EXPERT REVIEW

Epigenetic and epitranscriptomic regulation of axon regeneration

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Effective axonal regeneration in the adult mammalian nervous system requires coordination of elevated intrinsic growth capacity and decreased responses to the inhibitory environment. Intrinsic regenerative capacity largely depends on the gene regulatory network and protein translation machinery. A failure to activate these pathways upon injury is underlying a lack of robust axon regeneration in the mature mammalian central nervous system. Epigenetics and epitranscriptomics are key regulatory mechanisms that shape gene expression and protein translation. Here, we provide an overview of different types of modifications on DNA, histones, and RNA, underpinning the regenerative competence of axons in the mature mammalian peripheral and central nervous systems. We highlight other non-neuronal cells and their epigenetic changes in determining the microenvironment for tissue repair and axon regeneration. We also address advancements of single-cell technology in charting transcriptomic and epigenetic landscapes that may further facilitate the mechanistic understanding of differential regenerative capacity in neuronal subtypes. Finally, as epigenetic and epitranscriptomic processes are commonly affected by brain injuries and psychiatric disorders, understanding their alterations upon brain injury would provide unprecedented mechanistic insights into etiology of injury-associated-psychiatric disorders and facilitate the development of therapeutic interventions to restore brain function.

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INTRODUCTION

During development, neurons hold great growth competence, but gradually lose their capacity in neurite outgrowth during neuron maturation, especially in the mammalian central nervous system (CNS) [1, 2]. As a result, adult CNS neurons generally have limited regenerative competence after injury, which hinders the recovery of patients with spinal cord injury (SCI), stroke, or neurodegenerative diseases. To date, several interventions have been identified to enable injured adult neurons to regrow, revealing that certain signaling pathways can awaken the regenerative capacity of adult neurons [3–6]. In contrast to those in the CNS, neurons in the mature peripheral nervous system (PNS) attain a large degree of regenerative capacity after axonal injuries, which provides alternative avenues to identify intrinsic and extrinsic factors underpinning regenerative capacity. Gene regulatory circuits [7], protein translation control [3], metabolic regulation [8], and microenvironment [9] have been found to contribute to the neural repair through studies in several PNS and CNS injury models. For example, system-level analysis of temporal gene expression changes in rodent PNS neurons during nerve regeneration has identified a large set of regeneration-associated genes (RAGs) that form a complex gene regulatory network to determine the regeneration competence [7]. Moreover, other cell types, such as glial cells and resident immune cells, can alter their cellular states after injury, constituting either a beneficial or detrimental microenvironment to greatly influence axon regrowth [9–15].

Not all neuron subtypes regain the growth competency to the same extent [16], suggesting that their dormant regenerative capacity is differentially controlled by distinct mechanisms. Epigenetic and epitranscriptomic modifications that can directly modulate gene expression and protein synthesis may underlie distinct injury responses in a cell-type-dependent manner. Specifically, epigenetic modifications, including DNA and histone modifications, can lead to chromatin remodeling and transcription factor (TF) accessibility without changing the DNA sequence. RNA modifications, on the other hand, regulate RNA metabolism and protein synthesis without altering the RNA sequence. DNA, histone, and RNA modifications are critical regulatory mechanisms manifested in neurodevelopment, learning, and memory [17–19]. Dysregulated epigenetic and epitranscriptomic machinery due to genetic variances or mutations have been linked to etiology and pathophysiology of psychiatric disorders [20–22]. In addition to genetic dysregulations, environmental challenges, including stressful life events and physical trauma, are often found to be associated with comorbidities of mental health issues. Particularly, brain injury, such as mild traumatic brain injury (mTBI) or concussion, appears to increase susceptibility to a spectrum of neuropsychiatric disorders [23]. While causal mechanisms linking

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physical conditions to mental illness remain elusive, intriguingly, accumulating genes and signaling pathways affected by brain injury responses, which include epigenetic and epitranscriptomic regulators, are also found to be risk factors for development of psychiatric disorders [14, 15, 24–28].

In this review, we summarize recent advances in our knowledge of roles and mechanisms of epigenetic and epitranscriptomic modifications in regulating axon regeneration in the mammalian nervous system. We also discuss how single-cell technologies can facilitate the identification of previously underappreciated mechanisms for differential regenerative capacity in neuronal subtypes. We further provide an outlook for how the understanding of epigenetic and epitranscriptomic mechanisms can encourage axon regeneration and mitigate brain injury-associated psychiatric disorders.

DNA METHYLATION IN AXON REGENERATION

Chemical modifications to DNA bases are one of the major epigenetic mechanisms that regulate chromatin architecture and gene expression. The most widespread and prevalent DNA modification in eukaryotes is the methylation at 5′-carbon of the pyrimidine ring of cytosine nucleotide (5mC). 5mC modification primarily happens in 60–80% of CpG dinucleotides [29] and ~25% of CpHs (non-CpG DNA methylation, where H is C, A or T) [30, 31], which together modifies ~4% of cytosines in the mammalian genome [32]. CpG methylation is catalyzed by a family of DNA methyltransferases (DNMTs), of which DNMT1 maintains the DNA methylation pattern to daughter cells during replication, while DNMT3a and DNMT3b establish *de novo* methylation for unmodified DNAs [33–35]. In contrast, CpH methylation is restricted to certain tissues and cell types, such as embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs) and mature neurons [36], where its establishment is mediated by DNMT3a or DNMT3b and recognized by Methyl-CpG binding protein 2 (MeCP2), the only reader known so far for non-CpG DNA methylation, leading to transcriptional repression in mature neurons [31]. MeCP2 is highly expressed in the brain and its mutations are the primary cause of Rett syndrome [37]. The roles of MeCP2 in modulating neuronal function have been extensively studied in the context of transcriptional regulation, chromatin organization, and RNA splicing [38–41]. A recent study highlighted that the dynamic binding of MeCP2 to DNA methylation could be another paradigm to influence the onset of Rett syndrome [42]. The dynamics of CpG and CpH methylation landscape have been characterized in normal brain development [31, 43] and several neurological disorders [42, 44, 45]. During brain development, CpH methylation (especially CpA methylation), enriched in low CpG-density regions, markedly accumulates during neuronal maturation, while methylated CpG (mCpG) level is relatively unchanged [42, 46]. Through in vivo analyses of MeCP2 binding in the adult mouse brain, it has been proposed that MeCP2 binds early on to CpG methylation and then CpH methylation to influence transcription [42]. These results not only underscore the importance of dynamic CpG and non-CpG methylation landscapes in gene regulation but also suggest that aberrant methylation patterns can incorporate MeCP2 binding to affect the timing of onset for Rett syndrome [42].

DNA methylation was generally believed to be associated with transcriptional repression by repelling transcription factor binding in proximal promoter regions [47, 48]. However, the application of protein-microarray-based approach and methylation-sensitive SELEX (systemic evolution of ligands by exponential enrichment) that enabled researchers to systematically survey the binding affinity of chemically-modified nucleic acids to the entire human TF family or extended DNA binding domains, has revealed that certain TF preferentially bind to mCpGs [49–51]. For example, mCpG can create new binding sites for a repertoire of TFs, such as homeobox proteins and Krüppel-like factor 4 (KLF4), leading to augment of transcriptional activity [49–51]. Furthermore, whole-genome analysis uncovered 5mC can dynamically occur in enhancers, promoters, and gene bodies [30, 52, 53] and pose differential position effects on gene regulation. For instance, in male mouse germline, the methylation on gene bodies was shown to be positively correlated with transcriptional activity in actively transcribed genes, whereas the methylation on transcription start sites (TSS) was inversely associated with transcription initiation [54]. Similarly, in postnatal neural stem cells (NSCs), DNMT3a-dependent nonproximal promoter methylation was found to promote transcriptional activity of neurogenic genes through antagonizing polycomb repression [55]. Intriguingly, in contrast to promoting gene transcription, studies from the Bird group indicated that 5mC in the gene body can recruit MeCP2 to create "roadblocks", impeding the movement of RNA polymerase II to dampen the transcription in neurons [56]. Together, these results suggest that 5mC at different genomic regions can exert diversified functions in regulating gene activity but also have diametrically opposed functions on transcriptional states in a cell-type-specific manner [57].

5mC can be reversed either passively or actively to an unmodified state. Passive DNA demethylation mostly occurs during cell proliferation, though this does not apply to postmitotic neurons as they are non-proliferative. Active DNA demethylation elaborates ten-eleven translocation (TET) proteins for iterative oxidation of 5mC to 5hmC, 5fC, and 5caC, followed by thymine DNA glycosylase (TDG)-dependent base excision repair (BER) for replacement of eventual unmodified cytosine [58–62]. While 5hmC is the first oxidation intermediate in active DNA demethylation, merging evidence suggests it may hold additional roles in regulating gene activities itself.

Like 5mC, 5hmC occupancy was also found to vary among tissues and dynamically distributed across different genomic regions through whole-genome sequencing (i.e., gene bodies, promoters, and intergenic regions) [63, 64]. The genomic region-specific 5hmC seems to have distinct regulatory roles in modulating gene activity. In ESCs and neural progenitor cells (NPCs), 5hmC in the proximal promoter region is inversely associated with gene expression where actively transcribed genes show depleted 5hmC in TSS regions, and low-expressing genes displayed abundant 5hmC modification at promoters. In contrast, 5hmC levels in gene bodies are positively associated with actively transcribed genes in ESCs and other cell types [65–67]. Mechanistically, it was proposed that accumulated 5hmC in gene bodies of active genes cooperate with loss of H3K27me3 to promote brain development during neurogenesis [65]. Another study in postmitotic neurons suggests the accumulated 5hmC in the gene bodies of actively transcribed genes can lead to "functional demethylation" and prevent MeCP2 binding, subsequently facilitating transcription [66]. Thus, proper DNA methylation and hydroxymethylation patterns in promoter, gene body, and other genomic regions are crucial for the establishment and maintenance of transcriptional program to regulate cell function and responses. Mutations or altered expression of DNA modification enzymes in neurons can impair synaptic plasticity and cause cognitive and social deficits [68]. Furthermore, aberrant DNA methylation and hydroxymethylation patterns are often associated with different psychiatric disorders [69–72] and recognized as potential epigenetic risk factors to cause cognitive and social deficits.

External challenges, such as stress and injury, can lead to genome-wide alternations of DNA modifications and subsequent gene expression changes. Intervention or facilitation of these epigenetic changes can promote neuron protection and growth competence in a context-dependent manner. Widespread alternation of DNA methylation was observed in injured dorsal root ganglia (DRG) [73]. DNA hypermethylation prevails in the early

phase of epigenetic reprogramming after nerve injury [74], suggesting gain of DNA methylation may influence neuroregenerative capacity. Indeed, administration of folate, an essential nutrient required to generate S-Adenosyl methionine (SAM), which is a universal donor for DNA methylation, increases DNA methylation and facilitates axon regeneration in the injured spinal cord [75]. Moreover, genome-wide DNA methylation and hydroxymethylation profiles in a CNS injury model have revealed numbers of CpG differentially methylated regions (DMRs), CpH differentially methylated regions (DmCH), and differentially hydroxymethylated regions (DmCHs) that accompany with axonal regeneration processes [76]. Future studies examining those differentially methylated/hydroxymethylated regions and the binding proteins on those sites may render new targets to promote axon regeneration.

The mechanisms of reconfiguring DNA methylation patterns dedicated to enhancing regeneration processes remain elusive [75]. Recent studies suggest that epigenetic regulators, including ubiquitin-like containing PHD ring finger 1 (UHRF1) [77], SET domain bifurcated 1 (SETDB1) [78], and MeCP2 [79], can coordinate the recruitment of DNMT3a/b to establish methylation patterns at specific genomic regions. Thus, it is plausible that some of these epigenetic regulators may play a role in enhancing regenerative capacity. Indeed, in the context of peripheral axon regeneration, it was shown that UHRF1 promotes axon regeneration. Mechanistically, UHRF1 can interact with DNMTs and H3K9me3 for transcriptional repression of genes that are inhibitory or detrimental to regeneration. For instance, UHRF1 can be recruited to the promoter region of phosphatase and tensin homolog (PTEN) for gene inactivation, thereby increasing protein translation for axon regeneration. UHRF1 was also found to inhibit transcription of RE1 silencing transcription factor (REST) and thereby preventing prolonged elevated levels of REST that have adverse effects on the regenerative program [77].

In complement to methylation and gene silencing, injury-induced active DNA demethylation (or epigenetic reactivation) targets different loci and the resultant induced gene activation has been shown to promote regeneration processes (Fig. 1a). For example, 5hmC levels were found to be augmented in genomic loci of many RAGs, including activating transcription factor 3 (ATF3), brain-derived neurotrophic factor (BDNF), and SMAD Family Member 1 (SMAD1), in DRG neurons in the adult mouse PNS upon sciatic nerve injury [80, 81]. Blocking TET3 inhibits axon regeneration of DRG neurons and behavioral recovery, underscoring the necessity of DNA demethylation in regaining intrinsic growth competency [81]. Moreover, epigenetic reprogramming is also important for CNS neurons to sustain their regeneration capacity as knockdown TET1 limited axon growth of PTEN-deleted retinal ganglion cells (RGCs) after optic nerve injury [81]. These results also indicate neuron subtypes may have distinct epigenetic barriers to restrain their intrinsic growth competence and require manipulation of specific epigenetic regulators to breach regeneration inhibition.

Determining which epigenetic alternations are truly functional and causal for the phenotypic changes requires epigenome editing tools to systematically validate candidate epigenetic regulatory elements. As such, several programmable epigenome editing technologies have been developed via tethering a DNA binding domain, including zinc finger proteins (ZFPs), transcription activator-like effectors (TALEs) and nuclease-dead CRISPR/Cas systems (dCas), with an epigenetic modifier to reconfigure epigenetic state at designated genomic loci, followed by interrogation of the mechanistic links between epigenetic states, gene activity and phenotypic traits [82, 83]. For instance, the fusion of catalytically inactive Cas9 (dCas9) with the catalytic core of the histone acetyltransferase p300 has been reported to induce robust gene expression by catalyzing H3K27 acetylation at target proximal and distal enhancers [84]. Similarly, engineered CRISPR/dCas9-Dnmt3a or dCas9-Tet1 system has been established and successfully demonstrate DNA methylation or demethylation of targeted loci, respectively [85]. More importantly, these epigenome editing tools not only can enable dissecting the functional significance of epigenetic alternations in a locus-specific manner, but also provide potential therapeutic strategies to restore gene expression program in diseases involving epigenetic dysregulation. For instance, Fragile X syndrome (FXS), which results from CCG trinucleotide repeat expansion in the 5′-untranslated region of the Fragile X Messenger Ribonucleoprotein 1 (FMR1), exhibits DNA hypermethylation, aberrant histone modifications, and silencing of FMR1. Through exploiting the CRISPR/dCas9-TET1 system, Jaenisch lab demonstrated that targeted demethylation of CGG repeats in FXS patient iPSCs can reactivate FMR1 expression and rescue the electrophysiological abnormalities of FXS neurons [86]. Application of these epigenome editing tools creates unprecedented possibilities for programmable DNA demethylation via CRISPR/dCas systems on cis-regulatory elements (CREs) of RAGs in injured CNS neurons. This would enable a permissive environment for gene reactivation, which in turn provides a promising avenue for promoting axon regeneration as well as treatment of injury-associated psychiatric disorders. It is worth noting that the large number of epigenetic alterations upon axon injury may preclude easy identification of functional epigenetic alternations that are responsible for regenerative capacity. Recently, single-cell sequencing technology adopting CRISPR/Cas9 screens and RNA sequencing (RNA-seq) may overcome this limitation and pave the way for the dissection of candidate epigenetic regulatory elements [87].

HISTONE MODIFICATIONS IN AXON REGENERATION

In addition to DNA modifications, histones wrap DNA into nucleosomes, where different histone modifications aid the remodeling of chromatin and further direct the associated gene activation or inactivation. Each nucleosome is composed of an octamer of four core histones (H2A, H2B, H3, and H4). The N-terminal tails of histones are enriched by different post-transcriptional modifications (PTMs), among which acetylation and methylation are the most well studied. In general, histone acetylation is associated with open chromatin and increased TF accessibility [88]. Histone methylation, in contrast, can lead to either transcriptional activation or repression depending on the targeted histone residues and types of methylation. For instance, H3K4me1 and H3K4me3 represent active transcription, while H3K9me3 and H3K27me3 are generally repressive markers [89, 90]. Alterations of histone modifications due to genetic mutations or environmental insults can influence the gene expression program in specific brain regions and particular cell types, leading to substantial cognitive impairment and pathogenesis of neuropsychiatric diseases [91]. For example, Sirtuin 1 (SIRT1), a histone deacetylase that regulates histones H3 and H4 acetylation, plays a critical role in modulating neuroplasticity and was identified as a major depressive disorder (MDD) risk gene [92].

Cumulative data have shown that histone modifications modulate the expression of RAGs and contribute to axon regeneration. p300/CBP-associated factor (PCAF), the cognate enzyme of H3K9ac, exhibited increased levels after peripheral but not central axonal injury [93] (Fig. 1b). H3K9ac induction was found in the promoter region of several RAGs in DRG neurons following a peripheral nerve lesion, suggesting that the augment of PCAF activity or H3K9ac level is associated with regenerative capacity. In support of this notion, overexpression of PCAF increases growth competency of CNS neurons and encourages regeneration after SCI [93]. Due to the instructive roles of histone acetylation in promoting axon outgrowth, several histone deacetylase inhibitors (HDACi) that prevent histone deacetylation have been investigated for their potential to increase regeneration in both CNS and PNS injury models. In a rodent model of SCI, rats administered valproic acid, a potent HDACi, exhibited increased

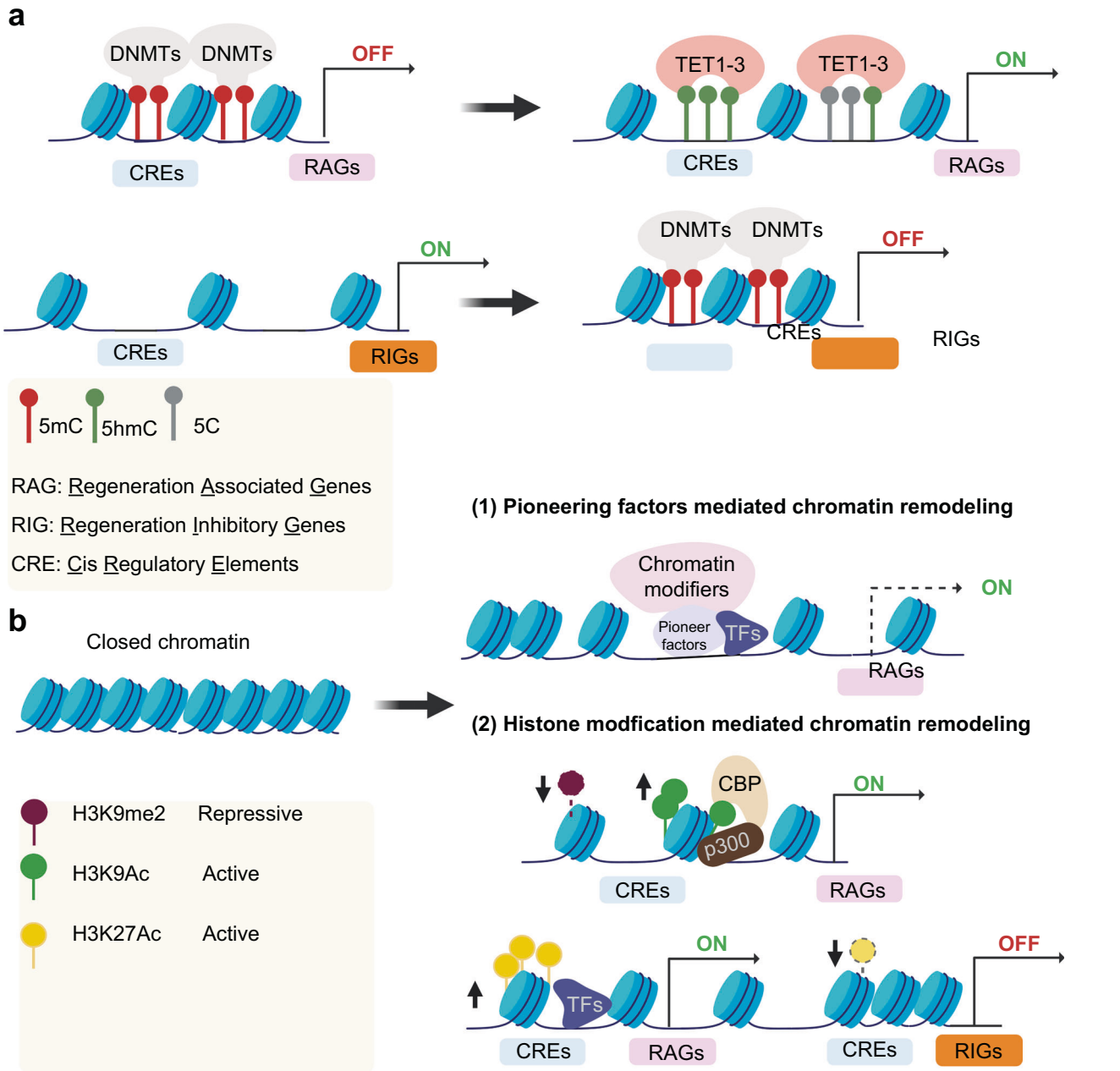

Fig. 1 Epigenetic regulation of regenerative capacity. a DNMTs establish and maintain 5mC on cis-regulatory elements (CREs) to deactivate pro-regenerative program in normal conditions. Upon peripheral injury, activated TETs can execute DNA demethylation, awakening RAGs expression to encourage axon regeneration. **b** Chromatin states regulate the transition of pro-regenerative program. Pioneer factors can target closed chromatin and initiate chromatin remodeling. The changes of histone modifications after injury control the pro-regenerative gene expression program. The augment of H3K9Ac and H3K27Ac or reduction of H3K9me2 can occur on RAGs for gene activation while the reduction of H3K27Ac can occur on regeneration inhibiting genes (RIGs) for gene inactivation.

locomotor function [94]. Other broad-spectrum Class I/II HDACi, such as trichostatin A (TSA) and MS-275, are also shown to promote axon regeneration by accelerating RAG expression in adult sensory neurons after injury [95]. To date, it remains elusive which specific cell types and downstream genes are affected by systemic administrations of HDACi. Genetic ablation of specific HDACs, followed by single-cell nuclei sequencing, would pinpoint which cell populations are heavily influenced by histone acetylation and help identify which target genes are responsible for the increased intrinsic growth capacity.

In contrast to the function of histone acetylation in promoting axon regeneration, very few studies have explored the role of repressive histone modifications in restricting growth capacity. Notably, Puttagunta et al. showed that H3K9me2, which is involved in gene silencing and chromatin repression, is downregulated in certain RAGs after sciatic nerve axotomy, but is increased upon dorsal column axotomy [93]. These changes of H3K9me2 levels are inversely correlated to RAG activation (Fig. 1b). Reopening the inaccessible chromatin regions by erasure of H3K9me2 may plausibly reactivate those RAGs. As proper genomic occupancy of active and repressive histone makers is required to establish a robust regenerative program, future studies are needed to identify the interplays of different histone modifications and changes of histone landscape after injury. Current omics techniques, such as

CUT&Tag and CUT&RUN [96, 97], which enable efficient and accurate profiling of histone modifications/DNA accessibility/TFs even at the single-cell level, have begun to reveal the regulatory gene circuitry controlling CNS and PNS growth competency. The advanced applications of single-cell histone modification profiling provide unique lenses to investigate epigenomic landscapes of cell subpopulations in specific organs/tissues.

Histone modifications can be amended by internal or extrinsic signals, which, in turn, recruit a distinct protein repertoire to alter chromatin states and activities. Pioneer factors are relatively newly defined TFs that can prime the closed chromatins and engage the nucleosome remodeling complexes and histone modifiers to initiate the formation of active DNA regulatory elements [98]. The function of pioneer factors varies in different cell type, and largely depends on the cellular state and availability of cofactors in target genomic regions. Two pioneer neurogenic basic helix-loop-helix TFs, achaete-scute family BHLH transcription factor 1 (ASCL1) and neuronal differentiation 1 (NEUROD1), have been shown to reprogram fibroblasts into neurons, where their occupancy is associated with increased DNA accessibility, induction of active histone marks H3K4me1 and H3K27Ac, and decreased levels of the repressive marker H3K9me3 [99]. In the adult brain, c-FOS is a neuronal activity-induced pioneer factor that exhibits increased occupancy to chromatin regions to enhance gene activity [100]. Thus, it is plausible that manipulation of proper pioneer factors can alter the chromatin states in favor of pro-regenerative program and subsequently endow enhanced regenerative capacity **(**Fig. 1b**)**. Furthermore, the pioneer factor-primed regions may also potentiate TF accessibility and maintain the epigenome status that leads to prolonged gene activation to escalate axon regeneration.

RNA MODIFICATIONS IN AXON REGENERATION

Different RNA types, including mRNA, rRNA, and tRNA, contain a variety of modified nucleotides, namely epitranscriptomics, and elaborate their own control on protein synthesis (Fig. 2a). Similar to DNA epigenetics, RNA modifications are alternations to the chemical moiety of RNA molecules that do not result in sequence changes, but do lead to distinct characteristics for RNA metabolism or structural features. While environmental challenges, including traumatic injury and stress, prompt epigenetic, chromatin, and gene expression changes, merging evidence indicates that stress exposure can also alter RNA modifications across the transcriptome [22]. Dysregulation of the epitranscriptomic responses results in maladaptive synaptic plasticity and ultimately contributes to the etiology and pathogenesis of stress-related psychiatric disorders [22, 101, 102].

Of multifaceted roles in regulating RNA metabolism, one critical function of RNA modifications is to modulate protein translation efficiency. Translation machinery which orchestrates mRNA, tRNAs, ribosomes, and translation factors for protein synthesis plays an indispensable role in modulating neuronal function, synaptic plasticity as well as regenerative capacity. For example, activation of the phosphatidylinositol-3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) pathway is one of many regulatory mechanisms required to increase protein synthesis [3, 103, 104]. Upon injury, PNS neurons augment PI3K/AKT/mTOR activity for protein translation and thus promote axon regeneration. In contrast, injured CNS neurons exhibit decreased mTOR signaling and reduced protein synthesis [3]. Restoration of mTOR signaling by *PTEN* deletion encourages axon regeneration in both retinal and spinal cord injury paradigms, highlighting the therapeutic promise of targeting the translation control apparatus [3, 104]. Thus, identifying and manipulating molecular targets or pathways that can robustly increase translational efficiency and enact another route to enhance regenerative competency would be needed.

RNA modifications occurring in tRNA, rRNA, and mRNA can fine-tune the translation machinery albeit through different mechanisms. Among these modifications, N1-methyladenosine (m^1A), N6-methyladenosine (m^6A), 5-methylcytosine (m^5C), and pseudouridine (Ψ) have been identified in mRNA with differential levels and displayed propensities to different positions of target transcripts [105, 106]. In contrast to m^5C and Ψ that potentially impedes mRNA translatability [107, 108], m^6A RNA methylation is the most prevalent internal modification on mRNAs that can facilitate protein translation [109].

m^6A RNA methylation was established by METTL3/METTL14 methyltransferase complex together with other regulatory subunits (e.g., Wilms' tumor 1-associating protein (WTAP), zinc finger CCCH-type containing 13 (ZC3H13) and vir like m^6A methyltransferase associated (VIRMA)) [110–113]. Transcriptome-wide analysis of m^6A has revealed that the m^6A landscapes are subjected to change after external challenges or cell-state transitions [114, 115]. By modulating RNA secondary structures or through recruitment of different m^6A-specific binding proteins ("readers"), including the YT521-B homology (YTH) domain family, heterogeneous nuclear ribonucleoproteins (HNRNPs), and insulin-like growth factor 2 mRNA-binding proteins (IGF2BPs) [109, 116–120], m^6A modifications exert diversified effects on RNA transcripts, spanning from regulation of mRNA degradation/stability to protein translation. For instance, the YTH family consists of YTH domain family proteins 1–3 (YTHDF1-3) and YTH domain-containing proteins 1-2 (YTHDC1-2). Binding of YTHDF2 to m^6A decreases mRNA stability [119], while YTHDF1 promotes translation of m^6A methylated RNA by the interaction of translation initiation factor eukaryotic initiation factor 3 (EIF3) [109]. IGF2BPs, on the other hand, facilitate mRNA translation by protecting m^6A-containing mRNAs from degradation [117]. Together, the changes of m^6A occupancy among the transcripts and the interplays between cognate binding proteins constitute a new layer of regulation for protein translation in the nervous system.

A recent study has illustrated that m^6A RNA methylation plays roles in regulating regenerative capacity (Fig. 2b). Following peripheral nerve lesion, m^6A levels were increased in DRG neurons, including multiple RAG transcripts. Depletion of m^6A by *METTL14* or *YTHDF1* knock-out perturbed protein translation, leads to attenuated axon regeneration [121]. Intriguingly, m^6A RNA methylation and its cognate binding proteins, YTHDFs, were recently found to regulate subcellular RNA localization. Loss of *METTL3* or *YTHDFs* reduces subset methylated RNAs transporting to neurite [122]. m^6A modification has been shown to control the local translation of mRNA in axons [123] and local translation in injured axons is crucial to elicit retrograde injury signaling and provide spatially restricted proteins for neurite outgrowth [124]. Therefore, it is plausible that the changes of m^6A RNA methylation after injury can redirect certain transcripts to axons for effective regenerative responses (Fig. 2b).

There is scarce research that addresses if m^6A augmentation is sufficient to increase growth competency of neurons after injury, which is probably due to a lack of approach to increase m^6A complex activity. Recent studies began to address this question and indicated that several signaling pathways can alter the m^6A RNA methylation levels. For instance, mammalian target of rapamycin complex 1 (mTORC1) signaling increased levels of SAM and WTAP expression, which in turn promoted the m^6A modification on targeted mRNA and, subsequently, protein synthesis [125]. The ERK pathway phosphorylates METTL3, followed by ubiquitin specific peptidase 5 (USP5)-mediated deubiquitination, leading to the stabilization of METTL3 and increased levels of mRNA methylation [126]. Transforming growth factor beta (TGF-β) signaling can facilitate recruitment of the m^6A methyltransferase complex and induce methylation on a subset of transcripts [127]. Moreover, post-translational modification, SUMOylation, could also affect m^6A enzyme activity [128]. Given the essential role of protein synthesis for axon regeneration, it would be interesting to examine if those signaling pathways

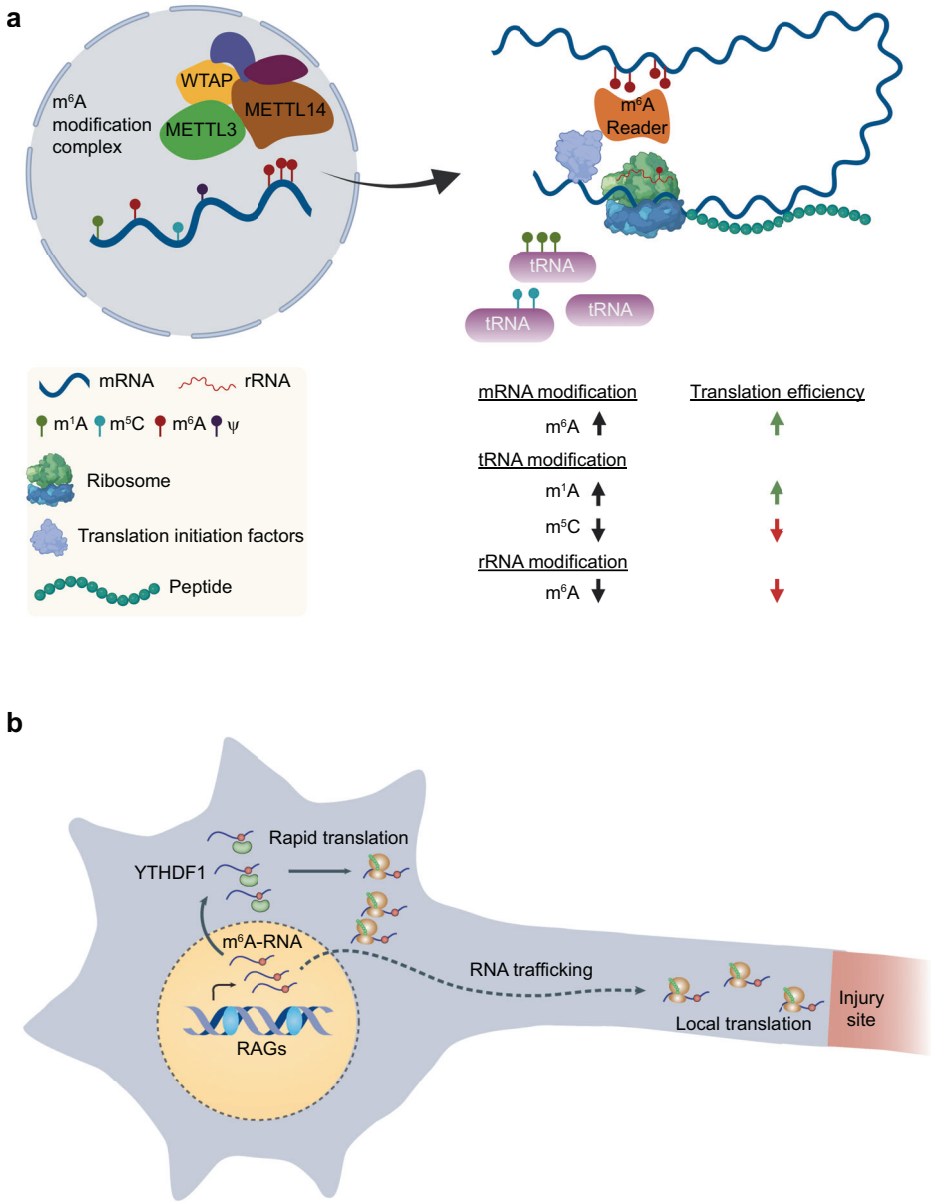

Fig. 2 Epitranscriptomic regulation of regenerative capacity. a RNA modifications, such as m^1A, m^6A, m^5C and Ψ are widely distributed on mRNA, tRNA and rRNA to fine-tune RNA stability and protein translation. **b** m^6A RNA methylation possesses regulatory effects on translation efficiency and, in turn, promotes the rapid activation of a pro-regenerative program for axon regeneration. m^6A residues may influence the subcellular localization of mRNA for local translation.

can augment m^6A levels for enhanced protein translation and stimulate CNS/PNS axon regeneration.

Besides m^6A mRNA methylation, other modifications that occur on rRNAs and tRNAs can also fine-tune the translation machinery [129, 130]. For example, tRNA is highly modified and decorated by other modifications, such as m^1A [131]. Enhanced expression of alkB homolog 1 (ALKBH1), a tRNA demethylase of m^1A, decreases the usage of tRNAs for protein synthesis [131]. Other modifications such as m^3C, m^5C and Ψ in tRNA were also found to control translation efficiency [132–134]. For instance, a recent study from our laboratory showed that Mettl8-dependent m^3C modification of mitochondrial tRNA promotes mitochondrial protein translation and regulates cortical neurogenesis [134]. Future studies of the crosstalk or combinatorial effects of RNA modifications on different RNA species [135] may offer an opportunity to significantly enhance protein synthesis for axon regeneration.

EPIGENETIC AND EPITRANSCRIPTOMIC REGULATION OF OTHER CELL TYPES

While cumulative data have identified intrinsic mechanisms for axon growth competence of mature neurons, recent studies have unveiled contributions of glial cells to extrinsic mechanisms of regeneration processes, including neuroprotective and neurotrophic effects, debris clearance, and myelin formation. Glial cells account for a large fraction of cell populations in both CNS and PNS. Specifically, the major glial cell types presented in the CNS are astrocytes, microglia, oligodendrocytes, and ependymal cells

[136, 137], while the glial populations in the PNS consist of Schwann cells, enteric glial cells, and satellite glial cells [138]. Each glial cell type has a unique origin, cell lineage, and molecular signature, and exhibits distinct cellular responses following injury.

CNS injury triggers a complex, multiphasic glial response, with both beneficial and detrimental effects. Astrocytes are a key glial component subjected to activation after CNS injury. Once becoming reactive, astrocytes elicit inflammatory responses and increase the production of glycosaminoglycans, such as chondroitin sulfate proteoglycan (CSPG), to form a glial scar. CSPGs have been shown to negatively regulate different aspects of the nerve repair process, including neuronal survival, axonal sprouting, regeneration, and conduction [14]. Although glial scars are historically regarded as the reason for the failure of axon regeneration, Mark et al. showed that attenuating scar-forming astrocytes instead fails to promote the spontaneous axonal regrowth in descending corticospinal tract (CST), ascending sensory tract and descending serotonergic (5HT) tract after SCI [15]. This finding highlights the possibility that the astrocyte heterogeneity constitutes diametrically opposed functions, which include both beneficial and detrimental effects, to influence regenerative responses [139]. The ability of astrocytes to use the same genetic information, but present distinct responses to extrinsic challenges, is in part attributed to epigenetic or epitranscriptomic differences among astrocyte subtypes. It is worth noting that HDACs, the epigenetic regulators of histone acetylation, are known to regulate astrocyte activation and inflammation responses. Pharmacological inhibitions of HDACs suppress the astrocytic cytokine and chemokine gene expression [140, 141]. Currently, our understanding of epigenetic mechanisms in regulating astrocyte function primarily results from the studies of bulk cell populations. Application of single-cell multi-omics technologies in astrocytes in the context of CNS injury will fill our knowledge gap of the epigenetic and epitranscriptomic mechanisms underpinning heterogeneous astrocyte activation and function.

Besides astrocytes, microglia are activated and infiltrate the injury site to execute a series of events, including phagocytosis, clearance of cellular debris, promotion of angiogenesis, and release of inflammatory mediators and trophic factors to impact tissue healing and regeneration within minutes after injury [142]. Microglia appear to promote corralling, wound compaction, and recovery after SCI [143], however, hyperactivation of adult microglia can escalate inflammation and cytotoxicity, facilitating glial scar formation. Intriguingly, a recent discovery of neonatal microglia-mediated scar-free healing in SCI indicates that, unlike adult microglia, neonatal microglia exhibit unique molecular signatures to enhance phagocytosis, resolve inflammation and prevent fibrotic scar formation [144]. While the underlying mechanisms render the protective effects of neonatal microglia remain to be investigated, a recent study of adult cortical and striatal microglia suggests that the polycomb repressive complex 2 (PRC2) and its mediated repressive chromatin modification H3K27me3, appear to epigenetically restrict the activation of clearance-related gene-expression programs and control microglia clearance activity to neuronal damage [145]. Further examination of such epigenetic regulations in adult microglia after SCI and other CNS injuries may provide a potential avenue for epigenetic interventions and facilitate the regeneration processes.

CNS injury often leads to the death of oligodendrocytes and demyelination, which accelerates axonal loss and degeneration. To compromise such deleterious effects, oligodendrocyte progenitor cells (OPCs) rapidly increased in number post-injury and differentiated into new oligodendrocytes to remyelinate axons [146]. Cumulative studies have identified several intrinsic and extrinsic factors, including epigenetic and epitranscriptomic mechanisms to instruct OPCs differentiation during development and remyelination [147]. For example, absence of m^6A RNA methylation can prevent maturation of oligodendrocytes and lead to hypomyelination [114]. Likewise, the cell functions and responses of other non-neuronal cell types to injury are coordinately controlled by various factors including TFs, histone modification, chromatin remodeling, and RNA modification. For example, injury-associated microglia, and macrophages (IAMs) exhibited increased levels of HDAC3 after SCI [148, 149]. In a rat TBI model, astrocytes exhibited decreased histone H3 acetylation after injury, which may contribute to the induction of astrogliosis and cognitive impairment [150]. Furthermore, although DNA methylation/hydroxymethylation of glial cell types has not been systematically investigated along the regeneration processes, several studies from mouse models of Alzheimer's disease and traumatic brain injury have suggested that global DNA methylation alterations occur in the microglia [151, 152].

In contrast to the CNS, the predominant glial cells in the PNS are Schwann cells. Upon PNS injury, Schwann cells become supportive of nerve repair by secreting neurotrophic factors, guiding axons back to their former target, and remyelinating regenerated axons. Following PNS injury, global alterations of H3K27Ac in enhancer regions and demethylation of H3K27me3 in promoters were found in Schwann cells to activate injury-induced gene expression for nerve repair [153].

Together, these studies suggest that glial cells may be equipped with distinct chromatin landscapes, resulting in differential responses to injury. There is no doubt that glial cells are a key player to support axon regeneration. Understanding how glial cells respond to injury and how epigenetic and epitranscriptomic modulators contribute to the state transition of glial cells after injury and during axon regeneration may provide new strategies to boost axon regeneration.

As epigenetic and epitranscriptomic regulatory elements could control cell-type-specific gene expression circuits and instructively define the cellular function of each cell, it is critical to understand the contribution of those regulatory elements to the diversified response of different cell types to nerve injury. Through single-cell sequencing, which enables massively parallel measurements of molecular signatures in thousands to millions of individual cells, diverse cell types have been discovered and comprehensively characterized in the nervous system [154–157]. Advanced single-cell technology also allows for the simultaneous detection of gene expression and chromatin states (e.g., DNA accessibility, DNA methylation, and histone modifications) and reveals multiple regulatory modalities in single cells [158, 159]. Numerous algorithms have been developed to perform cell clustering, pseudo time analysis, and decipher cell-cell interactions and communication of different cell types [160–162]. It has been recognized that neuronal subtypes possess different regenerative capacities [163]. For example, serotonergic neurons in the raphe nuclei, which project axons from the brainstem to the spinal cord, exhibit regenerative ability, in contrast to other CNS neurons that are unable to regrow after injury [163, 164]. RGCs are normally unable to regenerate after optical nerve injury but under experimental interventions, certain RGC subtypes (e.g., αRGCs and M1) have higher survival rates and regenerative capacity [16]. Most likely, these neurons hold a unique gene regulatory signature to enable them to regain axon growth potency. The application of single-cell sequencing has helped identification of several core transcriptional programs modulating neuronal survival and regeneration [165–167].

Single-cell RNA sequencing also uncovered that microglia, astrocytes, oligodendrocytes, and other non-neuronal cell types hold different gene signatures throughout the mouse lifespan [168]. Upon brain or spinal cord injury, the composition and expression profiles of these non-neuronal cells exhibit temporal changes [139, 168, 169], and therefore possibly result in disparate impacts on regeneration processes. Cumulative evidence has indicated that subsets of microglia, astrocytes, oligodendrocytes, and others exhibit distinct responses to injury or other extrinsic challenges [145, 148, 170]. These diversified responses within these glial cell types may result from differential levels of epigenetic regulators

that substantially alter gene expression program and in turn, ultimately alter cell function and behavior. For instance, PRC2 is recently identified to exhibit differential enrichment in microglia subpopulations which epigenetically restricts the microglial clearance activity to apoptotic neurons [145].

As the highly heterogenic (sub)cell types and complex cellular interactions influence neural repair, using single-cell sequencing to chart the transcriptomic, epigenetic, and epitranscriptomic landscapes may provide mechanistic insight into the gene regulatory machinery underpinning "supportive" or "detrimental" states of glial cells. Recently, multimodal profiling of chromatin accessibility, histone marks, and gene expression of the same cell has been successfully achieved in tissues of interest [158, 159]. In contrast, transcriptome-wide profiling of m^6A resides at single-cell levels is underway with several limitations. For example, deamination adjacent to RNA modification targets (DART-seq), which utilizes YTH domain to guide the fused cytidine deaminase Apolipoprotein B MRNA Editing Enzyme Catalytic Subunit 1 (APOBEC1) to target m^6A sites following the C-to-U conversion at the nearby cytidine residues, has been successfully applied to identify m^6A landscape at single-cell levels in cell lines [171, 172]. However, this technology requires the presence of APOBEC1-YTH in cells and the robust C-to-U converting rates around m^6A residues, which may challenge the feasibility of accurately detecting and quantifying single-cell m^6A sites in tissues. Future invention of single-cell sequencing technologies in quantitively detecting m^6A residues as well as other modifications at the full-length, single-molecule level may facilitate the determination of aberrant RNA modifications and their consequence in cellular dysfunction, regeneration failure, and disease susceptibility. Integrative analysis of multimodal single-cell data, including RNA modifications, histone marks, TFs occupancy, and gene expression will further advance our understanding of cell-type-specific differences in injury responses and reveal pivotal epigenetic and epitranscriptomic loci in determining regenerative capacity. In turn, we could take advantage of CRISPR/(d)Cas9 platforms to modulate epigenetic or epitranscriptomic states to promote regeneration.

CONCLUSION AND PERSPECTIVE

Cell intrinsic and extrinsic mechanisms are required to encourage successful axon regeneration. While DNA, histone, and RNA modifications are recognized to regulate gene expression and protein translation machinery, only a few modifications have been systematically studied in the context of axon regeneration. How the interplays between the epigenetic and RNA modifications constitute a complex modulatory network for the establishment and maintenance of growth competence during the regeneration phase remains to be addressed. Furthermore, subtypes of neurons, astrocytes, microglia, and other cells respond to injury/insult signals differentially and some contribute to inhibition of neural repair. Thus, it is crucial to examine and compare the intrinsic molecular signatures of neuronal subtypes to reveal the underpinning mechanisms of regenerative capacity. Likewise, identifying glial cells or resident immune cells with different signatures would enable us to better understand how the pro- or anti-regeneration microenvironment is established. Single-cell multi-omics sequencing provided insights into functional regulatory landscape and elucidated the fundamental mechanisms of epi-regulation, thereby providing us with the potential targets to manipulate. Engineering those regenerative refractory neurons or detrimental glial cells with sophisticated molecular tools would promote more neuron cells to regenerate with higher efficiency.

Experiences of brain injury, including mTBI or concussion, appear to associate with increased risks to various neuropsychiatric disorders [23]. While the causal mechanisms underpinning mTBI to confer susceptibility in psychiatric disorders remain elusive, potential determinants, including mechanical damage of neurocircuitry, neuroinflammation, and excitotoxicity [173], have been proposed to initiate or aggravate psychiatric disorders after brain injury. It is also plausible that brain injury causes psychiatric illnesses through dysregulating common genetic factors or converged signaling pathways that are highly associated with pathogenesis of psychiatric disorders. Several epigenetics or epitranscriptomic regulators (including TETs, METTL3, and HDACs), which play pivotal roles in neuronal function and plasticity, are subject to change after the injury. Dysregulation of these genes increases susceptibility to neuropsychiatric disorders [24–28]. Thus, understanding the epigenetic and epitranscriptomic mechanisms underlying injury responses not only enables us to identify an avenue to encourage axonal growth and neurocircuitry repair but also allows reversal of pathological signaling to attenuate injury-associated-psychiatric disorders.

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COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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