Review

Chromatin and nuclear architecture in the nervous system

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Neurons are arguably the most varied cell type both morphologically and functionally. Their fate during differentiation and development and the activity of mature neurons are significantly determined and regulated by chromatin. The nucleus is compartmentalized and the arrangement of these compartments, termed the nuclear architecture, distinguishes one cell type from another and dictates many nuclear processes. Nuclear architecture determines the arrangement of chromosomes, the positioning of genes within chromosomes, the distribution of nuclear bodies and the interplay between these different factors. Importantly, chromatin regulation has been shown to be the basis for a variety of central nervous system processes including grooming and nursing, depression and stress, and drug abuse, among others. Here we review the regulation and function of nuclear architecture and chromatin structure in the context of the nervous system and discuss the potential use of histone deacetylase inhibitors as chromatin-directed therapy for nervous system disorders.

Introduction

Eukaryotic nuclei are well-organized structures with distinct nuclear compartments. The arrangement of these compartments inside the nuclear space is termed nuclear architecture. Nuclear bodies are functionally and/or morphologically discrete subnuclear regions accommodating usually distinct resident proteins, and are devoid of membranes [1] (Figure 1). Examples include the nucleoli, sites of rRNA transcription; nuclear speckles, splicing factor compartments; Cajal bodies (CBs), thought to act as sites for snRNP assembly; and promyelocytic leukemia (PML) bodies, whose role is yet to be defined, despite their implication in a variety of cellular processes ranging from transcription and cell-cycle progression to apoptosis and DNA repair [2].

The main resident of eukaryotic nuclei is the genetic material itself, packaged in the form of chromatin. Chromatin is composed of DNA, histones and additional architectural proteins such as heterochromatin protein 1 (HP1). Two copies each of the four core histones, H2A, H2B, H3 and H4, are wrapped inside 147 bp of genomic DNA, forming the core nucleosome. The DNA bridging two adjacent nucleosomes is normally bound by the linker histone H1 and is termed linker DNA [3] (Figure 2). Although the core histones are bound relatively tightly to DNA, chromatin is largely maintained by the dynamic association with its architectural proteins [4–7]. In addition, histones are subject to a wide variety of posttranslational modifications regulating chromatin accessibility and gene expression (Box 1).

Akin to the nucleus itself, chromatin is also organized into well-defined domains. The chromosomes themselves occupy distinct subvolumes of the nuclear space, termed chromosome territories [8] (Figure 1), and the position of gene loci within each chromosome is also subject to strict regulation. The nuclear positioning of both chromosome territories and gene loci within territories are nonrandom and are tissue, cell-type and developmental-stage specific [1]. Within chromosomes, two distinct features of repetitious sequences are present – the centromeres and the telomeres. The former are the anchor points that control the separation of chromosomes during cell division and the latter are elements located at the ends of chromosomes that protect them from destruction. Because both centromeres and telomeres contain repetitive elements, they can be easily identified and visualized during mitosis or interphase (defined as any cell-cycle stage other than mitosis) by fluorescent microscopy (Figure 1).

Chromatin is classified as either euchromatin or heterochromatin. Euchromatin refers to regions of decondensed and usually active chromatin, and is normally marked with characteristic histone modifications such as histone H3 trimethylated on lysine 4 (H3K4me3) and H3 acetylated on lysine 9 (H3K9ac), whereas heterochromatin refers to condensed and normally inactive chromatin regions such as centromeric regions, and is marked by typical heterochromatic histone marks including H3K9me3 and H4K20me3 (Figure 1; Box 1). The term facultative heterochromatin refers to heterochromatic regions that can be activated when needed. Such regions are commonly marked by H3K27me3.

An additional important chromatin mark is DNA methylation. Methylation occurs specifically on cytosines inside CpG dinucleotides and is normally associated with gene silencing (Box 1). Heterochromatic regions, therefore, such as centromeric satellite repeats, are heavily methylated (Figure 1). Interestingly, whereas most CpG dinucleotides in the genome are methylated, regions that bear higher concentrations of CpGs (CpG islands), normally found in promoters of housekeeping genes, are usually hypomethylated, corresponding to gene activity [9].

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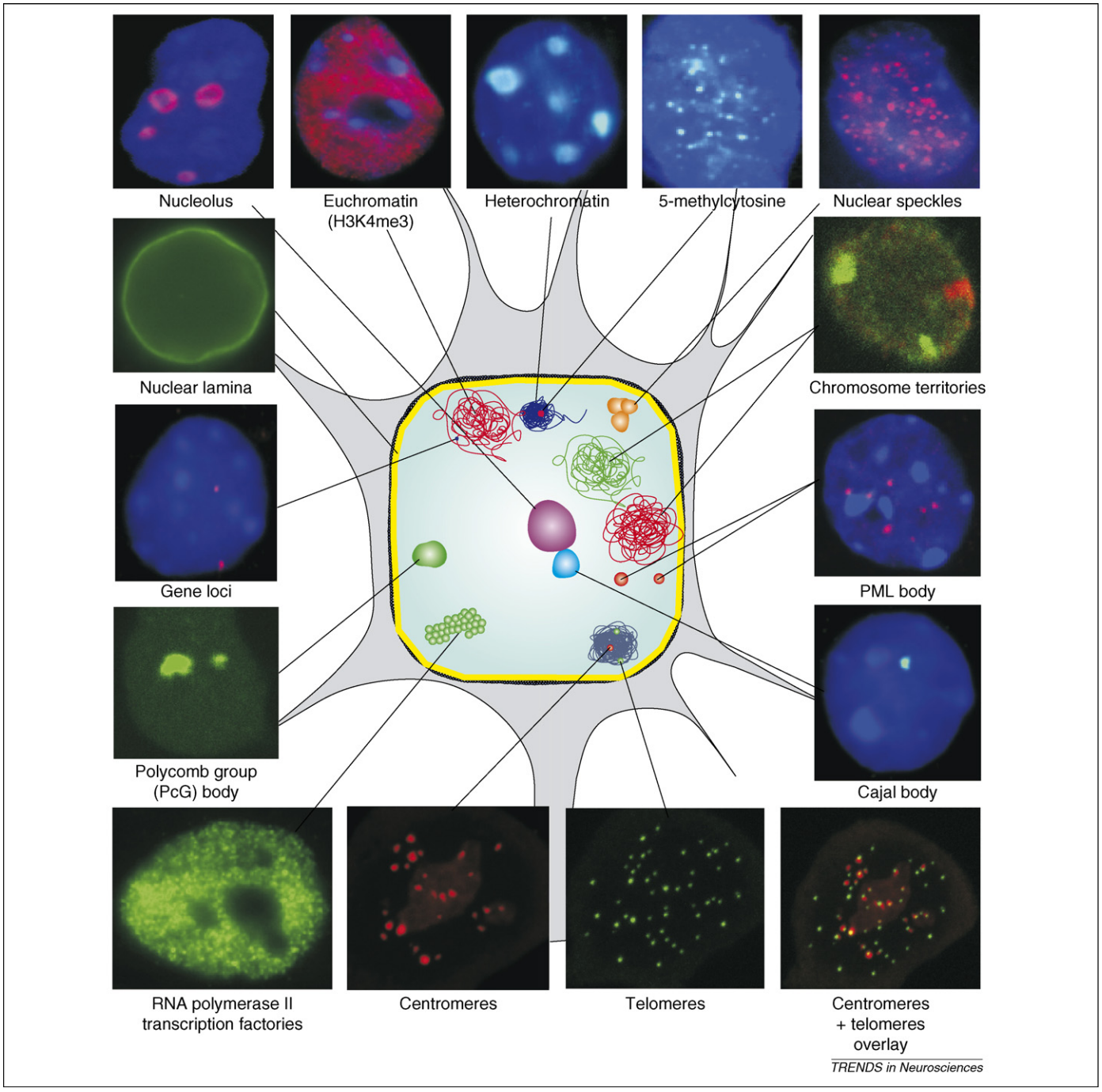

Figure 1. Nuclear architecture. Examples of common nuclear bodies are depicted. Neuronal immunofluorescence images are shown for the various nuclear domains.

This intricate organization of the mammalian nucleus poses important questions regarding the functional consequences of nuclear architecture and chromatin regulation. Here we discuss the relationship between nuclear organization and function and between chromatin structure and neuronal processes. Most of the examples provided are of mammalian systems, as we go on to demonstrate chromatin regulation in higher brain functions. This will lead to a final discussion on potential epigenetic-targeted therapy in the central nervous system.

Nuclear architecture: from global to local regulation

In the nervous system, nuclear architecture has been shown to be distinct among different types of cells and during different stages of differentiation or development [10–12]. We demonstrate this point by describing changes in global features of nuclear arrangements including nuclear bodies and global chromatin structure and proceed with a more fine-tuned type of regulation on chromosomal elements and gene loci.

Nuclear bodies

The arrangement of nuclear bodies correlates with function in interphase nuclei of neurons. For instance, several nucleoli are found close to the nuclear periphery in immature Purkinje neurons but converge into one or two larger nucleoli that are relocated to the center of the nucleus during neuronal differentiation [13]. CBs are often found

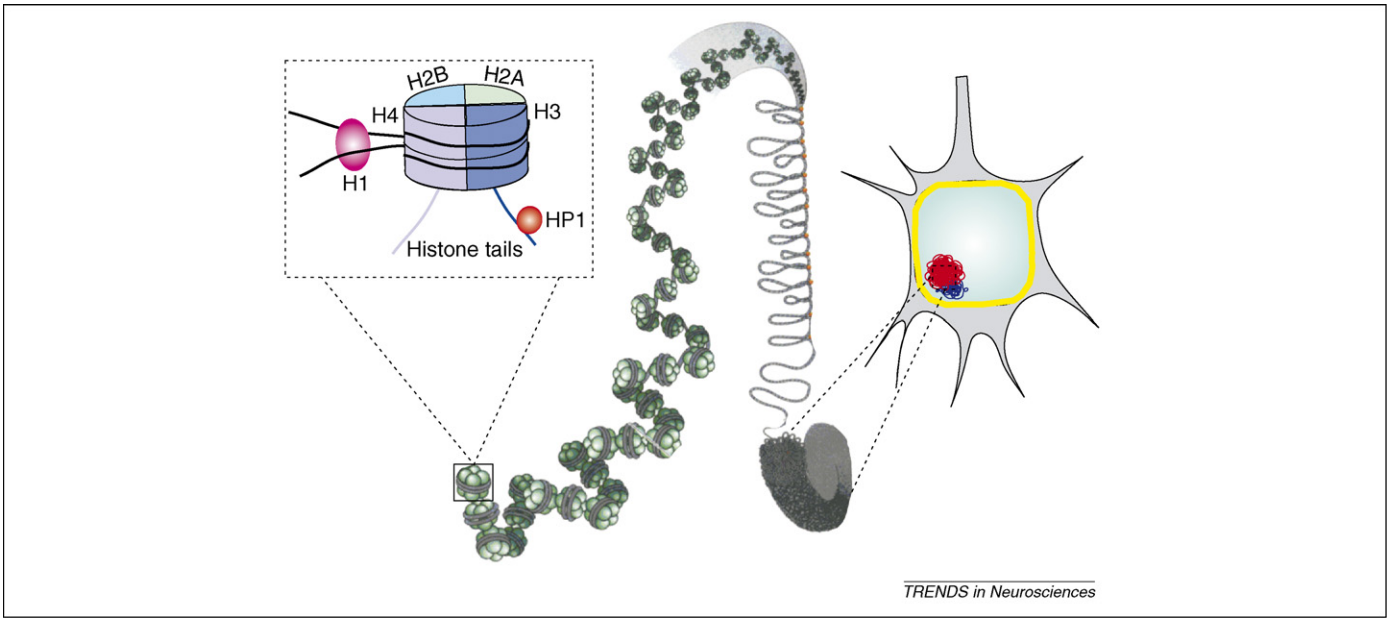

Figure 2. Nucleosome structure. The black line denotes the DNA wrapped around a nucleosome, composed of two copies each of H2A, H2B, H3 and H4 core histones. The linker histone H1 (pink) is bound to linker DNA and the heterochromatin protein HP1 (red) is bound to a specific modification of histone H3 (H3K9me3).

adjacent to nucleoli in mature neurons [14,15], and the number of CBs increases during neuronal differentiation [16] and is regulated by neuronal transcriptional activity following differentiation [15,17]. By contrast, PML bodies decrease in number during neural differentiation [18], whereas their number increases in dorsal root ganglion neurons in patients with acute inflammatory demyelinating polyneuropathy, extending the putative roles of PML bodies to regeneration and axonotomy [19].

A direct link between synaptic activity and nuclear architecture has been recently documented in primary rat hippocampal neurons [20]. In this study, a component of postsynaptic density (PSD), AIDA-1d, was identified as a molecule mediating synapse-to-nucleus signaling and neuronal activity by altering nucleolar numbers and global protein synthesis. Upon stimulation of NMDA receptors, AIDA-1d translocates into the nucleus in a calcium-dependent manner and binds to CBs, leading to the association between CBs and nucleoli. This mechanism enables neurons to increase the number of nucleoli and influence the rate of global protein synthesis through persistent synaptic activity.

Taken together, these data demonstrate the close relationship between neuronal activity and nuclear architecture and argue that the organization of nuclear bodies within the neuronal nucleus is regulated and functionally important.

Global chromatin changes

Global changes in chromatin organization and structure can be readily visualized by fluorescent staining methods (Figure 1) or assayed by enzymatic digestion of nucleases. Heterochromatin rearrangement occurs during neuronal differentiation of embryonic stem (ES) cells. Heterochromatin foci appear larger, fewer and decondensed in undifferentiated ES cells, numerous and condensed in ES cell-derived neuronal progenitor cells and again fewer but more compact in terminally differentiated neurons [18,21,22] (Figure 3a). Neurons in general possess fewer heterochromatin foci and a larger volume of euchromatin than mature glial cells [23] (Figure 3b), an arrangement which was suggested to relate to the higher transcriptional activity in neurons [24,25].

One of the global structural properties of chromatin is the nucleosome repeat length (NRL), the spacing between two adjacent nucleosomes. This fundamental property of chromatin structure was shown to change during differentiation and development and to be associated with changes in gene expression [26]. Interestingly, NRL is modified in cortical neurons during early postnatal development [27], during the course of neuronal differentiation in the rat brain [28] and during aging [29], demonstrating global regulation of chromatin structure in CNS development.

Global changes in chromatin structure were also observed in Alzheimer's disease (AD) patients. Brain nuclei of AD patients were less sensitive to digestion with micrococcal nuclease – an enzyme that digests chromatin in the linker region between two adjacent nucleosomes – and their histone protein composition was unique [30,31]. This demonstrates large-scale condensation of chromatin in AD.

Together, these studies demonstrate that large-scale chromatin structural changes occur when cells or tissues undergo global morphological and/or functional changes, such as during neuronal differentiation and development, or in pathophysiological states such as neurodegenerative diseases. But before such substantial chromatin transformations occur, more subtle changes can be observed. Some of these cases are discussed below.

Chromosomes and gene loci

The spatial positioning of discrete regions of chromosomes such as centromeres and telomeres is nonrandom and changes in accordance with cell differentiation and functions [11]. For example, centromeres are clustered in

Box 1. Chromatin modifications

Chromatin structure and function are largely regulated by the activities of histone-modifying enzymes [50–52]. These mostly include histone acetyltransferases (HATs) [92], histone deacetylases (HDACs) [93], histone methyltransferases [94] and the recently discovered family of histone demethylases [95]. In general, histone acetylation is correlated with transcriptional activity, whereas histone methylation is associated with suppressed chromatin and transcriptional silencing with the exception of H3 methylated on lysine 4 (H3K4me) and lysine 36 (H3K36me), which are regarded as hallmarks of actively transcribing chromatin [96] (Figure I). In addition to acetylation and methylation, histones are also modified by poly-ADP ribosylation [97], phosphorylation [98], ubiquitination [99] and sumoylation [100], often on multiple residues, adding significant complexity to chromatin regulation. Whether these modifications form a coherent 'histone code' is a matter of debate [101–103], as both redundancy [104] and opposing actions [105] seem to be commonplace among several of the histone modifications. Nonetheless, these modifications act to regulate gene expression, cell-cycle dynamics, DNA replication and more. In the central nervous system, histone-modifying enzymes have been implicated in several disorders as well as in higher brain functions such as learning and memory.

In addition to modifications of histone residues, methylation of cytosine in DNA acts as an additional mode of chromatin regulation. In mammalian cells, two different types of DNA methyltransferases – the enzymes that catalyze the addition of a methyl group to cytosine – exist. Dnmt1 is a maintenance DNA methyltransferase, whereas both Dnmt3a and Dnmt3b are *de novo* DNA methyltransferases [106]. Whether an enzyme that catalyzes the reverse reaction – DNA demethylation – exists is a matter of long-standing debate, although a recent report suggests that at least one protein, Gadd45a, actively participates in repair-mediated DNA demethylation [107]. Its function in the CNS remains to be identified.

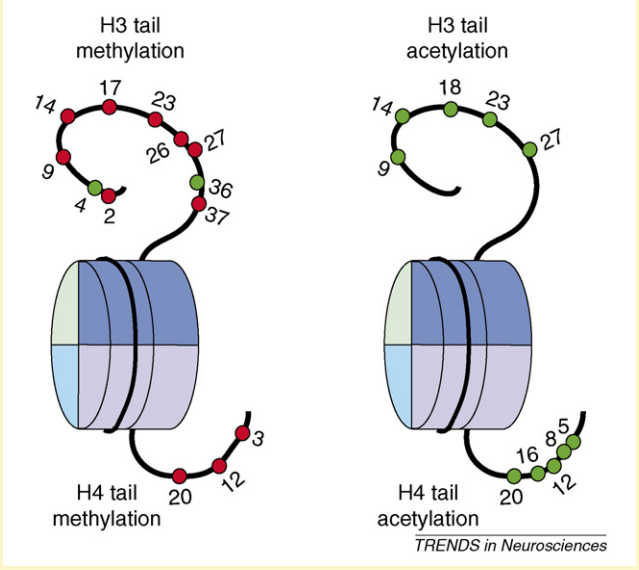

Figure I. Tail methylations (left) and acetylations (right) of histones H3 (top) and H4 (bottom). Numbers indicate positions of lysines along the tails. Green denotes modifications that are usually correlated with transcriptional activity whereas red denotes marks normally associated with transcriptional repression. Only lysine residues are shown for clarity.

mouse Purkinje neurons but not in fibroblasts [32]. The extent of clustering changes along with development and is believed to be established around postnatal day (P) 15 in the mouse cerebellum [32]. The characteristic distribution of centromeres in Purkinje neurons, where half the volume of centromeric heterochromatin surrounds the nucleoli and the other half is located on the periphery, is established

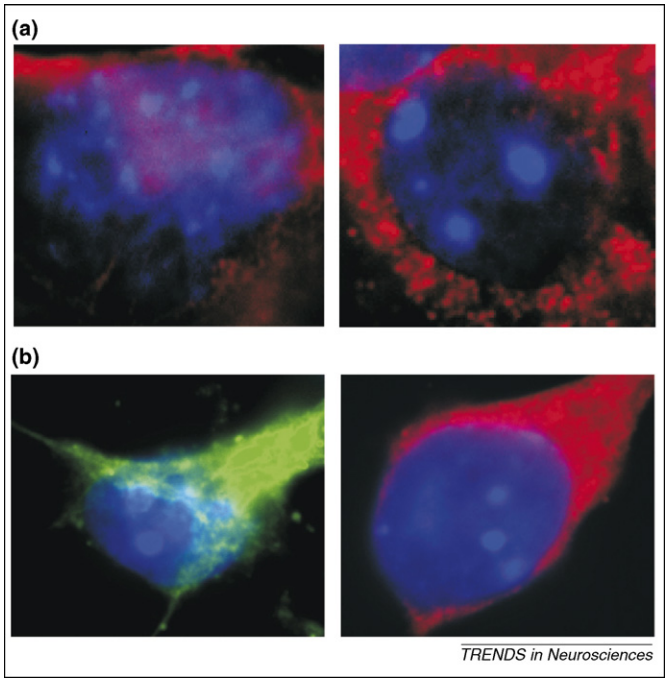

Figure 3. Heterochromatin in different CNS cell types. **(a)** Heterochromatin foci, visualized by DAPI staining (blue) in an embryonic stem cell-derived neuronal progenitor cell (left) immunolabeled with nestin (red) and in an embryonic stem cell-derived postmitotic dopaminergic neuron (right) immunolabeled with tyrosine hydroxylase (red). **(b)** Heterochromatin foci (DAPI, blue) in a primary astrocyte (left) immunolabeled with GFAP (green) and in a primary P14.5 postmitotic neuron (right) immunolabeled with MAP2 (red).

around P5 [32]. These observations are particularly interesting because the noted changes in nuclear architecture occur following exit from the cell cycle. Centromeres from particular chromosomes have favorable partners for clustering. In one example, homologues of chromosome 2 and 11 frequently cluster together in cerebellar Purkinje neurons compared to other chromosomal pairs [33]. Individual chromosomes were also shown to move positions during nervous system neuropathology, as was demonstrated in a seminal study for the X chromosome in both males and females during epilepsy [34]. These results demonstrate the importance of the nuclear locations of chromosomes themselves and suggest that chromatin domains are mobile within the nucleus.

Spatial positioning of gene loci in the nucleus is also regulated. In Purkinje neurons, *Plcβ3*, which gradually increases its expression between P2 and P7, is repositioned from the nuclear periphery toward the nuclear interior between P3 and P5. By contrast, the spatial positioning of a constantly expressed gene, *Rorα*, remains constant [32]. Along the same lines, an astrocyte-specific gene, encoding glial fibrillary acidic protein (GFAP), is repositioned toward the nuclear center during astrocyte differentiation. Furthermore, GFAP is monoallelically expressed and its active alleles are mapped more internally than inactive alleles in primary astrocytes, supporting the concept of internal localization of active gene loci [35]. Not all genes follow the same rule. For example, during oligodendrocyte differentiation, radial distribution of an oligodendrocyte-specific gene, *Plp*, does not change albeit its differentiation-mediated upregulation [36]. The direct proof that repositioning of genes to the nuclear periphery

can repress their expression came recently using a system which enabled the tethering of an internal gene locus to the nuclear lamina. Tethering usually resulted in decreased expression, histone deacetylation and the accumulation of laminar proteins at the tethered site [37], although some genes escape silencing [38,39]. These studies strongly link nuclear positioning of gene loci with activity and function.

Recently, pairing between different or homologous gene loci has been reported to play important roles in gene regulation. Homologous pairing of imprinted regions suggests a requirement for positioning in this process [40]. Homologous pairing of the 15q11–13 imprinted domain, which is known to be deficient in maternal alleles in Angelman syndrome (AS), increases from infantile to juvenile normal human brains, but is disrupted not only in brains of AS patients but also in other diseases with similar phenotypes such as Rett syndrome and autism [41].

Collectively, these studies illustrate the significance of nuclear architecture and spatial positioning of genes and chromosomes in neurons and during neuronal differentiation, but at the same time demonstrate our limited knowledge in this field. The mechanisms linking neuronal nuclear architecture with function remain to be elucidated.

Chromatin in physiological processes and psychosis

Most of the studied cases where chromatin plays a regulatory role in the nervous system describe local, not global, changes of chromatin structure. In this section, we bring several examples where local chromatin structure of specific gene loci was shown to be involved in physiological processes in the CNS.

One of the best-studied cases where local chromatin architecture was demonstrated to play a role in a psychiatric CNS disorder is the case of the genes encoding GAD67 and Reelin in schizophrenia. Reduction of both these genes occurs in the prefrontal cortex of schizophrenia patients as well as in a methionine-induced mouse model of schizophrenia [42]. Promoters of both these genes display elevated recruitment of the methylated DNA-binding proteins MeCP2 and MBD2, associated with GAD67 and Reelin promoter hypermethylation. Because DNA methylation is usually linked with histone deacetylation and reversely, DNA demethylation is often associated with histone acetylation, the authors tested the effect of histone deacetylase (HDAC) inhibitors on DNA methylation. These reversed promoter hypermethylation in a time-dependent manner and increased transcription of the genes encoding GAD67 and Reelin [43]. These results demonstrate that DNA methylation is a dynamic process in the central nervous system and that the recruitment of specific DNA-modifying proteins at gene promoters might influence the levels of cytosine DNA methylation.

One of the interesting questions regarding chromatin regulation is whether chromatin marks can persist through generations. To answer this question, promoter DNA methylation was studied at the gene encoding glucocorticoid receptor (GR) across generations [44]. In this study examining licking and grooming (LG) and arched-back nursing (ABN) behavior in rats, hippocampal GR DNA methylation patterns differed in pups of mothers that displayed high levels of LG and ABN (LG/ABN) as compared with offspring of mothers that showed low LG/ABN [44], resulting in different behavioral response to stress. The methylation patterns and stress response persisted throughout adulthood, and are basically maintained through generations unless intervened. The methylation levels seemed to be reversible upon cross-fostering between low LG/ABN and high LG/ABN within the first 12 h of birth. This demonstrates environment-induced specific changes in chromatin regulation and a nearly lifetime persistence of chromatin modifications under natural conditions. As we have seen for the genes encoding GAD67 and Reelin, the HDAC inhibitor TSA, administered intracerebroventricularly, reversed DNA methylation status at the GR promoter, leading to restoration of GR expression. These facts support the notion that, in some cases, DNA methylation is a potent regulator of chromatin but can be manipulated by drug administration.

The interplay between different histone modifications leading to stable chromatin regulation was studied at the *Bdnf* promoter (Figure 4) in defeat stress, a mouse model of depression [45]. In this work, mice were subjected to a brief daily exposure of a highly aggressive resident mouse for 10 consecutive days, after which the test mouse and the aggressor were separated by a transparent physical divider for the remaining 24 h period, allowing both visual and olfactory stimulations. The stress protocol was followed by a chronic 4 week treatment with the tricyclic antidepressant imipramine. Defeat stress induced a threefold down-regulation in specific alternatively spliced *Bdnf* variants, namely *Bdnf*III and *Bdnf*IV, accompanied by a corresponding increase in H3K27 dimethylation (H3K27m2) – a repressive histone mark usually associated with facultative heterochromatin – at their corresponding promoters [45] (Figure 4b). Interestingly, as is the case with GAD67 and Reelin, some changes observed for *Bdnf* were reversible, and specific for the noted *Bdnf* alternative transcripts upon treatment with imipramine. The latter induced promoter hyperacetylation as well as a significant decrease in the levels of one specific chromatin-modifying enzyme, histone deacetylase 5 (Hdac5) (Figure 4c). Indeed, overexpression of Hdac5 blocked the ability of imipramine to ameliorate chronic stress-related symptoms, demonstrating the functional relevance of this particular HDAC to the depression model used. Hdac5, but not other HDACs, was later found to be particularly involved in chronic but not acute stress, as well as in cocaine addiction, attesting to the specificity of Hdac5 in controlling behavioral adaptations [46]. Intriguingly, although imipramine treatment restored histone acetylation and transcription of *Bdnf*, it was unable to reverse H3K27 methylation. This suggests that the gene is marked with a 'depressant' histone modification, H3K27m2, for stable regulation of chromatin structure, and might provide the underlying mechanisms for clinical depression where withdrawal of medicine restores symptoms of depression.

This study implies that regulation of chromatin structure might be the underlying molecular mechanism for other noted examples of stress-induced alternative splicing in the CNS, such as stress-associated variants of acetylcholinesterase (AChE) in the mouse brain [47,48]. In these studies, chronic psychological stress caused marked

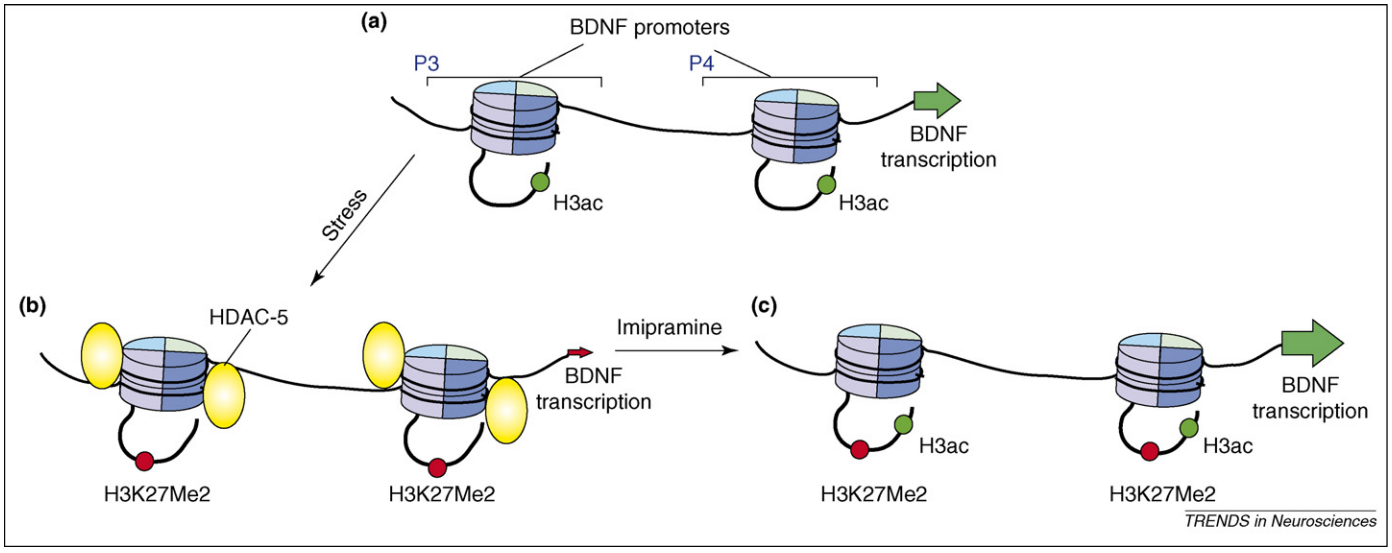

Figure 4. Chromatin regulation at the BDNF promoter following chronic stress and antidepressant treatment. **(a)** In normal situations, the gene encoding BDNF is expressed at basal levels. **(b)** Following chronic defeat stress, HDAC5 is recruited to BDNF promoters III and IV, elevating the levels of histone H3 dimethylated at lysine 27 (H3K27m2), inhibiting their transcription. **(c)** Following treatment with the antidepressant imipramine, histone acetylation is increased and expression of BDNF variants is restored.

overexpression of one specific AChE variant, AChE-R. Because overexpression of this stress-induced variant was retained for several weeks in the mouse brain [49], it is tempting to speculate that a stable change in the chromatin architecture of the gene encoding AChE is responsible for the long-lasting effect. This idea, however, remains to be tested.

These studies illustrate both acute and chronic, short-term and long-term, local regulation of chromatin structure in the central nervous system and establish the role that chromatin regulation plays in neuronal adaptation. They also demonstrate that DNA methylation is a reversible process, affected by both drugs and animal physiology.

Mechanisms of chromatin regulation

The mechanisms that underlie global chromatin structural changes and regulation of nuclear architecture remain unknown. However, local chromatin structure and function are mainly regulated by the activities of histone-modifying enzymes [50–52] (Box 1), as we have seen in the case of the *Bdnf* gene (Figure 4). Modifying enzymes are usually associated with complexes that bind chromatin through specific modifications, such as methylated DNA or histone residues. Discussed below are several examples that demonstrate the different types of chromatin regulation and their dynamic interplay.

Histone acetylation and deacetylation

Histone acetylation plays pivotal roles in essentially all cell types, but several examples demonstrate the specific involvement of histone acetyltransferases (HATs) in neuronal processes. HAT activity was shown to participate in normal brain development in the mouse. A genetic disruption of the *Querkopf* gene, a Myst family histone acetyltransferase, causes inhibition of cerebral cortex neurogenesis, craniofacial abnormalities and premature death during weaning [53]. Circadian rhythm also involves HAT activity, demonstrated by the periodic changes of histone H3 acetylation in the mouse brain [54]. Importantly, HAT activity was shown to be involved in higher brain functions. This was shown in a model of novel taste learning in the mouse insular cortex – a brain region involved in the formation of novel taste memories [55] and in mice which express a mutated form of CREB-binding protein (CBP) with depleted HAT activity. In these mice, the consolidation of long-term memory was impaired [53]. Histone acetylation was also observed during reestablishment of learning and memory during environmental enrichment in a mouse model of neurodegeneration [56]. In mice exposed to an enriched environment, acetylation of different histone tails was increased in the hippocampus after 24 h, including H3K14ac, H3K9ac, H4K5ac, H4K8ac and H4K12ac. Interestingly, in some, but not all, of these cases (e.g. H3K9ac, H4K5ac) elevated acetylation levels persisted for as long as 2 weeks, indicating that different phases of memory involve different modes of chromatin remodeling.

HAT activity is profoundly implicated in CNS-related diseases. For example, histone acetylation was shown to be inhibited by the action of caspases during apoptosis in both *in vitro* and *in vivo* models of neurodegeneration [57]. α Synuclein, a protein implicated in Parkinson's disease, was shown to bind histones directly and to subsequently reduce the levels of histone acetylation and neurotoxicity [58]. But most apparent and well-studied is Rubinstein-Taybi syndrome (RTS). RTS is caused by heterozygous mutations in the gene encoding CBP and is characterized by severe mental retardation, retarded growth and higher risk of tumorigenesis [59], demonstrating the vital function that histone acetylation plays in the brain. These examples establish the crucial association between histone acetylation and neuronal processes including neurogenesis, neuronal proliferation, circadian rhythm, memory formation and neuronal activity.

The enzymes that catalyze the reverse reaction of histone acetylation are histone deacetylases. HDAC activity is strongly implicated in the regulation of neuronal genes within the nervous system as well as in the silencing

of neuronal gene expression in nonneuronal cell types. HDAC regulation is involved in the differentiation of neural stem cells into both oligodendrocytes [60] and neurons [61,62], depending on the exact conditions and the precise differentiation stage, but is also implicated in neuronal cell death [63]. Hdac4 specifically shuttles from the nucleus to the cytoplasm in a stimulation-dependent manner, thereby activating specific pathways of neuronal death triggering apoptosis [64].

HDAC suppression of neuronal genes plays an important role in nonneuronal tissues. One of the best-studied cases is the suppression of neuronal genes which harbor specific repressor element 1 (RE1; also known as neuronal restrictive silencer element, NRSE) at their promoter region. RE1-binding protein REST (RE1-silencing transcription factor; also known as neuronal restrictive silencer factor, NRSF) represses neuronal expression through its specific association with Sin3a and an HDAC [65,66]. Additional proteins involved in the suppression of neuronal genes were identified, including the structural DNA-binding protein BRAF35, which as part of the BRAF-histone deacetylase complex (BHC) mediates the repression of neuronal genes by means of its HDAC activity [67]. These studies strongly implicate histone deacetylation as a major component of CNS functions in both health and disease, and prompted the use of HDAC inhibitors as potential therapeutic agents in central nervous system disorders (see below).

Histone methylation and demethylation

Examples of histone methylation and demethylation specifically associated with neural gene expression are still rare, but their nature implies vital significance in neuronal cells. For instance, disruption of the gene encoding euchromatin histone methyltransferase 1 (*Eu-HMTase1*), which methylates lysine 9 on histone H3 (H3K9), causes severe mental retardation and behavioral problems in patients [68], suggesting an imperative role in CNS development and function. In one elegant study, the histone methyltransferase G9a was shown to be recruited by the neuronal restrictive silencer factor NRSF to silence target genes in nonneuronal cells by H3K9 methylation [69]. Interestingly, the same protein can recruit an alternative histone methyltransferase, MLL, which methylates the transcription-associated H3K4, thereby activating, rather than suppressing, neuronal target genes [70]. But at the same time, the NRSF complex can also contain a histone demethylase, LSD1, which acts to demethylate H3K4, thus opposing the action of MLL. This type of mechanism might ensure specific activation and inactivation of different subsets of neuronal genes during differentiation, depending on the recruited enzyme. These studies implicate the involvement of histone methylation and demethylation in the central nervous system and call for further investigation of the mechanisms and proteins involved, especially as histone demethylation has only recently been discovered.

Drugs that alter chromatin structure as therapeutic agents

Given that changes in chromatin and histone modifications underlie many pathological conditions of the nervous system, drugs targeting chromatin are potential therapeutic agents. The initial and principle use of HDAC inhibitors in CNS pathologies focused on polyglutamine (polyQ) diseases, where reduced levels of histone acetylation were found [71]. Polyglutamine diseases include at least nine neurodegenerative disorders, including Huntington's disease (HD), which are caused by expansions of CAG repeats in different genes [72–74]. HDAC inhibitors were shown to ameliorate polyglutamine-mediated neurotoxicity in a mammalian model of HD [75], as well as in a variety of additional polyglutamine diseases [76], such as Dentatorubral-pallidoluysian atrophy (DRPLA), an autosomal dominant neurological disorder caused by a CAG repeat expansion in the gene encoding DRPLA. In this study, hydroxamic acid residue, which is shared by the HDAC inhibitor TSA, was shown to be a potent inhibitor of HDAC activity in a cell-culture model of differentiated PC12 cells [77]. Although neuronal rescue in this system was limited, the derivation of novel HDAC inhibitors with specific functions is a promising avenue for polyQ-related diseases.

Apart from polyglutamine diseases, HDAC inhibitors seem to also be effective in other nervous system disorders. Of special interest is their recent use in autoimmune encephalomyelitis (EAE), a model for the autoimmune disease multiple sclerosis (MS). MS patients and EAE mice suffer extensive demyelination in the central nervous system, leading to subsequent neurodegeneration and cognitive and physical malfunction [78]. Promisingly, TSA reduced spinal cord inflammation, demyelination and neuronal loss and considerably improved disability in the relapsing phase of EAE [79], demonstrating the involvement of HDACs in the pathology of MS and EAE. Cerebral ischemia was also shown to benefit from HDAC inhibition. The HDAC inhibitor valproic acid (VPA), commonly used for anticonvulsions and mood stabilization, proved effective in reducing brain damage in a transient focal cerebral ischemia model in rats [80], and the HDAC inhibitor SAHA prevented H3 deacetylation and reduced ischemic injury in a mouse model of middle cerebral artery occlusion [81]. In a mouse model of neurodegeneration [56], mentioned above, HDAC inhibitors induced dendrite sprouting and synaptogenesis and reestablished access to long-term memories. These studies together pave the way for successfully employing HDAC inhibitors and understanding their mechanism of action in the CNS.

The link between mood and HDAC inhibition has recently been established further, when the HDAC inhibitor sodium butyrate was shown to have antidepressant activities in mice, especially when administered together with the serotonin selective reuptake inhibitor (SSRI) fluoxetine [82]. Acetylation in this study was most significantly affected in the frontal cortex, a brain area associated with cognition, emotion and stress [83]. Finally, HDAC inhibitors recently proved useful in the treatment of spinal muscular atrophy (SMA), a disease caused by insufficient production of the survival motor neuron (SMN) protein, both in a rat model of SMA [84] and in fibroblast cells derived from SMA patients, where an elevation of up to sevenfold was observed for the SMN2 protein [85].

In addition to HDAC inhibitors, there are indications that DNA-demethylating agents, such as 5-aza-deoxycytidine

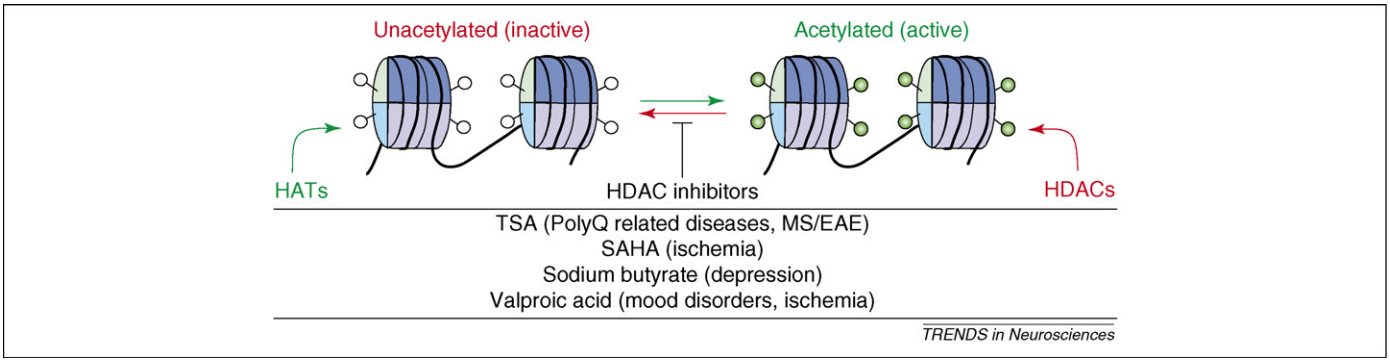

Figure 5. Histone acetylation and deacetylation and HDAC inhibition. Histone acetyltransferases (HATs) catalyze the addition of an acetyl group to a histone tail, allowing transcriptional activity. Histone deacetylases (HDACs) catalyze the reverse reaction, removing acetyl groups from histones, rendering them inactive. HDAC inhibitors thus retain histone acetylation and prevent gene silencing. Examples for HDAC inhibitors mentioned in the text are indicated below. Associated neuropathologies are mentioned in parentheses.

(5-Aza), which demethylates DNA passively by either incorporating into DNA instead of cytosine or by inhibiting DNA methyltransferases (DNMTs), could potentially benefit patients by restoring normally low baseline levels of 5′-cytosine methylation of target genes. This was shown, for example, for the gene encoding Reelin, which, as noted above, is reduced in brains of schizophrenic patients. Treatment of NT2 cells, in which the Reelin promoter was intentionally hypermethylated, with 5-Aza increased Reelin mRNA expression by over 50-fold [86]. The mechanism for this change is not altogether clear, as 5-Aza normally requires cells to go through the cell cycle to bring about demethylation. Although DNA-demethylating drugs have not been tested systematically in the central nervous system, they might assist in other cases where DNA hypermethylation plays a role, such as Alzheimer's disease, where promoters of genes responsible for the production of amyloid-β are hypermethylated [87]. Although HDAC inhibitors and DNA-demethylating agents primarily target chromatin, their pleiotropic effects are gaining increased attention, especially in treating cancer [88], but in many cases their mode of action remains obscure.

These results together demonstrate the key role that chromatin plays in the nervous system in general and in CNS pathology in particular. Importantly, our ability to control chromatin structure and function by retaining the levels of histone acetylation with HDAC inhibitors (Figure 5) or the levels of DNA methylation with DNA-demethylating agents should prove useful in combating polyglutamine- and other chromatin-related diseases in the central nervous system [89–91].

Concluding remarks

Nuclear architecture and chromatin structure play important roles in the nervous system, directing differentiation and development, regulating neuronal activity and assisting in the maintenance of neuronal memory. The established link between disease and histone modifications provides the scaffold for novel therapeutic opportunities in the CNS. Particularly, many chromatin-mediated events in the CNS are likely to be manipulated by HDAC inhibitors, suggesting potential therapeutic application of these compounds to neurological disorders. At the same time, there is much to be learned to understand the intimate relationship between nuclear organization and chromatin structure and neuronal function, especially now that most, if not all, modifications, some of which were previously regarded as stable, have been shown to be reversible. How stable then are chromatin modifications in postmitotic cells? What are the mechanisms that establish and regulate neuronal nuclear architecture? How do these processes relate to neuronal activity? How is chromatin structure modified to accommodate changes in neuronal plasticity? These are just a few of the fundamental questions that remain to be answered in this emerging field of chromatin and nuclear architecture in the nervous system.

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