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Review

Histone Variants and Histone Modifications in Neurogenesis

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During embryonic brain development, neurogenesis requires the orchestration of gene expression to regulate neural stem cell (NSC) fate specification. Epigenetic regulation with specific emphasis on the modes of histone variants and histone post-translational modifications are involved in interactive gene regulation of central nervous system (CNS) development. Here, we provide a broad overview of the regulatory system of histone variants and histone modifications that have been linked to neurogenesis and diseases. We also review the crosstalk between different histone modifications and discuss how the 3D genome affects cell fate dynamics during brain development. Understanding the mechanisms of epigenetic regulation in neurogenesis has shifted the paradigm from single gene regulation to synergistic interactions to ensure healthy embryonic neurogenesis.

Highlights

Mammalian neurogenesis is a dynamically regulated process with diverse factors. Epigenetic mechanisms, including histone variants and histone modifications, are involved in the expression of many genes to regulate central nervous system (CNS) dynamics.

Histone variants are nonallelic isoforms of core histones that incorporate into nucleosomes and regulate the dynamic changes necessary for neurogenesis.

Histone modifications can be dynamically regulated by sets of enzymes that act as epigenetic marks to support cell fate decisions and ensure robust embryonic neurogenesis.

The interactions of histone variants and histone modifications are typically characterized in chromatin states that have important roles in the memory and switching of gene expression states during brain development.

Hi-C-based high-throughput chromatin conformation capture techniques provide important insights into 3D genome architecture. Multiple factors, such as A/B compartment changes, heterochromatin organization, and enhancer–promoter interactions, affect chromatin interaction dynamics in neuronal development.

Histone Variants and Histone Modifications Are Critical for the Dynamic Modulation of Neurogenesis

In eukaryotes, nucleosomes form the basic repeating units of chromatin and comprise core histones (H2A, H2B, H3, and H4). The nucleosomes provide functional complexity via the incorporation of histone variants, which in turn regulate chromatin architecture and gene expression. Histone variants contribute to extending the information potential of the genetic code. They also regulate normal brain function, and other forms of histone modifications have been linked to neurogenesis and neural plasticity [1]. Incorporation of timing-specific histone variants generally leads to DNA–protein interactions, which allow changes in chromosomal architecture in global replacement. Recently, H2A.Z and H3.3 were found to have specialized functions that regulate lineage commitment during embryonic neurodevelopment [2,3]. Histones are decorated by a variety of modifications, including methylation, acetylation, phosphorylation, ubiquitination, crotonylation, and glycosylation. These modifications affect the access of transcription factors (TFs) and are critical for the dynamic modulation of chromatin function, contributing to influence gene activation and repression. A study of epigenetic regulation showed that histone modification functions are linked to histone chaperones and DNA methyltransferases (DNMTs) in brain development [4]. These interactions have different abilities to regulate nucleosome assembly or chromatin remodeling. Crosstalk can also occur between histone modifications and reinforce existing histone modifications. Therefore, histone variants and histone modifications establish chromatin states, which have important roles in the memory and switching of gene expression states during brain development.

The diversity of neocortical cell types and the remarkable functional capacities of neurogenesis have made the study of brain development a hot topic. The mechanisms by which one cell either transitions to a differentiated cell or retains its self-renewal are regulated by many epigenetic signals, including histone variants and histone modifications, which work synergistically to support this transition and ensure robust embryonic neurogenesis. Moreover, 3D genome studies have revealed high-order chromatin structure and have shown that chromatin accessibility is associated with neuronal differentiation processes [5,6]. In this review, we first discuss the epigenetic

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aspects of histone variants and histone modifications with reference to insights gained from neurodevelopment studies, which have revealed the multiple properties that histone variants have acquired to carry out their functions. We then address how the histone modification network is built through histone chaperones and different histone-modifying enzymes. Building on this molecular insight, we discuss how 3D genomic organization regulates gene expression and neural development. For in-depth discussions of histone variants and histone modification mechanisms in neurogenesis, it is necessary to understand the process of neural development fully.

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Histone Variants in Neurogenesis

Histone variants are structural components of chromatin; they are deposited onto chromatin by specific histone chaperones and interact with other chromatin modifiers (Table 1) [7,8]. Replacing canonical histones with histone variants affects the stability of nucleosomes and contributes to the production of functional chromatin domains [9]. Here, we mainly focus on variants of the H2A family and H3 family, which participate in the regulation of development (Figure 1).

The histone H2A family has four main histone variants: H2A.X, H2A.Bbd, macroH2A, and H2A.Z [10]. In particular, H2A.Z is strongly related to neurogenesis. During embryonic development, H2A.Z and SOX2 (see Glossary) colocalize on the promoter of Wnt5B, which recruits polycomb repressor complex 2 (PRC2) to regulate gene expression in human embryonic stem cell (hESC) lines [11]. In neurogenesis, H2A.Z regulates cortical development by interacting with SET Domain Containing 2 (Setd2) to facilitate H3K36me3 modification of the Nkx2–4 promoter and regulate the transcription of Nkx2–4 in the developing brain [2]. Moreover, Tet3 promotes efficient neuronal differentiation of mouse embryonic stem cells (mESCs) by increasing the expression of the neuron-specific TF BRN2 through differential 5-hydroxymethylcytosine (5hmC) enrichment and H2A.Z occupancy at the POU Class 3 homeobox 2 (BRN2) promoter [12]. Other studies showed that hippocampal deletion of histone variant H2A.Z enhances fear memory, suggesting that histone variants have an important role in delayed learning [13]. In addition, histone variants can regulate neural plasticity. H2A.Z increases with age and can also inhibit memory [14].

The histone variant H2A.Z has two hypervariants, H2A.Z.1 and H2A.Z.2, which have only three amino acid differences. First, H2A.Z.1 is important for the development of multipotent NSCs and has specialized functions in gliogenesis. The deletion of H2A.Z.1 inhibits the generation of non-neuronal glial populations and contributes to decreased astrocyte differentiation. Meanwhile, H2A.Z.1 regulates H3K56ac modification of the promoter region of folate receptor 1 (FOLR1) by interacting with its

Table 1. Mechanisms of Histone Variants Regulating Neurogenesis.

Histone variant	Interacting partner	Epigenetic mark	Target	Function in neurogenesis
H2A.Z	SOX2	H3K4me3 H3K27me3	Wnt5B	Strongly related to neurogenesis, promoting neuronal differentiation
	Setd2	H3K36me3	Nkx2–4	
	Tet3	5hmC	BRN2	
	ASF1a	H3K56ac	FOLR1	
H3.3	MOF	H4K16ac	Gli1	Controlling neuronal and glial-specific gene expression patterns, mediating not only proliferation but also differentiation
	Hira and UBN1/2	H3K4me3	Lmx1b, Zic2, and Zfp521	
	Hira and PRC2	H3K27me3	Tbx3	

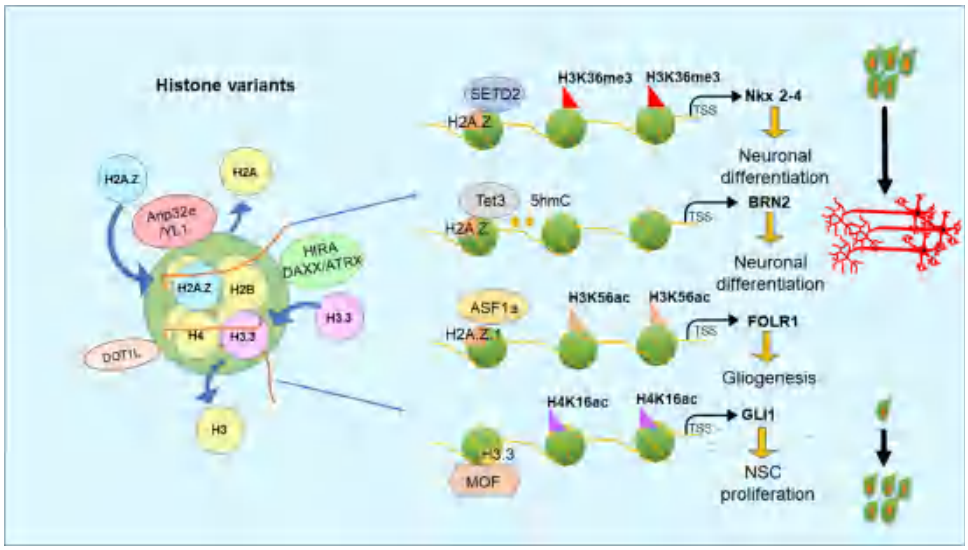

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Figure 1. Histone Variants in Neurogenesis. DOT1 binds to nucleosomes together with the ubiquitination of H2B, greatly destabilizing the nucleosome structure. H2A.Z facilitates H3K36me3 modification of the Nkx2–4 promoter by interacting with Setd2 to regulate the transcription of Nkx2–4. H2A.Z interacts with Tet3 to promote the expression of BRN2. H2A.Z.1 regulates H3K56ac modification of the promoter region of FOLR1 by interacting with ASF1a, and H3.3 directly interacts with the H4K16 acetyltransferase MOF. Abbreviations: ASF1a, anti-silencing function 1 a; BRN2, POU Class 3 Homeobox 2; DOT1, lysine methyltransferases are enyzmes that catalyze the transfer of methyl groups from S-adenosylmethionine (SAM) to the lysine residues on histones, particularly histones H3 and H4. The dysregulation of this methylation is critical in the development of cancer; FOLR1, folate receptor 1; MOF, Lysine Acetyltransferase 8; Nkx2–4, NK2 Homeobox 4; Setd2, SET Domain-Containing 2; Tet3, ten-eleven translocation 3.

chaperone antisilencing function 1a (ASF1a), thereby directly affecting the expression of FOLR1, which is involved in signal transduction of the JAK-STAT signaling pathway in gliogenesis [15]. In neural progenitor cells (NPCs), the depletion of H2A.Z.2 contributes to an abnormal increase in microglia in the ventricular zone/subventricular zone of the developing cortex. In addition, the specific loss of H2A.Z.2 in microglia itself has no noticeable effect on the development of early cortical microglia [16].

In the histone H3 family, there are six variants: CENP-A, H3.X, H3.Y, H3.1t, H3.5, and H3.3 [17,18]. H3.3 is involved in neurogenesis, and other variants also have special functions in development and chromosome stability modulation. H3.3 has five amino acids variations compared with H3. H3F3A and H3F3B are reported to encode H3.3. In addition, unlike canonical H3, H3.3 is constantly expressed throughout the cell cycle, which indicates that it has multiple functions in many biological processes [19]. Recently, studies revealed that neurogenesis, as an important part of brain development, is also precisely controlled by H3.3. For example, the exchange of nucleosomal histones is thought to remain stationary in postreplicative cells, but the level of histone variant H3.3 in nucleosomes is dynamic in the brain, thereby controlling neuronal- and glial-specific gene expression patterns, which have an important role in brain development [20]. In addition, deficiency in H3.3 in the mouse cortex results in abnormal cell distribution [3]. During neurogenesis, H3.3 can directly interact with the H4K16 acetyltransferase MOF. When H3 is replaced by H3.3 in the Gli1 promoter area, MOF can be recruited to modify H4K16ac and enhance the transcription of Gli1, a neural stem cell proliferation-related gene [21]. Some studies have also shown that H3.3 mediates not only proliferation, but also differentiation. In NSCs, the promoter regions of many lineage-specific genes (e.g., Lmx1b, Zic2, and Zfp521) have abundant H3 modifications, H3K4me3 for gene activation and H3K27me3 for gene repression [22]. The balance of these two modifications is precisely controlled by H3.3 [23]. For example, the

Glossary

Antisilencing function 1 a (ASF1a): gene encoding a member of the H3/H4 family of histone chaperone proteins; is similar to the antisilencing function-1 gene in yeast. The protein is a key component of a histone donor complex that functions in nucleosome assembly. It interacts with histones H3 and H4 and functions together with a chromatin assembly factor during DNA replication and repair.
Assay for transposase-accessible chromatin with high-throughput sequencing (ATAC-seq): useful method to map genome-wide chromatin accessibility and nucleosome positioning. It detects DNA accessibility with hyperactive Tn5 transposase, which inserts sequencing adapters into chromatin-accessible regions.
Autism spectrum disorder (ASD): a developmental disability that causes problems with social interaction and communication.
CCCTC-binding factor (CTCF): a multiple zinc finger protein that exerts diversified functions under different genomic contexts.
Chromatin Conformation Capture (3C) technique: an important technique used to study chromatin structure, as well as the basis for several other derivative techniques.
DNA methylation: epigenetic mechanism that occurs by the addition of a methyl group to DNA, thereby often modifying the function of the genes and affecting gene expression.
Epitranscriptomic modification: chemical modifications of RNA molecules. RNA modifications in the brain regulate the fate and function of both coding and noncoding RNAs and are emerging as a critical element of cellular function.
Folate receptor 1 (FOLR1): members of this gene family bind folic acid and its reduced derivatives and transport 5-methyltetrahydrofolate into cells. This gene product is a secreted protein that either anchors to membranes via a glycosyl-phosphatidylinositol linkage or exists in a soluble form.
Hes-related family BHLH transcription factor with YRPW motif like (HEYL): member of the hairy and enhancer of split related (HESR) family of basic helix-loop-helix (bHLH)-type transcription factors.
Heterochromatin protein 1 (HP1): transcriptional repressor that binds to

H3.3 distribution changes during the process of ESCs differentiating to NPCs. Increased H3.3 levels are found in the transcription start site and gene body of some NPC-related genes. Further studies showed that H3.3 interacts with PRC2 to modulate the H3K27me3 level in target gene regions, such as Tbx3, to modulate neurogenesis [23,24].

The latest and exciting advances in studies of H2A.Z and H3.3 in their functions and specific exchange complexes have enriched our understanding of gene expression transformation during neurogenesis. Further research is needed to determine the effects of the interaction between other variants and their partners on neurogenesis, and the discovery of the mechanisms of new histone variants will provide us with a more comprehensive understanding of the diversity and prescriptiveness of this process.

Histone Modifications in Neurogenesis

While histone variants affect neurogenesis, histone modifications, such as histone methylation, acetylation, phosphorylation, ubiquitination, crotonylation, and glycosylation, also directly or indirectly affect neurodevelopmental processes through different mechanisms. Abnormal histone modifications cause a series of neurological diseases and seriously endanger human health. Common histone modifications include methylation, acetylation, phosphorylation, and ubiquitination. Lysine (Lys or K) and arginine (Arg or R) residues are the most common acceptor sites of histone methylation. Methylation on H3K4 and H3K36 usually activates gene transcription to influence neurogenesis [25–27]. A recently published study indicated that loss of ASH2L, the COMPASS family histone methyltransferase co-factor, in NPCs impairs H3K4me3 levels and inhibits proliferation at late stages of neurogenesis [28] (Figure 2A). Thus, removal of H3K4me3 may suppress previously activated genes, and H3K4me3 must also be added to neurogenesis-related genes. In addition, H3K36 methylation is associated with transcription and splicing regulation [29,30]. A recent study suggested that, unlike SET-containing protein SETD2, SETD5 can also methylate H3K36 up to the trimethylated form. SETD5 haploinsufficiency impairs the correct structural and functional maturation of the mouse CNS [31] (Figure 2A).

Conversely, some histone methylation is associated with gene silencing, such as H3K27me3 [25,27]. H3K27me3 is a major marker of the transition of neuroepithelial cells into apical radial glia [32]. H3K27 methylation is catalyzed by enhancer of zeste homolog 2 (EZH2). EZH2 is highly expressed in NSC, targets many neuron-specific genes, and is downregulated during their differentiation [33] (Figure 2B).

Similar to histone methylation, acetylation is also widely studied, including in the nervous system, and histone acetylation is generally considered to be a marker of gene transcription activity and can directly regulate gene transcription (Figure 2A). Histone acetylation mainly occurs at the more conserved lysine at the N terminus of H3 and H4 and is coordinated by histone acetyltransferases (HATs) and histone deacetylases (HDACs) [34]. For example, lysine acetyltransferase 8 (KAT8) has a key role in the acetylation of H4K16. The deletion of KAT8 showed abnormal development of NPCs, and mutant NPCs formed poorer neurospheres [35]. Furthermore, histone deacetylation can silence transcription of many cognitive proteins, thereby impairing learning and memory. HDAC2 is an important negative regulator that participates in chronic stress cognitive impairment by modifying the phosphoinositide 3 kinase/protein kinase B signaling pathway [36].

In addition to histone methylation and acetylation, histone phosphorylation and ubiquitination also influence neurogenesis. Studies on histone phosphorylation have focused on the connection of histone and core histone H3, the most common being H3S10 phosphorylation (H3S10p). In addition, histone H3 phosphorylation (Ser10)-acetylation (Lys14) (H3S10phK14ac) is a major

the methylated lysine 9 residue of histone H3 (H3K9me), which is a hallmark histone modification for transcriptionally silenced heterochromatin.

Histone crotonylation: modification produced by histone crotonyltransferase (HCT) using crotonyl-CoA (Cr-coA) as a substrate to transfer crotonyl to lysine residues.

Histone glycosylation: one of the most common post-translational modifications of proteins; transfer of sugars to proteins and special amino acid residues on the proteins to form glycosidic bonds under the action of glycosyltransferases.

Liebenberg syndrome: condition that involves abnormal development of the arms, resulting in characteristic arm malformations that can vary in severity.

Liquid–liquid phase separation (LLPS): comprises proteins that self-organize into liquid-like condensates that recruit or exclude certain molecules and are related to multiple biological processes.

Polycomb repressor complex 2 (PRC2): multiprotein chromatin modifying complex that is essential for vertebrate development and differentiation. It comprises a trimeric core of SUZ12, EED, and EZH1/2 and is responsible for catalyzing both dimethylation and trimethylation of histone H3 at lysine 27 (H3K27me2/3).

SOX2: this intronless gene encodes a member of the SRY-related HMG-box (SOX) family of transcription factors involved in the regulation of embryonic development and in the determination of cell fate. The product of this gene is required for stem cell maintenance in the CNS and regulates gene expression in the stomach.

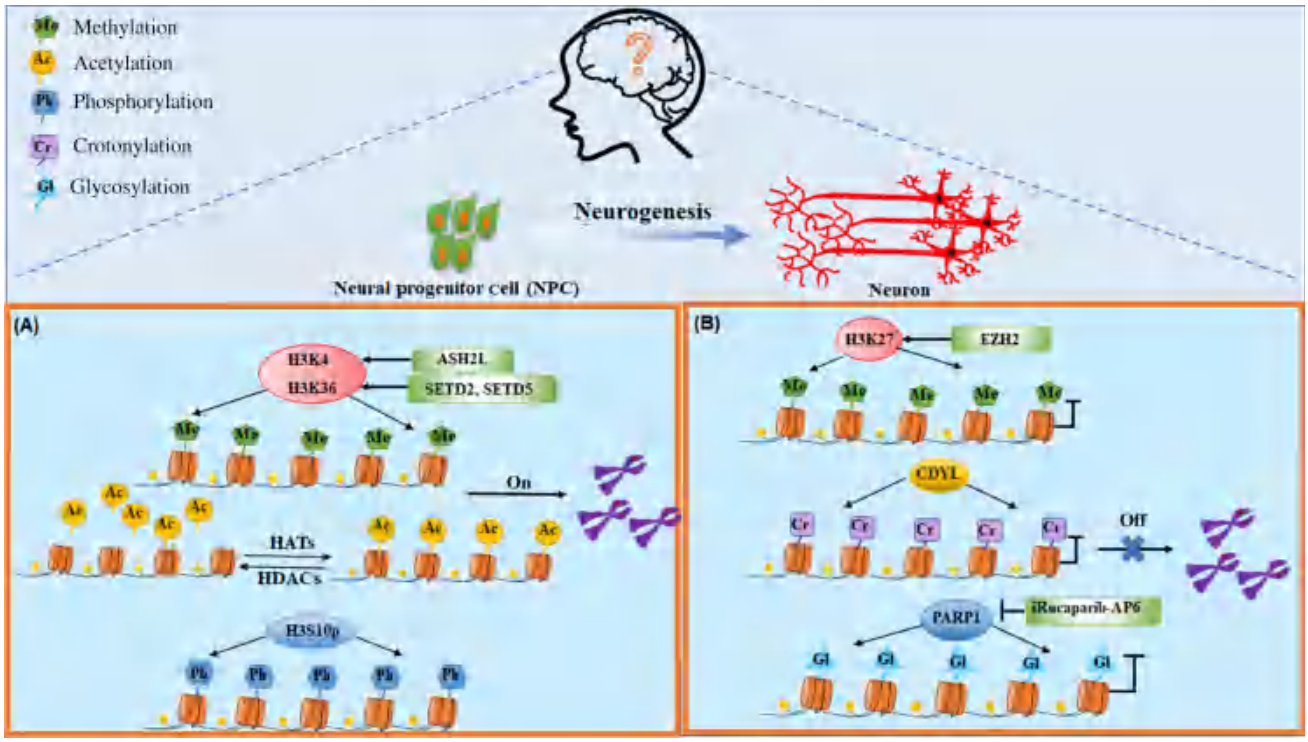

Figure 2. Histone Modifications in Neurogenesis. (A) Histone modification can influence the process of neurogenesis by activating gene transcription. Under the influence of regulators ASH2L, SETD2, and SETD5, methylation of H3K4 and H3K36 can activate gene transcription and promote neurogenesis. Similarly, histone acetylation may affect neurogenesis in coordination with histone acetyl transferases (HATs) and deacetylases (HDACs). In addition, H3S10 phosphorylation can also promote neurogenesis by affecting transcription. (B) Histone modification can influence the process of neurogenesis by inhibiting gene transcription. H3K27 methylation, catalyzed by EZH2, can silence genes and influence neural differentiation. In addition, overexpression of CDYL and PARP1 inhibits histone crotonylation and glycosylation, thereby inhibiting neurogenesis and leading to a series of neurological diseases. The inhibition can be alleviated when some inhibitors, such as IRucaparib-AP6, are added. Abbreviations: ASH2L, absent, small, or homeotic 2-like; CDYL, chromodomain y-like; EZH2, enhancer of zeste homolog 2; PARP1, polyADP-ribose polymerase 1; SETD2/5, SET domain containing 2/5.

regulator of the response of nerve cells to systemic lipopolysaccharides, providing an important basis for the treatment of neuroinflammation [37]. Histone ubiquitination is also closely linked to neurogenesis. For example, during development, the monoubiquitination (Ub) of histone H2A keeps the developmental genes of the polycomb repressive complex 1 (PRC1) in balance. The E3 ubiquitin ligase activity of the PRC1 core component (Ring1B) is necessary to temporarily inhibit key neuron genes in NPCs, but it is optional to inhibit the neurogenic potential of late-stage NPCs [38].

In recent years, the development of mass spectrometry has led to the discovery of new histone modifications, such as histone crotonylation and histone glycosylation, which have greatly expanded the potential complexity of the histone code. Lysine crotonylation (Kcr) is a highly evolutionarily conserved protein modification that is biologically distinct from histone lysine acetylation (Kac). In postmeiotic sperm cells, a high abundance of Kcr concentrates on the sex chromosomes to mark testicular-specific genes, which include a large number of sex chromosome-active genes [39]. For example, the protein chromodomain y-like (CDYL) on the human Y chromosome can be used as a hydrase to regulate the level of histone crotonylation modification and finally regulate the process of spermatogenesis. Based on proteome modification analysis, nonhistone replication protein A1 (RPA1) is a downstream-regulated CDYL-acylated modified

protein that affects DNA damage repair [40,41]. In addition, other studies have found that histone crotonylation and epigenetic CDYL have an important role in the occurrence and development of depression [42]. Furthermore, CDYL can affect the plasticity of synapses by inhibiting the expression of the neuropeptide VGF through transcriptional inhibition, thus regulating the occurrence and development of stress-mediated depression [42] (Figure 2B).

Sugar chains are involved in many physiological processes, such as neural cell adhesion, migration, synapse formation, and nerve conduction, so glycosylation modification of histones is closely related to neurogenesis [43,44]. PolyADP-ribose polymerase 1 (PARP1), a nuclear protein belonging to the PARP protein family that catalyzes the glycosylation of ADP ribosylation, is involved in some neurodegenerative diseases and is overactivated under these pathological stimuli to eventually activate the PARP1-dependent programmed cell death pathway [45]. Therefore, PARP1 inhibitors are needed to treat pathological diseases caused by overactivation of PARP1. Some studies have shown that iRucaparib-AP6 can specifically induce the degradation of PARP1 without changing the expression of other protein molecules [45,46] (Figure 2B).

Lysosomal storage diseases are caused by the absence of lysosomal enzymes that metabolize specific wastes within the body, leading to the accumulation of substrates in lysosomes and resulting in organ dysfunction and disease [47]. At present, the main treatment method for lysosomal storage disease is enzyme replacement therapy. However, the short half-life of glycosylation modification with enzyme replacement limits the therapeutic effect. Moreover, it is difficult to ensure that medicinal enzyme replacement targets specific organs. Researchers have designed a new N-linked glycosylation modification by using gene-editing technology. This modification can not only extend the circulation time of alternative enzymes in the body, but also ensure better targeting to the organs [48]. In addition, targeting the brain is difficult because of the blood–brain barrier (BBB), and drugs are urgently needed to cross this barrier and reach targets in the brain. We hypothesize that the design concept of glycosylation modification can also be applied to other drugs to overcome the BBB and accurately target the brain. With further study of protein function and biological mechanism, the understanding of the importance of post-translational protein modification is increasing. Although many new histone modifications have been discovered, how these new contribute to neurogenesis and whether they interact differently with each other to affect brain development is unknown and will need to be explored further.

Interactions between Histone Modification and other Epigenetic Modifications

In the earlier sections, we described the different types of histone variant and histone modification in neurodevelopment. In terms of regulatory functions, these epigenetic mechanisms are seldom isolated but interact. We take H3K36me3, an important histone modification described earlier, as an example.

In the section on histone variants, we mentioned that H2A.Z regulates the differentiation of NPCs into neurons by targeting the Nkx2-4 promoter through interaction with H3K36me3 methyltransferase-SETD2 [2]. In addition, another epigenetic modification, m^6A, is a typical type of epitranscriptomic modification that is essential for the differentiation of embryonic cells and the regulation of NPC capacity [49]. The expression level of H3K36me3 is positively correlated with the level of m^6A in mESCs. H3K36me3 guides m^6A modification by directly combining methyltransferase-like 14 (METTL14), a critical component of the m^6A methyltransferase complex, to inhibit pluripotency and promote differentiation of mESCs [50]. The interaction between these two modifications is conserved among species, which has been proven in studies on plants [51]. In addition to these two types of modification, research shows that H3K36me3

affects the location and activity of DNMTs; therefore, the DNA methylation level is positively correlated with the H3K36me3 level in ESCs [52]. Furthermore, this kind of interaction has been proven to be differentially expressed in ESCs and neurons. DNMT3b preferentially interacts with H3K36me3 in ESCs, abundant in actively transcribed gene-body regions, whereas DNMT3a showed little preference in neurons [53]. Furthermore, current studies on SETD5 prove that SETD2 is not the only H3K36 trimethylamine transferase that leads to more unknown and complex H3K36me3 regulation [31,54].

Here, we list the interactions between H3K36me3 and histone variants, RNA modifications, and DNA modifications. The earlier-discussed examples show that H3K36me3 is extensively found in all cells, from ESCs to NPCs and neurons. Along with the process of cell differentiation, how the interactions of H3K36me3 change is still unknown. H3K36me3 is only a representative example, and there is a great deal of crosstalk in histone modifications in neurodevelopment, such as protein arginine methyltransferase 6 (PRMT6), which catalyzes asymmetric dimethylation of H3R2me2a [55] and interplay between the 5hmC and H3K27me3 pathways during neural lineage commitment [56]. Many interplays are also based on changes in histone spatial structure. Analysis of the structure showed that DOT1-like protein (DOT1L), an H3K79 methyltransferase, binds to nucleosomes together with the ubiquitination of H2B and greatly destabilizes the nucleosome structure [57]. Therefore, epigenetic regulation can work in a coordinated manner, and this regulation can be present at a more macroscopic level.

Dynamic Regulation of the 3D Genome in Neurogenesis

Genome architecture has a key role in gene transcription regulation and neural development. As sequencing-based technologies have developed, 3D genome studies have revealed high-order chromatin structures, including A/B compartments, topologically associated domains (TADs), and chromatin loops (Box 1) [6]. What is the role of 3D genomic organization in neural development? Chromatin dynamic changes are crucial in cell fate commitment. Recent studies showed chromatin global compaction during neural differentiation. The assay for transposase-accessible chromatin with high-throughput sequencing (ATAC-seq) revealed chromatin accessibility progressive changes associated with neuronal differentiation processes [58–60]. Studies have shown that active region (A compartment) interactions decrease and inactive region (B compartment) interactions increase during NPC differentiation into neurons [5] (Figure 3A). The binding of the TF Prospero (Pros) to Heterochromatin protein 1 (HP1) and histone H3K9 methyltransferase SUV39h ensures neuronal differentiation by driving heterochromatin domain condensation and expansion [61,62] (Figure 3B). Polycomb groups (PcGs) are key factors for maintaining developmental gene balance in stem cells. Interactions mediated by PcG protein

Box 1. Overview of High-Order Chromatin Structure

Hi-C-based high-throughput Chromatin Conformation Capture (3C) techniques are an efficient approach for capturing spatial interactions across the whole genome. Hi-C analysis shows that A compartments as active regions and B compartments as inactive regions are interlaced within chromosomes. A and B interact preferentially in their own compartments [81]. Nuclear laminB1 interactions with heterochromatin have a key role in driving genomic compartmentalization [82,83]. Studies suggest that phase separation regulates chromatin compartmentalization. Heterochromatic H3K9me3 recognition complexes regulate chromatin compartmentalization by triggering liquid–liquid phase separation (LLPS) [61,62]. By contrast, transcriptional regulators, RNA, and RNA-binding proteins can form LLPS condensates in active chromatin compartments [84]. For example, the transcription effector Tafazzin (TAZ) compartmentalizes DNA-binding cofactor TEA domain family member (TEAD) and promotes transcriptional activation through phase separation [85]. Hi-C analysis indicates that topologically associated domains (TADs) are a common structural feature in genome organization. DNA interactions occur predominantly within TADs but not between different TADs. The constitutive CTCF sites have an important role in organizing and maintaining the formation of TADs [86–88]. Chromatin loops regulate interactions between enhancers and promoters, which influences gene expression within TADs [65,89].

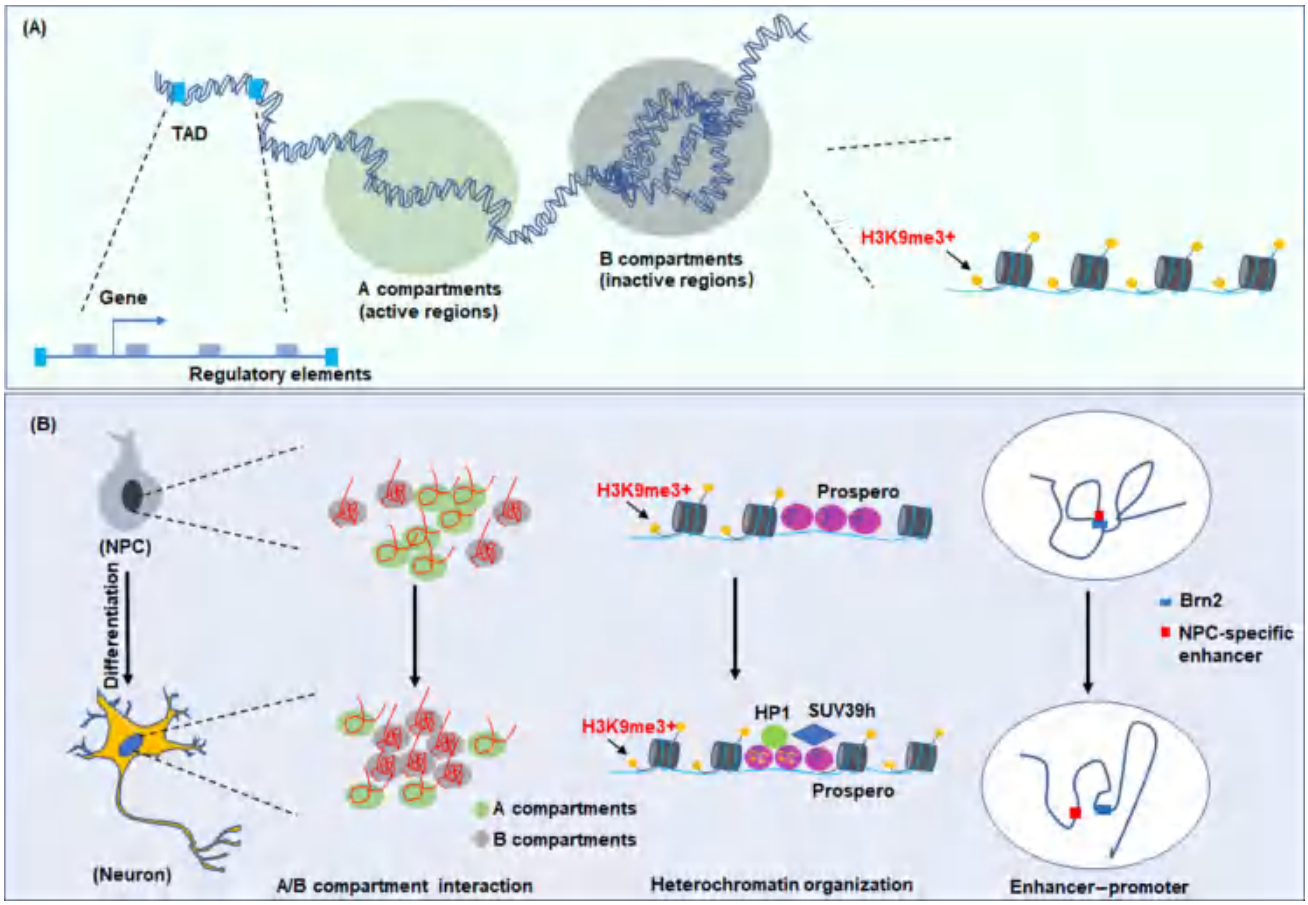

Figure 3. Dynamic Regulation of the 3D Genome in Neurogenesis. (A) Schematic of the high-order chromatin structure, including A/B compartments and topologically associated domains (TADs). (B) Multiple factors affect the dynamic regulation of the 3D genome in neural development. These include A/B compartment changes, heterochromatin organization, and enhancer–promoter interaction dynamics.

gradually weaken with the process of neural differentiation [5,63]. These studies provide strong support that chromatin is involved in global changes associated with the differentiation process.

Not only inter-TAD interactions, but also chromatin interactions within TADs (sub-TAD and intra-TAD) change during differentiation. Recent studies showed that enhancer–promoter interactions are constrained primarily in sub-TADs by using aggregate Hi-C maps or promoter capture Hi-C [5,64]. Enhancers can regulate the promoters of located proximal transcription factor (TF) or distant chromatin–protein complexes, which manage cell type-specific gene expression [65]. The contacts between neuronal transcription factor Brn2 promoters and NPC-specific enhancers increase in NPCs, and the interaction is broken in neurons, which indicates that enhancer–promoter interactions are dynamic during neural development [5] (Figure 3B). The Mediator complex regulates gene transcription by providing communication between active enhancers and promoters. The E-box transcription factor Tcf4 interacts with Mediator, which regulates neurogenic transcription factor genes by targeting superenhancers and extensive promoters with the H3K4me3 domain [66]. PcG proteins are involved in the interaction between enhancer promoters. Ring1, a component of PRC1, regulates the connection between tissue-specific enhancer and promoter of Meis2. Ring1-self dissociation causes the activation of Meis2, which contributes to midbrain development

[67,68]. In summary, dynamic changes in 3D genome architecture affected by multiple factors, including A/B compartment changes, heterochromatin organization, and enhancer–promoter interactions, are important for gene expression regulation and neural development.

Histone Dysregulation in Neurological Disorders

Mutations in histone variants are associated with various neurological disorders. For example, mutations in macroH2A have been identified as causing intellectual disability syndrome and Liebenberg syndrome, which is characterized by microcephaly and limb malformations [69]. In addition, mutations in H2BC13 and H2BC21 are associated with intellectual disability, which causes delayed development and intellectual disability [70]. Genetic variations of histone variant H4-16 are associated with neuronal ceroid lipofuscinosis, which is characterized by visual loss, seizures, and cognitive dysfunction [71].

Genetic mutations of histone modifiers essential for early embryonic neurogenesis can lead to the development of autism spectrum disorder (ASD), intellectual disability, epilepsy, or microcephaly. For example, mutations of histone acetyltransferase KAT6A are associated with an extremely rare neurodevelopment disorder, which features intellectual disability, speech delay, and microcephaly [72]. Mutations of KDM5C lead to compromised stability with protein production and, thus, reduce the removal rate of methyl groups from H3K4me2/3, resulting in frequent ASD phenotypes as well as aggressive behavior [73,74]. LSD1 is associated with the promoter of Hes-related family BHLH transcription factor with YRPW motif like (HEYL) and catalyzes the demethylation of H3K4me2, thus further repressing HEYL expression in primate NPCs [75]. SETD5 is associated with RNA polymerase II dynamics and the transcriptional landscape in neural progenitors. Mutations in the SETD2 and SETD5 alleles have been identified as a frequent cause of intellectual disability and ASD [31,76,77].

Moreover, mutations in genes involved in 3D chromatin architecture have also been associated with neurodevelopmental syndromes. Mutations of CCCTC-binding factor (CTCF), an insulator protein that has enhancer-blocking and barrier functions, are associated with intellectual disability [78]. In addition, the chromodomain protein CDYL is required for neuronal migration during brain development by repressing the expression of RHOA [79]. Patients with CDYL mutations are characterized by intellectual disability, language delay, learning difficulties, seizures, and delayed motor development [40].

Concluding Remarks and Future Perspectives

Recently, epigenetic factors were found to be increasingly involved in modulating neurogenesis and the pathogenesis of neurodevelopmental disorders. There is now an urgent need to reveal how histone variants and modifications, two main parts of epigenetic factors, affect neurogenesis.

Among the various histone variants, H2A.Z and H3.3 are closely related to neurogenesis. H2A.Z and H3.3 separately recruit histone-modifying enzymes to regulate the transcriptional activity of downstream neurogenesis-related genes and further affect brain development. Many gene sites on chromatin have histone variant replacement during neurogenesis. How these genes are related and modulated with suitable expression levels through histone variants is far from understood (see Outstanding Questions).

The replacement of histone variants is sometimes accompanied by histone modification. Some variants, such as H2A.Z and H3.3, could recruit modification enzymes and then regulate histone post-translational modifications. Furthermore, these modifications directly or indirectly affect chromatin structure through the chromatin-remodeling complex and then adjust the transcription of

Outstanding Questions

What are the molecular mechanisms by which histone variants and histone modifications regulate neurogenesis during brain development?

Why is neural development heavily influenced by epigenetic regulation? How do histone variants and histone modifications regulate NSC fate specification?

What is the exact process of histone variant replacement and deposition in chromatin?

Does histone modification have an important role during brain development?

Are there novel ways to detect states of chromatin in live cells? Could this help in understanding the relationship between chromatin dynamic changes and neurogenesis?

What is the regulatory mechanism for initiating phase separation in neurogenesis?

neurogenesis-related genes. Dysfunction of histone modification is strongly related to many diseases, such as intellectual disability, ASD, and microcephaly.

Different modifications modulate the structure of chromosome to make it easy to transcript and enhance gene expression or tighten the chromatin and repress gene expression. Chromatin changes in tightness are preliminary explained through phase separation research. This method is powerful enough to reveal the characteristic of regulating molecular assembled or disassembled from chromatin in neurogenesis. In addition, various novel histone modifications, including crotonylation, glycosylation, lactylation, succinylation, 2-hydroxyisobutyryl acylation, and benzoylation, are rarely explored for their functions in neurogenesis [80]. To determine their functions, conditional knockout mice or other model animals can be generated to explore the phenotype caused by modification dysfunction. With the use of ChIP-seq or ATAC-seq techniques, it is possible to determine which gene sites are rich in modifications, and further structural biology research will determine the exact process by which histone variants are deposited to a specific site. Finally, such information will enable us to draw a precise map of how histone variants and modifications modulate neurogenesis.

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