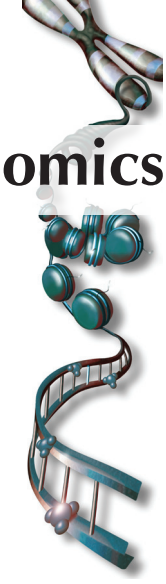
Review

*For reprint orders, please contact: reprints@futuremedicine.com*

Epigenomics



# Epigenetic regulation of axonal regenerative capacity

The intrinsic growth capacity of neurons in the CNS declines during neuronal maturation, while neurons in the adult PNS are capable of regeneration. Injured mature PNS neurons require activation of an array of regeneration-associated genes to regain axonal growth competence. Accumulating evidence indicates a pivotal role of epigenetic mechanisms in transcriptional reprogramming and regulation of neuronal growth ability upon injury. In this review, we summarize the latest findings implicating epigenetic mechanisms, including histone and DNA modifications, in axon regeneration and discuss differential epigenomic configurations between neurons in the adult mammalian CNS and PNS.

First draft submitted: 12 May 2016; Accepted for publication: 15 August 2016; Published online: 19 September 2016

**Keywords:** axon regeneration • DNA demethylation • DNA methylation • DNA methyltransferase • HDAC • histone deacetylase inhibitor • histone modification • TET family protein

Yi-Lan Weng[1,2], Jessica Joseph[1,3], Ran An[1,2], Hongjun Song[1,2,3,4] & Guo-li Ming*[,1,2,3,4,5]

[1]Institute for Cell Engineering, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA

[2]Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA

[3]Graduate Program in Cellular & Molecular Medicine, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA

[4]The Solomon H Snyder Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA

[5]Department of Psychiatry & Behavioral Sciences, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA

*Author for correspondence: gming1@jhmi.edu

Successful axon regeneration hinges on the growth competence of injured neurons and a permissive environment that enables severed axons to regrow and recognize their appropriate synaptic targets. The intrinsic growth capacity of neurons in both the PNS and CNS depends on gene expression that supports growth, which normally declines during neuronal maturation [1]. For example, prenatal immature CNS neurons in retina, brainstem and cerebellum exhibit robust axon regeneration, but these neurons possess a limited ability for axonal growth after birth [2–4]. In parallel with this shift in regenerative capacity, gene-expression profiling of retinal ganglion cells (RGCs) over the course of development reveals distinct transcriptomes between embryonic and adult stages, suggesting that changes of a transcriptional program may control the developmental loss of the intrinsic growth ability [5]. Indeed, transcription factors (TFs) in the Krüppel-like factor (KLF) family are developmentally regulated and have been shown to modulate regenerative potential in adult CNS neurons [6,7]. Given the observed global gene-expression changes and the need for TFs to gain access to suppressed genomic loci, epigenetic mechanisms that can modulate chromatin may play a pivotal role in determining the regenerative capacity in CNS neurons. In support of this notion, several epigenetic modifications, including histone acetylation and methylation, and DNA methylation, have been shown to exhibit dynamic patterns during RGC development [8–10]. While these epigenetic changes have been well documented to regulate cell fate determination and maintain cell function and survival in adults, recent studies suggest that manipulation of epigenetic states also enables adult RGCs to regain growth competence, highlighting the importance of epigenetic regulation in reprogramming the neuronal growth state and axon regeneration [9,11].

Future Medicine part of fsg

Although the expression of genes promoting growth in mature neurons decreases over time in both the PNS and CNS, adult PNS neurons are able to regain growth competence via transcriptional activation of a large repertoire of regeneration-associated genes (RAGs) upon injury [12,13]. Genome-wide profiling in axotomized PNS neurons have led to the hypothesis that injury-induced activation of specific TFs may serve as key hub components in gene regulatory networks that switch PNS neurons into a regenerative and growth state [14,15]. These TFs include CREB, c-Jun, Smad1, STAT3 and ATF3. Reactivation of individual TF-RAGs, however, has been shown to only marginally increase the intrinsic growth capacity, leading to modest axon regeneration in the adult CNS [16–18]. These observations illustrate that a robust regenerative response may require coordination of multiple transcriptional regulatory pathways to establish a pro-regenerative program. Ongoing work has begun to reveal how epigenetic modifications interact with TFs to contribute to differential injury responses in the PNS and CNS. Understanding epigenetic mechanisms responsible for regulating regenerative capacity and developing strategies to reprogram neurons into a regenerative state will provide another route to enhance axon regeneration in a variety of neurological disorders, including traumatic brain injury, spinal cord injury and stroke [19]. Here, we first provide a brief overview of epigenetic responses to nerve injury and highlight distinct epigenomic configurations between mammalian neurons in the adult CNS and PNS, and then we discuss in detail various epigenetic mechanisms that can be harnessed to promote axon regeneration in CNS injury.

## Histone modifications

The distribution of dynamic histone modifications across the genome defines discrete chromatin regions and TF accessibility [20]. Histones are grouped by eight subunits into a nucleosome, which consists of two units each of H2A, H2B, H3 and H4. Each subunit has an associated N-terminus tail that can be modified on certain residues. Lysine modifications include methylation and acetylation, while serine can be phosphorylated. Each modification changes the configuration of histone, which then alters DNA accessibility. The complexity of these modification interactions is staggering; not only do individual histone modifications affect the genes immediately surrounding the area, but they can affect distant genes as well. Coordinated patterns of histone modifications could in principle constitute a regulatory circuit that temporally controls gene expression of RAGs to enable the regenerative capacity. Among different histone modifications, histone acetylation is the most well-studied in regard to axonal regeneration.

### Histone acetylation & CNS regeneration

Epigenetic information encoded by histone modifications is regulated by three classes of regulatory proteins: 'writers' that attach modifications to histones; 'erasers' that remove modification for reversible regulation; and 'readers' that interpret the epigenetic codes. In recent years, there has been a rapid advance in our knowledge about the involvement of histone acetylation in neuronal plasticity, memory and neurodegenerative disorders [21]. The status of histone acetylation is determined by the opposing activities of histone acetyltransferase (HAT) and histone deacetylase (HDAC) and is shown to exhibit regulatory roles in gene regulation. In general, the presence of histone acetylation mediated by HATs increases chromatin accessibility for transcription factor binding, resulting in gene activation, whereas histone deacetylation induced by HDACs yields a more compact chromatin structure and represses gene activity (Figure 1). In mammalian cells, the HAT family is comprised of three subfamilies: the GNAT-family, the MYST-family and p300/CBP (CREB-binding protein) [22].

Mammalian HDACs are subdivided into four subfamilies (class I-IV) according their domain organization and homology [23]. Expression of HDACs exhibits temporally and spatially distinct patterns in the developing CNS, suggesting they may have regulatory roles in neuronal development and maturation [24]. One intriguing question is whether histone acetylation exerts transcriptional regulation over RAGs and in part governs the intrinsic growth capacity during neuronal maturation. Investigation of H3K9 and H3K14 acetylation in purified cortical and cerebellar neurons reveals that the level of histone H3 acetylation is developmentally downregulated [25]. Inhibition of deacetylation by an HDAC inhibitor, trichostatin A (TSA) can induce histone H3K9/14 hyperacetylation, resulting in *Gap43* gene expression and axon outgrowth *in vitro* [25]. Notably, TSA causes transcription-dependent effects on neurite outgrowth because Actinomycin D, a transcriptional inhibitor, blocks these effects. Thus, HATs/HDACs dictate diverse histone acetylation patterns to control gene expression, and are likely playing an important role in regulating intrinsic axon growth capacity in CNS neurons.

The direct role of HATs in epigenetic transcriptional regulation in neuronal regenerative capacity has been the subject of several recent studies. Exogenous expression of the histone acetyltransferases p300, CBP and P/CAF drives neurite outgrowth in primary neurons *in vitro* [25]. Additionally, overexpression of p300 results in axon

regeneration, but not RGC survival, after optic nerve crush *in vivo* [9]. Chromatin immunoprecipitation from injured retina tissue with p300 overexpression further reveals increased occupancy of p300 and histone acetylation on the promoters of proregenerative gene targets, including *Gap43*, *Coronin 1b* and *Sprr1*. Importantly, direct promoter occupancy and modulation of histone acetylation are associated with elevated levels of gene expression. Together, these findings suggest that manipulation of epigenetic states at the chromatin level may be able to reactivate a silenced developmental program and allow mature neurons to regain their growth capacity.

Different types of neurons may employ distinct epigenetic regulators to control their regenerative programming. Reticulospinal neurons (RS) in the lamprey brain exhibit heterogeneous regenerative abilities after spinal cord injury. A recent study characterizing those regenerative RS neurons revealed that *HDAC1* is downregulated at 2 weeks and 4 weeks after spinal cord injury, consistent with the notion that increased histone acetylation is important for CNS regeneration [26]. Interestingly, *HDAC1* exhibits temporally dynamic expression patterns, but distinct expression levels in low- and high-regenerative capacity RS

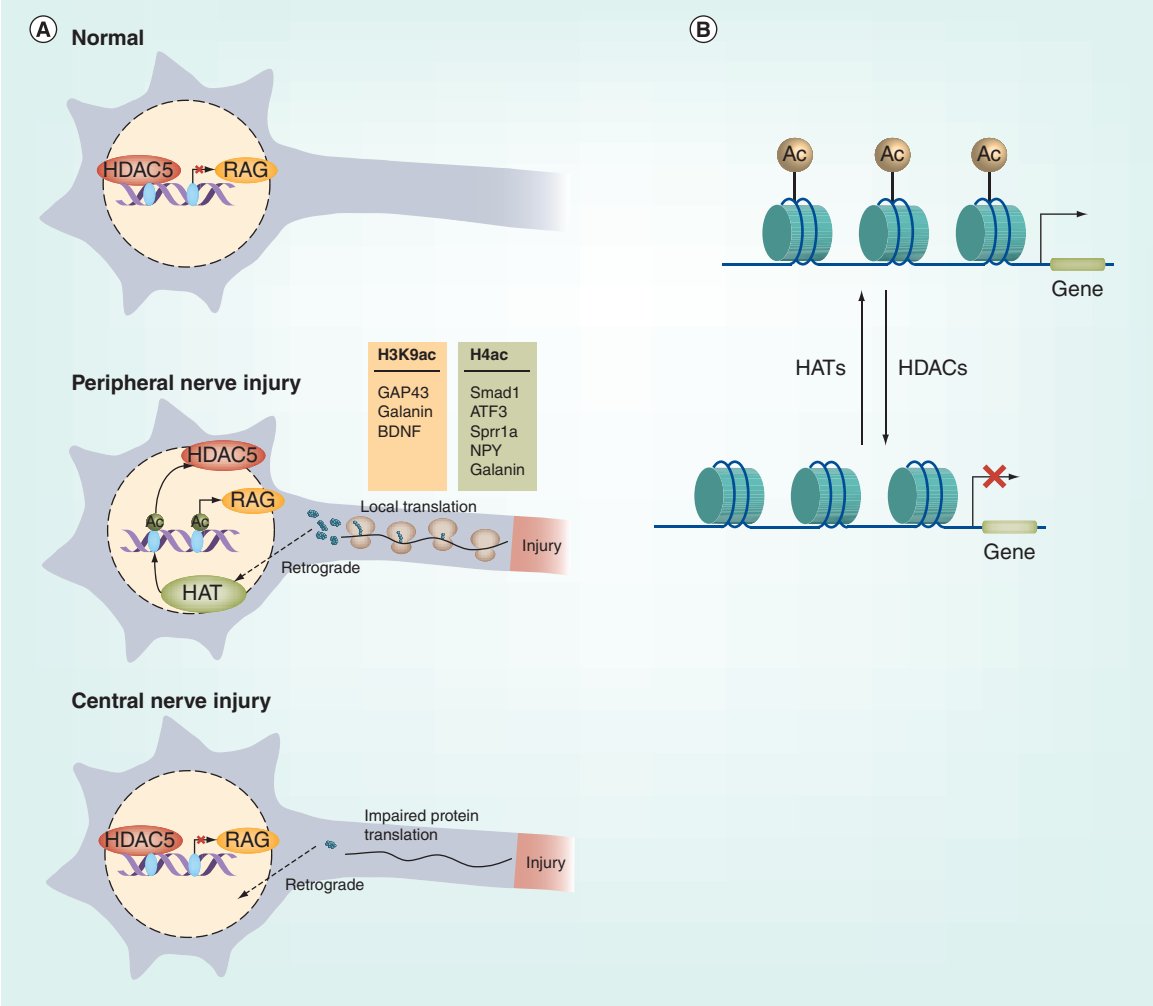



**Figure 1. Histone and DNA modifications modulate expression of regeneration-associated genes. (A)** RAGs are expressed minimally in mature neurons in PNS and CNS. Upon injury, locally translated proteins play an important function in signaling axon regeneration by relaying injury information to the cell body. In peripheral nerve lesions, retrograde injury signals can influence HAT and HDAC5 activity, leading to a distinct epigenetic landscape and RAG expression. In contrast, failure to induce a regenerative program after central nerve lesion can result from impaired local mRNA translation and a non-permissive epigenome for the expression of RAGs. **(B)** HATs and HDACs regulate histone acetylation patterns to remodel chromatin architecture. Induction of a 'loose or open chromatin' state by histone acetylation can increase DNA accessibility to transcriptional regulatory proteins and consequently lead to gene activation.
Ac: Acetyl modifications; HAT: Histone acetyltransferase; HDAC: Histone deacetylase; RAG: Regeneration-associated gene; TET: Ten-eleven translocation methylcytosine dioxygenase.

neurons. In particular, elevated HDAC1 at 10 weeks post-spinal cord injury is only observed in high regenerative-capacity RS neurons. These findings suggest that dynamic epigenetic modifications are required to fine-tune gene-expression programs for better growth capacity. Future studies will be needed to better understand how HATs and HDACs coordinate to define gene-expression pattern during and after axonal injury.

## Histone acetylation in PNS regeneration

After axonal damage, PNS neurons exhibit an intrinsic capacity to regrow whereas CNS neurons exhibit poor regenerative ability. What are the key modulators that determine the differential injury responses between CNS and PNS neurons? Dorsal root ganglion (DRG) neurons are unique in that they have both central and peripheral axonal projections. Interestingly, peripheral axon branch lesions, but not central axon branch lesions, increase global acetylation of histone H3 and H4 in DRG neurons (Figure 1A) [27,28]. *In vitro*, axonal injury of DRG neurons induces a back-propagating calcium wave to soma, which, in turn, elicits nuclear export of HDAC5 and leads to augmentation of acetylated H3 and stimulates gene expression [29]. Among these HDAC5-dependent genes, several are known TF-RAGs, such as *Jun*, *Fos* and *Klf*. This study suggests an intriguing model that translocation of HDAC5 may play an important role in shaping the epigenetic landscape to initiate a regenerative program. Axotomized CNS neurons, on the contrary, appear to be unable to establish such a mechanism, suggesting potential differences in changing the epigenetic states of CNS and PNS in responses to injury (Figure 1A) [29].

In addition to chromatin remodeling and gene regulatory activity in the nucleus, several HDAC members, such as HDAC5, HDAC6 and SIRT2, have been identified to have to cytoplasmic function in deacetylating tubulins and microtubules and regulate axon outgrowth in a context-dependent manner [30]. For example, elevated HDAC5 after peripheral lesion results in tubulin deacetylation proximal to the injury site, thereby destabilizing the microtubules [31]. As a result of the decreased stability, this paradigm encourages growth cone dynamics and axon regeneration. To address how HDAC5 is transported to the tips of injured axons, a recent study identified that Filamin A, an actin-binding protein organizing the actin filaments into an orthogonal network, is capable of binding HDAC5 *in vitro*. Further *in vivo* experiments demonstrated that Filamin A is locally translated in the injured axons, and its interaction with HDAC5 is important for tubulin deacetylation and axonal outgrowth [32]. By contrast, HDAC6 does not play a prominent role in tubulin deacetylation or in regulation of the intrinsic growth capacity in DRG neurons [31]. Instead, HDAC6 is a key effector for mediating the inhibition of neurite extension when DRG neurons are cultured in the presence of inhibitory substrates, such as MAG or CSPG [33]. Consistently, pharmacological inhibition of HDAC6 promotes neurite outgrowth on inhibitory substrates. Additional investigation are needed to determine whether the beneficial effects of HDAC6 inhibitors involve changes of the epigenetic landscape to encourage neurite outgrowth.

In search of key histone modifications that could contribute to regenerative program activation, ChIP assays reveal that H3K9ac is enriched in promoters of a subset of RAGs and positively correlates with gene expression. In conjunction with the elevated level of H3K9ac, PCAF, an H3K9ac-specific acetyltransferase, is upregulated upon peripheral lesion and recruited to promoters of RAGs with enriched H3K9ac (Figure 1A). The instrumental role of H3K9ac in regulating regenerative capacity has been further shown by overexpression of PCAF in DRGs, where neurons without a preconditioning lesion can initiate a regenerative program and induce axonal regeneration in spinal cord [27]. Given the selective H3K9ac enrichment in only a subset of RAGs, additional epigenetic regulation is likely to exist, such as changes of DNA epigenome or additional histone modifications. Indeed, peripheral lesion leads to enrichment of histone H4 acetylation (H4ac) on another repertoire of RAGs that predominantly do not have H3K9ac enrichment [28]. Augmented H4ac also appears to correlate with gene activity; application of MS-275, an HDAC1-specific inhibitor, sufficiently increases H4ac levels, concomitant with the induction of several RAGs. It is worth noting that MS-275 also increases histone H3 acetylation. Thus, whether increased H4ac induced by peripheral lesion or by MS-275 exerts an instructive role in regulating RAGs requires further investigation.

## Nerve injury signaling & epigenetic switches

Upon injury, changes in cellular state require injury signals to be relayed to the soma to elicit differential gene expression. Several mechanisms have been found to regulate retrograde injury signaling. These include $Ca^{2+}$ influx, local synthesis and retrograde of axoplasmic proteins, and loss of trophic substances from the periphery [34]. Elevated $Ca^{2+}$ activates multiple signaling cascades to initiate regeneration. For instance, $Ca^{2+}$ is known to activate adenylate cyclase to increase intracellular cAMP levels and subsequently lead to CREB-dependent gene expression [35]. In addition to regulating activators of transcription, $Ca^{2+}$ signaling can alter epigenetic states to reshape the transcriptome. Studies in non-neuronal cell types have shown that elevated $Ca^{2+}$

can promote nuclear export of HDAC4/5/7/9 by activation of CaMKs [36]. Indeed, the calcium-responsive nuclear export of HDAC5 is found after peripheral axotomy and increases histone acetylation in DRG neurons to initiate regenerative gene expression *in vitro* [29].

Several proteins synthesized or activated by axonal lesion can act as injury signaling components, but need to be transported to the cell body to increase intrinsic growth capacity. These include STAT3, JNK, MAPKs and other kinases. These injury signals can activate downstream TFs through complex pathways to change gene-expression patterns in injured neurons. For example, retrograde transport of phosphorylated ERK1/2 activates ELK1, while JNK leads to c-JUN phosphorylation and ATF3 induction [37]. It is not known how the arrival of injury signals reorganizes the transcriptional hierarchy to establish axon growth competence in the neurons. One possibility is that epigenetic configurations are more amenable to change by specific signaling cascades to allow temporal control of gene expression. In support of this notion, recent data have shown that ERK-mediated retrograde signaling is required for PCAF-mediated histone acetylation on promoters of several RAGs [27]. Future studies are needed to determine whether other signaling pathways are responsible and how these signals are interpreted for transcriptional changes upon injury.

Differential responses to injury between the PNS and CNS could be due to cell-specific epigenomes that induce regenerative pathways in PNS cells and apoptotic pathways in CNS cells. Several sets of data have emerged to support this notion. For example, in contrast to nuclear export of HDAC5 in DRG neurons, nuclear translocation of HDAC3 was found in retinal ganglion cells (RGCs) following nerve injury [38]. Nuclear localization of HDAC3 and the lack of PKCμ phosphorylation for induction of nuclear export of HDAC5 in axotomized RGC neurons consequently lead to widespread histone deacetylation that is thought to encode a different transcriptome for injury responses [29,38]. Furthermore, protein synthesis is diminished after CNS injury, which may impair generation of injury signals. As retrograde injury signaling can in principle change the behavior of some epigenetic modifiers, absence of proper injury signals may also confer different configurations of the epigenome. To better understand how epigenetic mechanisms regulate growth capacity, further studies are necessary to discover the different epigenetic landscapes between the CNS and PNS in the context of nerve injury and axon regeneration.

## DNA modifications

DNA methylation landscapes, known as methylomes, are distinct in different cell types and developmentally regulated. Originally, 5-methylcytosine (5mC) in the mammalian genome was considered to be a stable repressive DNA modification to downregulate gene expression. With the development of new technologies allowing genome-wide profiling of modified DNAs, recent studies have revealed that 5mC exhibits complex regulatory roles in gene expression, and its function is dependent on the genomic position of modifications, such as the promoter, gene body, regulatory elements or intergenic regions [39,40]. For example, methylation in promoter regions represses gene transcription whereas methylation in the gene body positively correlates with expression levels and modulates alternative splicing in specific cell types (Figure 2A) [41,42]. Owing to its important role in regulating cell type-specific gene expression, genomic imprinting and other biological processes, aberrant regulation or recognition of DNA methylation has been associated with many human diseases, including disorders in the nervous system [43].

### DNA methylation & regeneration

Epigenetic information encoded by DNA methylation patterns requires specialized enzymes that add ('writers') or remove ('erasers') modifications to particular genomic loci. Cognate binding proteins, termed 'readers', can bind to epigenetically modified DNA sequences and translate this information to downstream cellular pathways and biological processes. Establishing and maintaining the mammalian DNA methylome is catalyzed by the DNA methyltransferase family proteins: DNMT1, DNMT3a and DNMT3b (Figure 2). During DNA replication, DNMT1 adds methyl groups to hemimethylated CpGs on the nascent strand, maintaining methylation status over multiple cell divisions. By contrast, DNMT3a and DNMT3b are responsible for *de novo* DNA methylation regardless of the methylation state [43]. In particular, DNMT3a has been shown to methylate nonCpGs in mammalian neurons [44]. These DNMTs cooperatively shape the DNA methylation landscapes in a cell type-specific manner. Notably, neurons abundantly express these DNA methyltransferases, albeit at different levels in different brain regions. This raises the possibility that DNMTs are capable of dynamically changing neuronal DNA methylation patterns in response to extrinsic stimuli and conferring plasticity in the nervous system. Indeed, a recent study has shown that the expression level of Dnmt3b is altered under chronic cocaine exposure or chronic stress, leading to changes in both neuronal gene expression and synaptic function [45].

Gene expression is regulated at multiple levels after nerve injury. DNA methylation dynamics constitute a regulatory unit in gene reprogramming and regenerative responses. An intriguing study in a rodent model

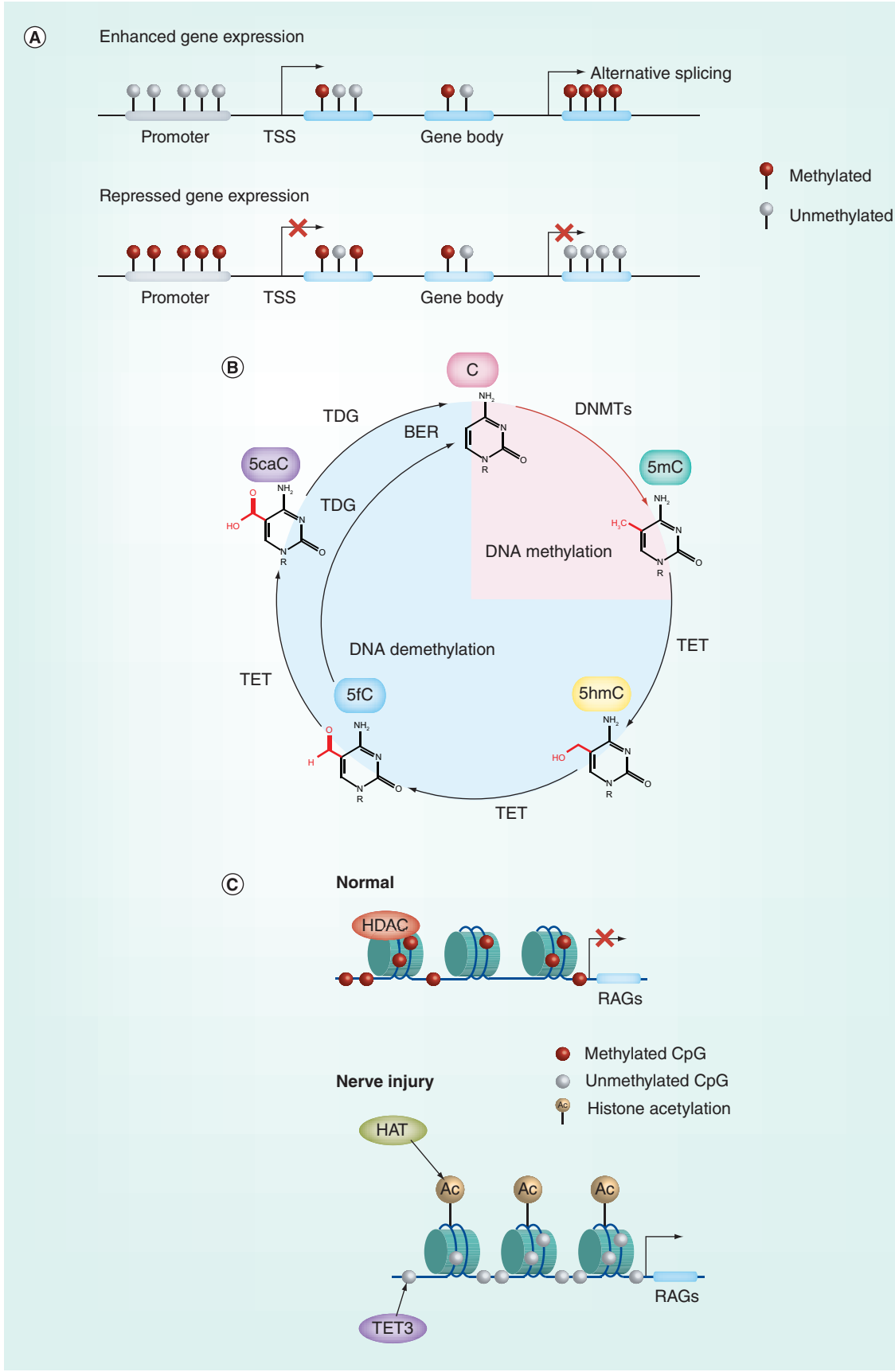

A
Enhanced gene expression
Promoter
TSS
Gene body
Alternative splicing
Repressed gene expression
Promoter
TSS
Gene body
Methylated
Unmethylated
B
C
TDG
BER
DNMTs
5caC
5mC
TDG
DNA methylation
DNA demethylation
TET
TET
5fC
5hmC
TET
C
Normal
HDAC
RAGs
Methylated CpG
Unmethylated CpG
Histone acetylation
Nerve injury
HAT
Ac
Ac
Ac
RAGs
TET3

**Figure 2. Functions of DNA methylation and histone acetylation. (A)** 5mC exerts distinct regulatory roles on gene activity depending on DNA methylation patterns. Promoter hypermethylation is usually associated with gene silencing. Methylation in the gene body is positively correlated with gene activity and can induce alternative splicing. **(B)** TET family proteins catalyze iterative oxidation of 5mC, yielding different 5mC derivatives (5hmC, 5fC and 5caC). TDG can recognize 5caC and elicit BER pathway activation replacing 5caC with unmethylated cytosine. **(C)** Potential coordinated roles of DNA demethylation and histone modifications in the activation of RAGs. Under normal conditions, DNA methylation and condensed chromatin represses RAG expression. Upon injury, DNA demethylases, such as TET proteins, may remove DNA methylation of expanded chromatins to activate RAG expression and initiate the regenerative program.
5caC: 5-carboxylcytosine; 5fC: 5-formylcytosine; 5hmC: 5-hydroxymethylcytosine; 5mC: 5-methylcytosine; Ac: Acetyl modifications; BER: Base excision repair; C: Cytosine; HAT: Histone acetyltransferase; RAG: Regeneration-associated gene; TDG: Thymine DNA glycosylase; TET: Ten-eleven translocation methylcytosine dioxygenase; TSS: Transcription start site.

of neuropathic pain shows that Dnmt3b is preferentially expressed in DRG neurons and substantially upregulated by peripheral nerve injury [46]. This suggests that the configuration of the DNA methylome in DRG neurons may be amenable to change in response to injury. Using DNA methylation microarrays, Puttagunta *et al.* assessed promoter and CpG DNA methylation in DRGs after dorsal column (CNS injury) or sciatic nerve axotomy (PNS injury) [27]. Surprisingly, despite the high-throughput format, only a modest number of genes were found to exhibit differential methylation between the two types of injuries, and none of the genes were RAGs. One potential limitation of this study is the use of whole DRGs for profiling. Because the ratio of glia to neurons in the DRG is approximately 10:1 [47], DNA methylation arrays or reduced representation bisulfite sequencing from DRG tissues would more likely reflect the methylation landscape of glia cells rather than neurons. Thus, the effect of Dnmt family proteins and DNA methylation in modulating regenerative capacity still requires further examination. Functional studies of Dnmts and a genome-wide DNA methylation analysis in axotomized neurons may help to reveal the link between DNA methylation patterns and expression changes in RAGs. In another study, it was shown that the folate pathway promotes axon regeneration coinciding with global and gene-specific DNA methylation changes in the injured spinal cord [48]. In this case, supplementation of folate after CNS injury was found to increase DNA methylation on the promoter region of Gadd45, a gene induced by axonal injury [49]. However, it is not clear whether the DNA methylation changes arise from neurons or glial cells and how specific modifications, such as hypermethylation of the Gadd45a promoter, can enhance CNS repair. It is worth noting that effects of folate may not be restricted to DNA, as S-adenosylmethionine (SAM) generated from the folate cycle is a universal methyl donor for methyltransferases to catalyze not only DNA, but also RNA and histone methylation. As discussed above, histone methylation in particular exhibits complex regulation of gene expression. Thus, whether global DNA methylation alone is responsible for increasing regenerative capacity, and the identity of its critical targets, awaits further investigation.

## DNA demethylation & axon regeneration

It is now clear that the DNA methylation landscape in mature neurons can be altered by a variety of external stimuli [50]. Dynamic changes of DNA methylation patterns result from combinatorial actions of *de novo* DNA methylation and active demethylation processes. Recent studies have uncovered molecular players in DNA demethylation and begun to delineate the underlying mechanisms. One of the key components that initiates the process is Ten-eleven translocation methylcytosine dioxygenase 1–3 (TET1–3), which iteratively oxidizes 5mC to 5hmC and further oxidation derivatives, including 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC) (Figure 2B) [51,52]. Thymine DNA glycosylase (TDG) has robust excision activity toward 5fC and 5caC to initiate base excision repair (BER) pathway for reintroduction of unmethylated cytosine (Figure 2B) [53]. The importance of active DNA demethylation in several aspects of neuronal function, including synaptic scaling, and memory formation and extinction, has been recently established [54,55]. Identifying the underlying molecular machinery may allow for the enhancement or preservation of these functions under neural injury or degenerative conditions.

TET enzymes and 5hmC have important roles in regulating proliferation, survival and differentiation of neural progenitor cells during neurogenesis [56,57]. Particularly, recent reports have illustrated the importance of 5hmC in neuronal differentiation and axonogenesis [58–60]. By comparing 5hmC distribution between cortical neural progenitor cells and neurons at E15.5, Hahn and colleagues revealed that the level of 5hmC is reduced in active enhancers (p300 binding sites) and is enriched in gene bodies [59]. The gain of intragenic 5hmC appears to be partnered with a loss of H3K27me3 in a repertoire of genes that are required for neuronal differentiation and axonogenesis. It is also worth noting that several histone modifications, including H3K4me3 and H3K36me3, also occur in

different gene regions, such as promoter and intergenic regions, during neuronal development. These results suggest that a mechanism controls the interplay between DNA and histone modifications and ultimately governs specific transcriptional programming for neural development and axonal projection. Understanding how these epigenetic switches govern intrinsic growth capacity may help us develop strategies to enhance the regenerative capacity of mature neurons in adulthood.

The intrinsic growth capacity of neurons depends on the growth-promoting molecular program during development, which declines dramatically after maturation and synapse formation. Cellular triggers and molecular transitions responsible for this programmatic change are poorly understood. A recent study shows that neuronal 5hmC increases in the brain with age [61], highlighting the possibility that the gain of 5hmC may lead to neuronal maturation and loss of growth capacity. Indeed, retinal RGCs at the late postnatal stage exhibit a higher level of TET3 expression and acquire 5hmC over the course of development [8]. In this case, 5hmC is particularly enriched in gene bodies and results in neuronal gene activation. On the other hand, there is a portion of 5hmC enriched in 5′UTR and promoters, which may downregulate gene expression, as it has been suggested that 5hmC in the promoter region may function as a general repressive mark [62]. Thus, 5hmC patterns, depending on their genomic location, could exert epigenetic regulation of gene activity, and in turn, contribute to regenerative capacity. Future studies are needed to directly test the hypothesis that epigenetic modification induces reprogramming of mature neurons to a regenerative state.

## DNA methylation & cell death

Cell death is a major contributor to the permanent loss of function from spinal cord injury and brain trauma. Therefore, regeneration in the adult CNS not only depends on increased neuronal growth capacity of surviving neurons, but could also be achieved through neuroprotective mechanisms to prevent cell loss after injury. Recent studies of cerebral ischemia revealed a spectrum of epigenetic processes that have fundamental influences on the pathophysiology of cell death. Among these epigenetic modifications, augmented DNA methylation was found after brain injury and is detrimental for cell survival [63]. *Dnmt1*-haploinsufficient mice exhibit neuroprotection and ameliorated damage following mild ischemic brain injury. These observations highlight the possibility that manipulation of DNA methylation patterns can alter injury responses, yet the underlying mechanisms remain unclear. Using a model of sciatic nerve avulsion in rodents to induce robust apoptosis of spinal motor neurons, emerging evidence indicates that DNA methylation also exerts a regulatory role in axotomy-induced cell death [64]. Both DNMT1 and DNMT3a are found to be enriched in apoptotic motor neurons and DNA methylation increases during apoptosis. Pharmacological inhibition of DNMTs by RG108, an inhibitor that blocks the enzyme active site, prevents injury-induced DNA methylation and rescues spinal motor neurons from axotomy-induced cell death. Since active DNA demethylation counterbalances DNA methylation levels, one may postulate that TET family proteins have a potent neuroprotective function. Gain- and loss-of-function studies of TETs in different injury models will help determine effects of these genes in regenerative responses of axotomized neurons.

## DNA & histone methylation/acetylation interactions

While independent studies on DNA and histone modifications can elucidate components of a complete axon regrowth program, a more holistic view can begin to take form by recognizing the influence that these marks have on each other and the result on transcriptional regulation (Figure 2C).

Proteins with methyl-CpG-binding domain and BTB/POZ families bind to methylated CpG dinucleotides, where they associate with various enzymes, including histone deacetylases and methyltransferases, and affect histone modifications. As a result, these interactions lead to transcriptional repression and heterochromatin formation, matching the repressed state of the methylated DNA. For instance, during embryonic development, pluripotency genes must be downregulated, while lineage-specific genes need to be activated. One recent study showed that the Lsd1-Mi2/NuRD complex both demethylates and deacytelates H3K4 near pluripotency gene enhancers made up of CpG islands. This demarcation recruits Dnmt3 to the histone tail to form *de novo* DNA methylation in the enhancer region and reduce pluripotency [65].

In tandem, H3K9me2 has been shown to protect DNA from demethylation, which supports a cyclical relationship to continuously downregulate transcription of areas with methylated CpG. For example, PGC7 (a maternal factor also known as Dppa3) has been shown in early mouse embryonic development to inhibit the conversion of 5mC to 5hmC by binding to H3K9me2 [66]. The balance between the two states of cytosine is correlated with pluripotency and lineage determination, which suggests that cellular state determination is reliant on DNA and histone methylation interactions.

CpG dinucleotide methylation has important implications for histone methylation, but these areas are

different from CpG islands, the majority of which are nonmethylated, and mainly located in gene promoters and enhancers. Importantly, nonmethylated CpG islands are correlated with certain histone lysine methylation sites, such as H3K4me3 and H3K27me3, and specifically nonmethylated H3K36 [67]. In fact, H3K4 methyltransferase enzymes can be recruited to nonmethylated CpG islands, which suggests the role of the nonmethylated DNA region in helping to methylate the histone lysine. On the other hand, the trimethylation of H3K4me3 blocks Dnmt3a from binding to the histone tails and prevents DNA methylation, leaving the enhancer/promoter available for transcriptional purposes. Overall, the complexity of this system suggests a specific and targeted means of defining discrete chromatin regions for gene regulation in development, and could potentially be recapitulated during neuronal regeneration.

## miRNA in neural regeneration

Although not generally associated with classical epigenetic mechanisms, miRNA are important epigenetic mediators for transcriptional and translational control during neuronal development, maintenance, injury response and regeneration. In animals, miRNAs are small endogenously encoded segments of RNA that work as a part of the RNA induced silencing complex to target, in general, the 3′UTR region of mRNA [68]. This causes either the degradation of the mRNA, or decreased levels of translation, which results in decreased protein levels. In addition to direct effects on specifically targeted proteins, if used to target a transcription factor, it may have a broad influence on cellular function. Although miRNAs have been studied for decades, there has been a recent surge in research implicating miRNAs in disease and therapeutics [69].

While successful regeneration requires expression of various miRNAs concomitantly, each miRNA can have multiple targets that are specific to different cell types. Table 1 highlights some of the most well-studied miRNAs and their targets in both the CNS and PNS, although this list is by no means exhaustive. In line with histone modifications, miRNA-138 forms a negative feedback loop with a nicotinamide adenine dinucleotide (NAD)-dependent histone deacetylase after injury [70]. This miRNA acts as a molecular repressor by targeting SIRT1 in both development and regeneration, which is known to induce axonal outgrowth in the PNS. However, SIRT1 acts as a transcriptional repressor to downregulate miRNA-138, forming a mutual negative-feedback loop. One week after sciatic nerve injury, miRNA-138 was shown to be endogenously downregulated, as a result of increased SIRT1 expression upon regenerative pathway activation. This study suggests that in a naive state, HDAC is constitutively inhibited to prevent regenerative genes from being expressed, but a marked increase of SIRT1 transcription and translation as a result of injury leads to gene activation and regeneration in DRGs.

Another recent study showed that overexpression of miR-210 led to transcriptional downregulation of *ephrin-A3*, an apoptosis inducing receptor protein-tyrosine kinase, leading to increased survival

Table 1. miRNAs involved in neural regeneration.

| miRNA | Location | Target | Effect | Ref. |
|---|---|---|---|---|
| miR-21 | DRG | *Spry2* | Blocks inhibitor of axonal outgrowth/ promote regeneration | [72] |
| miRNA-30b | RGC | *Sema3A* | Blocks downstream anti-regenerative factors | [73] |
| miRNA-26a | DRG | *Gsk3Beta* | Controls Smad1 expression to allow regeneration | [74] |
| miRNA-133b | Cortical neurons | *RhoA* | Activates MEK/ERK and PI3K/Akt signaling for regeneration | [75] |
| miRNA-138 | DRG | *Sirt1* | Downregulated miRNA138 ensures more efficient SIRT1 up-regulation | [70] |
| miRNA-210 | DRG | *Ephrin-A3* | Promotes axonal outgrowth; blocks apoptotic signal after injury | [76] |
| miRNA-222 | DRG | *Pten* | Reduces expression of PTEN to allow nerve regeneration | [77] |
| miRNA-431 | DRG | *Kremen1* | Silences antagonist of Wnt/β-catenin signaling to allow regeneration | [78] |

DRG: Dorsal root ganglion; RGC: Retinal ganglion cell.

and regeneration of DRGs both *in vitro* and *in vivo* [76]. miR-210 was even found to permit CNS neurogenesis in the adult mouse brain after injury through upregulation of *Vegf* as well as downregulation of *Ephrin-A3* in astrocytes [79,80]. Interestingly, peripheral axon length after recovery increased with overexpression of miR-210, but was not observed when the target, *Ephrin-A3*, was endogenously knocked down [76]. In the PNS, inhibiting let-7 miRNAs in spinal cord co-cultured with DRGs has been shown to upregulate NGF, leading to increased axon outgrowth following injury, as well as in the sciatic nerve *in vivo* [81]. Under oxidative stress conditions, the let-7 miRNA family decreases apoptosis after injury, while inhibiting the miRNA increases apoptosis. However, knockdown of the target, NGF, increases apoptosis [81]. In studies of both miRNAs, knockdown of the target does not have the expected results associated with activity of the miRNA, which suggests that their respective miRNAs might play a different role in the activity of caspase-3 and other apoptotic factors. So far, the molecular mechanisms other than direct targeting of mRNA have yet to be studied. While many specific miRNA pathways have led to basic and translational applications, many more pathways have yet to be elucidated to understand the whole picture of the most important miRNAs in regeneration.

## Conclusion & future perspective

Epigenetic mechanisms, including DNA methylation and histone modifications, are likely to work cooperatively to affect accessibility of the genome to TFs, and to unlock the silenced genomic loci in order to reprogram injured neurons into a growth-competent cellular state for successful regeneration (Figure 1). While we are still in the early stages of understanding the complexity and the extensiveness of the neuronal epigenomes, it is clear that distinct epigenetic regulatory differences exist between PNS and CNS neurons in terms of their response to injury and the regenerative capacity. Future studies need to interrogate epigenetic patterns at different stages to decipher differential regenerative responses between neurons in the adult mammalian CNS and PNS. Many questions remain to be answered, including what injury signaling cascades regulate the epigenetic state of specific subsets of RAGs, and which epigenetic modifications would allow CNS neurons to regain their regenerative capacity. Genome-wide epigenetic studies, such as ChIP-Seq for histone modifications, whole-genome bisulfite sequencing and TET-assisted bisulfite sequencing (TAB-seq), in a cell type-specific manner will begin to fill the gaps in our knowledge and help us to understand how growth competence is re-established or lost after injury.

Identification of active DNA demethylation mechanisms indicates that DNA methylation in postmitotic neurons is modulated by environmental stimuli. Given the detrimental effects of DNA hypermethylation on cell survival, manipulation of active DNA demethylation mechanisms may elicit neuroprotective effects and prevent cell loss after CNS injury. Several regulators, such as GADD45 and TET family proteins, have been identified that facilitate DNA demethylation [54,82]. Employing epigenetic editing [83] using CRISPR-based TETs or Gadd45 alterations at defined genomic regions may provide proof-of-principle evidence that modulating DNA methylation could lead to reactivation of genes important for axon regeneration.

A growing body of evidence suggests that epigenetic changes of histone modifications are capable of increasing regenerative capacity, even in the absence of the initiating cue. For example, overexpression of PCAF, without a preconditioning lesion, allows regrowth of spinal axons beyond the site of spinal cord injury. Additionally, administration of different HDAC inhibitors such as TSA, Valproic acid and MS-275, has been shown to promote axon outgrowth in both CNS and PNS neurons (Table 2) [25,28,71].

Table 2. Effects of histone deacetylase inhibitors in axon regeneration.

| HDAC inhibitors | Injury models | Specificity | Effects | Mechanism | Ref. |
|---|---|---|---|---|---|
| TSA | Optic nerve crush | HDAC I/II | Promote cell survival | Unknown | [9] |
| TSA | Primary cell culture | HDAC I/II | Enhance axon outgrowth | Activation of RAGs | [25] |
| Valproic acid | SCI | HDAC I/II | Promote the recovery of SCI | Modulation of neurotrophic factors | [71] |
| Valproic acid | Optic nerve crush | HDAC I/II | Enhance axon outgrowth and survival | Activation of transcription factors | [11] |
| MS-275 | Sensory + SCI | HDAC I | Enhance spinal axon regeneration | Activation of RAGs | [28] |

HDAC: Histone deacetylase; RAG: Regeneration-associated gene; SCI: Spinal cord injury; TSA: Trichostatin A.

Because mammalian HDAC superfamily encodes 11 members that are not redundant in function, certain cell types in particular CNS regions may utilize different HDACs to specify their function. Thus, identification of specific HDACs that can reshape the epigenetic landscape for regeneration will open up a new avenue for the treatment of injury in the CNS and other neurological disorders. Although existing HDAC inhibitors with broader target specificity have proven effective for promotion of axon regeneration, they may have off-target effects on neural function. Novel HDAC inhibitors with greater target specificity would be important for therapeutic applications.

In addition to the intrinsic growth capacity, the microenvironment around the injured axon affects the axon's ability to regenerate. For example, diminished Schwann cell plasticity has been associated with the age-dependent decline of axon regeneration ability in the PNS, rather than axonal limitations [84]. Expression profiling revealed that aged Schwann cells fail to activate transcriptional repair pathways. However, the underlying mechanism for how inactivity emerges with age has yet to be discovered. DNA methylation and histone modifications have also been suggested to regulate Schwann cell function [85,86]. Particularly, H3K27 acetylation exhibits dynamic changes in Schwann cells after peripheral injury and is enriched in several TFs, including c-JUN and RUNX2, which are vital for myelin debris clearance and axon regeneration after injury [86]. Together, these findings highlight the possibility that epigenetic mechanisms may also control the transcriptional activation of repair pathways in Schwann cells and are responsible for age-related changes in injury responses. In combination with the enhancement of intrinsic growth capacity, harnessing extrinsic neuronal mechanisms to increase regenerative potential may render better functional recovery after traumatic nerve injury.

In addition to modifications on DNA and histones, RNA can be marked by more than 100 chemical modifications that may alter the RNA structure and recruit specific cognate proteins to regulate RNA stability, splicing, transportation and translation [87]. Among these modifications, N6-methyl-adenosine (m6A) is the most prevalent epigenetic mark in eukaryotic mRNA. Remarkably, recent transcriptome-wide mapping revealed that m6A distribution can be altered by a subset of stimuli, resulting in differential gene expression and protein translation [80,88], thus representing another layer of epigenetic regulation. RNA modifications rapidly reshape the transcriptome

## Executive summary

**Fundamentals of intrinsic growth capacity**

- Axonal regenerative capacity depends on the transcriptional program and declines with age.
- In contrast to CNS injury, peripheral lesions activate a repertoire of regeneration-associated genes (RAGs) to initiate a regenerative program in mature mammalian neurons.
- Manipulation of epigenetic configurations could allow CNS neurons regain growth capacity.

**DNA methylation & demethylation in neural regeneration**

- DNA methylation is established by DNMTs, while DNA demethylation is catalyzed by TET family proteins via iterative oxidation reaction of 5-methylcytosine followed by base-excision repair.
- Changes in DNMTs upon peripheral lesion have been implicated in the regulation of gene reprogramming and injury responses.
- Inhibition of DNA methylation by pharmaceutical inhibitors of DNMTs elicits neuroprotection and increases cell survival after injury.

**Histone acetylation in neural regeneration**

- Histone H4 acetylation is enriched in certain RAGs concomitant with increased gene activity after peripheral lesion. Application of MS-275, a histone deacetylase1-specific inhibitor (HDAC1-specific inhibitor), sufficiently increases AcH4 levels and increases the intrinsic growth capacity.
- H3K9ac is also enriched in certain RAGs concomitant with increased gene activity after peripheral lesion. Overexpression of histone acetyltransferase PCAF, without a preconditioning lesion, can promote spinal axon regeneration in spinal cord injury.
- Injury-induced nuclear export of HDAC5 is a unique mechanism in the PNS to reshape the epigenetic landscape and induce the regenerative transcriptional program.
- Inhibition of HDACs or increase of histone acetyltransferases can promote CNS regeneration.

**Future perspective**

- Fully understanding epigenetic regulation of regenerative capacity requires comprehensive analysis of different epigenetic modifications in a cell type-specific manner.
- The role of the epitranscriptome in axon regeneration warrants further study.
- Differential injury signals between CNS and PNS may confer distinct epigenomes and transcriptomes that determine regenerative capacity.

and induce protein level changes, permitting a fast response to external stimuli. Whether these post-transcriptional modifications on RNA also play a role in axon regeneration merit future study. In summary, epigenetic marks at histone, DNA and RNA appear to be plastic and the plasticity among readers, writers and erasers could be harnessed for the development of therapeutic regimens to engineer regenerative reprogramming.

### Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

## References

1 Goldberg JL, Klassen MP, Hua Y, Barres BA. Amacrine-signaled loss of intrinsic axon growth ability by retinal ganglion cells. *Science* 296(5574), 1860–1864 (2002).

2 Blackmore M, Letourneau PC. Changes within maturing neurons limit axonal regeneration in the developing spinal cord. *J. Neurobiol.* 66(4), 348–360 (2006).

3 Dusart I, Airaksinen MS, Sotelo C. Purkinje cell survival and axonal regeneration are age dependent: an *in vitro* study. *J. Neurosci.* 17(10), 3710–3726 (1997).

4 Chen DF, Jhaveri S, Schneider GE. Intrinsic changes in developing retinal neurons result in regenerative failure of their axons. *Proc. Natl Acad. Sci. USA* 92(16), 7287–7291 (1995).

5 Wang JT, Kunzevitzky NJ, Dugas JC, Cameron M, Barres BA, Goldberg JL. Disease gene candidates revealed by expression profiling of retinal ganglion cell development. *J. Neurosci.* 27(32), 8593–8603 (2007).

6 Moore DL, Blackmore MG, Hu Y *et al.* KLF family members regulate intrinsic axon regeneration ability. *Science* 326(5950), 298–301 (2009).

7 Blackmore MG, Wang Z, Lerch JK *et al.* Kruppel-like Factor 7 engineered for transcriptional activation promotes axon regeneration in the adult corticospinal tract. *Proc. Natl Acad. Sci. USA* 109(19), 7517–7522 (2012).

8 Perera A, Eisen D, Wagner M *et al.* TET3 is recruited by REST for context-specific hydroxymethylation and induction of gene expression. *Cell Rep.* 11(2), 283–294 (2015).

9 Gaub P, Joshi Y, Wuttke A *et al.* The histone acetyltransferase p300 promotes intrinsic axonal regeneration. *Brain* 134, 2134–2148 (2011).

10 Rao RC, Tchedre KT, Malik MT *et al.* Dynamic patterns of histone lysine methylation in the developing retina. *Invest. Ophthalmol. Vis. Sci.* 51(12), 6784–6792 (2010).

11 Biermann J, Grieshaber P, Goebel U *et al.* Valproic acid-mediated neuroprotection and regeneration in injured retinal ganglion cells. *Invest. Ophthalmol. Vis. Sci.* 51(1), 526–534 (2010).

12 Ma TC, Willis DE. What makes a RAG regeneration associated? *Front. Mol. Neurosci.* 8, 43 (2015).

13 Smith DS, Skene JH. A transcription-dependent switch controls competence of adult neurons for distinct modes of axon growth. *J. Neurosci.* 17(2), 646–658 (1997).

14 Chandran V, Coppola G, Nawabi H *et al.* A systems-level analysis of the Peripheral Nerve Intrinsic Axonal Growth Program. *Neuron* 89(5), 956–970 (2016).

15 Stam FJ, MacGillavry HD, Armstrong NJ *et al.* Identification of candidate transcriptional modulators involved in successful regeneration after nerve injury. *Eur. J. Neurosci.* 25(12), 3629–3637 (2007).

16 Storer PD, Dolbeare D, Houle JD. Treatment of chronically injured spinal cord with neurotrophic factors stimulates β-II-tubulin and GAP-43 expression in rubrospinal tract neurons. *J. Neurosci. Res.* 74(4), 502–511 (2003).

17 Bomze HM, Bulsara KR, Iskandar BJ, Caroni P, Skene JH. Spinal axon regeneration evoked by replacing two growth cone proteins in adult neurons. *Nat. Neurosci.* 4(1), 38–43 (2001).

18 Pernet V, Joly S, Jordi N *et al.* Misguidance and modulation of axonal regeneration by Stat3 and Rho/ROCK signaling in the transparent optic nerve. *Cell Death Dis.* 4, e734 (2013).

19 Lindner R, Puttagunta R, Di Giovanni S. Epigenetic regulation of axon outgrowth and regeneration in CNS injury: the first steps forward. *Neurotherapeutics* 10(4), 771–781 (2013).

20 Bannister AJ, Kouzarides T. Regulation of chromatin by histone modifications. *Cell Res.* 21(3), 381–395 (2011).

21 Peixoto L, Abel T. The role of histone acetylation in memory formation and cognitive impairments. *Neuropsychopharmacology* 38(1), 62–76 (2013).

22 Lee KK, Workman JL. Histone acetyltransferase complexes: one size doesn't fit all. *Nat. Rev. Mol. Cell Biol.* 8(4), 284–295 (2007).

23 Haberland M, Montgomery RL, Olson EN. The many roles of histone deacetylases in development and physiology: implications for disease and therapy. *Nat. Rev. Genet.* 10(1), 32–42 (2009).

24 Broide RS, Redwine JM, Aftahi N, Young W, Bloom FE, Winrow CJ. Distribution of histone deacetylases 1–11 in the rat brain. *J. Mol. Neurosci.* 31(1), 47–58 (2007).

25 Gaub P, Tedeschi A, Puttagunta R, Nguyen T, Schmandke A, Di Giovanni S. HDAC inhibition promotes neuronal outgrowth and counteracts growth cone collapse through CBP/p300 and P/CAF-dependent p53 acetylation. *Cell Death Differ.* 17(9), 1392–1408 (2010).

26 Chen J, Laramore C, Shifman MI. Differential expression of HDACs and KATs in high and low regeneration capacity neurons during spinal cord regeneration. *Exp. Neurol.* 280, 50–59 (2016).

27 Puttagunta R, Tedeschi A, Soria MG *et al.* PCAF-dependent epigenetic changes promote axonal regeneration in the central nervous system. *Nat. Commun.* 5, 3527 (2014).

28 Finelli MJ, Wong JK, Zou H. Epigenetic regulation of sensory axon regeneration after spinal cord injury. *J. Neurosci.* 33(50), 19664–19676 (2013).

29 Cho Y, Sloutsky R, Naegle KM, Cavalli V. Injury-induced HDAC5 nuclear export is essential for axon regeneration. *Cell* 155(4), 894–908 (2013).

30 Cho Y, Cavalli V. HDAC signaling in neuronal development and axon regeneration. *Curr. Opin. Neurobiol.* 27, 118–126 (2014).

31 Cho Y, Cavalli V. HDAC5 is a novel injury-regulated tubulin deacetylase controlling axon regeneration. *EMBO J.* 31(14), 3063–3078 (2012).

32 Cho Y, Park D, Cavalli V. Filamin A is required in injured axons for HDAC5 activity and axon regeneration. *J. Biol. Chem.* 290(37), 22759–22770 (2015).

33 Rivieccio MA, Brochier C, Willis DE *et al.* HDAC6 is a target for protection and regeneration following injury in the nervous system. *Proc. Natl Acad. Sci. USA* 106(46), 19599–19604 (2009).

34 Abe N, Cavalli V. Nerve injury signaling. *Curr. Opin. Neurobiol.* 18(3), 276–283 (2008).

35 Teng FY, Tang BL. Axonal regeneration in adult CNS neurons-signaling molecules and pathways. *J. Neurochem.* 96(6), 1501–1508 (2006).

36 West AE, Griffith EC, Greenberg ME. Regulation of transcription factors by neuronal activity. *Nat. Rev. Neurosci.* 3(12), 921–931 (2002).

37 Mar FM, Bonni A, Sousa MM. Cell intrinsic control of axon regeneration. *EMBO Rep.* 15(3), 254–263 (2014).

38 Schmitt HM, Pelzel HR, Schlamp CL, Nickells RW. Histone deacetylase 3 (HDAC3) plays an important role in retinal ganglion cell death after acute optic nerve injury. *Mol. Neurodegener.* 9, 39 (2014).

39 Shin J, Ming GL, Song H. Decoding neural transcriptomes and epigenomes via high-throughput sequencing. *Nat. Neurosci.* 17(11), 1463–1475 (2014).

40 Jones PA. Functions of DNA methylation: islands, start sites, gene bodies and beyond. *Nat. Rev. Genet.* 13(7), 484–492 (2012).

41 Hellman A, Chess A. Gene body-specific methylation on the active X chromosome. *Science* 315(5815), 1141–1143 (2007).

42 Shukla S, Kavak E, Gregory M *et al.* CTCF-promoted RNA polymerase II pausing links DNA methylation to splicing. *Nature* 479(7371), 74–79 (2011).

43 Weng YL, An R, Shin J, Song H, Ming GL. DNA modifications and neurological disorders. *Neurotherapeutics* 10(4), 556–567 (2013).

44 Guo JU, Su Y, Shin JH *et al.* Distribution, recognition and regulation of non-CpG methylation in the adult mammalian brain. *Nat. Neurosci.* 17(2), 215–222 (2014).

45 Laplant Q, Vialou V, Covington HE 3rd *et al.* Dnmt3a regulates emotional behavior and spine plasticity in the nucleus accumbens. *Nat. Neurosci.* 13(9), 1137–1143 (2010).

46 Pollema-Mays SL, Centeno MV, Apkarian AV, Martina M. Expression of DNA methyltransferases in adult dorsal root ganglia is cell-type specific and up regulated in a rodent model of neuropathic pain. *Front. Cell. Neurosci.* 8, 217 (2014).

47 Delree P, Leprince P, Schoenen J, Moonen G. Purification and culture of adult rat dorsal root ganglia neurons. *J. Neurosci. Res.* 23(2), 198–206 (1989).

48 Iskandar BJ, Rizk E, Meier B *et al.* Folate regulation of axonal regeneration in the rodent central nervous system through DNA methylation. *J. Clin. Invest.* 120(5), 1603–1616 (2010).

49 Ma DK, Guo JU, Ming GL, Song H. DNA excision repair proteins and Gadd45 as molecular players for active DNA demethylation. *Cell Cycle* 8(10), 1526–1531 (2009).

50 Guo JU, Ma DK, Mo H *et al.* Neuronal activity modifies the DNA methylation landscape in the adult brain. *Nat. Neurosci.* 14(10), 1345–1351 (2011).

51 Ito S, Shen L, Dai Q *et al.* TET proteins can convert 5-methylcytosine to 5-formylcytosine and 5-carboxylcytosine. *Science* 333(6047), 1300–1303 (2011).

52 Tahiliani M, Koh KP, Shen Y *et al.* Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. *Science* 324(5929), 930–935 (2009).

53 He YF, Li BZ, Li Z *et al.* Tet-mediated formation of 5-carboxylcytosine and its excision by TDG in mammalian DNA. *Science* 333(6047), 1303–1307 (2011).

54 Yu H, Su Y, Shin J *et al.* Tet3 regulates synaptic transmission and homeostatic plasticity via DNA oxidation and repair. *Nat. Neurosci.* 18(6), 836–843 (2015).

55 Rudenko A, Dawlaty MM, Seo J *et al.* Tet1 is critical for neuronal activity-regulated gene expression and memory extinction. *Neuron* 79(6), 1109–1122 (2013).

56 Zhang RR, Cui QY, Murai K *et al.* Tet1 regulates adult hippocampal neurogenesis and cognition. *Cell Stem Cell* 13(2), 237–245 (2013).

57 Li T, Yang D, Li J, Tang Y, Yang J, Le W. Critical role of Tet3 in neural progenitor cell maintenance and terminal differentiation. *Mol. Neurobiol.* 51(1), 142–154 (2015).

58 Papale LA, Zhang Q, Li S, Chen K, Keles S, Alisch RS. Genome-wide disruption of 5-hydroxymethylcytosine in a mouse model of autism. *Hum. Mol. Genet.* 24(24), 7121–7131 (2015).

59 Hahn MA, Qiu R, Wu X *et al.* Dynamics of 5-hydroxymethylcytosine and chromatin marks in Mammalian neurogenesis. *Cell Rep.* 3(2), 291–300 (2013).

60 Xu Y, Xu C, Kato A *et al.* Tet3 CXXC domain and dioxygenase activity cooperatively regulate key genes for *Xenopus* eye and neural development. *Cell* 151(6), 1200–1213 (2012).

61 Szulwach KE, Li X, Li Y *et al.* 5-hmC-mediated epigenetic dynamics during postnatal neurodevelopment and aging. *Nat. Neurosci.* 14(12), 1607–1616 (2011).

62 Wu H, D'Alessio AC, Ito S *et al.* Genome-wide analysis of 5-hydroxymethylcytosine distribution reveals its dual function in transcriptional regulation in mouse embryonic stem cells. *Genes Dev.* 25(7), 679–684 (2011).

63 Endres M, Meisel A, Biniszkiewicz D *et al.* DNA methyltransferase contributes to delayed ischemic brain injury. *J. Neurosci.* 20(9), 3175–3181 (2000).

64 Chestnut BA, Chang Q, Price A, Lesuisse C, Wong M, Martin LJ. Epigenetic regulation of motor neuron cell death through DNA methylation. *J. Neurosci.* 31(46), 16619–16636 (2011).

65 Petell CJ, Alabdi L, He M, San MP, Rose R, Gowher H. An epigenetic switch regulates *de novo* DNA methylation at a subset of pluripotency gene enhancers during embryonic stem cell differentiation. *Nucleic Acids Res.* doi:10.1093/nar/gkw426 (2016) (Epub ahead of print).

66 Nakamura T, Liu YJ, Nakashima H *et al.* PGC7 binds histone H3K9me2 to protect against conversion of 5mC to 5hmC in early embryos. *Nature* 486(7403), 415–419 (2012).

67 Rose NR, Klose RJ. Understanding the relationship between DNA methylation and histone lysine methylation. *Biochim. Biophys. Acta* 1839(12), 1362–1372 (2014).

68 Im HI, Kenny PJ. MicroRNAs in neuronal function and dysfunction. *Trends Neurosci.* 35(5), 325–334 (2012).

69 Li Z, Rana TM. Therapeutic targeting of microRNAs: current status and future challenges. *Nat. Rev. Drug Discov.* 13(8), 622–638 (2014).

70 Liu CM, Wang RY, Saijilafu, Jiao ZX, Zhang BY, Zhou FQ. MicroRNA-138 and SIRT1 form a mutual negative feedback loop to regulate mammalian axon regeneration. *Genes Dev.* 27(13), 1473–1483 (2013).

71 Lv L, Han X, Sun Y, Wang X, Dong Q. Valproic acid improves locomotion *in vivo* after SCI and axonal growth of neurons *in vitro*. *Exp. Neurol.* 233(2), 783–790 (2012).

72 Strickland IT, Richards L, Holmes FE, Wynick D, Uney JB, Wong LF. Axotomy-induced miR-21 promotes axon growth in adult dorsal root ganglion neurons. *PLoS ONE* 6(8), e23423 (2011).

73 Han F, Huo Y, Huang CJ, Chen CL, Ye J. MicroRNA-30b promotes axon outgrowth of retinal ganglion cells by inhibiting Semaphorin3A expression. *Brain Res.* 1611, 65–73 (2015).

74 Jiang JJ, Liu CM, Zhang BY *et al.* MicroRNA-26a supports mammalian axon regeneration *in vivo* by suppressing GSK3beta expression. *Cell Death Dis.* 6, e1865 (2015).

75 Lu XC, Zheng JY, Tang LJ *et al.* MiR-133b Promotes neurite outgrowth by targeting RhoA expression. *Cell Physiol. Biochem.* 35(1), 246–258 (2015).

76 Hu YW, Jiang JJ, Yan G, Wang RY, Tu GJ. MicroRNA-210 promotes sensory axon regeneration of adult mice *in vivo* and *in vitro*. *Neurosci. Lett.* 622 61–66 (2016).

77 Zhou S, Shen D, Wang Y *et al.* MicroRNA-222 targeting PTEN promotes neurite outgrowth from adult dorsal root ganglion neurons following sciatic nerve transection. *PLoS ONE* 7(9), e44768 (2012).

78 Wu D, Murashov AK. MicroRNA-431 regulates axon regeneration in mature sensory neurons by targeting the Wnt antagonist Kremen1. *Front. Mol. Neurosci.* 6, 35 (2013).

79 Zeng L, He X, Wang Y *et al.* MicroRNA-210 overexpression induces angiogenesis and neurogenesis in the normal adult mouse brain. *Gene Ther.* 21(1), 37–43 (2014).

80 Zhou J, Wan J, Gao X, Zhang X, Jaffrey SR, Qian SB. Dynamic m(6)A mRNA methylation directs translational control of heat shock response. *Nature* 526(7574), 591–594 (2015).

81 Li S, Wang X, Gu Y *et al.* Let-7 microRNAs regenerate peripheral nerve regeneration by targeting nerve growth factor. *Mol. Ther.* 23(3), 423–433 (2015).

82 Ma DK, Jang MH, Guo JU *et al.* Neuronal activity-induced Gadd45b promotes epigenetic DNA demethylation and adult neurogenesis. *Science* 323(5917), 1074–1077 (2009).

83 Hilton IB, D'Ippolito AM, Vockley CM *et al.* Epigenome editing by a CRISPR–Cas9-based acetyltransferase activates genes from promoters and enhancers. *Nat. Biotechnol.* 33(5), 510–517 (2015).

84 Painter MW, Brosius Lutz A, Cheng YC *et al.* Diminished Schwann cell repair responses underlie age-associated impaired axonal regeneration. *Neuron* 83(2), 331–343 (2014).

85 Varela-Rey M, Iruarrizaga-Lejarreta M, Lozano JJ *et al.* S-adenosylmethionine levels regulate the schwann cell DNA methylome. *Neuron* 81(5), 1024–1039 (2014).

86 Hung HA, Sun G, Keles S, Svaren J. Dynamic regulation of Schwann cell enhancers after peripheral nerve injury. *J. Biol. Chem.* 290(11), 6937–6950 (2015).

87 Fu Y, Dominissini D, Rechavi G, He C. Gene expression regulation mediated through reversible m(6)A RNA methylation. *Nat. Rev. Genet.* 15(5), 293–306 (2014).

88 Dominissini D, Moshitch-Moshkovitz S, Schwartz S *et al.* Topology of the human and mouse m6A RNA methylomes revealed by m6A-seq. *Nature* 485(7397), 201–206 (2012).