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Epigenetic Regulation of Neural Differentiation from Embryonic Stem Cells

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1. Introduction

During development, ESCs in the inner cell mass of the blastocyst undergo progressive fate restriction and sequentially give rise to tissue-specific multipotent progenitor cells [1-3]. In the nervous system, neural progenitor cells (NPCs) are defined as multipotent cells capable of differentiating into neurons and glia, the latter of which include astrocytes and oligodendrocytes. NPCs undergo sequential fate restriction during nervous system development [4-6]. During early embryonic development, NPCs give rise exclusively to neuronal cell types, however at later stages they differentiate into glial cell types [7, 8]. Understanding how ESCs differentiate into neuronal or glial cell types, thus, constitutes a fundamental aspect of nervous system development.

ESCs possess two cardinal cellular characteristics: self-renewal capacity and pluripotency [2, 3]. ESCs, when undifferentiated, maintain a unique gene expression profile which keeps themselves in a pluripotent state [3, 9-12]. When ESCs begin to differentiate, however, this profile is dramatically altered without changes in the DNA sequence, allowing initial cell lineage specification to take place [11, 13-15]. Studies on lineage choice and differentiation of stem cells suggest that the crosstalk between transcription factors and epigenetic mechanisms plays pivotal roles in maintaining the existing transcriptional profile or changing it into a new transcriptional profile [16, 17].

Chromatin in eukaryotes is organized into arrays of nucleosomes, which are comprised of DNA wrapped around a histone octamer containing 2 copies of individual core histones, H2A, H2B, H3, and H4 [18]. Chromatin can be tightly or loosely packed to alter its accessibility to transcription factors and RNA polymerases, thereby ultimately modulating the efficiency of gene transcription [19-23]. Epigenetic mechanisms act to change this accessibility both locally and globally through modifications of nucleosomes, and by remodeling of nu-

cleosomes [21, 24, 25]. In addition to epigenetic modifications and chromatin remodeling, epigenetic regulators have been recently extended to non-coding RNAs (ncRNAs), because ncRNAs can affect chromatin structure and transcriptional activation by regulating expression of key nucleosome modifiers [26]. These epigenetic controls appear to influence gene expression profiles, which are essential for self-renewal and differentiation capacities of ESCs. Thus, a clear understanding of the epigenetic mechanisms underlying gene expression patterns will provide significant and novel insights into cell fate specification of ESCs directed to differentiate into neurons. Furthermore, the epigenetic mechanisms are believed to be capable of responding to extrinsic signals such as morphogens and cytokines [8]. Therefore, knowledge of the epigenetic mechanisms is also important for our understanding of neural differentiation by extrinsic factors. In this review, we will describe the major epigenetic processes that underlie the acquisition of the NPC fate from ESCs as well as the subsequent neuronal subtype specification. The focus of this review is weighted on neuronal cell lineage specification, and not on glial cell specification.

2. Epigenetic mechanisms

2.1. Post-translational modifications of histones

Post-translational chemical modifications of histones in chromatin are diverse and include methylation, acetylation, phosphorylation, sumoylation, ubiquitination, and ADP-ribosylation [25, 27]. Here, we will focus on histone methylation and acetylation because these modifications are considered to be most important and widespread for influencing biological processes during neural differentiation.

Histone methylation occurs mainly at lysine and arginine residues on the tails of the histone H3 and H4 [28]. Histone methylation is reversible. Methylation at these sites has been associated with not only transcriptional activation and repression [27, 29] but also multiple biological processes, including heterochromatin formation and genomic imprinting [27, 28]. The differences in the effects of methylation depend on the lysine residues. For example, methylation of histone H3 lysine 9 (H3K9), H3K27, or H4K20 is generally linked to formation of gene silencing [30, 31], whereas methylation on H3K4, H3K36, and H3K79 is associated with actively transcribed regions and gene activation [29, 32, 33]. In addition, lysine residues can be mono-(me1), di-(me2), or tri-methylated (me3). These differentially methylated lysine residues lead to different levels of transcriptional activation or repression, resulting in diverse functional outcomes. For example, H4K20me1 plays an important role in transcriptional repression and X inactivation, while H4K20me2 and H4K20me3 are linked to DNA repair of double-stranded DNA damage [34].

All core histones can be dynamically acetylated on lysine residues in their tails and occasionally within the globular core. Histone acetylation removes the positive charge on the histones, thereby weakening the interaction with negatively charged DNA [25, 29]. As a consequence, chromatin is transformed into a more relaxed structure, which is associated with transcriptional activation. Like methylation, histone acetylation is reversible. This reversible

acetylation in histones is an important mechanism of controlling gene expression because histone acetylation and deacetylation are linked to transcriptional activation and inactivation, respectively [25, 27].

2.2. DNA methylation

DNA methylation is one of the major repressive epigenetic pathways. Methylation occurs at the cytosine residues followed by a guanine (CpG dinucleotides) in the DNA sequence. CpG DNA methylation of gene promoters is a well-known hallmark for transcriptionally inactive genes, and is generally associated with stable gene silencing, such as genomic imprinting and X chromosome inactivation [35, 36]. The DNA methylation state is established during embryogenesis by several DNA methyltransferases (DNMTs) [35]. In mammals, 2 types of DNMTs have been identified. DNMT3A and DNMT3B establish *de novo* DNA methylation, while DNMT1 maintains DNA methylation patterns during DNA replication [37]. These DNA methylation sites then recruit methyl-CpG-binding proteins, including methyl-CpG-binding domain (MBD) proteins [38, 39], which bind the histone deacetylase (HDAC)-containing repressor complex, and consequently repress transcription [40, 41].

2.3. Chromatin remodeling

Chromatin structure is not static, but subject to change in response to internal and external developmental signals [23]. Dynamic changes in the chromatin structure are regulated by ATP-dependent chromatin remodelers, which allow the transcriptional machinery to access its targets more or less effectively [42-44]. Using energy derived from ATP hydrolysis, ATP-dependent chromatin remodelers relocate nucleosomes either by mobilizing or restructuring nucleosomes [45, 46]. Thus, ATP-dependent chromatin remodelers can function both in transcriptional activation and repression via their nucleosome remodeling activity. Nearly all ATP-dependent chromatin remodelers are multi-protein complexes that contain an ATPase subunit, which belongs to the sucrose non-fermenting 2 (SNF2) family of ATPases. Based on the homology between their ATPase domains, ATP-dependent chromatin remodeling complexes are divided into 4 groups: switch/sucrose non-fermenting (SWI/SNF), imitation switch (ISWI), chromo helicase DNA binding (CHD), and inositol auxotroph 80 (INO80) [42-44].

Genetic and biochemical studies indicate that some ATP-dependent chromatin remodeling complexes contain epigenetic factors such as HDAC and MBD proteins. For example, nucleosome-remodeling deacetylase (NuRD) is a multi-subunit complex that includes a SWI2/SNF2 helicase/ATPase domain-containing Mi2 protein, HDAC1, HDAC2, and MBD3 [47]. The NuRD complexes promote the establishment of a specific chromatin structure at rRNA genes that are transcriptionally inactive but are poised for transcriptional activation and control transcription of these genes [48]. Thus, ATP-dependent chromatin complexes play essential roles in epigenetic regulation of transcription along with several histone-modifying enzymes and/or modified histone codes.

2.4. Non-coding RNAs

Apart from the role of histone modifications and DNA methylation, another form of epigenetic regulation involves non-coding RNAs (ncRNAs). A large variety of ncRNAs can be classified into 2 major classes based on their transcript size: small ncRNAs (less than 200 nucleotides) and long ncRNAs (greater than 200 nucleotides) [49]. Each of these classes can be further divided into subclasses. Micro RNA (miRNA) is a subgroup of small ncRNA molecules ~22 nucleotides in length. miRNAs post-transcriptionally regulate gene expression by binding to complementary or uncomplimentary sequences on target mRNA transcripts, which results in either mRNA degradation or inhibition of translation [50]. In animal cells, miRNA genes tend to be clustered in the genome, and are widely distributed [51]. Approximately half of the human miRNA genes are located in the introns of protein-coding genes. Expression studies have revealed that the clustered miRNA genes are often co-expressed, suggesting that they are jointly transcribed as a polycistron [52, 53].

3. Transition from ESCs to NPCS

3.1. Histone modifications

Expression of pluripotency genes, such as *OCT4* or *NANOG*, is a hallmark of undifferentiated ESCs. The promoters and/or enhancers of these pluripotency genes are marked by H3K4me₃, which is strongly associated with transcriptionally active genes. In contrast, the majority of genes, whose upregulation leads to differentiation, are inactivated or expressed at very low levels [54, 55]. These genes loci are maintained in a transcriptionally competent but inactive state characterized by both active (H3-K4me₃) and repressive (H3-K27me₃) histone marks, a configuration described as a “bivalent domain” (Figure 1) [54, 55]. Bivalent histone methylation in the promoters of proneural genes, such as Neurogenins (*Ngns*), *Pax6*, and *Mash1*, has been reported in undifferentiated ESCs [56]. H3K27 and H3K4 methylation is catalyzed by Polycomb-group (PcG) and Trithorax-group (TrxG) proteins, respectively [57]. PcG proteins form a complex referred to as the Polycomb Repressor Complex (PRC). PRCs can be biochemically subdivided into 2 groups: PRC1 and PRC2. PRC1 and PRC2 are essential for the repression of key developmental genes and maintenance of pluripotency in ESCs [58, 59]. The PRC2 complexes, which contain Enhancer of Zeste Homolog (EZH), a histone methyltransferase (HMTase), catalyze tri-methylation of H3K27 [60]. This histone mark leads to the recruitment of PRC1, thereby contributing to a repressive chromatin state [61, 62]. Consistent with this, ESCs deficient in a PRC2 component display de-repression of tissue-specific genes, including neural-associated genes [59]. TrxG proteins also act as large multimeric complexes [57]. TrxG complexes possess methyltransferase activity directed specifically towards H3K4, thereby leading to increased levels of H3K4me₃ [57]. *Dpy-30* is a mammalian homolog of the *Drosophila* TrxG protein, and a core component of myeloid/lymphoid or mixed-lineage leukemia (MLL) histone methyltransferase complexes. Depletion of *Dpy-30* in ESCs results in a defect in their neural lineage specification through reduced H3K4 methylation at bivalent domains of key developmental loci [63]. Collectively, these findings suggest that the bivalent domain

mediated by PcG and TrxG proteins plays a key epigenetic role in maintenance of the undifferentiated state and suppress neural differentiation.

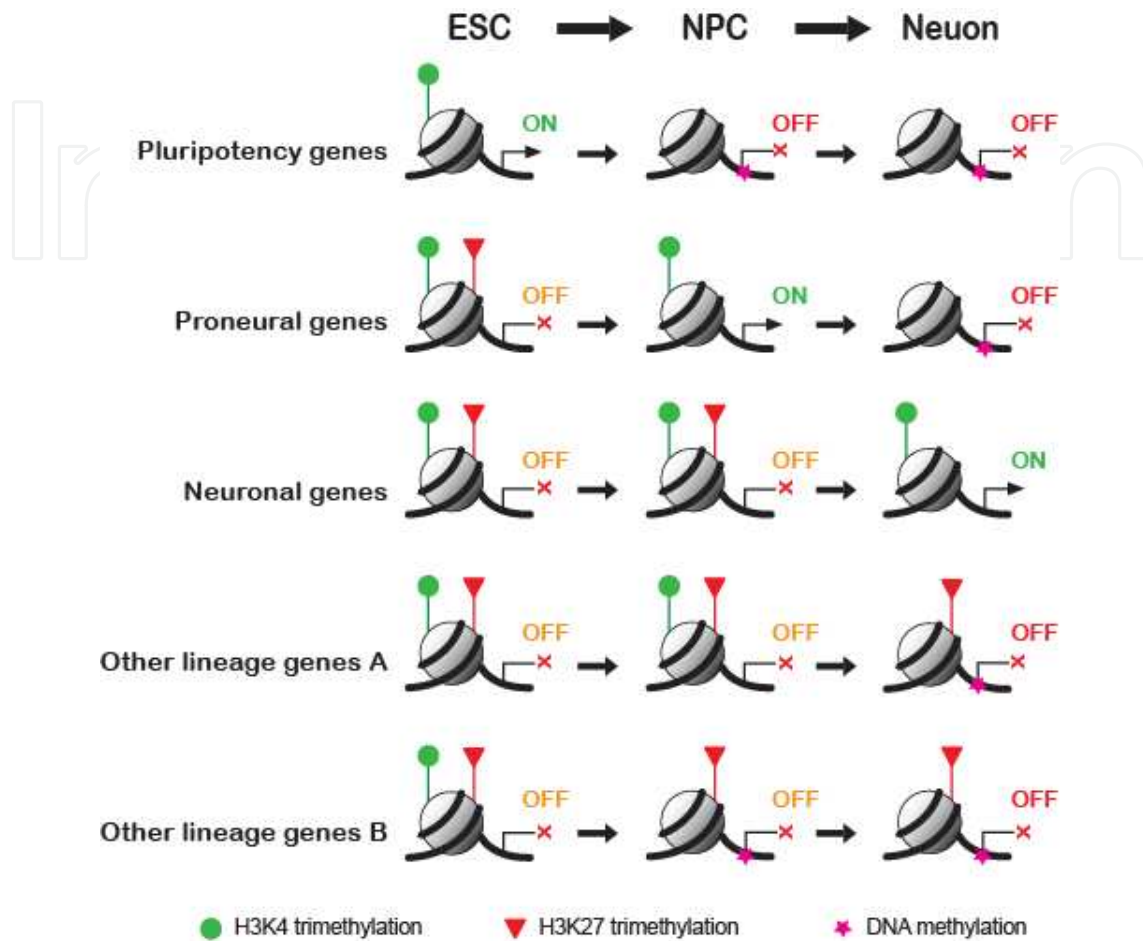


Figure 1. Schematic diagram of transcriptional states during neuronal fate acquisition from ESCs. In ESCs, pluripotency genes are transcriptionally activated (green "ON"). Key developmental genes are transcriptionally silent, yet competent for expression (orange "OFF"). This silencing ensures rapid reactions to extracellular inductive signals. Upon neural lineage choice, the transcriptional states of various developmental genes are altered. Pluripotency genes and genes associated with other lineages become repressed during the transition from ESCs to neurogenic NPCs (red "OFF"). Such a state of transcriptional repression is maintained over a long period. Gene activation and repression correlates with the presence of H3K4 tri-methylation (green flags) and H3K27 tri-methylation (red flags). DNA methylation (shocking pink stars) contributes to repression in combination with H3K27 tri-methylation.

Genome-wide mapping by chromatin immunoprecipitation has revealed that differentiation of ESCs is generally accompanied by global changes in histone methylation [55, 56]. In the course of neural lineage commitment, the promoters of many neural lineage genes have been shown to lose H3K27me3 from the bivalent domain, retain H3K4me3, and become activated. At the same time, the promoter loci of non-neural lineage genes maintain H3K27me3 in the bivalent domain, while removing H3K4me3, which results in a stable silent state. The promoters of some pluripotency genes such as *SOX2*, *POU5F1*, and *NANOG* shift from modification by H3K4me3 alone to neither H3K4 nor H3K27 methylation as they are repressed during differentiation. As ESCs differentiate into NPCs, H3K27 specific demethy-

lase JMJD3 is recruited to and resolves the bivalent domain at the promoters of neuron-specific genes such as nestin [64]. The majority of the JMJD3 target genes are key inducers of neurogenesis, including Pax6 and Sox1. Knockdown of the H3K4me2/3 demethylase *JARID1B* results in upregulation of stem cell-specific genes in ESC-derived NPCs [65]. Furthermore, *JARID1B* knockdown ESCs fail to progress beyond the NPC stage [65]. These results suggest that *JARID1B* promotes ESCs to differentiate towards a neural lineage by silencing genes associated with pluripotency. Thus, when ESCs are committed to a neural lineage, bivalent domains appear to be resolved in a lineage-specific fashion by leaving methyl marks either being activated or repressed. This mechanism is believed to allow rapid transcription of developmental genes in response to a variety of extrinsic cues.

Other repressive histone marks have also been reported to play important roles during differentiation. A sustained increase in silent chromatin marked by H3K9 methylation is observed in ESC-derived cells undergoing differentiation [66], suggesting that repressive histone marks H3K9me2/3 is essential for promoting differentiation. Additionally, it is possible that H3K9me2/3 marks play an important role in the establishment of an expression profile of neuron specific genes in response to extracellular signals [67]. Ciliary neurotrophic factor (CNTF), an astrocyte differentiation factor, is incapable of inducing expression of glial fibrillary acidic protein (*GFAP*), an astrocytes-specific marker gene, in NPCs, because the *GFAP* promoter is marked by H3K9 methylation. However, fibroblast growth factor 2 (FGF2) confer NPCs with responsiveness to CNTF by adding active H3K4 methylation and removing H3K9 methylation at the *GFAP* promoter. Thus, H3K9 methylation controls the timing of astrogliogenesis through regulation of CNTF-mediated signaling.

In addition to histone methylation, histone acetylation also appears to be an important epigenetic modification during neural differentiation from ESCs, as ESCs generally undergo striking changes in the global pattern of histone acetylation during neural differentiation [68]. Histone acetylation is catalyzed by histone acetyltransferase (HAT), while histone deacetylation is catalyzed by histone deacetylase (HDAC) [69]. Generally, HATs induce the transcriptional activation of their target genes. However, the 60-kDa HIV-Tat interactive protein (Tip60) histone acetyltransferase has been implicated in both transcriptional repression and activation [70]. Tip60-p400 chromatin remodeling complexes containing Tip60 are necessary to maintain characteristic features of ESCs [71]. Tip60-p400 complexes acetylate histone H4 on the promoters of both activated and repressed genes. Additionally, distribution patterns of p400 in ESCs strongly correlate with H3K4me3 marks on the promoters of both active and inactive genes. These results suggest that Tip60-p400 complex-mediated histone acetylation functions as an active mark in the bivalent domain together with the active H3K4me3 mark in ESCs.

3.2. DNA methylation

Genome-wide mapping of DNA methylation patterns has revealed dynamic DNA methylation states at gene promoters during ESC differentiation (Figure 1) [72, 73]. The most pronounced changes in the DNA methylation state occur during neural lineage commitment [74], suggesting that alterations in the DNA methylation state more strongly correlate with

neural lineage commitment and loss of pluripotency than with terminal neural differentiation. The promoters of highly expressed housekeeping and pluripotency genes in ESCs exhibit low methylation levels [75]. By contrast, most key developmental and tissue-specific genes exhibit high methylation levels, and are transcriptionally repressed [75]. As ESCs undergo differentiation, significant changes in DNA methylation patterns are observed. *De novo* methylation occurs on the promoter regions of pluripotency-associated factors [74].

DNA methylation potentially accompanies histone modifications during neural differentiation from ESCs. Cross-referencing DNA methylation patterns with mapping of histone H3K27me3 in ESCs and ESC-derived neurons has revealed that, upon differentiation, the regions marked by H3K27me3 acquire DNA methylation in a sequence-independent manner [74, 75]. H3K27me3 and DNA methylation are compatible throughout most of the genome [76]. Furthermore, deficiency of *DNMT* in ESCs causes widespread H3K27me3 genomic changes [76]. Taken together, these data suggest that DNA methylation, in cooperation with histone modifications, may function as a protective gear by repressing pluripotency and other lineage-specific genes during differentiation.

3.3. Chromatin remodeling

In addition to histone modifications and DNA methylation, the ATP-dependent chromatin remodeling complexes also play a pivotal role in the maintenance of ESC pluripotency. As previously described, Tip60-p400 chromatin remodeling complexes appear to be necessary for the maintenance of ESCs, including pluripotency [71]. Subunits of the NuRD complexes have also been shown to be important for ESC pluripotency and differentiation [77, 78]. Furthermore, ESCs contain another specialized ATP-dependent chromatin remodeling complex: the Brahma-associated factor (BAF) complex. The BAF complexes are characterized by 2 SWI2/SNF2-like ATPases, BRG1 and BRM. The BAF complexes in ESCs have a unique subunit composition (termed esBAF) that is not seen in other tissues, such as NPCs and post-mitotic neurons [79]. This specialized subunit composition is essential for establishment and maintenance of ESCs. The esBAF complexes contain BRG1 but not BRM, and BAF155 but not BAF170 (Figure 2). As ESCs differentiate into NPCs, these complexes undergo several subunit exchanges. The esBAF complexes incorporate BRM and excludes BAF60B, thereby forming the neural progenitor-specific BAF (npBAF) complexes in NPCs. The npBAF complex-specific subunit is necessary and sufficient for amplification of NPCs [80].

3.4. Non-coding RNAs

Increasing evidence demonstrates contributions of specific miRNAs in establishing ESC properties and their transitioning to NPCs. The miR-290-295 cluster codes for miRNAs are the most abundant in mouse ESCs and constitute over 70% of their entire miRNA population [81]. Consistent with their high expression levels, miR-290-295 miRNAs are involved in many functions in ESCs. For example, miR-290-295 miRNAs promote the transition from mitosis to S phase by targeting G1/S transition inhibitors such as *Cdkn1a* [82]. Furthermore, miR-290-295 miRNAs have been shown to target the *Rbl2* gene, which controls the expression of the DNA methyltransferases, DNMT3A and DNMT3B, thereby establishing *de novo*

DNA methylation in ESCs [83]. miR-9 is gradually upregulated during neural differentiation from ESCs [84, 85]. Furthermore, miR-9 performs diverse functions in different aspects of neuronal differentiation [86]. For example, during neural differentiation of human ESCs, miR-9 expression is not detectable in embryoid bodies, but is turned on in NPCs [84]. Inhibition of miR-9 activity has been found to suppress proliferation and simultaneously promote migration of NPCs [84]. Collectively, miRNAs could regulate multiple developmental processes at the post-transcriptional level.

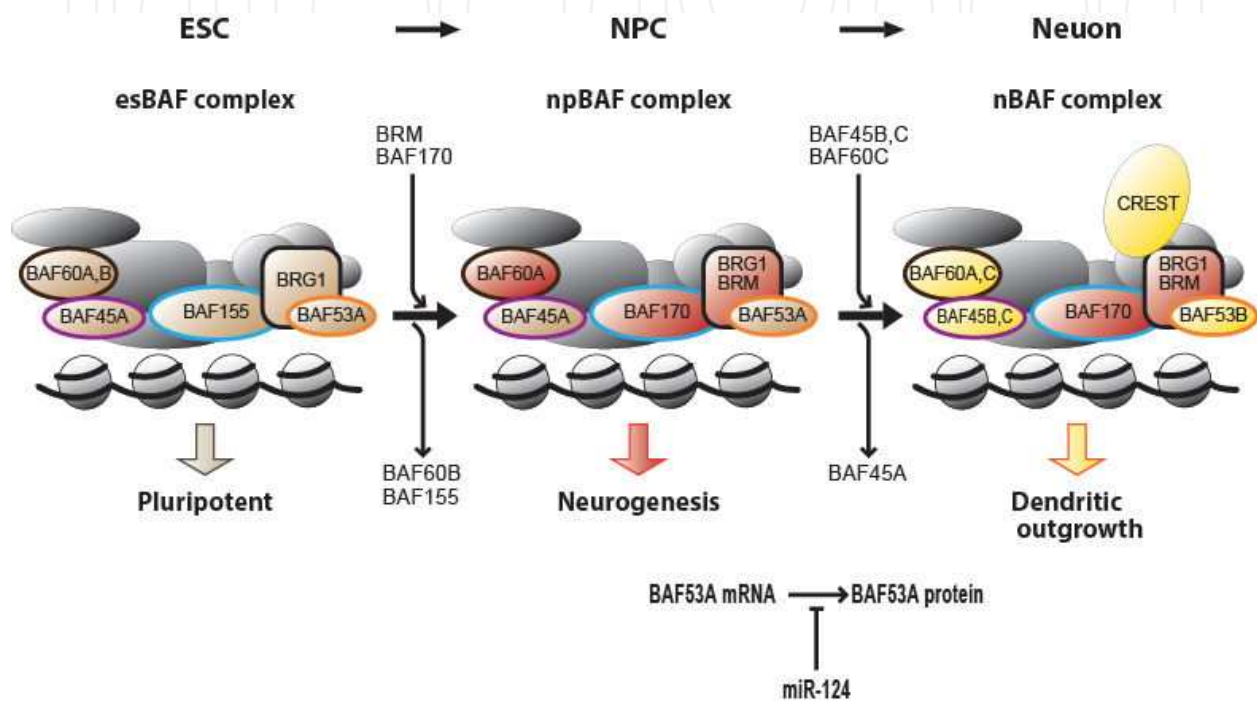


Figure 2. A switch in subunit composition of the BAF complexes during neuronal differentiation from ESCs. The exchange of the components is essential for the transition from ESCs to post-mitotic neurons. The exchangeable subunits are colored as follows: esBAF complex-specific subunit, ocher; npBAF complex-specific subunit, red; and nBAF complex-specific subunit, yellow. Cell type-specific BAF complexes have distinct functions that are indispensable for their properties. The BAF complex in ESCs, NPCs, and neurons are defined as esBAF, npBAF, and nBAF, respectively. The microRNA miR-124, binds to *BAF53A* mRNA transcripts to suppress its expression, thereby facilitating the replacement of BAF53 in the npBAF complexes.

4. Transition from NPCs to neurons

4.1. Histone modifications

After being committed to a neural lineage, NPCs exit the cell cycle and sequentially undergo neural and glial differentiation. Wnt signaling, which plays important roles in the maintenance of embryonic and adult stem cells, promotes differentiation of neurogenic ESCs to neurons, but does not promote differentiation of astrogenic NPCs into neurons [8]. In neurogenic NPCs, one of the target genes for Wnt signaling is the proneural gene *Ngn1*, which

promotes neurogenesis and inhibits astrocytic differentiation [87]. During the transition from neurogenic NPCs to astrogenic NPCs, the level of repressive H3K27me3 mark at the promoter of the *Ngn1* gene gradually increases, leading to gene silencing [88]. Therefore, activation of the Wnt signaling does not lead to transactivation of *Ngn1* at later developmental stages. This illustrates how histone methylation can modulate responsiveness of NPCs to extracellular cues, thereby rendering NPCs to switch from neurogenesis to astrogenesis.

CREB-binding protein (CBP), one of the most extensively studied HAT proteins, has been found to play an essential role in motor neuron differentiation by interacting with retinoic acid (RA) signaling [89]. Retinoic acid receptors (RARs) are DNA-binding proteins and form a complex with Neurogenin2 (*Ngn2*) on the promoter of a motor neuron enhancer gene. Binding of RA to RARs triggers recruitment of CBP on the promoter, which leads to acetylation of core histone proteins and activation of the motor neuron enhancer gene. These results indicate that neuronal subtype specification is regulated, at least in part, by the interplay between intrinsic epigenetic mechanisms and extrinsic cues.

Histone deacetylation by HDACs also plays essential roles in neuronal differentiation, as evidenced by studies using histone deacetylase inhibitors, such as valproic (VP) and trichostatin A (TSA) [90-92]. VPA promotes neuronal differentiation from adult hippocampal NPCs by inducing the proneural genes, *Ngn1*, *Math1*, and *NeuroD*, and histone H4 acetylation, while inhibiting glial differentiation [92]. A combination of TSA with sonic hedgehog (Shh), fibroblast growth factor 8 (FGF8) and Wnt instructs non-mesencephalic NPCs to give rise to dopaminergic neurons [93]. Inhibiting the activity of all HDAC1, 2, and 3 in NPCs leads to suppression of oligodendrocyte differentiation, while HDAC2 activity alone inhibits astrocyte differentiation. On the other hand, the HDAC1 activity is required for neural differentiation [94]. HDACs are generally present within large multi-subunit protein complexes in the nucleus [69]. Among them in association with neuronal differentiation is the HDAC/CoREST/REST repressor complex. The repressor element 1-silencing transcription factor (REST) is a vertebrate zinc finger transcriptional repressor protein, which plays a fundamental role in neurogenesis [95-97]. REST is expressed in both ESCs and NSCs, but is not expressed in ES-derived neurons [98]. REST binds to an evolutionally conserved DNA motif known as the repressor element 1 (RE1) [96, 97]. REST represses the expression of RE1-containing neuronal genes via recruitment of HDAC/CoREST complexes containing HDAC1 and HDAC 2 [97]. Thus, RE1 motif-associated neuronal genes in ESCs and NPCs appear to be suppressed by REST. This repression could block premature expression of genes associated with terminal differentiation at earlier stages than needed. Recently, a genome-wide binding site analysis revealed the target genes of REST during cholinergic, GABAergic, glutamatergic, and medium spiny projection neuronal specification from NPCs [99]. A large number of the identified REST target genes are unique for each neuronal subtype, strongly suggesting that histone deacetylase plays essential roles in epigenetic control of neuronal subtype specification as well as neuronal lineage commitment.

4.2. DNA methylation

DNA methylation has been shown to suppress astrocyte-specific genes in NPCs during early stages of development. Promoters of many astrocyte-specific genes contain the signal

transducer and activator of transcription (STAT) binding elements. With this, astrocyte-specific genes are transcriptionally activated through the Janus kinase (JAK)-STAT pathway, one of whose ligands is the cytokine leukemia inhibitory factor (LIF). However, neurogenic NPCs are not competent to differentiate into astrocytes even when they are grown with LIF, because the STAT-binding elements within astrocyte-specific genes promoters are methylated [100, 101]. This DNA methylation inhibits the association of activated STATs with the promoter of astrocyte-specific genes, thereby repressing their transcription. Conditional deletion of *DNMT1* in embryonic NPCs results in DNA hypomethylation and alteration of the timing of astrocytogenesis [101]. In addition, knockdown of *DNMT3B* in ESCs alters the timing of their neural differentiation [102]. These findings suggest that DNA methylation controls the timing and developmental switch from neurogenesis to astroglialogenesis of NPCs by altering responsiveness to their extracellular developmental cues.

4.3. Chromatin remodeling

As NPCs exit the cell cycle and differentiate into mature neurons, BAF60C is incorporated into the npBAF complexes [80, 103]. BAF45A and BAF53A in the complexes give way to BAF45B/C and BAF53B, respectively (Figure 2) [80, 103], establishing the post-mitotic neuron-specific nBAF complexes. Preventing the exchange of npBAF and nBAF components impairs neuronal differentiation, indicating that a switch in subunit composition of the BAF complexes is required for the transition from pluripotent ESCs to post-mitotic neurons [80, 103]. The nBAF complexes, along with Ca²⁺-responsive dendritic regulator CREST, also play a role in regulating the activation of genes essential for activity-dependent dendritic outgrowth, suggesting that the nBAF complexes are required for morphological/synaptic development of neurons [103, 104].

The BAF complexes incorporate the BAF57 subunit containing DNA-binding HMG-box domains [105]. In addition, the BAF complex subunits contain motifs known to bind to modified histones, including chromo-, bromo-, and PHD domains [103]. The bromodomain can bind acetylated histones [106]. The chromo- and PHD domains function as lysine-methylated histone-binding domains [106]. The esBAF and npBAF complexes contain different chromodomain proteins (BAF155 or BAF170) [103], whereas the npBAF and nBAF complexes contain different PHD domain proteins (BAF45a or BAF45b) [80, 103]. Thus, changes in subunit composition could alter targets of the BAF complexes, thereby causing changes in gene expression patterns during neuronal differentiation.

4.4. Non-coding RNAs

A number of miRNAs involved in cell fate decision during stem cell differentiation is also highly expressed in the nervous systems. Among these is miR-9, which is expressed specifically in neurogenic areas of the embryonic and adult brains [107, 108]. TLX, an orphan nuclear receptor, is essential for maintaining a self-renewable and undifferentiated state [109], as well as cell cycle progression [110] of NPCs in the developing brain. TLX is highly expressed in NPCs, but its expression is down-regulated upon neural differentiation [111]. Conversely, miR-9 expression increases during neural differentiation [111]. Furthermore,

miR-9 has been shown to negatively regulate NPC proliferation and accelerate differentiation through targeting *TLX* transcription [111]. These findings suggest that miR-9 can switch from undifferentiated to differentiated state of NPCs by downregulating *TLX* expression. Another miRNA, miR-124, has been reported to promote neuronal differentiation by targeting several genes involved in selection of non-neuronal cell fates. For example, miR-124 regulates the BAF complex subunit composition during differentiation from NPCs to neurons (Figure 2) by binding and thus suppressing *BAF53A* mRNA transcripts, which allows facilitation of a switch between BAF53 subunits [112]. These studies indicate that miRNA expression should be strictly controlled to ensure proper differentiation of ESCs into neurons. Consistent with this assumption, miR-124 is expressed in neurons, but not in astrocytes [113], and the miR-124 level increases during neural differentiation [84]. Remarkably, the control of miR-124 expression itself is mediated by an epigenetic mechanism. The promoter of the *miR-124* gene contains a functional RE1 site. In ESCs and NPCs, REST occupies the miR-124 gene locus and represses its expression [114], allowing persistent expression of non-neuronal mRNAs. However, once NPCs start to differentiate into neurons, REST is downregulated, thereby disinhibiting miR-124 expression. miR-124 then triggers degradation of non-neuronal mRNA transcripts, which promotes differentiation towards a neuronal lineage. One of the known target genes for miR-124 is C-terminal domain phosphatase 1 (*SCP1*), which represses transcription of RE1-containing neuronal genes by REST, thereby preventing cells from adopting a neuronal lineage and producing non-neural tissues [115]. Together, *miR-124* is a target for REST, but at the same token also targets the REST co-repressor. This represents the presence of a negative feedback loop between miRNA and a REST silencing complex, and such a mechanism may be broadly used to ensure proper cell fate transitions during development.

Neurons derived from different human ESC lines exhibit distinctive cellular properties due to the fact that human ESC lines were established under diverse conditions and from embryos with different genetic backgrounds. Comparison of neurons derived from HSF1 and HSF6 ESC human lines has revealed that the HSF1 line produces forebrain neurons with GABAergic and dopaminergic neurotransmitter phenotypes, while HSF6-derived neurons produce midbrain/hindbrain neurons bearing dopaminergic, cholinergic, serotonergic, non-forebrain GABAergic, and glutamatergic phenotypes [116]. Significant differences in the miRNA expression profile was noted between these 2 human ESC lines [116], suggesting that miRNA expression patterns might dictate in defining various neuronal subtypes arisen from ESCs.

5. Conclusions

Epigenetic mechanisms are regulatory processes that control gene expression via changes in chromatin structure without alterations in the DNA sequence. Changes in chromatin structure alter the accessibility of transcription factors and RNA polymerase to genes packed into chromatin, thereby modulating the efficiency of gene transcription. Epigenetic mechanisms act to control this accessibility through histone modifications, DNA methylation, chromatin

remodeling, and non-coding RNAs. Each of these epigenetic events interacts with intrinsic (ex. transcription factors) and/or extrinsic factors (ex. developmental cues such as morphogens and cytokines). Studies so far have suggested that, during sequential transitions from pluripotent ESCs to terminally differentiated neurons, epigenetic mechanisms play critical roles in not only maintaining self-renewal capacity and pluripotency of ESCs, but also restricting cell lineage choices. Further investigation will therefore help clarifying the mechanisms that control pluripotency and neuronal/glial fate specification. Furthermore, the knowledge will be used in harnessing ESCs safely and effectively for clinical applications.

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