to remarkable regenerative capacity. Decades of studies in regeneration, however, have described few such genes. *Prod1* is the only reported species-specific gene that is present in the salamander, an animal with a

remarkable ability to regenerate amputated limbs. Initial evidence of the involvement of *Prod1* in regeneration came from an assay for retinoic acid (RA)-regulated genes (da Silva et al., 2002). *Prod1*, a member of the Ly6 protein superfamily, is found on the surfaces of blastema, a mass of progenitor cells. *In situ* hybridization assays detected *Prod1* expression in some blastema cells as early as 1 day post-amputation (dpa) and in the majority of mesenchymal blastema cells by 16 dpa. RA treatment increased *Prod1* expression in the blastema and even in uninjured limbs in a dose-dependent manner, suggesting a potential role for *Prod1* in tissue respecification (da Silva et al., 2002).

Another clue to the role of *Prod1* was provided with the discovery of a *Prod1* ligand, newt Anterior Gradient (nAG). nAG was identified as a binding protein of Prod1 through a yeast two-hybrid screen. nAG is a secreted protein, and its expression depends on the presence of a nerve. After amputation, *nAG* was initially expressed in Schwann cells along the amputation plane; later, *nAG* expression was observed in gland cells of the blastema. In the absence of a nerve, *nAG* expression is diminished in both the distal nerve sheath and the wound epidermis, resulting in complete loss of regenerative capacity of the limb. Interestingly, ectopic *nAG* expression in this denervation context was sufficient to allow reestablishment of blastema, leading to the regeneration of the amputated limb (Kumar et al., 2007b). These findings support the hypothesis that nAG secreted by the nerve sheath interacts with Prod1 on the surface of blastema cells to promote cell proliferation during regeneration.

Like many regeneration genes (Sehring et al., 2016), *Prod1* is an important player during development. An expression gradient of *Prod1* in Schwann cells along the proximal-distal (PD) axis of uninjured adult limbs supports the *Prod1* roles in PD identity

(Kumar et al., 2007a). In contrast to *Meis2*, another gene implicated in PD identity, *Prod1* is highly expressed at the proximal structure with an exponential decrease at more distal structures. Coexpression of *Prod1* and *Bmp2*, an important cytokine for digit formation in amniotes, in the developing limb bud suggests the developmental roles of *Prod1* (Kumar et al., 2007a). Although disruption of *Prod1* function via TALEN mRNA injection did not appear to affect early limb development, a lack of zeugopod and digit formation was observed (Kumar et al., 2015). Further regeneration studies with a *Prod1*-null model will provide insight into the specific roles of *Prod1* in limb regeneration.

Since its discovery, *Prod1* has been identified in at least nine species of salamanders spanning four families (Geng et al., 2015) and appears to be an orphan gene specific to salamanders (Garza-Garcia et al., 2010). An orthologue of *Prod1* does not exist in other vertebrate organisms, including *Xenopus* and zebrafish. Interestingly, whole-genome sequencing of the axolotl has revealed a Ly6 protein superfamily surface receptor that is upregulated in axolotl limb blastema (Nowoshilow et al., 2018). It would be interesting to compare the role of this newly unearthed axolotl Ly6 protein with the known functions of salamander Prod1.

Advances in genome sequencing and mapping hold many promises for the discovery of other species-specific regeneration genes. Mining of these genome sequences will be invaluable in the search for how certain species evolved to have higher regenerative capabilities than other species. Moreover, it would be beneficial to examine whether these salamander-specific genes can be utilized in injured mammalian appendages to improve tissue repair.

Differentially regulated conserved gene expression as a key of diverse regenerative capacities

Regeneration studies have revealed many regeneration-driving genes that are shared among species. Often, these genes are differentially expressed in the same tissues among and within species, resulting in varying regenerative abilities.

Almost planarians species possess outstanding regenerative capacity, yet some planarian species, including *Procotyla fluviatilis, Dugesia japonica,* and *Dendrocoelum lacteum* show restricted ability to replace missing body parts (Newmark and Sanchez Alvarado, 2002; Liu et al., 2013; Sikes and Newmark, 2013; Umesono et al., 2013). When *P. fluviatilis, D. japonica,* and *D. lacteum* are amputated posterior to the pharynx, the posterior fragment fails to regenerate anterior structures. Comparative transcriptomic analysis defined differentially expressed genes between regenerating and non-regenerating tissues, demonstrating that the non-regenerating posterior stump retains aberrant activation of the Wnt signaling pathway. (Liu et al., 2013; Sikes and Newmark, 2013; Umesono et al., 2013). Interestingly, downregulating the β-catenin level, the key transducer of Wnt signaling pathway, in regeneration-deficient tissues restored the head regeneration. Although the regulatory mechanisms driving aberrant Wnt signaling activation or inhibiting Wnt signaling antagonist expression are unknown, these results indicate that differential gene expression within species can result in distinct regenerative abilities.

Appendages, such as limbs and fins, are distinctive regeneration systems depending on species. Although mammals poorly regenerate lost limbs, salamander, *Xenopus*, and zebrafish spectacularly regenerate limbs or fins. In addition to the

presence of *Prod1* salamander-specific limb regeneration genes, evolutionarily conserved genes are implicated in limb or fin (appendage) regeneration. Multiple studies demonstrated that appendage regeneration in zebrafish, *Xenopus*, and axolotl are regulated by the Wnt/β-catenin signaling pathway, a well-conserved signaling pathway across species (Kawakami et al., 2006; Stoick-Cooper et al., 2007; Yokoyama et al., 2007). During embryogenesis, limb bud outgrowth is driven by the apical ectodermal ridge (AER), a distally localized signaling center that secretes FGF8, an essential gene for limb bud initiation. Posterior localization of Sonic Hedgehog (SHH) is associated in limb bud development, including outgrowth and patterning (Petit et al., 2017). Although various morphological discrepancies are observed among species, these key genes and developmental processes are largely conserved. Similar to limb development, limb amputation re-induces the expression of *Fgf8* and *Shh* in anterior and posterior blastema cells, respectively. Cross-talk between anterior FGF8 and posterior SHH sustains their expression, which governs both limb regrowth and positioning (Nacu et al., 2016). These results suggest that species retaining regenerative capacity of appendages can trigger the transcription of evolutionarily conserved genes upon injury and that the failure of transcription could cause deficient regeneration.

Enhancers involved in the activation of the regeneration program

Transcription of eukaryotic genes is an intricate process that requires the precise orchestration of interactions between *trans*-acting protein factors and *cis*-regulatory DNA elements. Among *cis*-regulatory elements, enhancers are key elements controlling spatial and temporal patterns of gene expression (Lagha et al., 2012). Active enhancer

candidates can be identified and annotated by various methodologies. Profiling histone markers of active enhancers using chromatin immunoprecipitation followed by sequencing (ChIP-seq) with H3K27ac and H3K4me1 antibodies are a common method to predict active enhancer candidates. Mapping open chromatin regions, obtained by assay for transposase-accessible chromatin using sequencing (ATAC-seq), DNase-seq, or formaldehyde-assisted isolation of regulatory elements sequencing (FAIRE-seq), is another widely used method (Buenrostro et al., 2013; Shlyueva et al., 2014). Chromosome conformation capture approaches also predict the enhancer candidates by analyzing the interactions between promoter and regulatory elements, such as enhancers, at individual gene loci (Long et al., 2016). The histone variant H3.3 actively marks active enhancers; thus, H3.3 deposited regions can be considered as active enhancers (Jin et al., 2009; Goldberg et al., 2010; Goldman et al., 2017). Identification of conserved non-coding elements across species is another way to uncover functional enhancers (Braasch et al., 2016; Dickel et al., 2018). Because active enhancers are often transcribed bidirectionally to produce enhancer RNA (eRNA), eRNA profiles can be utilized to detect active enhancers (Elkon and Agami, 2017, Leveille et al., 2015).

These methodologies are powerful in the prediction of candidate enhancers but appear to have limitations in the validation of the *in vivo* functionality. Functional validation is often performed using transgenic assays, in which a candidate enhancer sequence coupled with a minimal promoter and a reporter gene is introduced into an animal and reporter gene expression is tested in the context of interest (Kvon, 2015). An emerging alternative method is to delete candidate enhancers using genome editing techniques and then determine target gene expression and functional outcomes (Hewitt et al., 2017;

Mehta et al., 2017; Dickel et al., 2018; Osterwalder et al., 2018). These methods have helped identify numerous developmental or disease-related enhancers.

Despite the existence of these methods, there are several challenges to identifying regeneration enhancers. First, many mammalian tissues regenerate poorly. Injury to mammalian tissues prompts wound-healing and some extent of proliferation, but these tissues often cannot reach the adequate proliferation required for the progression of regeneration (Tanaka, 2016). However, there have been multiple attempts to identify injury-responsive enhancer elements, which are described in the following sections. Second, transgenic assays to confirm regeneration enhancer activity require tremendous resources and effort (Kvon, 2015). Developmental enhancers can be readily determined by examining reporter gene expression at the developmental stage of interest. In contrast, regeneration enhancers are inactive in uninjured tissues. Thus, to obtain regenerating tissue samples requires additional procedures, such as amputation or damage, some of which are not easy depending on the tissue type or require specific genetic ablation models. Considerable resources and efforts have also been diverted towards raising transgenic animals to the adult stage to examine regeneration-specific activity but not developmental activity. Third, regenerating tissues comprise diverse cell types and simultaneously contain both proliferating and differentiating cells. Thus, without sorting specific cell types, this complexity can hamper the identification of regeneration-associated enhancers.

Despite these challenges, several regeneration-associated enhancers have been discovered (Harris et al., 2016; Kang et al., 2016; Goldman et al., 2017). The *Drosophila* imaginal disc, a larval epithelial structure that gives rise to adult body parts, such as the

wings and eyes, is capable of regenerating lost portions during early larval stages. After injury, early larval imaginal discs ectopically induce the expression of WNT genes, including *wingless* (*wg*) and *wnt6*, to promote regeneration (Smith-Bolton et al., 2009; Harris et al., 2016). Transgenic assay with a collection of transgenic reporter lines containing overlapping non-coding regions near *wg* revealed that injury-induced *wg* expression is regulated by enhancer BRV118 (Schubiger et al., 2010). The BRV118 enhancer is activated by multiple injuries to drive both WNT genes, including *wg* and *wnt6*, suggesting that BRV118 is a regeneration-associated enhancer (Harris et al., 2016). Interestingly, the wg^{1} homozygote files, in which most of the BRV118 enhancer is deleted, markedly reduce the injury-dependent *wg* and *wnt6* induction and exhibit compromised recovery from the injury (Harris et al., 2016). These data provide evidence that there are regeneration-associated enhancer elements controlling the regeneration gene activation and regenerative ability.

Enhancers shared between development and injury/regeneration

The epicardium envelops vertebrate hearts and functions as a signaling center for cardiac development and repair (Cao and Poss, 2018). To delineate the transcriptional mechanisms governing epicardial activation, Huang et al. developed a mouse embryonic heart organ culture and gene expression system, with which they screened epicardial enhancer elements (Huang et al., 2012). They investigated evolutionarily conserved regions associated with common epicardial factors, including *Tcf21, Tbx18, Raldh2*, and *Wt1.* This analysis identified two epicardial enhancers, *Raldh2* (*Raldh2* CR2; 746 bp) and *Wt1* (*Wt1* CR14; 635 bp), that have the potential to drive epicardial expression in a mouse

embryo (Fig. 1). Further analysis to define the minimal sequences and their binding factors revealed 160-bp and 53-bp core sequences of *Raldh2* CR2 and *Wt1* CR14, respectively, and a C/EBP transcription factor that mediates epicardial activation. Notably, these two epicardial enhancers are also activated after cardiac injury in adult stages, indicating that they are injury-responsive enhancers. These results indicate that developmental enhancers can be reused as injury or regeneration enhancers in adult tissues (Fig. 1). Investigating the relationship between developmental and regeneration enhancers will elucidate the transcriptional mechanisms to reactivate developmental gene expression upon injury.

Regeneration-specific enhancers and regulatory elements

While development and regeneration share many features, including sequential events of growth (cell proliferation), differentiation, and patterning, transcriptional regulations and associated enhancers of both contexts could be different due to different input signals (developmental cue vs. injury). Recent zebrafish studies proved this difference based on discoveries of regeneration-specific enhancers (Kang et al., 2016). Transcriptome analysis using regenerating fin and cardiac tissues revealed that the zebrafish gene *leptin b (lepb)*, one of two zebrafish paralogues related to mammalian *leptin* (Zhang et al., 1994), is robustly induced during regeneration in both tissues. An unbiased genome-wide chromatin profiling study of H3K27ac defined putative regeneration enhancers distally located to *lepb*. Subsequent extensive transgenic assays with multiple promoters and different fragments of the candidate sequence have confirmed that a 1.3-kb fragment localized 6 kb upstream of *lepb* has the potential to drive regeneration-dependent

expression. Interestingly, this *lepb*-linked regeneration enhancer (*LEN)* directs regeneration-specific gene expression without developmental activity, indicating the presence of a regeneration-specific enhancer (Fig. 2A). Notably, this specificity of the *LEN* can be utilized to modulate regenerative ability. *LEN*-driven expression of pro- or anti-regenerative factors led to their strong induction upon injury, with a concomitant positive or negative modulation of regeneration, but did not alter normal zebrafish development (Kang et al., 2016). These studies suggest that deploying regeneration-specific enhancers could provide a therapeutic approach to stimulating tissue repair. Further attempts to define essential sequences in *LEN* to drive fin and heart regeneration-dependent expression demonstrated that fin and heart regeneration modules are located in different regions in the *LEN*. These results suggest that different tissue injuries activated tissue-specific regulatory elements to drive the same target gene expression.

Bone morphogenetic protein 5 (BMP5) is a key signaling molecule required for skeletal development and repair. *Bmp5* is expressed in skeletal structures and soft tissues during embryonic development as well as during adult bone regeneration (Green, 1958; King et al., 1996). A large array of mouse transgenic assays to identify *cis*-regulatory elements in *Bmp5* expression during development revealed that *Bmp5* expression at particular anatomical locations is controlled by multiple separate regulatory elements (DiLeone et al., 1998; DiLeone et al., 2000). Recently, Guenther et al. uncovered the *Bmp5* injury response element activated by bone fracture, skin wound, and lung injury in adult mice (Guenther et al., 2015). Interestingly, this *Bmp5* injury response element does not direct developmental expression, demonstrating that distinct regulatory pathways regulate *Bmp5* expression during embryonic development and adult

regeneration (Fig. 2B). However, due to the large size (17.5 kb) of regulatory sequences containing an injury response module, it is unclear whether *Bmp5* injury response element also composes of tissue- or injury-specific regulatory elements.

Different organs often utilize common genetic factors to facilitate regeneration (Qin et al., 2009; Sehring et al., 2016). Pursuing regulatory elements that become active in regenerating fin and heart tissues of zebrafish determined that a 3.18-kb upstream regulatory sequence of *connective tissue growth factor a* (*ctgfa*) drove the regeneration-specific expression in fin and heart (Fig. 2C) (Pfefferli and Jazwinska, 2017). Reporter lines harboring this regulatory sequence direct expression during development in the notochord, the lateral line systems (a sensory system in fish and amphibians on the body surface), certain junctional cells between bones and mesenchyme, and the outer heart layer (a primordial cardiac layer) (Chiou et al., 2006; Mateus et al., 2015; Pfefferli and Jazwinska, 2017). However, various injuries ectopically upregulated the reporter expression in the notochord, fin and heart. The upregulated reporter gene expression became reduced when regeneration was completed, suggesting the existence of regeneration-specific regulatory elements within this *ctgfa* upstream sequence. Further analysis demonstrated that there is a potential binding site of Smad3, a downstream effector of TGFβ/Activin-β signaling pathway, in the *ctfga* regulatory sequence and that activity of the *ctgfa* regulatory sequence is dependent on TGFβ/Activin-β signaling. These findings provide evidence of common regeneration-specific regulatory elements activated in multiple tissues.

Injury re-activates developmental genes used earlier in life, by which tissue regeneration is achieved. The existence of regeneration/injury-specific enhancers

suggests that different enhancers control the transcription of a subset of genes expressed in both developmental and regenerative contexts. Identifying the essential motifs of regeneration-specific enhancers, their binding partners and upstream regulators will uncover how injury signals are transformed to trigger regeneration programs.

Silencing of regeneration enhancers during maturation

The activity of regeneration enhancers declines during development or maturation. For instance, while damaged *Drosophila* imaginal discs remarkably regenerate in the early larval stage, they progressively lose the ability to direct the WNT gene expression upon injury and to recover the lost parts during the late larval instar 3 (L3) stage (Smith-Bolton et al., 2009; Harris et al., 2016). Although the BRV118 regeneration enhancer contains a regeneration-associated (or damage-responsive) module, the BRV118 includes another age-dependent silencing element. The regeneration-associated module within the BRV118 enhancer is mainly regulated by the JNK/AP-1 pathway to trigger *wg* and *wnt16* expression upon injury, facilitating regeneration in the early larval stage. However, during the late L3 stage, the BRV118 enhancer, but not WNT gene bodies, are marked by H3K27 tri-methylation (H3K27me3). a hallmark of epigenetic silencing. When a subset of BRV118 enriched by H3K27me3 is deleted, the H3K27me3 level throughout the whole BRV118 is significantly reduced and the BRV118 restores the ability to induce injury-dependent *wg* expression. In null mutants of the Polycomb repressive complex (PRC), a protein complex that primarily tri-methylates H3K27 (Cao et al., 2002), BRV118 restores its damage-dependent activation, suggesting that the PRC silences the regeneration enhancer through the interaction with the adjacent repressive domain during maturation

(Fig. 3). This epigenetic silencing during maturation implies that repression is one of the main mechanisms limiting the activation of regeneration genes and regenerative capacity in the adult stage.

Regeneration enhancers in cancer

The skin epidermis is continuously renewed under homeostatic conditions and rapidly healed upon injury through the activation of resident stem cells. Ge et al. isolated stem cell populations from uninjured and wounded skin and tumors from adult mice and performed ATAC-seq to identify global open chromatin landscapes of homeostatic, wound-induced, and tumorigenic stem cells (Ge et al., 2017). Comparing the ATAC-seq profiles of homeostatic and tumorigenic stem cells revealed distinct open chromatin landscapes in these two groups. In contrast, ATAC peaks and gene expression changes in wound-induced and tumorigenic stem cells are very similar, suggesting that the proliferative abilities of regenerating and tumorigenic skin originate from common mechanisms. While the ATAC peaks shared by wound and tumor cells are transient in wound-induced stem cells, they are not decommissioned in tumorigenic stem cells ("decommission" means that active enhancers return to a naïve state). Tumorigenic stem cells also exhibit additional ATAC peaks, which may represent oncogenic enhancers and tumor-specific features (Fig. 4). These results suggest that tumorigenic stem cells not only have their unique transcriptional networks, but also utilize regeneration-associated transcriptional programs to continuously proliferate.

In homeostatic uninjured skin, *Klf5* and *Sox9* are exclusively expressed in epidermal and hair follicle stem cells, respectively, and epidermal *Klf5* restricts the

epidermal lineage by suppressing *Sox9* expression. Upon injury, wound regulatory factors, such as *Ets2,* prevent *Klf5*-mediated *Sox9* suppression so that wound-induced stem cells produce both *Klf5* and *Sox9*. Dual expression of *Klf5* and *Sox9* is not only found in wound tissue but also in tumors. Similar to ATAC peaks, dual expression is transient in wound-induced stem cells but not in tumorigenic stem cells. Moreover, the tumor-specific ATAC peaks are enriched with KLF, Sox, and ETS2 binding motifs, highlighting that tumor cells can gain new regulatory networks by hijacking wound-induced regulatory factors. These results provide molecular evidence of the hijacking hypothesis that “cancer is a wound that never heals” (Ge and Fuchs, 2018). In summary, this study identified enhancer candidates that are responsible for skin wound repair and demonstrated how aberrantly regulated regeneration-associated regulatory mechanisms can be utilized for tumorigenesis.

Evolutionary perspective of regeneration enhancers

Emergence or loss of regeneration enhancers as a way to evolve regenerative capacities

Enhancers evolve rapidly, leading to phenotypic differences among species (Nord et al., 2013; Rebeiz et al., 2015; Villar et al., 2015; Long et al., 2016). For example, comparative analyses of limb and brain developmental enhancers have uncovered that modifications in functional enhancers can reconstruct transcriptional networks and subsequent disparities in phenotype (Boyd et al., 2015; Kvon et al., 2016; Leal and Cohn, 2016; Vermunt et al., 2016). Regeneration enhancers are also subject to rapid changes during evolution via positive or negative selection, contributing to diverse regenerative capacities across species. Positive selection could lead to the advent of new regeneration

enhancers, while negative selection could eliminate regeneration enhancers from the genome. Analysis of regions upstream of *leptin* in mammalian genomes revealed limited DNA sequence conservation to zebrafish *LEN*. As an alternative approach to finding functional TREEs in mammals, Kang et al. introduced zebrafish *LEN* into mice and examined its activity. Surprisingly, zebrafish *LEN* can direct injury-dependent gene expression in mammalian damaged hearts and amputated digit tips (Kang et al., 2016). This interesting observation suggests that TREEs may have been lost during evolution, although mammalian tissues retain injury-induced gene regulatory networks that could activate zebrafish TREEs. Thus, the evolutionary loss of TREEs might have influenced the deficiencies of regeneration in mammals.

Chromosome rearrangements such as inversion and translocation can separate (or join) two genetic loci, resulting in miscoupling between enhancers and their target genes. From this miscoupling, a new pattern of gene expression can emerge, which may lead to changes in phenotypes. This type of event has been reported in developmental disorders and tumors (Groschel et al., 2014; Herranz et al., 2014; Yamazaki et al., 2014; Giorgio et al., 2015; van den Heuvel et al., 2015). As shown by studies of the *Gata2* enhancer and *Evi1* transcription factor (Groschel et al., 2014; Yamazaki et al., 2014), chromosome rearrangements cause mis-localization of the *Gata2* enhancer close to *Evi1*, inducing ectopic EVI1 expression in hematopoietic cells. This new transcriptional circuit eventually alters the proliferative ability of hematopoietic cells, causing leukemia. Likewise, chromosome rearrangements in the course of evolution can create novel pairings or lead to the uncoupling of regeneration enhancers and genes, generating different transcriptional networks for tissue regeneration. It would be interesting to

investigate whether patterns of shared synteny or synteny breaks between species are associated with different regenerative capacities.

Epigenetic silencing to limit regenerative capacity

Epigenetic silencing is another molecular mechanism that may limit regenerative capacity. In the case of the *Drosophila* enhancer BRV118 (Harris et al., 2016), the active regeneration enhancer can be silenced by epigenetic suppressors via the adjacent repressive element during maturation (Fig. 3). Larval imaginal discs lose their regenerative ability in the L3 stage, the largest and final larval form (Tyler, 2000). L3 larvae molt into pupae in preparation for metamorphosis, which requires a large amount of energy. L3 larvae may allocate all available resources to prepare for metamorphosis rather than for regeneration, presumably because allocation to regeneration is not cost-effective. Thus, regenerative capacity could be lost as a trade-off for a more valuable event (in this case, metamorphosis), and epigenetic silencing may be a strategy employed by *Drosophila* larvae.

Transcriptional repressor-mediated regenerative failure is also reported in mammalian tissue. Although immature neurons in the mammalian central nervous system (CNS) robustly regenerate axons, neurons in adult CNS are unable to regenerate. One reason for regenerative failure in the CNS neurons is the increased expression of transcriptional repressors, such as *Klf4* and *Klf9* (Moore et al., 2009; Apara et al., 2017). In fact, *Klf4* and *Klf9* expression in retinal ganglion cells (RGCs) increases postnatally, suggesting their negative influence on regenerative ability. Overexpression of KLF4 can suppress axon growth, while conditional knock-out (KO) of *Klf4* in RGCs enhances axon

growth ability at embryonic stages and during axon regeneration in adult mice (Moore et al., 2009). In addition to *Klf4*, knock-down of *Klf9* in postnatal RGCs promotes optic nerve regeneration after injury. Notably, the strong expression of *Klf9* is not altered by optic nerve injury in adults, suggesting that the mammalian adult nerve is unable to de-repress the expression of regeneration suppressors (Apara et al., 2017). Uncovering the molecular mechanisms to inhibit suppressor expression could lead to the discovery of a therapeutic strategy to increase the intrinsic regenerative capacity of post-mitotic cells after injury.

Sun and colleagues recently provided evidence that manipulating the chromatin remodeling complex can be a promising approach to transcriptional de-repression of regenerative ability, thereby enhancing tissue repair after injury (Sun et al., 2016). *Arid1a* is a member of the SWI/SNF ATP-dependent chromatin remodeling complex, which functions to maintain the differentiated cell state (Edbrooke et al., 2011). *Arid1a* expression is lacking in neonatal mouse liver but present 10 days after birth and in adult mice. Interestingly, *Arid1a* expression is down-regulated in regenerating liver tissues, and liver-specific *Arid1a* KO mice exhibited improved regeneration after severe liver loss, demonstrating that *Arid1a* is a regeneration suppressor. Further analysis revealed that *Arid1a* loss alters the chromatin remodeling ability of the SWI/SNF complex by replacing *Arid1a* with *Arid1b*, a functionally distinct factor compared to *Arid1a* (Nagl et al., 2007). In uninjured liver, the ARID1A-SWI/SNF complex enables transcriptional access of the transcriptional factors, such as *Cebpa* and *Hnf4a,* to maintain terminally differentiated states. In injured tissues, ARID1A-SWI/SNF interacts with E2F4, a repressive factor of E2F (Chen et al., 2002), to inhibit the cell cycle and mitosis gene expression. In *Arid1a*-

deficient liver, however, attenuated SWI/SNF blocked C/EBPa and E2F4 from accessing chromatin in uninjured and injured tissues, respectively, resulting in altered target gene expression. In particular, *Arid1a* depletion in injured tissues derepressed the expression of cell cycle genes by inactivating the repressive factor *E2f4,* thus improving tissue repair. These results suggest that eliminating epigenetic suppressor could be a way to enhance regenerative capacity.

Conclusions

There are two major epigenetic approaches to enhancing regenerative capacity. First, introducing transcriptional activators of regeneration genes is a potential method to regain the regenerative capacity. Second, eliminating suppressors, which contribute to the maintenance of a more valuable attribute at the expense of regenerative capacity, may be another way to recover regenerative capacity. Although many regeneration-associated genetic factors have been uncovered, their regulatory mechanisms remain largely unknown. Elucidating these mechanisms will reveal fundamental insights into regenerative capacity and lead to the development of novel therapies for tissue regeneration.

Acknowledgements

We thank Jingli Cao for comments and editing on the manuscript. We apologize to authors whose important studies cannot be cited due to space constraints. This work was supported by the Vice Chancellor for Research and Graduate Education start-up fund (AAC6429), the UW-Madison Stem Cell and Regenerative Medicine Center start-up fund

(AAC8979), the School of Medicine and Public Health start-up fund (AAC8355), the Cell and Regenerative Biology start-up fund, and an American Heart Association grant (AHA16SDG30020001) to J.K.

References

Apara A, Galvao J, Wang Y, Blackmore M, Trillo A, Iwao K, Brown DP, Jr., Fernandes KA, Huang A, Nguyen T, Ashouri M, Zhang X, Shaw PX, Kunzevitzky NJ, Moore DL, Libby RT, Goldberg JL. 2017. KLF9 and JNK3 Interact to Suppress Axon Regeneration in the Adult CNS. J Neurosci 37:9632-9644.

Boyd JL, Skove SL, Rouanet JP, Pilaz LJ, Bepler T, Gordan R, Wray GA, Silver DL. 2015. Human-chimpanzee differences in a FZD8 enhancer alter cell-cycle dynamics in the developing neocortex. Curr Biol 25:772-779.

Braasch I, Gehrke AR, Smith JJ, Kawasaki K, Manousaki T, Pasquier J, Amores A, Desvignes T, Batzel P, Catchen J, Berlin AM, Campbell MS, Barrell D, Martin KJ, Mulley JF, Ravi V, Lee AP, Nakamura T, Chalopin D, Fan S, Wcisel D, Canestro C, Sydes J, Beaudry FE, Sun Y, Hertel J, Beam MJ, Fasold M, Ishiyama M, Johnson J, Kehr S, Lara M, Letaw JH, Litman GW, Litman RT, Mikami M, Ota T, Saha NR, Williams L, Stadler PF, Wang H, Taylor JS, Fontenot Q, Ferrara A, Searle SM, Aken B, Yandell M, Schneider I, Yoder JA, Volff JN, Meyer A, Amemiya CT, Venkatesh B, Holland PW, Guiguen Y, Bobe J, Shubin NH, Di Palma F, Alfoldi J, Lindblad-Toh K, Postlethwait JH. 2016. The spotted gar genome illuminates vertebrate evolution and facilitates human-teleost comparisons. Nat Genet 48:427-437.

Brockes JP, Kumar A, Velloso CP. 2001. Regeneration as an evolutionary variable. Journal of Anatomy 199:3-11.

Buenrostro JD, Giresi PG, Zaba LC, Chang HY, Greenleaf WJ. 2013. Transposition of native chromatin for fast and sensitive epigenomic profiling of open chromatin, DNA-binding proteins and nucleosome position. Nat Methods 10:1213-1218.

Cao J, Poss KD. 2018. The epicardium as a hub for heart regeneration. Nat Rev Cardiol.

Cao R, Wang L, Wang H, Xia L, Erdjument-Bromage H, Tempst P, Jones RS, Zhang Y. 2002. Role of histone H3 lysine 27 methylation in Polycomb-group silencing. Science 298:1039-1043.

Chen CR, Kang Y, Siegel PM, Massague J. 2002. E2F4/5 and p107 as Smad cofactors linking the TGFbeta receptor to c-myc repression. Cell 110:19-32.

Chiou MJ, Chao TT, Wu JL, Kuo CM, Chen JY. 2006. The physiological role of CTGF/CCN2 in zebrafish notochond development and biological analysis of the proximal promoter region. Biochem Biophys Res Commun 349:750-758.

da Silva SM, Gates PB, Brockes JP. 2002. The newt ortholog of CD59 is implicated in proximodistal identity during amphibian limb regeneration. Dev Cell 3:547-555.

Dickel DE, Ypsilanti AR, Pla R, Zhu Y, Barozzi I, Mannion BJ, Khin YS, Fukuda-Yuzawa Y, Plajzer-Frick I, Pickle CS, Lee EA, Harrington AN, Pham QT, Garvin TH, Kato M, Osterwalder M, Akiyama JA, Afzal V, Rubenstein JLR, Pennacchio LA, Visel A. 2018. Ultraconserved Enhancers Are Required for Normal Development. Cell 172:491-499 e415.

DiLeone RJ, Marcus GA, Johnson MD, Kingsley DM. 2000. Efficient studies of long-distance Bmp5 gene regulation using bacterial artificial chromosomes. Proc Natl Acad Sci U S A 97:1612-1617.

DiLeone RJ, Russell LB, Kingsley DM. 1998. An extensive 3' regulatory region controls expression of Bmp5 in specific anatomical structures of the mouse embryo. Genetics 148:401-408.

Edbrooke DL, Minelli C, Mills GH, Iapichino G, Pezzi A, Corbella D, Jacobs P, Lippert A, Wiis J, Pesenti A, Patroniti N, Pirracchio R, Payen D, Gurman G, Bakker J, Kesecioglu J, Hargreaves C, Cohen SL, Baras M, Artigas A, Sprung CL. 2011. Implications of ICU triage decisions on patient mortality: a cost-effectiveness analysis. Crit Care 15:R56.

Elkon R, Agami R. 2017. Characterization of noncoding regulatory DNA in the human genome. Nat Biotechnol 35:732-746.

Garza-Garcia AA, Driscoll PC, Brockes JP. 2010. Evidence for the local evolution of mechanisms underlying limb regeneration in salamanders. Integr Comp Biol 50:528-535.

Ge Y, Fuchs E. 2018. Stretching the limits: from homeostasis to stem cell plasticity in wound healing and cancer. Nat Rev Genet.

Ge Y, Gomez NC, Adam RC, Nikolova M, Yang H, Verma A, Lu CP, Polak L, Yuan S, Elemento O, Fuchs E. 2017. Stem Cell Lineage Infidelity Drives Wound Repair and Cancer. Cell 169:636-650 e614.

Geng J, Gates PB, Kumar A, Guenther S, Garza-Garcia A, Kuenne C, Zhang P, Looso M, Brockes JP. 2015. Identification of the orphan gene Prod 1 in basal and other salamander families. Evodevo 6:9.

Giorgio E, Robyr D, Spielmann M, Ferrero E, Di Gregorio E, Imperiale D, Vaula G, Stamoulis G, Santoni F, Atzori C, Gasparini L, Ferrera D, Canale C, Guipponi M, Pennacchio LA, Antonarakis SE, Brussino A, Brusco A. 2015. A large genomic deletion leads to enhancer adoption by the lamin B1 gene: a second path to autosomal dominant adult-onset demyelinating leukodystrophy (ADLD). Hum Mol Genet.

Goldberg AD, Banaszynski LA, Noh KM, Lewis PW, Elsaesser SJ, Stadler S, Dewell S, Law M, Guo X, Li X, Wen D, Chapgier A, DeKelver RC, Miller JC, Lee YL, Boydston EA, Holmes MC, Gregory PD, Greally JM, Rafii S, Yang C, Scambler PJ, Garrick D, Gibbons RJ, Higgs DR, Cristea IM, Urnov FD, Zheng D, Allis CD. 2010. Distinct factors control histone variant H3.3 localization at specific genomic regions. Cell 140:678-691.

Goldman JA, Kuzu G, Lee N, Karasik J, Gemberling M, Foglia MJ, Karra R, Dickson AL, Sun F, Tolstorukov MY, Poss KD. 2017. Resolving Heart Regeneration by Replacement Histone Profiling. Dev Cell 40:392-404 e395.

Green MC. 1958. Effects of the short ear gene in the mouse on cartilage formation in healing bone fractures. J Exp Zool 137:75-88.

Groschel S, Sanders MA, Hoogenboezem R, de Wit E, Bouwman BA, Erpelinck C, van der Velden VH, Havermans M, Avellino R, van Lom K, Rombouts EJ, van Duin M, Dohner K, Beverloo HB, Bradner JE, Dohner H, Lowenberg B, Valk PJ, Bindels EM, de Laat W, Delwel R. 2014. A single oncogenic enhancer rearrangement causes concomitant EVI1 and GATA2 deregulation in leukemia. Cell 157:369-381.

Guenther CA, Wang Z, Li E, Tran MC, Logan CY, Nusse R, Pantalena-Filho L, Yang GP, Kingsley DM. 2015. A distinct regulatory region of the Bmp5 locus activates gene expression following adult bone fracture or soft tissue injury. Bone 77:31-41.

Harris RE, Setiawan L, Saul J, Hariharan IK. 2016. Localized epigenetic silencing of a damage-activated WNT enhancer limits regeneration in mature Drosophila imaginal discs. Elife 5.
Herranz D, Ambesi-Impiombato A, Palomero T, Schnell SA, Belver L, Wendorff AA, Xu L, Castillo-Martin M, Llobet-Navas D, Cordon-Cardo C, Clappier E, Soulier J, Ferrando AA. 2014. A NOTCH1-driven MYC enhancer promotes T cell development, transformation and acute lymphoblastic leukemia. Nat Med 20:1130-1137.
Hewitt KJ, Katsumura KR, Matson DR, Devadas P, Tanimura N, Hebert AS, Coon JJ, Kim JS, Dewey CN, Keles S, Hao S, Paulson RF, Bresnick EH. 2017. GATA Factor-Regulated Samd14 Enhancer Confers Red Blood Cell Regeneration and Survival in Severe Anemia. Dev Cell 42:213-225 e214.
Hon GC, Rajagopal N, Shen Y, McCleary DF, Yue F, Dang MD, Ren B. 2013. Epigenetic memory at embryonic enhancers identified in DNA methylation maps from adult mouse tissues. Nat Genet 45:1198-1206.
Huang GN, Thatcher JE, McAnally J, Kong Y, Qi X, Tan W, DiMaio JM, Amatruda JF, Gerard RD, Hill JA, Bassel-Duby R, Olson EN. 2012. C/EBP transcription factors mediate epicardial activation during heart development and injury. Science 338:1599-1603.
Ito K, Morioka M, Kimura S, Tasaki M, Inohaya K, Kudo A. 2014. Differential reparative phenotypes between zebrafish and medaka after cardiac injury. Dev Dyn 243:1106-1115.
Jin C, Zang C, Wei G, Cui K, Peng W, Zhao K, Felsenfeld G. 2009. H3.3/H2A.Z double variant-containing nucleosomes mark 'nucleosome-free regions' of active promoters and other regulatory regions. Nat Genet 41:941-945.
Kang J, Hu J, Karra R, Dickson AL, Tornini VA, Nachtrab G, Gemberling M, Goldman JA, Black BL, Poss KD. 2016. Modulation of tissue repair by regeneration enhancer elements. Nature 532:201-206.
Kawakami Y, Rodriguez Esteban C, Raya M, Kawakami H, Marti M, Dubova I, Izpisua Belmonte JC. 2006. Wnt/beta-catenin signaling regulates vertebrate limb regeneration. Genes Dev 20:3232-3237.
King JA, Storm EE, Marker PC, Dileone RJ, Kingsley DM. 1996. The role of BMPs and GDFs in development of region-specific skeletal structures. Ann N Y Acad Sci 785:70-79.
Kumar A, Gates PB, Brockes JP. 2007a. Positional identity of adult stem cells in salamander limb regeneration. C R Biol 330:485-490.
Kumar A, Gates PB, Czarkwiani A, Brockes JP. 2015. An orphan gene is necessary for preaxial digit formation during salamander limb development. Nat Commun 6:8684.
Kumar A, Godwin JW, Gates PB, Garza-Garcia AA, Brockes JP. 2007b. Molecular basis for the nerve dependence of limb regeneration in an adult vertebrate. Science 318:772-777.
Kvon EZ. 2015. Using transgenic reporter assays to functionally characterize enhancers in animals. Genomics 106:185-192.
Kvon EZ, Kamneva OK, Melo US, Barozzi I, Osterwalder M, Mannion BJ, Tissieres V, Pickle CS, Plajzer-Frick I, Lee EA, Kato M, Garvin TH, Akiyama JA, Afzal V, Lopez-

Rios J, Rubin EM, Dickel DE, Pennacchio LA, Visel A. 2016. Progressive Loss of Function in a Limb Enhancer during Snake Evolution. Cell 167:633-642 e611.
Lagha M, Bothma JP, Levine M. 2012. Mechanisms of transcriptional precision in animal development. Trends Genet 28:409-416.
Lai SL, Marin-Juez R, Moura PL, Kuenne C, Lai JKH, Tsedeke AT, Guenther S, Looso M, Stainier DY. 2017. Reciprocal analyses in zebrafish and medaka reveal that harnessing the immune response promotes cardiac regeneration. Elife 6.
Leal F, Cohn MJ. 2016. Loss and Re-emergence of Legs in Snakes by Modular Evolution of Sonic hedgehog and HOXD Enhancers. Curr Biol 26:2966-2973.
Leveille N, Melo CA, Rooijers K, Diaz-Lagares A, Melo SA, Korkmaz G, Lopes R, Akbari Moqadam F, Maia AR, Wijchers PJ, Geeven G, den Boer ML, Kalluri R, de Laat W, Esteller M, Agami R. 2015. Genome-wide profiling of p53-regulated enhancer RNAs uncovers a subset of enhancers controlled by a lncRNA. Nat Commun 6:6520.
Liu SY, Selck C, Friedrich B, Lutz R, Vila-Farre M, Dahl A, Brandl H, Lakshmanaperumal N, Henry I, Rink JC. 2013. Reactivating head regrowth in a regeneration-deficient planarian species. Nature 500:81-84.
Long HK, Prescott SL, Wysocka J. 2016. Ever-Changing Landscapes: Transcriptional Enhancers in Development and Evolution. Cell 167:1170-1187.
Maginnis TL. 2006. The costs of autotomy and regeneration in animals: a review and framework for future research. Behavioral Ecology 17:857-872.
Mateus R, Lourenco R, Fang Y, Brito G, Farinho A, Valerio F, Jacinto A. 2015. Control of tissue growth by Yap relies on cell density and F-actin in zebrafish fin regeneration. Development 142:2752-2763.
Matias Santos D, Rita AM, Casanellas I, Brito Ova A, Araujo IM, Power D, Tiscornia G. 2016. Ear wound regeneration in the African spiny mouse Acomys cahirinus. Regeneration (Oxf) 3:52-61.
Mehta C, Johnson KD, Gao X, Ong IM, Katsumura KR, McIver SC, Ranheim EA, Bresnick EH. 2017. Integrating Enhancer Mechanisms to Establish a Hierarchical Blood Development Program. Cell Rep 20:2966-2979.
Moore DL, Blackmore MG, Hu Y, Kaestner KH, Bixby JL, Lemmon VP, Goldberg JL. 2009. KLF family members regulate intrinsic axon regeneration ability. Science 326:298-301.
Nacu E, Gromberg E, Oliveira CR, Drechsel D, Tanaka EM. 2016. FGF8 and SHH substitute for anterior-posterior tissue interactions to induce limb regeneration. Nature 533:407-410.
Nagl NG, Jr., Wang X, Patsialou A, Van Scoy M, Moran E. 2007. Distinct mammalian SWI/SNF chromatin remodeling complexes with opposing roles in cell-cycle control. EMBO J 26:752-763.
Newmark PA, Sanchez Alvarado A. 2002. Not your father's planarian: a classic model enters the era of functional genomics. Nat Rev Genet 3:210-219.
Nord AS, Blow MJ, Attanasio C, Akiyama JA, Holt A, Hosseini R, Phouanenavong S, Plajzer-Frick I, Shoukry M, Afzal V, Rubenstein JL, Rubin EM, Pennacchio LA, Visel A. 2013. Rapid and pervasive changes in genome-wide enhancer usage during mammalian development. Cell 155:1521-1531.

Nowoshilow S, Schloissnig S, Fei JF, Dahl A, Pang AWC, Pippel M, Winkler S, Hastie AR, Young G, Roscito JG, Falcon F, Knapp D, Powell S, Cruz A, Cao H, Habermann B, Hiller M, Tanaka EM, Myers EW. 2018. The axolotl genome and the evolution of key tissue formation regulators. Nature 554:50-55.

Osterwalder M, Barozzi I, Tissieres V, Fukuda-Yuzawa Y, Mannion BJ, Afzal SY, Lee EA, Zhu Y, Plajzer-Frick I, Pickle CS, Kato M, Garvin TH, Pham QT, Harrington AN, Akiyama JA, Afzal V, Lopez-Rios J, Dickel DE, Visel A, Pennacchio LA. 2018. Enhancer redundancy provides phenotypic robustness in mammalian development. Nature 554:239-243.

Petit F, Sears KE, Ahituv N. 2017. Limb development: a paradigm of gene regulation. Nat Rev Genet.

Pfefferli C, Jazwinska A. 2017. The careg element reveals a common regulation of regeneration in the zebrafish myocardium and fin. Nat Commun 8:15151.

Poss KD, Wilson LG, Keating MT. 2002. Heart regeneration in zebrafish. Science 298:2188-2190.

Qin Z, Barthel LK, Raymond PA. 2009. Genetic evidence for shared mechanisms of epimorphic regeneration in zebrafish. Proc Natl Acad Sci U S A 106:9310-9315.

Rebeiz M, Patel NH, Hinman VF. 2015. Unraveling the Tangled Skein: The Evolution of Transcriptional Regulatory Networks in Development. Annu Rev Genomics Hum Genet 16:103-131.

Sanchez Alvarado A. 2000. Regeneration in the metazoans: why does it happen? Bioessays 22:578-590.

Scadding SR. 1977. Phylogenic Distribution of Limb Regeneration Capacity in Adult Amphibia. Journal of Experimental Zoology 202:57-67.

Schubiger M, Sustar A, Schubiger G. 2010. Regeneration and transdetermination: the role of wingless and its regulation. Dev Biol 347:315-324.

Sehring IM, Jahn C, Weidinger G. 2016. Zebrafish fin and heart: what's special about regeneration? Curr Opin Genet Dev 40:48-56.

Seifert AW, Kiama SG, Seifert MG, Goheen JR, Palmer TM, Maden M. 2012. Skin shedding and tissue regeneration in African spiny mice (Acomys). Nature 489:561-565.

Shlyueva D, Stampfel G, Stark A. 2014. Transcriptional enhancers: from properties to genome-wide predictions. Nat Rev Genet 15:272-286.

Sikes JM, Newmark PA. 2013. Restoration of anterior regeneration in a planarian with limited regenerative ability. Nature 500:77-80.

Simkin J, Gawriluk TR, Gensel JC, Seifert AW. 2017. Macrophages are necessary for epimorphic regeneration in African spiny mice. Elife 6.

Smith-Bolton RK, Worley MI, Kanda H, Hariharan IK. 2009. Regenerative growth in Drosophila imaginal discs is regulated by Wingless and Myc. Dev Cell 16:797-809.

Stoick-Cooper CL, Weidinger G, Riehle KJ, Hubbert C, Major MB, Fausto N, Moon RT. 2007. Distinct Wnt signaling pathways have opposing roles in appendage regeneration. Development 134:479-489.

Sun X, Chuang JC, Kanchwala M, Wu L, Celen C, Li L, Liang H, Zhang S, Maples T, Nguyen LH, Wang SC, Signer RA, Sorouri M, Nassour I, Liu X, Xu J, Wu M, Zhao Y, Kuo YC, Wang Z, Xing C, Zhu H. 2016. Suppression of the SWI/SNF

Component Arid1a Promotes Mammalian Regeneration. Cell Stem Cell 18:456-466.
Tanaka EM. 2016. The Molecular and Cellular Choreography of Appendage Regeneration. Cell 165:1598-1608.
Tyler MS. 2000. Developmental Biology: A Guide for Experimental Study. MA: Sunderland. p 85-106.
Umesono Y, Tasaki J, Nishimura Y, Hrouda M, Kawaguchi E, Yazawa S, Nishimura O, Hosoda K, Inoue T, Agata K. 2013. The molecular logic for planarian regeneration along the anterior-posterior axis. Nature 500:73-76.
van den Heuvel A, Stadhouders R, Andrieu-Soler C, Grosveld F, Soler E. 2015. Long-range gene regulation and novel therapeutic applications. Blood.
Vermunt MW, Tan SC, Castelijns B, Geeven G, Reinink P, de Bruijn E, Kondova I, Persengiev S, Netherlands Brain B, Bontrop R, Cuppen E, de Laat W, Creyghton MP. 2016. Epigenomic annotation of gene regulatory alterations during evolution of the primate brain. Nat Neurosci 19:494-503.
Villar D, Berthelot C, Aldridge S, Rayner TF, Lukk M, Pignatelli M, Park TJ, Deaville R, Erichsen JT, Jasinska AJ, Turner JM, Bertelsen MF, Murchison EP, Flicek P, Odom DT. 2015. Enhancer Evolution across 20 Mammalian Species. Cell 160:554-566.
Yamazaki H, Suzuki M, Otsuki A, Shimizu R, Bresnick EH, Engel JD, Yamamoto M. 2014. A remote GATA2 hematopoietic enhancer drives leukemogenesis in inv(3)(q21;q26) by activating EVI1 expression. Cancer Cell 25:415-427.
Yokoyama H, Ogino H, Stoick-Cooper CL, Grainger RM, Moon RT. 2007. Wnt/beta-catenin signaling has an essential role in the initiation of limb regeneration. Dev Biol 306:170-178.
Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. 1994. Positional cloning of the mouse obese gene and its human homologue. Nature 372:425-432.

Figure Legends

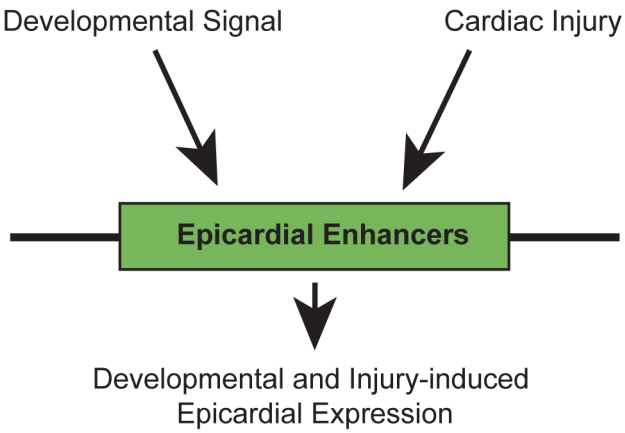

Figure 1. Enhancers shared between development and injury/regeneration

Epicardial enhancers of *Raldh2* and *Wt1* are activated during heart development. The same enhancer elements are activated by cardiac injury in damaged adult hearts.

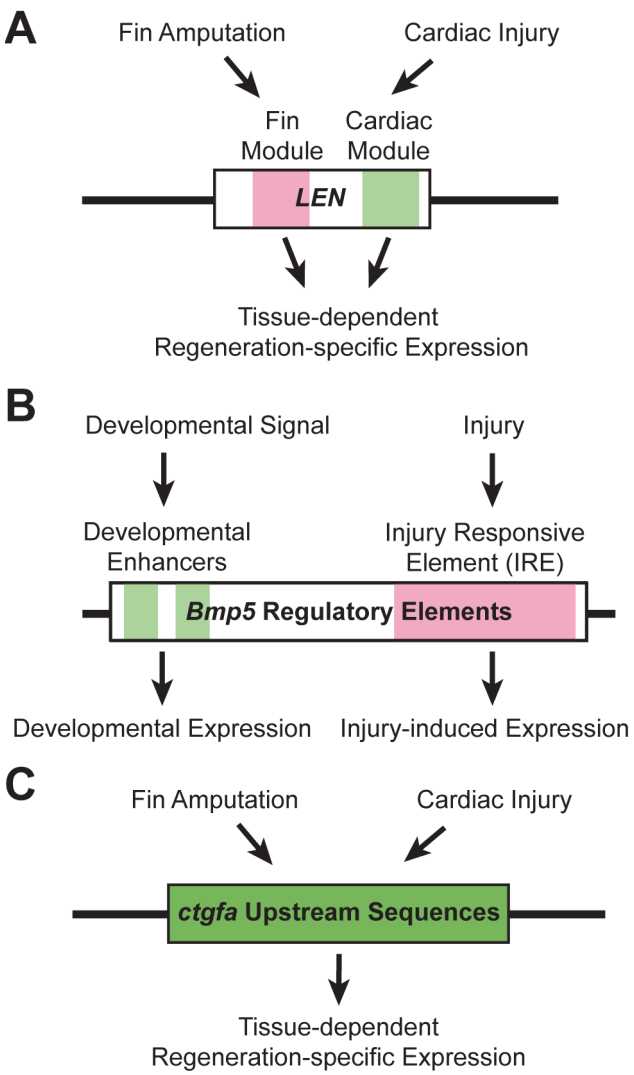

Figure 2. Regeneration-associated enhancers

(A) In zebrafish, the *lepb* TREE (*LEN*) contains tissue-specific regeneration modules that are activated by different injuries, including fin and heart injuries. *LEN* activity is not detected during development or in uninjured tissues. (B) In mice, developmental *Bmp5* expression is controlled by multiple separate regulatory enhancer elements. Injury-induced *Bmp5* expression is not driven by these developmental enhancers but is driven by *Bmp5* injury-responsive elements (IRE). (C) The zebrafish 3.18 kb *ctgfa* upstream sequence is activated by fin and cardiac injuries to direct strong regeneration-specific

expression. Note that this *ctgfa* regulatory element drives homeostatic expression in some cells of the uninjured fins and hearts. In addition, the endogenous *ctgfa* expression pattern does not match the reporter expression pattern of transgenic fish carrying this *ctgfa* regulatory element, suggesting that additional genomic sequences regulate endogenous *ctgfa* expression.

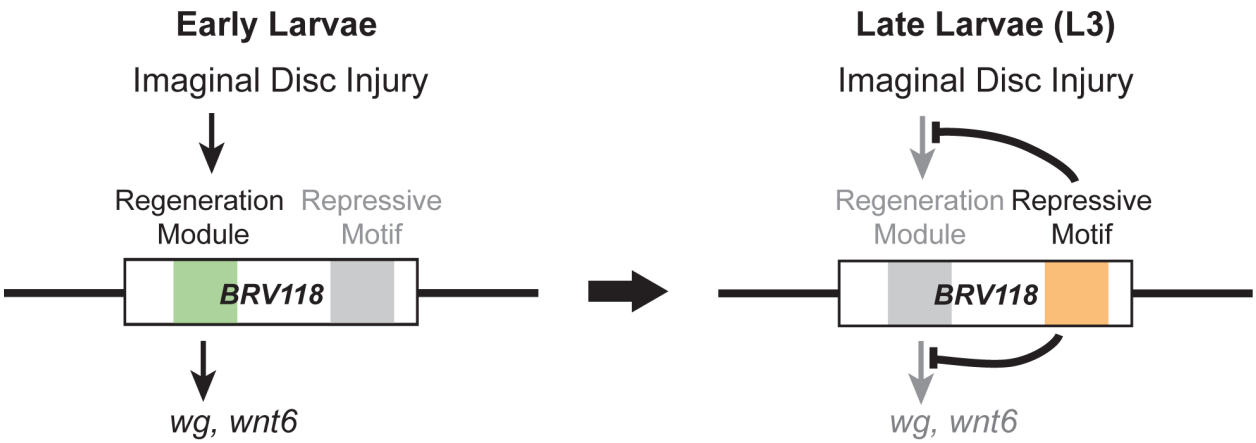

Figure 3. Silencing of regeneration enhancers during maturation

The regeneration enhancer BRV118 in *Drosophila* controls the expression of *wg* and *wnt6*, which allow the imaginal disc to regenerate lost tissues in the early larval stage. In the late larval stage (L3), silencing elements in BRV118 repress the activation of BRV118, resulting in a lack of *wg* and *wnt6* expression upon injury.

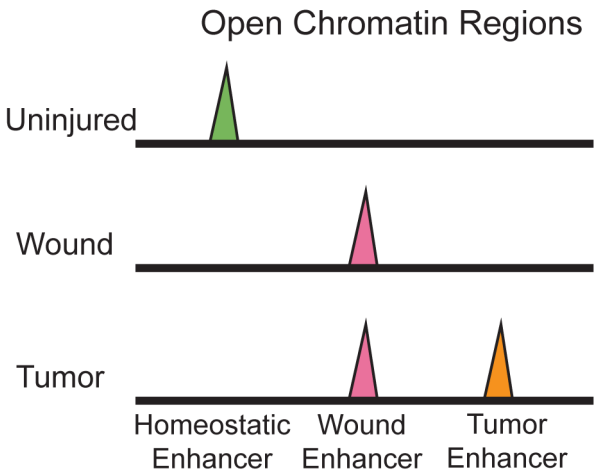

Figure 4. Regeneration enhancers in cancer

ATAC-seq peaks in uninjured, wounded, and tumorigenic skin stem cells of mice. Wounded and tumorigenic stem cells display similar global open chromatin profiles, but tumorigenic stem cells show additional tumor-specific ATAC-seq peaks.