Review

Epigenetic reprogramming during tissue regeneration

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\textbf{A B S T R A C T}

Epigenetic control of gene regulation is fundamental to the maintenance of cellular identities during all stages of metazoan life. Tissue regeneration involves cellular reprogramming processes, like dedifferentiation, re-differentiation, and trans-differentiation. Hence, in these processes epigenetic maintenance of gene expression programs requires a resetting through mechanisms that we are only beginning to understand. Here we summarize the current status of these studies, in particular regarding the role of epigenetic mechanisms of cellular reprogramming during tissue regeneration.

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1. Introduction

The capacity to regenerate lost or damaged body parts is very diverse among species in the animal kingdom, and the respective abilities can even change at different developmental stages in the same animal [1,2]. Among vertebrates, for example, urodele amphibians like newts and salamanders as well as some fish exhibit outstanding regeneration ability in many body parts including limbs or fins [3,4]. Anuran amphibians like frogs and toads are representative examples that change their regenerative ability during the development; in the African clawed frog, for example, the developing tadpole limbs can be completely restored upon amputation, whereas the froglet limbs cannot be reconstructed [5,6]. Regeneration is also observed in arthropods. Some insects and crustaceans, like crabs and shrimps, can regenerate legs and antennae. Nymphs of hemimetabolous insects such as cockroaches possess a remarkable ability to regenerate the amputated legs [7]. In arthropods the regeneration process is often associated with molting. In contrast, mammals have not retained similar extraordinary regeneration abilities for extremities or large organs [8]. Recent studies on cardiac muscle regeneration, for example, have revealed that the regenerative capacity of the embryonic and neonatal mouse heart is lost within the first week of postnatal life [9].

As demonstrated thorough studies on regeneration models like urodele amphibians, a successful regeneration generally involves (1) wound healing of epithelium after transection or injury, followed by (2) regeneration blastema formation consisting of the mass of regenerative progenitor cells, and (3) morphogenesis including differentiation and patterning to give rise to the appropriate structure and function of the damaged body parts. The regeneration blastema, which is the definitive characteristic of epimorphic regeneration, could be derived by dedifferentiation and proliferation of local tissues of the amputated limb stump, where some quiescent stem cells might also be sequestered [3,10].

Besides many pioneering studies based on extensive histological observation of regeneration processes, accumulating knowledge on the underlying molecular mechanisms has led to the concept that regeneration recapitulates the original development of the structures [11]. For example it has been shown that limb regeneration processes involve the reactivation of many genes originally expressed during limb development, yet, have diverse regeneration capacities [12,13]. However, vertebrates share almost identical mechanisms for limb development [14]. What, then, are the differences among the animals that either enable or prevent them from reutilizing the limb development program in regeneration?

Recent emerging technologies in stem cell biology, as for example represented by induced pluripotent stem (iPS) cells, have demonstrated that the cellular identity of differentiated mammalian cells can be artificially reprogrammed to the embryonic stem (ES) cell-like state, although mammals do not have a significant inherent regeneration ability [15]. The reprogramming factors are normally involved in both positive autoregulatory loops and
repressive activities preventing differentiation [16]. The orthologs of these factors exist also in fish and amphibians and recent studies have shown that some of them are expressed in regenerating appendages of newts and zebrafish [17,18]. Additionally, a functional assay knocking down two of the factors (Oct4 and Sox2) demonstrated their requirement for zebrafish fin regeneration [18]. During iPSC cell generation, the chromatin state of differentiated cells is reset to an embryonic type [19]. Many compounds and factors with properties to modify epigenetic control enhance the generation of iPSC cells [20–22], reflecting the importance of epigenetic reprogramming for the cellular reprogramming process. Regeneration induces considerable changes in the transcriptional programs of cells involved in the process. Many developmental and pattern signaling pathways need to be coordinately reactivated to allow for the appropriate reconstruction of lost tissue parts. Although the molecular mechanisms of regeneration are currently being studied in a variety of contexts in both vertebrates and invertebrates, the mechanistic details of epigenetic reprogramming during regeneration are only beginning to be understood.

This review we start by introducing epigenetic gene regulation mechanisms, with a focus on the role of Polycomb group (PcG) and Trithorax group (TrxG) proteins. We then summarize the current status of regeneration studies, particularly regarding mechanisms of epigenetic control and cellular reprogramming.

2. Epigenetic gene control

Control and maintenance of gene expression is not only dependent on regulatory circuits of transcription factors, but also on the epigenetic control – the heritable modulation of gene activity that is independent of the underlying DNA sequence. Epigenetic control mechanisms are fundamental in sustaining cellular identities in metazoa, which is for example required during growth processes. The importance of epigenetic gene control has now been well recognized for a wide variety of biological processes, including cell differentiation, stem cell plasticity, cell cycle control, dosage compensation, and stabilization of genome integrity [23–27]. Consistent with these pivotal roles, a growing number of human diseases including cancer or neurodegenerative diseases have been found to be associated with aberrant epigenetic control [28,29].

Epigenetic gene regulation involves the alteration of chromatin structure through histone modifications (e.g., methylation, acetylation) [29], exchange with histone variants [30], and DNA methylation [31]. In addition to histone modifications and DNA methylation, non-histone chromosomal proteins such as ATP-dependent chromatin remodelers including ISWI (imitation switch) and CHD (chromodomains–helicase–DNA binding) complexes, and HMG (high-mobility group) proteins are also essential controllers of chromatin structure and function [32,33]. These chromatin modifications and the interactions of non-histone chromosomal proteins are mitotically heritable, but still retaining the reversible characteristics allowing context-dependent changes of gene expression.

Many of the enzyme and protein complexes that write, erase, or read specific histone and DNA modifications have been extensively studied. Among the best-characterized regulators required to maintain cellular identities are the Polycomb group (PcG) and Trithorax group (TrxG) protein complexes. The PcG and TrxG proteins, respectively assemble into multimeric complexes and exert opposing gene regulatory functions [34,35]. The PcG proteins maintain the target genes in a silent state. Two major complexes called Polycomb repressive complexes 1 and 2 (PRC1 and PRC2) have been extensively characterized in the fruit fly Drosophila. PRC2 catalyses tri-methylation of lysine 27 of histone H3 (H3K27me3) by its enzymatic subunit Enhancer of zeste (E(z)) [36]. PRC1 can bind to H3K27me3 through the subunit Polycomb (Ps), and mono-ubiquitinates lysine 119 of histone H2A (H2AK119ub1) [37,38]. In contrast, the TrxG proteins counteract the PcG silencing to maintain the transcriptionally active state. In flies, among the most prominent members of the TrxG proteins are Trithorax (Trx) and Ash1 (absent, small or homeotic discs) that exert histone methyltransferase activity on lysine 4 of histone H3 (H3K4me3) [39,40].

PcG and TrxG complexes are recruited to their target genes through cis-regulatory DNA sequences called PcG response elements (PREs). Each PRE has the potential to drive epigenetic inheritance of active or inactive states, depending on their transcriptional history [41,42]. The active/inactive states are switchable by the change in the transcriptional status at the promoter [43] or by the change of balance of PcG and TrxG activities at either transcriptional or post-translational level [44,45]. Recent high-throughput mapping technology has shown genome-wide chromatin profiles of PcG and TrxG proteins and associated histone modifications. PcG and TrxG proteins target many developmental genes and locate close to their transcriptional start sites [35,46,47]. Moreover, these global pictures have provided new insight into the transcriptional state mediated by the PcG/TrxG system. At many of the target loci having zero-to-low levels expression, both PcG and Trx localized together at promoter regions containing stalled RNA polymerase II (RNA Pol II) [47,48]. It is known that many developmental control genes remain in a poised state by blocking (stalling) RNA Pol II at their promoter sites [49,50]. The dynamic equilibrium state of the poised chromatin seems to be easily displaced to become either active or permanently inactive by the balance of histone modification of the PcG/TrxG systems. The nature of the PREs as flexible switches is thought to be essential for their functions in various cellular transition processes during development [51].

Recent studies have shown that in mammalian embryonic stem (ES) cells more than 2000 lineage-specific developmental genes are simultaneously marked with the TrxG-mediated active H3K4me3 and PcG-mediated repressive H3K27me3 epigenetic modification [52–54]. The bivalency of subsets of the chromatin domains is retained also in more lineage-committed cells [52,55]. The bivalent chromatin domain is thought to play a role to predispose the cells to acquire a lineage specific expression upon differentiation cues. Interestingly, the bivalent H3K27me3/H3K4me3 mark is not found in the Drosophila genome, although many genes are simultaneously targeted by PcG and TrxG proteins [35,47,56]. DNA methylation is another major epigenetic modification, which involves a covalently attached methyl residue at the C-5 position of cytosine in the context of CpG dinucleotides. The CpG methylation is catalysed by DNA methyltransferases (DNMTs); de novo marked by DNMT3a/b, and maintained by DNMT1. The DNMT1/3 genes are conserved among most eukaryotes [57]. DNA methylation is associated with silenced chromatin by interfering with suppressive histone modifications. The methylated CpG dinucleotide is recognized by the proteins containing methyl-CpG binding domain (MBD), which recruit chromatin modifiers such as histone deacetylases or methyltransferases [58].

3. Drosophila imaginal discs as cellular reprogramming model

The fruit fly Drosophila, which belongs to holometabolous insects, is not able to regenerate damaged legs or wings in adults. However, the larval harbour regeneration abilities in primordial tissues called imaginal discs. Imaginal discs can regenerate to form normal adult appendages even after large lesions of disc cells are caused by X-rays irradiation at larval stages [59]. Regeneration of the imaginal discs is also observed under ex vivo conditions. When imaginal discs are manually fragmented, transplanted and cultured in the abdomen of an adult fly, where the immune-controlled
nutritious fluid (hemolymph) contains only low levels of ecdysteroids, the disc cells at the wound site undergo proliferation and regenerate the missing parts (Fig. 1A). Note that the imaginal disc cells consist of already differentially determined but undifferentiated cells until metamorphosis, hence they are not uniform cells. Each imaginal disc established during embryogenesis is destined to follow a specific developmental pathway, and the regional identities and specific cell fates in each disc are precisely determined in stepwise manner throughout the larval stage [60].

The steps in the regeneration process of fragmented imaginal discs are in principle analogous to those of amphibian limb regeneration, consisting of wound healing (closure), localized cell proliferation (regeneration blastema formation), and pattern formation [61–63]. Collectively, these observations demonstrate that disc regeneration induces limited cellular reprogramming, enabling the reconstitution of the lost tissue while the disc identity is maintained independently of the local environment.

Studies of imaginal disc regeneration have uncovered the process of ‘transdetermination’; i.e., neighbouring group of cells in transdetermination; i.e., neighbouring group of cells in regeneration blastema sometimes become more plastic and acquire alternative organ identities from different imaginal discs [64]. Intensive analyses on transdetermination performed in the 1960’s and 1970’s showed that all imaginal discs are capable to acquire alternative organ identities from different imaginal discs [61–63]. Collectively, these observations demonstrate that disc regeneration induces limited cellular reprogramming, enabling the reconstitution of the lost tissue while the disc identity is maintained independently of the local environment.

The healing at wound site involves the activation of Jun N-terminal kinase (JNK) signaling pathway in several rows of cells at the edge of the wound [72–74]. The blastema cells are derived from cells in which JNK has been activated [75]. When JNK signaling is impaired, the regeneration is blocked and transdetermination efficiency is reduced [67,74]. It was found that the JNK signaling pathway directly controls the down-regulation of PcG function. Clonal activation of the JNK pathway in imaginal discs was sufficient to reduce PcG-mediated silencing function, as visualized by ectopic expression of a Hox gene (N. Lee and RP, unpublished data). Hence, the down-regulation of PcG silencing by JNK signaling appears to result in a specific reactivation of PcG target genes, many of them being developmental regulators (Fig. 1B). However, it remains an open question how at the molecular level JNK signaling pathway leads to down regulation of PcG silencing in regeneration process.

4. Zebrafish fin regeneration: the importance of bivalent chromatin

Zebrafish, belonging to teleost fish, depicts remarkable regeneration ability in many tissues and organs, with caudal fin restoration being one of best-characterized models [4]. A recent study by Stewart et al. demonstrated that caudal fin cells maintain the bivalent chromatin domains at the promoter region of many genes involved in fin regeneration [76]. Bivalent domains were also found in non-regenerative tissue in zebrafish, suggesting that zebrafish may intrinsically keep particular regeneration-associated genes in a dormant state regardless of the regeneration ability of the tissue. Most of these bivalent domains of regeneration regulators were set towards an active state during fin regeneration.

The expression levels of JmjD3 (zf JmjD3 and zf JmjD3-like) were elevated during fin regeneration, whereas UTX (zf UTX and zf UTX-1) expressions remained almost constant [76]. The unique contribution of zf JmjD3 was suggested because the expression was detected only in regeneration blastema, whereas the other demethylases
were expressed both in the blastema and the epidermis of amputated fins. Fin regeneration was impaired when specific morpholino reagents against JmjD3 mRNA were applied. It appeared that JmjD3 and UTX do not demethylate H3K27me3 in a genome wide manner, but rather specifically target some loci depending on context [77,78]. UTX is found enriched at the transcription start sites of many Hox genes in human fibroblasts, where Hox genes are differentially expressed. However, UTX is selectively excluded from these loci in ES cells, where the Hox genes are silent [77]. A recent study on retinoic acid-induced neuronal differentiation of embryonic carcinoma cells has shown that the up-regulated JmjD3 is recruited to the Mash1 (neuronal marker gene) promoter by interacting with the transcriptional factor Hes1, and is demethylating H3K27me3 for a heritable Mash1 expression [78]. The bivalent domain of dbx4a (distal-less homeobox gene 4a) promoter was found to be a target of JmjD3 in fin regeneration. Although the transcriptional factors for dbx4a are still unknown, it is expected that JmjD3 will be recruited by such a factor to the bivalent dbx4a promoter to place the gene into an active state.

How might JmjD3 be activated in blastema cells? JmjD3 expression is activated upon a response to extracellular signals [70,79]. A recent study showed that JmjD3 is induced by the activation of Ras-Raf signaling [79]. The responsible cis-element for Ras-Raf mediated JmjD3 expression was mapped to a 70 bp DNA sequence in the JmjD3 promoter region. This element contains consensus binding sites for RAP1 (repressor activator protein 1), Sp1 (specificity protein 1), Krox-20/EGR2 (early growth response 2), Ets (transcription factor), C/EBPα (CCAAT enhancer binding protein α), and AP-1 (activator protein 1) [79]. Ras-Raf can activate downstream MAPK signaling via MEK (MAPK/ERK kinase) and ERK (extracellular signal-regulated kinase) [80]. Furthermore, ERK, JNK and p38 signaling is involved in the transcriptional and posttranslational regulation of AP-1 activity [81]. Taken together, Ras-Raf mediated MAPK signaling may have a function to induce JmjD3 through AP-1 and in regeneration, JNK signaling might be involved in the induction of JmjD3.

5. Epigenetic regulation of pattern formation during regeneration

Anuran amphibians like frogs and toads change their regenerative ability throughout development. For instance, in the African clawed frog Xenopus, the regeneration capability of developing tadpole limbs is gradually reduced with progress of metamorphosis, and froglets eventually restructure only a spike shaped structure from amputated limbs [5,6].

Accumulating knowledge on the underlying molecular mechanisms of the regeneration process has led to the theory that limb regeneration recapitulates the original limb development [11]. It has been shown that limb regeneration processes involve the reactivation of many genes originally expressed during limb development [12,13]. In vertebrate limb development, for example, the antero-posterior (AP) polarity is established by the localized expression of Sonic hedgehog (Shh) in the posterior margin of developing limb bud, which is known as the zone of polarizing activity (ZPA) [82]. The concentration gradient of Shh secreted from ZPA is the determinant for the limb AP pattern formation (e.g., digit identity). In limb regeneration, the polarizing Shh activity plays again essential roles in appropriate AP pattern formation. In newt and Axolotl limb regeneration Shh expression is reactivated at a group of the posterior cells after regeneration of blastema formation [83,84]. On the other hand, Xenopus froglet do not reactivate Shh in limb regeneration, resulting in forming only a non-patterned spike shape structure [13]. Therefore, the polarizing activity of Shh would have an important role that is linked to the regeneration ability of the limbs.

Recently, a study by Yakushiji et al. [85] shed light on the correlation between the extent of methylated CpG in the limb-specific shh enhancer region and shh reactivation in amphibian limb regeneration. The limb specific shh expression in both development and regeneration is regulated via a conserved long-range enhancer element, MFCS1 (mammal fish conserved sequence 1). It was found in mouse studies that the deletion of MFCS1 specifically abolishes shh expression in limb bud and causes severe defects only in limb morphogenesis [86]. MFCS1 is also conserved in amphibians [87]. Yakushiji et al. showed that in Xenopus froglet the MFCS1 is hypermethylated in the intact limb (84.3%) and also in the regenerating limb blastema (91.4%). In tadpole stage 53, when the amputated limb can be still fully regenerated, the methylation level of MFCS1 is low in the developing limb, especially in the posterior half (36.0%), where ZPA is included. At the same stage, in contrast, the MFCS1 was about two-fold more methylated in the anterior half of limb (70.7%), and almost fully in the eye (98.8%) and the heart (98.6%), where MFCS1 would never be used. The epigenetic states of target genes are regulated by the interplay of DNA methyltransferases, histone modification enzymes, and other chromatin regulators [58]. Indeed, further experiments using primary cultured cells from froglet limb blastema indicated that the hypermethylation of CpG in MFCS1 brings about the chromatin structural change by chromatin repressive complexes [88]. These results indicate the limb-specific shh enhancer MFCS1 is regulated by DNA methylation-mediated epigenetic mechanism and MFCS1 is gradually more methylated even in limb as development progresses and fixed in silenced state during metamorphosis.

What produces the difference in regenerative ability between urodele (e.g., newt, axolotl) and anuran (e.g., Xenopus) amphibians? In axolotl and newt, by contrast, the methylation levels of MFCS1 were kept at low-to-medium in the limbs (21.4% in axolotl, and 51.8% in newt), and remained at the same level in the regeneration blastema at 10 days after amputation (22.3% in axolotl, and 46.4% in newt) [85]. In urodele limb development, shh expression in ZPA is stopped by the two-to-three digit formation stage [83,84], and shh need not be reexpressed, unless limb amputation occurred. However, the weak DNA methylation states of MFCS1, which are still unknown, it is expected that JmjD3 will be recruited to the bivalent dbx4a promoter to place the gene into an active state.

6. Cellular reprogramming in regeneration: Does the capacity of cellular plasticity depend on the original tissue cell type?

Urodele amphibians such as newts and salamanders exhibit elevated regenerative ability in different types of body parts, like tail, limbs, jaws, lens, retina, spinal cord, intestine, and even parts of the brain [3,89]. They can rebuild a complete replica of the damaged part, which is morphologically and functionally indistinguishable from the original tissue. It has been a well ac-
cepted concept, based on histological analyses, that the tissue cells subjected to the emergency of regeneration undergo dedifferentiation to revert into pluripotent state and subsequently re-differentiate (trans-differentiate) to restore all the lost cell types [90]. One of the better-understood examples of trans-differentiation is lens regeneration in newts, where after lens excision, the pigment epithelial cells (PECs) of the tip of the dorsal iris dedifferentiate and give rise to a new lens [91]. However, the observation that the dorsal iris PECs trans-differentiate to lens even after being transplanted to regenerating limbs, suggests that PECs are not reverted to pluripotent cells but rather reprogrammed to a specific progenitor cell like state [92].

Limb regeneration is more complex because the blastema cells are formed as a result of dedifferentiation from a multitude of types of tissue cells at the wound site. In addition, to exactly restore the lost part of the limb, the three-dimensional positional information, especially along the proximal-distal (PD) axis, has to be read out and interpreted depending on the amputated position. Recently, Kragl et al. proposed that the cells in the blastema are not pluripotent, but heterogeneous progenitor cells with distinct, and largely restricted, regenerative abilities [93]. They accurately tracked the lineages of cells from each major tissue during axolotl limb regeneration. Among the tissue cells they examined, dermal cells turn out to be only multipotent, forming cartilages and tendons, but never switched the fate across embryonic germ layers, to become for example muscles. Other blastema cells derived from epidermal cells, muscles, Schwann cells, and cartilage cells, retained their original tissue identity in regeneration. These results suggest that the most of tissue cells maintain their original identity and hence cellular memory during regeneration.

Besides these fascinating findings, the cell lineage tracking experiments also provided new insights into the capacity of each limb tissue for reprogramming the positional identity along the PD axis during limb regeneration. It seems that positional information is also maintained differently in each tissue type and that this results in tissue specific cellular behaviour in limb regeneration.

During limb development, Meis, HoxA11, and HoxA13 expression specify the three major elements of the limb: upper-arm, lower-arm, and hand/foot-plate region, respectively. The orthologs of these genes in various species are regulated by the PcG and TrxG proteins. Hence, reactivation of these genes must involve an epigenetic reprogramming. When the limb was amputated at the upper-arm level, 95% of the cartilage-derived blastema still showed nuclear-localized Meis expression, although HoxA13 was detected in 15% of cartilage-derived blastema [93]. Consistent with these data, the cells of upper-arm cartilage origin largely located again at upper-arm skeleton in regenerated limbs. It was shown before that Meis expressing blastema cells are preferentially located in proximal region in regenerated limbs [94]. These results suggest that the majority of cartilage cells mostly remain in the original positional identity and do not disengage themselves from the home position. This is the case also in other parts of limb cartilages. If the finger cartilage is previously grafted onto the upper-arm, upon limb amputation at upper-arm level the finger-derived cartilage cells home to the restored hand-plate region. Therefore, the positional identity along the PD axis appears not to become substantially reprogrammed in cartilage derived blastema cells.

Then, what could rebuild the more distal skeletons when the limb was amputated at the upper-arm level? Surprisingly, large parts of distal cartilages (e.g., hand skeletons) were regenerated by dermis-derived blastema cells, as if dermal cells are able to cover the shortcoming of the cartilage cells [93]. In the dermal graft of upper-arm origin, more than 40% of the cells activated the HoxA13 expression and slightly over 25% of the cells retained the nuclear Meis expression (these ratios were estimated from data [bar chart] in [93]). The dermis cells and the cartilage cells have a common origin in an embryonic layer. Although the possibility of the existence and contribution of hidden reserve stem cells cannot be formally ruled out, the dermis-derived cells appear to be more plastic than cartilage-derived cells in the blastema. The regenerative ability of Axolotl would be in part attributed to the regenerative capacity of dermis-derived regenerating cells.

Similarly to dermis cells, regenerating muscle cells are capable of reprogramming the positional identity. In the regenerating muscle cells of upper-arm origin HoxA13 was detected in about 70% of cells, and Meis positive cells are only less than 30% (these ratios were estimated from data [bar chart] in [93]). Since muscle tissue contains reserve satellite cells, it can be assumed that the newly generated muscle progenitor cells may have substantial capacity to adopt the positional value.

Taken together, each limb tissue has a different potential to reprogram during limb regeneration, not only the cell type identity but also the positional identity (Fig. 2). These results suggest that the epigenetic state is differently maintained in each tissue type and that this results in tissue specific cellular behaviour during limb regeneration. New techniques will allow not only to track the cell fate, but also to isolate particular dedifferentiated progenitor cells and investigate their molecular and cellular characteristics through the regeneration process. For example, DNA methylation profile is specific to each cell type like a fingerprint, and it can be used to identify the cellular state. The epigenome profiling of each tissue type will provide further insights towards a better understanding of the cellular reprogramming process during regeneration.

### 7. Concluding remarks

Regeneration is the dynamic cellular reprogramming process including the formation of regeneration blastema and the subsequent reutilization of developmental programs to restructure the missing or damaged tissue part. Despite the longstanding studies in various regeneration models in order to elucidate the molecular and cellular mechanisms in regeneration, the underlying epige-
The gene expression in response to the emerging needs of tissue that can adapt the gene expression to needs of cellular regeneration (Fig. 3). We assume that in the animal state that can adapt the gene expression to needs of cellular regeneration, how long in their life the tissue cells maintain a flexible chromatin structure. It is likely that the regeneration abilities of animals depend on identifying the core features that distinguish the animals that are able to regenerate the lost body parts from those which cannot. It is likely that the regeneration abilities of animals depend on how long in their life the tissue cells maintain a flexible chromatin state that can adapt the gene expression to needs of cellular reprogramming during regeneration. On the other hand, other animals including human, which do not possess the regenerative potential or lose the ability with the progression of development, might drop the flexible chromatin state much sooner during development.

Tissues that encounter the need to regenerate do not revert their cells to a pluripotent cell state like the ES type [92,93]. Unlike the exogenous expression of reprogramming factors to induce iPS cells, the natural regeneration signals can reprogram the chromatin state of the tissue cells to more lineage-restricted progenitor states. The regeneration capacity of each progenitor cell is different in each cell type, indicating the flexibility of chromatin state must be cell type dependent. The poised chromatin state is the consequence of the cooperative epigenetic regulation by PcG and TrxG proteins. More genome-wide studies are required to define the extent and the functions of epigenetic remodelling during regeneration. It will be an important issue to understand epigenetic mechanism in regeneration, for example, how such a flexible chromatin state is maintained in some animals throughout development and adulthood.

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