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Epigenetic regulation of oligodendrocyte identity

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Abstract

The interplay of transcription factors and epigenetic modifiers, including histone modifications, DNA methylation and microRNAs during development is essential for the acquisition of specific cell fates. Here we review the epigenetic “programming” of stem cells into oligodendrocytes, by analyzing three sequential stages of lineage progression. The first transition from pluripotent stem cell to neural precursor is characterized by repression of pluripotency genes and restriction of the lineage potential to the neural fate. The second transition from multipotential precursor to oligodendrocyte progenitor is associated with the progressive loss of plasticity and the repression of neuronal and astrocytic genes. The last step of differentiation of oligodendrocyte progenitors into myelin-forming cells is defined by a model of de-repression of myelin genes.

Epigenetic Modulation of Gene Expression Defines Cell Identity

One of the most exciting findings of the past few years has been the discovery that developmental processes are regulated by the crosstalk between transcription factors and epigenetic modulators of gene expression, including post-translational modifications of nucleosomal histones, changes in histone variants, chromatin remodeling enzymes, DNA methylation and microRNAs^{1–6}. Collectively, these other factors have been defined as “epigenetic regulators” (epi = above), because they control gene expression by regulating transcript levels, independently of changes in DNA sequence. The global effects of chromatin modifications on gene expression and the transmissibility of the information during cell division further delineate the importance of epigenetics in building a “memory” or “signature” of cell identity⁷. It has been proposed that the identity of each cell is dependent on “heritable memory” that is traced by epigenetic regulators and is associated with the activation of a lineage-specific program of gene expression⁸. The acquisition of this “memory” during development has been defined as “epigenetic programming”⁸. In this review we discuss these concepts within the framework of the molecular events characterizing the transition from pluripotent stem cells to myelin-forming oligodendrocytes.

Epigenetic programming and reprogramming

The concept of differentiation was originally envisioned as unidirectional progression through sequential states, each characterized by precise “epigenetic marks”. The discovery of enzymatic activities with the ability to “erase” these epigenetic marks^{9, 10}, however, revolutionized the field and introduced the idea of differentiation as “bidirectional” event. An original breakthrough study¹¹, provided the first experimental evidence that the process

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leading from ESCs to somatic cells could be reversed by over-expression of four transcription factors, including the Pou-family factor *Oct4*, the SRY (sex determining region Y)-box 2 factor *Sox2*, the homeodomain protein *Nanog* and either the Kruppel-like factor *Klf4* or *c-Myc*. After transfer of four of these genes, somatic cells exhibited almost indistinguishable patterns of histone modifications and DNA methylation as ESCs^{12–14}. They acquired the ability to produce viable chimeric mice when injected into blastocysts¹² and were named induced pluripotent stem (iPS) cells¹¹. Although it is not completely understood how these transcription factors are able to induce pluripotency, it is clear that reprogramming entails several modifications of histone marks and changes in DNA methylation. Indeed treatment with pharmacological agents affecting histone modifications or inhibiting DNA methylation, facilitate the induction of iPS cells^{15–17}. Together, these data suggest that the efficiency of reprogramming is dependent on histone modifications and DNA methylation.

This review introduces the epigenetic regulators of cell identity and then focuses on the steps characterizing the progression from ESCs to multipotential neural precursors to oligodendrocyte progenitors (OPC) and from OPCs to myelinating oligodendrocytes. Due to space constraints we apologize to colleagues whose work could not be cited^{4, 18,19}.

Chromatin remodeling, DNA methylation and microRNAs

Histone modifications

Chromatin modifiers comprise two large groups of enzymatic activities. One group is responsible for secondary modifications of the histones, while the other group includes enzymes using ATP to unwind nucleosomes and affect DNA/histone interactions. Post-translational modifications include the deposition or removal of acetyl, methyl, phosphoryl, sumoyl, ubiquitin, ADP-ribosyl groups from amino acids in the histone tails (Box 1). The most studied histone modifications are acetylation/deacetylation (Box 2) and methylation (Table 1). The functional consequences of these modifications on transcription depend on the type of change and on the position of the targeted amino acid residue within the tail of nucleosomal histones. Methylation of lysine residues on histone H3 have been particularly studied due to their important role for transcriptional activation or repression. For example, deposition of methyl groups on lysine 4 of histone H3 (H3K4me3) is associated with gene activation, while methylation of lysine 9 (H3K9me3) or 27 (H3K27me3) results in repression²⁰. Removal of methyl groups from the same residues has opposite effects^{1, 10, 21}. The second group of enzymes includes components of the SWI/SNF complex which mobilize the nucleosomes and provide transcription factors (either inhibitors or activators of gene expression) access to DNA^{22, 23}. Two principal forms have been identified: BAFs (i.e. BRG1/hBRM associated factors) and PBAF (Polybromo associated factors), although several additional denominations have been used to describe complexes formed by association with other subunits.

DNA methylation

DNA methylation is an important epigenetic mechanism regulating gene expression. It has been thoroughly characterized in X-chromosome inactivation in females²⁴ and genomic imprinting²⁵. DNA methylation occurs at the C-5 position of cytosine residues at CpG dinucleotides, due to the activity of the DNA methyltransferases Dnmt3a and Dnmt3b for *de novo* establishment of DNA methylation and Dnmt1 for its maintenance. These enzymes are expressed at high levels in undifferentiated ESCs²⁶. However, knockdown of all three enzymes in ESCs does not affect self-renewal²⁷, but prevents differentiation²⁸. These results are consistent with the reports of unmethylated DNA in undifferentiated ESCs and with the correlation of DNA methylation with lineage commitment^{29, 30}. Therefore DNA methylation is a mechanism that cells employ to induce a stable, although not irreversible repression of gene expression³¹. DNA demethylation, for instance, occurs during cell division and during

neural activity-induced neurogenesis in dentate gyrus³². Few DNA demethylase candidates have been proposed to date^{31,32} and it is likely that their characterization and the discovery of new candidates will further our understanding of reprogramming.

MicroRNAs (miRNAs)

MicroRNAs are small (19–25nt) endogenous non-coding RNAs that play a role in post-transcriptional regulation of gene expression. They decrease transcripts by base-pairing to target mRNAs, and either inhibiting mRNA translation or facilitating its degradation³³. In humans and rodents, miRNAs can be present in high or low copy number, with each miRNA having hundreds or even thousands of predicted mRNA targets.

An important relationship exists between microRNAs and DNA methylation, possibly mediated by the regulation of the levels of *Dnmt1*, *3a* and *3b*^{34,35}. Characterization of miRNA in undifferentiated and differentiated ESCs revealed the presence of clusters enriched in pluripotent cells³⁶ and others increasing during differentiation³⁷. These studies and others conducted in neural stem cells have begun to shed some light on their function^{38,39}.

Transition 1: from pluripotent ESCs to multipotent neural stem cells

Histone modifications

ESCs are characterized by the properties of self-renewal and pluripotency (i.e. the ability to generate all cell types of an organism), which depend on the interplay between the pluripotency-associated factors (i.e. *Oct4*, *Sox2* and *Nanog*) and epigenetic modulators. To gain a mechanistic insight on the chromatin structure in ESC, genome-wide studies were conducted using chromatin immunoprecipitation (see Box 1) and antibodies specific for pluripotency factors (i.e. Oct4, Sox2 or Nanog), for trimethylated K27 histone H3 (H3K27me3) and for the enzymatic activities responsible for this modification^{40–42}. The repressive histone marks were detected in chromatin regions that were also characterized by the presence of Oct4, Sox2 or Nanog and that included several hundreds of genes, important for lineage commitment and differentiation^{40,41}. These repressive marks co-localize with proteins possessing enzymatic activities specific for H3K27, such as histone methyltransferases of the “polycomb group” (PcG) proteins. The PcG group includes a family of transcriptional repressors regulating body patterning. This group was originally discovered in *Drosophila*^{43–45} and consists of two complexes: PRC2, with methyltransferase activity for H3K27 (i.e. Ezh2, Eed), and PRC1, which includes Ring-domain proteins with E3 monoubiquitin ligase activity (i.e. Ring1A, Ring1B, Bmi)⁴⁶. In agreement with the importance of K27 trimethylation, ESCs lacking one component of the PRC2 complex displayed inappropriate expression of genes associated with lineage commitment and cellular differentiation⁴². Therefore the undifferentiated state of ESCs is the result of a complex interplay between activating events and repressive mechanisms involving transcription factors and PcG-mediated deposition of repressive histone marks (i.e. H3K27me3).

The next challenge was to understand whether unique or similar mechanisms of repression of specific genes (for instance mesodermal genes), were shared by pluripotent cells (i.e. ESCs) and cells committed to a different lineage (i.e. neural precursors). The answer was provided by an interesting genome-wide ChIP study conducted in chromatin derived from ESCs or from lineage committed precursors, using antibodies specific for histone marks of transcriptional activation (i.e. H3K4me3) and of transcriptional repression (i.e. H3K27me3). In ESCs, the chromatin regions containing transcription factors involved in the regulation of developmental processes were characterized by the presence of both activating and repressive marks. These regions were therefore characterized as “transcriptionally ambivalent” in ESCs⁴⁷. The authors named bivalent domains the co-existence of activating and repressive marks on precise

chromatin regions. They also proposed that these domains could explain the repression of differentiation genes in ESCs (due to the presence of repressive H3K27me3), and their prompt activation (due to the presence of activating H3K4me3) during differentiation along distinct lineages⁴⁷. One suggestion was that ESCs retain pluripotency by maintaining lineage commitment and differentiation genes repressed but “poised” for transcription (i.e. ready to be transcribed). In contrast committed precursors actively transcribe genes related to their lineage and silence those related to other lineages. In other words, the progression from ESC to neural precursors is characterized by changes in the mode of regulation of gene expression. Neural precursors remove the H3K4me3 activation mark and retain the repressive H3K27me3 mark on mesodermal or endodermal genes thereby resulting in silencing of genes associated with mesodermal or endodermal lineage choice (Fig. 1). At the same time, they retain the activating H3K4me3, and remove H3K27me3 in regions containing neural genes⁴⁷ (Fig. 1). These studies suggested that the transition from pluripotent ES cell, (i.e. with the ability to generate endodermal, mesodermal and ectodermal derivatives) to multipotential neural “stem” cells (i.e. with the ability to generate only neuroectodermal derivatives), is characterized by the resolution of “bivalent domains” on lineage commitment and differentiation genes. However, “bivalent domains” on transcriptionally poised genes were detected not only in pluripotent ESCs, but also in neural multipotential precursors, embryonic fibroblasts and other undifferentiated cell lines^{48, 49} and therefore, could not represent a distinctive feature for ESCs. The recent discovery of acetylated lysine residue 56 of histone H3 on *Sox2*, *Oct4* and *Nanog* target genes in ESCs⁵⁰, identifies this modification as prominent in ESCs.

DNA methylation and microRNAs

Genome-wide studies of changes in DNA methylation during the differentiation of ESCs to glutamatergic neurons identified the occurrence of *de novo* DNA methylation at the transition from undifferentiated pluripotent cells to multipotential precursors³⁰. There is now general consensus regarding the presence of hypomethylated DNA in undifferentiated ESCs and the progressive increase in DNA methylation occurring at lineage commitment, but not during terminal differentiation^{29, 30}. In molecular terms, *de novo* DNA methylation occurred more frequently on promoters characterized by the presence of H3K27me3 mark and it was frequently associated with the resolution of the “bivalent domains” due to loss of the activating mark H3K4me3. Based on these results, a model was proposed, suggesting that DNA methylation may serve as a “protective” function in repressing genes that are incompatible with the chosen lineage. In addition, methylated DNA was also found on the promoters of differentiation genes lacking the “bivalent domains”^{29, 30}. Together these studies suggest that histone modifications and DNA methylation are part of the epigenetic mechanisms underlying pluripotency and therefore are essential for the establishment and maintenance of the “stem cell identity”. Finally it is important to add an additional level of regulation, which includes the function of microRNAs. A recent study has identified the microRNA miR-145, as a target of *Oct4* with the ability to repress *Sox2*, *Oct4* and *Klf4*⁵¹. The complex layers of regulation of the undifferentiated state by microRNAs have been nicely presented in other reviews⁵².

In summary, the ES state is characterized by the ability to generate all the cell types necessary to the organism. This elaborate task is accomplished by a combination of transcription factors, DNA methylation and histone modifications that suppress the expression of genes involved in lineage commitment and differentiation, while retaining them in a chromatin conformation that is poised for transcription. The progression towards multipotential precursors (also named “neural stem cells”) is characterized by more stable silencing of genes involved in other lineages (i.e. endoderm and mesoderm) and expression of neural genes.

Transition 2: From multipotent NSC to oligodendrocyte progenitors (OPC)

Histone modifications

The concept of progressive restriction of cell lineage potential during differentiation, applies not only to the transition from pluripotent ES cells to multipotent neural precursors, but also to the restriction of multipotent neural precursors to lineage committed oligodendrocyte progenitors (OPC). This choice is characterized by the progressive decrease of genes like *Sox2* and chromatin modifications on astrocytic and neuronal genes that is initiated by the activity of histone deacetylases⁵³ and is antagonized by Bcr1 and Brm⁵⁴. Inhibiting HDAC activity in OPCs, using pharmacological inhibitors⁵⁵, or culturing the cells for prolonged periods of time in the presence of mitogens⁵⁴, or keeping the cells in the presence of both HDAC inhibitors and mitogens⁵⁶ reverted these committed progenitors to multipotent cells characterized by *Sox2* expression.

The idea that commitment is associated with remodeling of chromatin on astrocytic and neuronal genes has not yet been formally demonstrated. However, it is important to mention here that several studies have reported the generation of neurons and astrocytes from oligodendrocyte progenitors^{53, 57–59}. If OPC identity requires the repression of neuronal genes, it is likely that this event may require the presence of chromatin remodeling complexes containing the RE1 silencing transcription factor (REST)/neuron restrictive silencing factor (NRSF). REST is a powerful repressor with the ability to recruit the co-repressors Sin3A and Co-REST and the histone deacetylases HDAC1/2^{60, 61}. This explanation would account for the increased number of cells expressing neuronal markers after treatment with HDAC inhibitors⁵³.

The expression of astrocytic specific genes, such as glial fibrillary acidic protein (*Gfap*) and *S100beta*, could similarly be down-regulated by deacetylation of nucleosomal histones (in this review, we shall consider *Gfap* expression as an astrocytic marker, even though it is acknowledged that it can also be expressed by adult neural stem cells⁶²). The involvement of histone acetylation/deacetylation in regulating astrocytic gene expression is supported by multiple lines of evidence. Differentiation of neural stem cells into astrocytes appears to be associated with the recruitment of the co-activator complexes including STAT1/3, histone acetyltransferases and Smad1 to specific regions of the *Gfap* promoter⁶³. Treatment with HDAC inhibitors increases the expression of *S100* and *Gfap* in cultured oligodendrocyte progenitors and in neonatal rats after transplantation⁵³. Finally, in developing astrocytes *Gfap* is repressed by complexes containing HDAC, the nuclear receptor co-repressor (N-CoR) and adaptor proteins⁶⁴, and N-CoR knockout mice are characterized by precocious expression of *Gfap*^{64, 65}. Similar mechanisms could occur in the cell after oligodendrocyte lineage choice.

The involvement of histone methylation in the transition from NSC to OPC is also suggested by experimental evidence on the role of PcG proteins, especially *Ezh2*^{66, 67}. *Ezh2* was down-regulated during commitment to neuronal and astrocytic lineage and up-regulated during commitment to the oligodendrocytic lineage. Overexpression of *Ezh2* in mouse neural stem cells (NSCs) increased the number of oligodendrocytes at the expenses of astrocytes, while silencing of *Ezh2* yielded opposite results⁶⁶. Another PcG protein, member of the PRC1 complex, the histone H2A E3 monoubiquitin ligase Ring1B, seems to be essential for oligodendrocyte differentiation⁶⁷. *Ring1B* deletion through Cre-mediated recombination *in vitro* selectively resulted in decreased number of oligodendrocytes from NSCs isolated from mouse embryonic olfactory bulb⁶⁷. Thus, components from both PRC2 and PRC1 complexes are critical for oligodendroglialogenesis from NSCs. One intriguing possibility is that these PcG proteins could play an essential role in down-regulating genes involved in neurogenesis and astrogenesis, thereby allowing oligodendroglialogenesis to take place. Another possibility is that oligodendroglialogenesis occurs only after PcG-mediated repression of the neurogenic basic

HLH transcription factor *neurogenin*⁶⁸, similar to what is described for astrogliogenesis⁶⁹. Indeed components of the PRC1 complex modulate timing of astrogenesis in the developing brain and were shown to repress the *neurogenin* promoter in a developmental stage-related manner⁶⁹.

Role of microRNAs in modulating neuronal and non-neuronal transcripts

Finally, recent reports have underlined the importance of miRNAs in modulating astrocytic and neuronal differentiation. Krichevsky et al. demonstrated that over-expression of miR-124a and miR-9 in mouse ESCs inhibited astrogliogenesis, possibly by interfering with STAT3 phosphorylation, which is critical for STAT3 activation. This was achieved by microRNA targeting of STAT3 upstream factors⁷⁰. MiR-124a also negatively regulates non-neuronal gene expression in neural progenitor cells and is regulated by REST/NRSF. During neural differentiation, the expression of REST decreases and leads to de-repression of miR-124a, thereby allowing the degradation of non-neuronal transcripts⁷¹.

Based on the experimental evidence discussed above, we propose that the transition from NSC to OPC requires a series of repressive events resulting from the cross-talk of histone modifications, DNA methylation and miRNAs.

Transition 3: From oligodendrocyte progenitors to myelin-forming cells

Histone modifications

Ultrastructural studies have described the characteristic appearance of compacted chromatin in myelinating and non-myelinating oligodendrocytes⁷². Because compaction is associated with transcriptional repression, we propose a model of oligodendrocyte differentiation characterized by the progressive decrease of transcriptional inhibitors, followed by the up-regulation of activators and then myelin gene expression (Fig. 2). This model is supported by the detection of transcriptional inhibitors in proliferating OPCs followed by their gradual decrease during maturation into myelin-forming cells^{55, 73}. An example is the expression of *Hes5*, a transcriptional inhibitor that not only inhibits myelin gene expression by direct recruitment of histone deacetylases to myelin gene promoters, but also decreases the effectiveness of the activators by direct repression of their promoters and protein sequestration⁷³. The expression of this inhibitor is regulated by histone deacetylase activity⁷⁴ and the progressive decline of its levels modulates myelin gene expression by decreasing inhibition on the myelin gene promoters and releasing the inhibition on the activators (such as *Mash1* and *Sox10*)⁷³.

Consistent with the model described above, multiple studies have demonstrated the requirement of HDAC activity for oligodendrocyte differentiation *in vitro* and *in vivo*. Systemic administration of rat pups with HDAC inhibitors prevented the differentiation of OPCs⁷⁵. Several members of class I HDAC, including HDAC1 and HDAC2, were shown to be expressed in oligodendrocyte cells *in vivo* and to be critical for oligodendrocyte differentiation in development and repair^{55, 75}. Silencing of HDAC1 and HDAC2 resulted in defective OPC differentiation *in vitro*, and was associated with high levels of *Sox2*⁵⁵. Hdac1 and Hdac2 were also recruited to the promoter of *Sox2* during repair of demyelinating lesions in adult rodents⁵⁵. Microinjections of *hdac1* antisense oligonucleotides in zebrafish and analysis of *hdac1* mutants revealed the absence of oligodendrocytes⁷⁶. Further supporting evidence was provided by the phenotypic analysis of the conditional *Hdac1* and *Hdac2* double knockout mice, generated by crossing floxed *Hdac1* and *Hdac2* mice with *Olig1-Cre* transgenic lines. The double knockout mice, developed severe hypomyelination and died during the second postnatal week⁷⁷.

In addition, HDAC1 and HDAC2 form complexes and repress the expression of transcription factors that inhibit myelin gene expression and oligodendrocyte differentiation, such as *Hes5*

and *Tcf4*^{55, 77}. A zinc-finger protein, Yin Yang 1 (YY1), recruits HDAC1 to the promoter region of *Tcf4* and *Id4*, thereby decreasing their levels due to transcriptional repression. An additional model of down-regulation of *Id2/4* transcript levels is proposed and associated with competition of Hdac1/2 with the downstream Wnt signaling molecule β -catenin for the binding of Tcf4⁷⁷. Together these results suggest that myelin gene expression requires the repression of transcriptional inhibitors and subsequent surge of transcriptional activators. Additional regulators of myelin genes are summarized in Table 2.

An alternative model has proposed a different role for the histone deacetylase isoform, Hdac11. This histone deacetylase is highly expressed during myelination and modulates deacetylation of H3K9/K14 on myelin genes during oligodendrocyte maturation. Expression of HDAC11 increases in maturing OL-1 oligodendrocytes and primary cultured OPCs, along with its recruitment to the MBP and PLP genes. Silencing HDAC11 with siRNA increased acetylated H3K9/K14, and led to reduced myelin gene expression due to arrested oligodendrocyte maturation⁷⁸. Although the mechanisms by which histone deacetylation actively regulates myelin gene expression remain elusive, collectively, these studies clearly demonstrate that histone deacetylation is critical for OPC differentiation.

The role of microRNAs during oligodendrocyte differentiation has been defined using specific microarrays³⁸. This analysis identified several miRNAs and 37 of them had a specific mRNA target bias, which is computationally determined by the co-expression of a certain miRNA with its predicted targets. In particular, the study demonstrated that miR-9 negatively regulated the expression of peripheral myelin protein *pmp22*, a protein expressed in Schwann cells, by interacting with the 3' untranslated region and preventing expression of this protein in oligodendrocytes³⁸. Additionally, miR-23 negatively regulates *laminB1*, a structural protein expressed in the nuclear envelope, whose overexpression characterizes autosomal dominant leukodystrophy (ADLD) patients. Over-expression of lamin B1 is associated with reduced myelin gene expression *in vitro* and *in vivo*, altered subcellular localization of MBP and PLP and reduced branching of oligodendrocyte, that can be rescued by co-expression of miR-23⁷⁹. Together, these studies provide compelling evidence for miRNA-mediated regulation in the differentiation of progenitors to mature oligodendrocytes.

Conclusion

The recent advances in regulation of gene expression have revealed the existence of an intricate regulatory network including transcription factors, chromatin modulators DNA methylation and microRNAs. This network defines the unique identity of each cell type, via a process of “epigenetic programming”. In this review we discuss the subsequent transitions from pluripotent ESCs to myelinating oligodendrocytes. The overall concept is that repressive epigenetic regulations play a critical role for the progressive restriction of the lineage potential, due to the presence of covalent histone modifications, DNA methylation and the emerging role of microRNA. A better understanding of the molecular events characterizing each transition may provide an important framework for future therapeutic studies aimed at repair of demyelinating conditions.

BOX

OUTSTANDING QUESTIONS

1. Oligodendrocytes are generated by a series of sequential transitions from stem cells through multipotential progenitors to oligodendrocyte progenitors that differentiate into myelin-forming oligodendrocytes. What are the main epigenetic events characterizing each of these transitions?

2. Does oligodendroglialogenesis entail the repression of transcriptional programs of neurogenesis and astroglialogenesis?
3. Does the functional state of myelinating oligodendrocyte coincide with the tail end of terminal differentiation? Is the differentiation process from stem cell to oligodendrocyte uni- or bi-directional?

BOX 1

Nucleosomal Histones

The basic unit of chromatin is the nucleosome, which is composed of a core octamer of histones (H2A, H2B, H3, and H4) surrounded by 146 base pairs of DNA. The post-translational modifications on specific amino acid residues on the tails of the histones modulate the transcriptional activity of genes and the access of the transcriptional machinery to DNA. These modulations include acetylation/deacetylation, methylation, sumoylation and ubiquitination of lysine residues, methylation and citrullination of arginine residues, phosphorylation of serines and threonines, ADP ribosylation and isomerization of prolines (reviewed by ⁸⁰). The addition or removal of chemical groups on specific residues of histones contributes to transcriptional activation or repression. The presence of histone modifications or specific transcription factor binding to defined chromatin regions can be assessed using a technique called ChIP (Chromatin Immunoprecipitation). The advantage of this technique is that proteins and DNA are cross-linked in cells prior to chromatin extraction and this allows the detection of the precise histone modifications or transcription factors present in a given chromatin region in a specific cell type at a precise developmental time point. The proteins in chromatin can be immunoprecipitated with antibodies that recognize post-translational modifications of histones, transcription factors or chromatin modifying enzymes. The DNA-protein complex can be then recovered, reverse-cross-linked and subject to further analysis.

BOX 12

Histone acetylation and deacetylation

Histone acetylation is functionally correlated with transcriptionally competent chromatin and is the result of a balance between the activity of histone acetyltransferases (HATs) and histone deacetylases (HDACs). HATs are categorized into two families: type A, which are nuclear and type B, which are cytoplasmic and contribute to acetylation of newly synthesized histones ⁸¹. Based on structural and functional differences, type A HATs are subdivided into five families. The GNAT (GCN5, PCAF and ELP3) and the p300/CBP family (p300 and CBP) acetylate various substrates, including histone and non-histone proteins, such as p53 and E1A. The MYST family (TIP60, MOZ, MOF, MORF and HBO1) has a MYST domain with the ability to interact with DNA. Other families of HATs include transcription factors with HAT activity (ATF2, TAF1 and TFIIC90) and the nuclear hormone-related HATs (SRC4 and ACTR). Most histone acetylation occurs at the N-terminal histone tails, with the exception of H3K56 ⁵⁰. HDACs have been categorized into four groups: Class I (HDAC-1, -2, -3, and -8), Class II (HDAC-4, -5, -6, -7, -9 and -10), Class III (SIRT 1-7) and Class IV (HDAC11), based on subcellular localization, tissue distribution and sequence homology ⁸². Class I and IV are mainly nuclear. Class II HDACs are cytoplasmic, with the exception of HDAC10, but have the ability to shuttle to the nucleus. Class III includes nuclear and cytosolic NAD-dependent enzymes called Sirtuins, of which only Sirt2 has been shown to regulate oligodendrocyte differentiation ⁸³. Class IV

HDACs include only HDAC11, which has been recently shown to positively modulate myelin gene expression ⁷⁸.

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References

1. Kim JK, et al. Epigenetic mechanisms in mammals. *Cell Mol Life Sci* 2009;66:596–612. [PubMed: 18985277]
2. Santenard A, Torres-Padilla ME. Epigenetic reprogramming in mammalian reproduction: contribution from histone variants. *Epigenetics* 2009;4:80–84. [PubMed: 19242119]
3. Copray S, et al. Epigenetic mechanisms facilitating oligodendrocyte development, maturation, and aging. *Glia*. 2009
4. Li H, et al. Two-tier transcriptional control of oligodendrocyte differentiation. *Curr Opin Neurobiol*. 2009
5. Namiyama M, et al. Epigenetic mechanisms regulating fate specification of neural stem cells. *Philos Trans R Soc Lond B Biol Sci* 2008;363:2099–2109. [PubMed: 18375376]
6. Shi Y, et al. Neural stem cell self-renewal. *Crit Rev Oncol Hematol* 2008;65:43–53. [PubMed: 17644000]
7. Reik W. Stability and flexibility of epigenetic gene regulation in mammalian development. *Nature* 2007;447:425–432. [PubMed: 17522676]
8. Hemberger M, et al. Epigenetic dynamics of stem cells and cell lineage commitment: digging Waddington's canal. *Nat Rev Mol Cell Biol* 2009;10:526–537. [PubMed: 19603040]
9. Cloos PA, et al. Erasing the methyl mark: histone demethylases at the center of cellular differentiation and disease. *Genes Dev* 2008;22:1115–1140. [PubMed: 18451103]
10. Shi Y. Histone lysine demethylases: emerging roles in development, physiology and disease. *Nat Rev Genet* 2007;8:829–833. [PubMed: 17909537]
11. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 2006;126:663–676. [PubMed: 16904174]
12. Wernig M, et al. In vitro reprogramming of fibroblasts into a pluripotent ES-cell-like state. *Nature* 2007;448:318–324. [PubMed: 17554336]
13. Mikkelsen TS, et al. Dissecting direct reprogramming through integrative genomic analysis. *Nature* 2008;454:49–55. [PubMed: 18509334]
14. Deng J, et al. Targeted bisulfite sequencing reveals changes in DNA methylation associated with nuclear reprogramming. *Nat Biotechnol* 2009;27:353–360. [PubMed: 19330000]
15. Huangfu D, et al. Induction of pluripotent stem cells by defined factors is greatly improved by small-molecule compounds. *Nat Biotechnol* 2008;26:795–797. [PubMed: 18568017]
16. Huangfu D, et al. Induction of pluripotent stem cells from primary human fibroblasts with only Oct4 and Sox2. *Nat Biotechnol* 2008;26:1269–1275. [PubMed: 18849973]
17. Shi Y, et al. Induction of pluripotent stem cells from mouse embryonic fibroblasts by Oct4 and Klf4 with small-molecule compounds. *Cell Stem Cell* 2008;3:568–574. [PubMed: 18983970]
18. Wegner M. A matter of identity: transcriptional control in oligodendrocytes. *J Mol Neurosci* 2008;35:3–12. [PubMed: 18401762]
19. Keenen B, de la Serna IL. Chromatin remodeling in embryonic stem cells: regulating the balance between pluripotency and differentiation. *J Cell Physiol* 2009;219:1–7. [PubMed: 19097034]
20. Shilatifard A. Chromatin modifications by methylation and ubiquitination: implications in the regulation of gene expression. *Annu Rev Biochem* 2006;75:243–269. [PubMed: 16756492]
21. Ruthenburg AJ, et al. Methylation of lysine 4 on histone H3: intricacy of writing and reading a single epigenetic mark. *Mol Cell* 2007;25:15–30. [PubMed: 17218268]

22. Gangaraju VK, Bartholomew B. Mechanisms of ATP dependent chromatin remodeling. *Mutat Res* 2007;618:3–17. [PubMed: 17306844]
23. Li B, et al. The role of chromatin during transcription. *Cell* 2007;128:707–719. [PubMed: 17320508]
24. Heard E, et al. X-chromosome inactivation in mammals. *Annu Rev Genet* 1997;31:571–610. [PubMed: 9442908]
25. Li E, et al. Role for DNA methylation in genomic imprinting. *Nature* 1993;366:362–365. [PubMed: 8247133]
26. Chen T, et al. A novel Dnmt3a isoform produced from an alternative promoter localizes to euchromatin and its expression correlates with active de novo methylation. *J Biol Chem* 2002;277:38746–38754. [PubMed: 12138111]
27. Tsumura A, et al. Maintenance of self-renewal ability of mouse embryonic stem cells in the absence of DNA methyltransferases Dnmt1, Dnmt3a and Dnmt3b. *Genes Cells* 2006;11:805–814. [PubMed: 16824199]
28. Jackson M, et al. Severe global DNA hypomethylation blocks differentiation and induces histone hyperacetylation in embryonic stem cells. *Mol Cell Biol* 2004;24:8862–8871. [PubMed: 15456861]
29. Fouse SD, et al. Promoter CpG methylation contributes to ES cell gene regulation in parallel with Oct4/Nanog, PcG complex, and histone H3 K4/K27 trimethylation. *Cell Stem Cell* 2008;2:160–169. [PubMed: 18371437]
30. Mohn F, et al. Lineage-specific polycomb targets and de novo DNA methylation define restriction and potential of neuronal progenitors. *Mol Cell* 2008;30:755–766. [PubMed: 18514006]
31. Ooi L, Wood IC. Regulation of gene expression in the nervous system. *Biochem J* 2008;414:327–341. [PubMed: 18717648]
32. Ma DK, et al. Neuronal activity-induced Gadd45b promotes epigenetic DNA demethylation and adult neurogenesis. *Science* 2009;323:1074–1077. [PubMed: 19119186]
33. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 2004;116:281–297. [PubMed: 14744438]
34. Benetti R, et al. A mammalian microRNA cluster controls DNA methylation and telomere recombination via Rbl2-dependent regulation of DNA methyltransferases. *Nat Struct Mol Biol* 2008;15:998. [PubMed: 18769471]
35. Sinkkonen L, et al. MicroRNAs control de novo DNA methylation through regulation of transcriptional repressors in mouse embryonic stem cells. *Nat Struct Mol Biol* 2008;15:259–267. [PubMed: 18311153]
36. Houbaviy HB, et al. Embryonic stem cell-specific MicroRNAs. *Dev Cell* 2003;5:351–358. [PubMed: 12919684]
37. Tay Y, et al. MicroRNAs to Nanog, Oct4 and Sox2 coding regions modulate embryonic stem cell differentiation. *Nature* 2008;455:1124–1128. [PubMed: 18806776]
38. Lau P, et al. Identification of dynamically regulated microRNA and mRNA networks in developing oligodendrocytes. *J Neurosci* 2008;28:11720–11730. [PubMed: 18987208]
39. Lehotzky A, et al. Tubulin polymerization-promoting protein (TPPP/p25) is critical for oligodendrocyte differentiation. *Glia*. 2009
40. Boyer LA, et al. Core transcriptional regulatory circuitry in human embryonic stem cells. *Cell* 2005;122:947–956. [PubMed: 16153702]
41. Lee TI, et al. Control of developmental regulators by Polycomb in human embryonic stem cells. *Cell* 2006;125:301–313. [PubMed: 16630818]
42. Boyer LA, et al. Polycomb complexes repress developmental regulators in murine embryonic stem cells. *Nature* 2006;441:349–353. [PubMed: 16625203]
43. Czermin B, et al. Drosophila enhancer of Zeste/ESC complexes have a histone H3 methyltransferase activity that marks chromosomal Polycomb sites. *Cell* 2002;111:185–196. [PubMed: 12408863]
44. Muller J, et al. Histone methyltransferase activity of a Drosophila Polycomb group repressor complex. *Cell* 2002;111:197–208. [PubMed: 12408864]
45. Schwartz YB, Pirrotta V. Polycomb silencing mechanisms and the management of genomic programmes. *Nat Rev Genet* 2007;8:9–22. [PubMed: 17173055]

46. Wang H, et al. Role of histone H2A ubiquitination in Polycomb silencing. *Nature* 2004;431:873–878. [PubMed: 15386022]
47. Bernstein BE, et al. A bivalent chromatin structure marks key developmental genes in embryonic stem cells. *Cell* 2006;125:315–326. [PubMed: 16630819]
48. Mikkelsen TS, et al. Genome-wide maps of chromatin state in pluripotent and lineage-committed cells. *Nature* 2007;448:553–560. [PubMed: 17603471]
49. Zhao XD, et al. Whole-genome mapping of histone H3 Lys4 and 27 trimethylations reveals distinct genomic compartments in human embryonic stem cells. *Cell Stem Cell* 2007;1:286–298. [PubMed: 18371363]
50. Xie W, et al. Histone h3 lysine 56 acetylation is linked to the core transcriptional network in human embryonic stem cells. *Mol Cell* 2009;33:417–427. [PubMed: 19250903]
51. Xu N, et al. MicroRNA-145 regulates OCT4, SOX2, and KLF4 and represses pluripotency in human embryonic stem cells. *Cell* 2009;137:647–658. [PubMed: 19409607]
52. Mehler MF. Epigenetic principles and mechanisms underlying nervous system functions in health and disease. *Prog Neurobiol* 2008;86:305–341. [PubMed: 18940229]
53. Liu A, et al. The glial or neuronal fate choice of oligodendrocyte progenitors is modulated by their ability to acquire an epigenetic memory. *J Neurosci* 2007;27:7339–7343. [PubMed: 17611286]
54. Kondo T, Raff M. Chromatin remodeling and histone modification in the conversion of oligodendrocyte precursors to neural stem cells. *Genes Dev* 2004;18:2963–2972. [PubMed: 15574597]
55. Shen S, et al. Age-dependent epigenetic control of differentiation inhibitors is critical for remyelination efficiency. *Nat Neurosci* 2008;11:1024–1034. [PubMed: 19160500]
56. Lyssiotis CA, et al. Inhibition of histone deacetylase activity induces developmental plasticity in oligodendrocyte precursor cells. *Proc Natl Acad Sci U S A* 2007;104:14982–14987. [PubMed: 17855562]
57. Belachew S, et al. Postnatal NG2 proteoglycan-expressing progenitor cells are intrinsically multipotent and generate functional neurons. *J Cell Biol* 2003;161:169–186. [PubMed: 12682089]
58. Rivers LE, et al. PDGFRA/NG2 glia generate myelinating oligodendrocytes and piriform projection neurons in adult mice. *Nat Neurosci* 2008;11:1392–1401. [PubMed: 18849983]
59. Nishiyama A, et al. Polydendrocytes (NG2 cells): multifunctional cells with lineage plasticity. *Nat Rev Neurosci* 2009;10:9–22. [PubMed: 19096367]
60. Ballas N, et al. Regulation of neuronal traits by a novel transcriptional complex. *Neuron* 2001;31:353–365. [PubMed: 11516394]
61. Ballas N, et al. REST and its corepressors mediate plasticity of neuronal gene chromatin throughout neurogenesis. *Cell* 2005;121:645–657. [PubMed: 15907476]
62. Doetsch F. The glial identity of neural stem cells. *Nat Neurosci* 2003;6:1127–1134. [PubMed: 14583753]
63. Nakashima K, et al. Synergistic signaling in fetal brain by STAT3-Smad1 complex bridged by p300. *Science* 1999;284:479–482. [PubMed: 10205054]
64. Sardi SP, et al. Presenilin-dependent ErbB4 nuclear signaling regulates the timing of astrogenesis in the developing brain. *Cell* 2006;127:185–197. [PubMed: 17018285]
65. Hermanson O, et al. N-CoR controls differentiation of neural stem cells into astrocytes. *Nature* 2002;419:934–939. [PubMed: 12410313]
66. Sher F, et al. Differentiation of neural stem cells into oligodendrocytes: involvement of the polycomb group protein Ezh2. *Stem Cells* 2008;26:2875–2883. [PubMed: 18687996]
67. Roman-Trufero M, et al. Maintenance of undifferentiated state and self-renewal of embryonic neural stem cells by Polycomb protein Ring1B. *Stem Cells* 2009;27:1559–1570. [PubMed: 19544461]
68. Guillemot F. Spatial and temporal specification of neural fates by transcription factor codes. *Development* 2007;134:3771–3780. [PubMed: 17898002]
69. Hirabayashi Y, et al. Polycomb limits the neurogenic competence of neural precursor cells to promote astrogenic fate transition. *Neuron* 2009;63:600–613. [PubMed: 19755104]
70. Krichevsky AM, et al. Specific microRNAs modulate embryonic stem cell-derived neurogenesis. *Stem Cells* 2006;24:857–864. [PubMed: 16357340]

71. Conaco C, et al. Reciprocal actions of REST and a microRNA promote neuronal identity. *Proc Natl Acad Sci U S A* 2006;103:2422–2427. [PubMed: 16461918]
72. Menn B, et al. Origin of oligodendrocytes in the subventricular zone of the adult brain. *J Neurosci* 2006;26:7907–7918. [PubMed: 16870736]
73. Liu A, et al. A molecular insight of Hes5-dependent inhibition of myelin gene expression: old partners and new players. *Embo J* 2006;25:4833–4842. [PubMed: 17006542]
74. Shen S, Casaccia-Bonnel P. Post-translational modifications of nucleosomal histones in oligodendrocyte lineage cells in development and disease. *J Mol Neurosci* 2008;35:13–22. [PubMed: 17999198]
75. Shen S, et al. Histone modifications affect timing of oligodendrocyte progenitor differentiation in the developing rat brain. *J Cell Biol* 2005;169:577–589. [PubMed: 15897262]
76. Cunliffe VT, Casaccia-Bonnel P. Histone deacetylase 1 is essential for oligodendrocyte specification in the zebrafish CNS. *Mech Dev* 2006;123:24–30. [PubMed: 16324829]
77. Ye F, et al. HDAC1 and HDAC2 regulate oligodendrocyte differentiation by disrupting the beta-catenin-TCF interaction. *Nat Neurosci* 2009;12:829–838. [PubMed: 19503085]
78. Liu H, et al. Histone deacetylase 11 regulates oligodendrocyte-specific gene expression and cell development in OL-1 oligodendroglia cells. *Glia* 2009;57:1–12. [PubMed: 18627006]
79. Lin ST, Fu YH. miR-23 regulation of lamin B1 is crucial for oligodendrocyte development and myelination. *Dis Model Mech* 2009;2:178–188. [PubMed: 19259393]
80. Kouzarides T. Chromatin modifications and their function. *Cell* 2007;128:693–705. [PubMed: 17320507]
81. Parthun MR. Hat1: the emerging cellular roles of a type B histone acetyltransferase. *Oncogene* 2007;26:5319–5328. [PubMed: 17694075]
82. Thiagalingam S, et al. Histone deacetylases: unique players in shaping the epigenetic histone code. *Ann N Y Acad Sci* 2003;983:84–100. [PubMed: 12724214]
83. Li W, et al. Sirtuin 2, a mammalian homolog of yeast silent information regulator-2 longevity regulator, is an oligodendroglial protein that decelerates cell differentiation through deacetylating alpha-tubulin. *J Neurosci* 2007;27:2606–2616. [PubMed: 17344398]
84. Barrand S, Collas P. Chromatin states of core pluripotency-associated genes in pluripotent, multipotent and differentiated cells. *Biochem Biophys Res Commun*. 2009
85. Sebastian S, et al. MLL5, a trithorax homolog, indirectly regulates H3K4 methylation, represses cyclin A2 expression, and promotes myogenic differentiation. *Proc Natl Acad Sci U S A* 2009;106:4719–4724. [PubMed: 19264965]
86. Benevolenskaya EV. Histone H3K4 demethylases are essential in development and differentiation. *Biochem Cell Biol* 2007;85:435–443. [PubMed: 17713579]
87. Lan F, et al. Mechanisms involved in the regulation of histone lysine demethylases. *Curr Opin Cell Biol* 2008;20:316–325. [PubMed: 18440794]
88. Schneider R, et al. Unsafe SETs: histone lysine methyltransferases and cancer. *Trends Biochem Sci* 2002;27:396–402. [PubMed: 12151224]
89. Nottke A, et al. Developmental roles of the histone lysine demethylases. *Development* 2009;136:879–889. [PubMed: 19234061]
90. Brown MA, et al. Identification and characterization of Smyd2: a split SET/MYND domain-containing histone H3 lysine 36-specific methyltransferase that interacts with the Sin3 histone deacetylase complex. *Mol Cancer* 2006;5:26. [PubMed: 16805913]
91. Tanaka Y, et al. Trithorax-group protein ASH1 methylates histone H3 lysine 36. *Gene* 2007;397:161–168. [PubMed: 17544230]
92. Klose RJ, et al. The transcriptional repressor JHDM3A demethylates trimethyl histone H3 lysine 9 and lysine 36. *Nature* 2006;442:312–316. [PubMed: 16732292]
93. Steger DJ, et al. DOT1L/KMT4 recruitment and H3K79 methylation are ubiquitously coupled with gene transcription in mammalian cells. *Mol Cell Biol* 2008;28:2825–2839. [PubMed: 18285465]
94. Karachentsev D, et al. PR-Set7-dependent methylation of histone H4 Lys 20 functions in repression of gene expression and is essential for mitosis. *Genes Dev* 2005;19:431–435. [PubMed: 15681608]

95. Yang H, Mizzen CA. The multiple facets of histone H4-lysine 20 methylation. *Biochem Cell Biol* 2009;87:151–161. [PubMed: 19234531]
96. Lu QR, et al. Common developmental requirement for Olig function indicates a motor neuron/oligodendrocyte connection. *Cell* 2002;109:75–86. [PubMed: 11955448]
97. Li H, et al. Olig1 and Sox10 interact synergistically to drive myelin basic protein transcription in oligodendrocytes. *J Neurosci* 2007;27:14375–14382. [PubMed: 18160645]
98. Sugimori M, et al. Ascl1 is required for oligodendrocyte development in the spinal cord. *Development* 2008;135:1271–1281. [PubMed: 18287202]
99. Samanta J, Kessler JA. Interactions between ID and OLIG proteins mediate the inhibitory effects of BMP4 on oligodendroglial differentiation. *Development* 2004;131:4131–4142. [PubMed: 15280210]
100. Marin-Husstege M, et al. Multiple roles of Id4 in developmental myelination: predicted outcomes and unexpected findings. *Glia* 2006;54:285–296. [PubMed: 16862533]
101. He Y, et al. The transcription factor Yin Yang 1 is essential for oligodendrocyte progenitor differentiation. *Neuron* 2007;55:217–230. [PubMed: 17640524]
102. Fancy SP, et al. Dysregulation of the Wnt pathway inhibits timely myelination and remyelination in the mammalian CNS. *Genes Dev* 2009;23:1571–1585. [PubMed: 19515974]
103. Stolt CC, et al. SoxD proteins influence multiple stages of oligodendrocyte development and modulate SoxE protein function. *Dev Cell* 2006;11:697–709. [PubMed: 17084361]
104. Chew LJ, Gallo V. The Yin and Yang of Sox proteins: Activation and repression in development and disease. *J Neurosci Res*. 2009
105. Stolt CC, Wegner M. SoxE function in vertebrate nervous system development. *Int J Biochem Cell Biol*. 2009
106. Barres BA, et al. A novel role for thyroid hormone, glucocorticoids and retinoic acid in timing oligodendrocyte development. *Development* 1994;120:1097–1108. [PubMed: 8026323]
107. Pombo PM, et al. Stimulation of the myelin basic protein gene expression by 9-cis-retinoic acid and thyroid hormone: activation in the context of its native promoter. *Brain Res Mol Brain Res* 1999;64:92–100. [PubMed: 9889331]
108. Wang SZ, et al. An oligodendrocyte-specific zinc-finger transcription regulator cooperates with Olig2 to promote oligodendrocyte differentiation. *Development* 2006;133:3389–3398. [PubMed: 16908628]
109. Nielsen JA, et al. Myelin transcription factor 1 (Myt1) modulates the proliferation and differentiation of oligodendrocyte lineage cells. *Mol Cell Neurosci* 2004;25:111–123. [PubMed: 14962745]
110. Romm E, et al. Myt1 family recruits histone deacetylase to regulate neural transcription. *J Neurochem* 2005;93:1444–1453. [PubMed: 15935060]
111. Emery B, et al. Myelin gene regulatory factor is a critical transcriptional regulator required for CNS myelination. *Cell* 2009;138:172–185. [PubMed: 19596243]
112. Qi Y, et al. Control of oligodendrocyte differentiation by the Nkx2.2 homeodomain transcription factor. *Development* 2001;128:2723–2733. [PubMed: 11526078]
113. Wei Q, et al. Stage-specific expression of myelin basic protein in oligodendrocytes involves Nkx2.2-mediated repression that is relieved by the Sp1 transcription factor. *J Biol Chem* 2005;280:16284–16294. [PubMed: 15695521]
114. Vallstedt A, et al. Multiple dorsoventral origins of oligodendrocyte generation in the spinal cord and hindbrain. *Neuron* 2005;45:55–67. [PubMed: 15629702]

Figure 1. Epigenetic mechanisms during the transition from embryonic stem cells (ESC) to neural stem cells (NSCs)

The progression of pluripotent ESCs to multipotent neural precursors or “neural stem” cells (NSCs) is characterized by the repression of critical transcription factors involved in pluripotency, such as Oct4, Sox2 and Nanog. (a) In ESCs, these genes are actively transcribed (green light) and this correlates with the presence of active histone marks, such as methylation of lysine residue K4 (yellow flag) and acetylation of lysine K9 and lysine 14 (blue triangle). (b) Lineage-specific genes (neural, endodermal or mesodermal genes), in contrast are maintained in a transcriptionally competent, but inactive state (“poised state”), due to the presence of “bivalent domains” that are characterized by the simultaneous presence of histone marks of activation (yellow flag) and repression (orange flag). (c) Upon neural lineage choice, a massive rearrangement of chromatin occurs. Regulatory regions of genes involved in pluripotency and genes related to other lineages become repressed. The repression is achieved by a combination of DNA methylation (white circles), through the recruitment of *de novo* DNA methyltransferase DNMT3a/3b and repressive histone methylation (red and orange flags).^{42, 47, 84} Neural genes, in contrast, become “active” by removing the repressive marks and retaining activating methylation of K4.

Figure 2. Epigenetic mechanisms modulating the transition from neural stem cell (NSCs) to oligodendrocyte progenitor (OPCs) and from OPC to myelin-forming cells

Schematic diagram of oligodendrocyte lineage choice in two steps. The top panel (a) depicts a central role of HDAC in oligodendrogliogenesis, due to repression of alternative fates (i.e. repression of neuronal or astrocytic lineage genes). The bottom panel (b) shows a schematic representation of the process of differentiation of OPCs into myelin-forming cells. OPCs express high levels of transcriptional inhibitors of myelin genes. Differentiation is characterized by the increased expression of transcriptional activators and by the progressive down-regulation of the inhibitors due to the removal of active histone marks (pink triangles) and addition of repressive histone marks on their promoters, including deacetylation⁵⁵ and repressive methylation (red flag). For myelin genes, two scenarios are presented. The first one is a hypothetical model showing acetylated histones during active transcription of myelin genes. The second one refers to a study identifying HDAC11 as a positive regulator of myelin gene expression⁷⁸.

Table 1

Histone methylation modifying enzymes

Mark	Methyltransferase	Refs	Demethylase	Refs
H3K4	Mll-1	21, 85	LSD1	86, 87
	Mll-2		JRAID1A	
	Mll-3		JARID1B	
	Mll-4		JARID1C	
	Mll-5		JARID1D	
	SET1A		JHDM1B	
	SET1B			
	ASH1			
	SMYD3			
	SET7/9			
	PRDM9			
H3K9	Suv39h1	80, 88	LSD1	87, 89
	Suv39h2		JHDM2A	
	G9a		JHDM2B	
	EuHMTase		JHDM2C	
	ESET		JMJD2A	
	RIZ1		JMJD2B	
			JMJD2C	
			JMJD2D	
H3K27	EZH2	88	JMJD3	87, 89
			UTX	
H3K36	SET2	88, 90, 91	JHDM1A	87, 89, 92
	NSD1		JHDM1B	
	SMYD2		JMJD2A	
	ASH1		JMJD2B	
			JMJD2C	
H3K79	DOT1L	84, 93		
H4K20	Pr-SET7/8	94, 95		
	SUV4-20H1			
	SUV4-20H2			

Green = transcription activation; Red = transcription repression, or heterochromatin formation and silencing. For the enzymes in grey boxes, please refer to the references for further information.

Table 2

Transcription regulators of myelin genes

Molecules	Effect	Family	References
Hes5	–	basic helix-loop-helix (bHLH)	73
Olig1/2	+	basic helix-loop-helix (bHLH)	96, 97
ASCL1 (Mash1)	+	basic helix-loop-helix (bHLH)	98
Id2, Id4	–	helix-loop-helix (HLH)	99, 100
Tcf7L2	–	high mobility group (HMG)	77, 101, 102
Sox4, Sox5, Sox6, Sox11	–	high mobility group (HMG)	103, 104
Sox8, Sox9, Sox10, Sox17	+	high mobility group (HMG)	97, 103–105
Nuclear hormone receptor	+		106, 107
YY1	+	Zinc-finger protein	101
Zfp488	+	Zinc-finger protein	108
MYT1	+/-	Zinc-finger protein	109, 110
MRF	+	NDT80 domain	111
Nkx2.2	+/-	Homeodomain	112, 113
Nkx6.1, NKx6.2	+	Homeodomain	114