Trends in Neurosciences

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REVIEWS

Review

Epigenetic Regulation in Neurodegenerative Diseases

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Mechanisms of epigenetic regulation, including DNA methylation, chromatin remodeling, and histone post-translational modifications, are involved in multiple aspects of neuronal function and development. Recent discoveries have shed light on critical functions of chromatin in the aging brain, with an emerging realization that the maintenance of a healthy brain relies heavily on epigenetic mechanisms. Here, we present recent advances, with a focus on histone modifications and the implications for several neurodegenerative diseases including Alzheimer's disease (AD), Huntington's disease (HD), and amyotrophic lateral sclerosis (ALS). We highlight common and unique epigenetic mechanisms among these situations and point to emerging therapeutic approaches.

Epigenetic Regulation of Chromatin in the Brain

Eukaryotic genomic DNA must be packaged to fit inside the nucleus, the diameter of which is roughly 100 000 times smaller than the length of the DNA. By maintaining specific loci at a more open state and other loci tightly packed, chromatin structure regulates various processes that require access to DNA. The nucleosome, which is the basic unit of DNA packaging, consists of 147 bp of DNA wrapped around a histone octamer (made of two copies of histones H2A, H2B, H3, and H4). Multiple mechanisms that regulate the interaction between histones and DNA control access and recruitment of factors critical for DNA replication, transcription, or repair.

Several epigenetic regulatory mechanisms – including DNA methylation, histone post-translational modifications, chromatin remodeling, histone protein variants, and long noncoding RNA – have all been shown to control chromatin structure and regulate a plethora of cellular and organismal processes (Box 1). Established and emerging techniques for the study of chromatin structure enable genome-wide characterization of protein–DNA interactions at the single cell and single base resolution [1,2]. Epigenetic regulation has critical implications in human health, with alterations in chromatin known to be involved in multiple illnesses, most notably cancer, in which drugs that inhibit DNA methylation and histone deacetylation have been approved for clinical use by the FDA [3]. With specific relevance to the brain, mutations in several chromatin-associated factors lead to neurological disorders, including autism spectrum disorder, mental retardation, intellectual disability, and epilepsy [4], highlighting the important roles of epigenetic mechanisms for brain development and function. The protein levels of multiple epigenetic factors are also altered by mutations in the translational regulator *FMR1* in Fragile X syndrome [5], the leading inherited cause of intellectual disability and autism. A shared histone acetylome profile characterizes cortical chromatin in autism spectrum disorders [6]. These recent findings suggest a unifying underpinning in the heterogeneous group of neurological disorders encompassed by intellectual disability and autism.

Additional mechanisms link specific chromatin modifications with neuronal physiology. DNA CpG demethylation occurs in brain-specific genes related to neuronal plasticity following

Highlights

Genome-wide studies have begun to characterize epigenetic changes in neurodegenerative diseases. Both global and local alterations in the levels of multiple histone marks have been identified.

The impact of these alterations on gene expression is not clear in all situations, and other mechanisms likely contribute to transcriptional dysregulation in the degenerative brain.

Loss of chromatin dynamics occurs in aging and neurodegenerative diseases. However, these are separate states and the chromatin landscape of the neurodegenerative brain is distinct from that of the healthy aged brain.

Advances in brain chromatin technology now include single-cell analysis of DNA methylation and histone modifications.

In multiple animal models of neurodegenerative diseases, including Alzheimer's disease, Huntington's disease, and amyotrophic lateral sclerosis, reversing aberrant chromatin structure mitigates toxicity of disease-associated proteins.

Epigenetic editing may prove a useful tool to alter locus-specific chromatin structure and avoid non-histone targets of small molecule inhibitors.

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Box 1. Mechanisms of Chromatin Regulation

DNA Methylation

DNA can be methylated on cytosine residues at the carbon 5 position (5mC) by a family of methyltransferases. Methylation occurs in the context of CpG or CpHpG (H denoting A, T, or C). Classic functions of DNA methylation include X-chromosome inactivation in mammalian females, genomic imprinting and gene silencing. 5mC can be converted to 5-hydroxymethylcytosine which is particularly abundant in the brain [82]. Demethylation occurs through a series of deamination or oxidation reactions.

Histone Acetylation

Acetylation occurs on the ε amino group of lysine residues in the N-terminal region of histone proteins (referred to as the histone tails). Generally, acetylation is associated with gene activation and is mediated by histone acetyltransferase enzymes, while removal of the mark is catalyzed by histone deacetylases. Several mechanisms may explain the positive effect of acetylation on transcription: the acetyl group removes the positive charge of lysine side chains, thus reducing electrostatic interactions between the positively charged histones with the negatively charged DNA backbone. In addition, chromatin readers that bind acetylated histones can mediate chromatin remodeling and allow a more open chromatin structure.

Histone Methylation

The effect of histone methylation on transcription is context specific with several histone methylated marks promoting gene activation and others enhancing heterochromatization and reducing access to specific loci. Methylation can occur on lysine or arginine residues and does not alter the charge of the affected residues. Methylation, as with other histone modifications, is dynamic with histone methyltransferases adding the methyl group and histone demethylases removing it. Arginine residues can be methylated in one or two locations on the guanidine group and the methylation could be symmetric or asymmetric resulting in four possible states. Lysine methylation can add mono-, di-, or trimethyl groups (me1, me2, and me3), with each state conferring unique structural alterations that are recognized by appropriate reader proteins.

Histone Phosphorylation

Phosphorylation of serine, threonine, or tyrosine side chains is catalyzed by protein kinases and can be dephosphorylated by phosphatases. This well-studied protein modification also takes place in histone tails and regulates multiple processes, the best known of which is the DNA damage response where the phosphorylated H2A(X) histone variant (γH2AX) accumulates at DNA damage sites. Histone phosphorylation also regulates gene expression and has been linked to acetylation events. In addition, phosphorylation is associated with chromatin condensation during mitosis and meiosis [83].

Histone Variants

Canonical histones can be replaced by histone variants that introduce sequence variations, with all histones except histone H4 having multiple gene variants in humans. Incorporation of histone variants occurs both during replication or in a replication-independent manner. Histone variants promote unique interactions with chromatin-associated proteins, such as chromatin remodeling factors, or alter chromatin structure, and play important roles during mammalian development, X-chromosomal inactivation, and gene expression in the brain [84].

Chromatin Remodeling

The association of DNA with histones, which serves as a barrier to transcription and other processes, can be altered by chromatin remodeling factors that use ATP hydrolysis to mobilize nucleosomes [85]. Nucleosome sliding, ejection or insertion, change chromatin structure and the interaction with auxiliary factors. The ATPase motor is accompanied by additional domains that are characteristic of specific chromatin remodeling subfamilies [86]. Four major families of chromatin remodeling factors are: SWI/SNF, ISWI, INO80/SWR1, and NuRD.

Histone Chaperones

Histone chaperones are a diverse set of proteins regulating histone storage, transport, post-translational modifications, and nucleosome assembly and turnover [87]. Newly synthesized histones, as well as replacement variants and recycled histones, may be deposited on DNA following replication, transcription, DNA damage repair, and other nuclear processes. Major histone chaperones include HIRA, DAXX, CAF1 complex, and ASF1.

Long Noncoding RNAs (lncRNAs)

lncRNAs allow allele-specific chromatin alterations by tethering RNA–protein complexes to a specific locus [88]. One of the best examples is *Xist*, a lncRNA transcribed from the X-inactivation center (*Xic*), which covers the entire mammalian inactive X chromosome and promotes silencing.

3D Organization

Within the nucleus, chromatin is organized in a 3D structure that brings selective regions to close proximity and sets other regions apart. Genome-wide techniques that defined such interactions (e.g., Hi-C), led to the identification of topologically associated domains (TADs) [89], which are remarkably conserved between cell types and mammalian species. Regulatory interactions, such as those between enhancers and promoters, mainly occur within the same TAD. Similarly, genes within the same TAD can show co-regulatory properties, suggesting a functional and regulatory role for TADs [90].

neuronal activation [7], and non-CG methylation accumulates in neurons but not glia during development [8]. The brain has unique metabolic characteristics [9], and metabolism controls important aspects of epigenetic regulation [10]. Interestingly, production of acetyl-CoA, a substrate for histone acetylation, is carried out in neurons in proximity of genomic loci that are critical for learning and memory. This on-site production likely allows efficient acetyltransferase reaction and supports neuronal gene expression [11].

A critical emerging question is therefore whether chromatin structure is also altered during neurodegenerative processes, and if such changes are causally involved in disease. Preliminary analyses identified common changes in DNA methylation in several neurodegenerative diseases [12], pointing to shared regulatory programs. Human neurodegenerative diseases including Alzheimer's disease (AD), Huntington's disease (HD), and ALS are associated with dramatic changes to the transcriptional profile [13–15], suggesting that altered chromatin regulation might be involved.

In this review, we summarize recent advances in our understanding of chromatin-related mechanisms in the pathogenesis of neurodegenerative diseases, primarily AD, HD, and ALS (Figure 1). Because aging is the strongest risk factor for neurodegenerative diseases, we start by describing how chromatin might be affected during aging in the brain and how these changes may sensitize neurons to disease.

Aging as a Risk Factor for Epigenetic Alterations Leading to Neurodegeneration

The most notable risk factor for neurodegenerative diseases is age, and aging itself is associated with a decline in cognitive capacities. Chromatin alterations that occur as the brain ages might therefore be important targets to prevent cognitive deterioration [16]. In addition, such alterations could contribute to the development of degenerative diseases. Altered levels of histone acetylation and methylation have been associated with advanced age. In general, an increase in repressive marks of H3K9me2, H3K9me3, and H3K27me3 and a decrease in activating marks of H3K36me3 and H3K27ac have been observed in cerebral cortex and hippocampus of aged animal models (reviewed in [17]). Studies in *Drosophila* heads however revealed loss of H3K9me3 and heterochromatin protein 1 (HP1)-associated heterochromatin structures and increased expression of genes that are normally silenced such as transposable elements [18]. Additionally, in *Drosophila* heads, genes with high H3K36me3 levels show relative stability in their mRNA expression, while genes with low H3K36me3 levels have a higher frequency of drastic gene expression changes during aging [19]. In aged mice, impaired memory functions measured using fear-conditioning paradigms

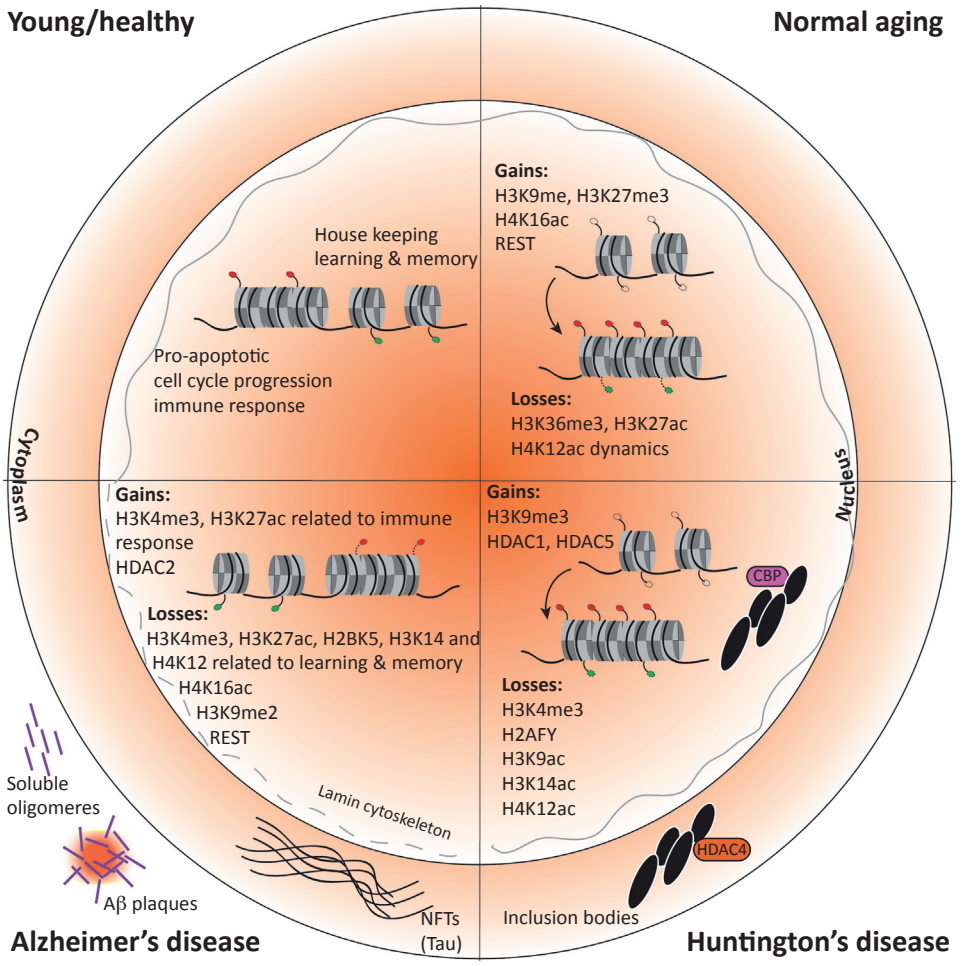

Figure 1. Chromatin Alterations in Brain Aging and Disease. We summarize global changes to histone modifications and related alterations that occur in aging, AD, and HD. We note that due to the complexity of the genome, with both losses and gains usually reported for histone marks in these conditions, this schematic represents a simplified model of more intricate changes. From the studies highlighted here, a general theme emerges in which aging and HD are primarily characterized by reduced levels of modifications usually associated with open chromatin (in aging, H3K36me3 and H3K27ac [17]; in HD, H3K4me3, H3K9ac, H3K14ac, and H3K12ac [54,91]) and increases in marks associated with closed chromatin (in aging, H3K9me and H3K27me3 [17]; in HD, H3K9me3 [43,45]). In *Drosophila* heads however, reduced levels of H3K9me3 and HP1 with age are associated with increased expression of genes that are normally silenced [18]. In AD, the alterations appear to be distinct with global losses of heterochromatin marks (H3K9me2 in *Drosophila* τ model [36]), as well as locus-specific losses and gains of activating marks (H3K4me3 and H3K27ac in a mouse model [27], and H4K16ac in the human AD brain [33]). Changes to the nuclear architecture, for example loss of the lamin cytoskeleton in tauopathies, may also contribute to reduced levels of heterochromatic marks and gene expression imbalances. In HD, the pathological accumulations of nuclear and cytoplasmic inclusion bodies interact with several chromatin factors including CBP and HDAC4, providing a direct mechanism by which these pathologies promote alterations to chromatin structure. Abbreviations: AD, Alzheimer's disease; CBP, CREB-binding protein HD, Huntington's disease; HDAC, histone deacetylase; NFTs, neurofibrillary tangles; REST, Repressor element 1-silencing transcription factor.

correlate with inability to upregulate acetylation of H4K12 [20]. Hippocampal gene expression analysis demonstrates minor changes in gene expression in old mice. Remarkably, however, fear conditioning induces large-scale changes to gene expression of young mice, but the transcriptome of old mice remains mostly unchanged 1 h after the stressful stimuli.

Therefore, aging may block transcriptome dynamics in a mechanism dependent on H4K12ac [20].

An important candidate gene that could be involved in age-associated reduced cognitive capacities is *Bdnf*. Brain-derived neurotrophic factor (BDNF) is critical for learning and memory, and *Bdnf* mRNA levels are reduced in hippocampi of aged mice. In the *Bdnf* promoter, reduced H3K27ac and increased H3K27me3 levels were observed in aged mice, suggesting that a shift from open to closed chromatin underlies the reduced transcriptional output [21]. Reduced levels of the histone acetyltransferase (HAT) CREB-binding protein (CBP) and increased levels of the histone deacetylase (HDAC)4 at *Bdnf* promoter regions in aged mice support this notion. CBP is recruited by active CREB following N-methyl-d-aspartate receptor (NMDAR) activation [22]. Interestingly, age-associated reduction in membrane cholesterol in the hippocampus leads to reduced NMDAR signaling and low H3K27ac levels at the *Bdnf* promoter. Prevention of age-associated cholesterol loss rescues *Bdnf* transcription and enhances cognitive performance of old mice [21], linking H3K27ac to age-associated neuronal physiology and cognitive performance.

Could epigenomic analysis of the aging brain point to pathways that are relevant to neurodegenerative diseases? Transcriptomic analysis of the human brain as it ages identified reduced expression of targets of repressor element 1-silencing transcription factor (REST), predicting that REST levels should increase in normal aging [23]. Indeed, REST levels are high during neurodevelopment but remain low until advanced age, when they increase again. In neurodegenerative diseases including AD and frontotemporal dementia, REST levels fail to increase with age, leading to reduced levels of neuroprotective genes such as *FOXO*, which mediates oxidative stress resistance, and the antiapoptotic gene *BCL2* [23]. Conversely, increased levels of genes that promote AD pathology (e.g., *PSEN2*) and cell death (e.g., the proapoptotic *BID*, *PUMA*, and *BAX*) result from reduced REST expression [23], and could promote neuronal fragility in these diseases. These alterations may involve altered histone modifications as REST recruits histone deacetylases [24] and levels of H3K9ac are reduced in normal aging but not in the AD prefrontal cortex [23].

AD and Tauopathies

AD is the leading cause of dementia in elderly people and a major public health concern with a current estimation of 5.5 million patients in the US alone [25]. Both beta-amyloid (Aβ) plaques and neurofibrillary tangles composed of hyperphosphorylated Tau are pathological hallmarks of the disease, and soluble oligomers as well as aggregated proteins contribute to neuronal toxicity [26]. A study of the CK-p25 AD mouse model [27] showed increased expression of genes associated with immune response functions, and reduced expression of genes involved in synaptic and learning functions. Corresponding immunoprecipitation followed by sequencing (ChIP-seq) analyses have revealed changes in promoter (H3K4me3) or enhancer (H3K27ac) marks that correlate with gene expression alterations, while few alterations in heterochromatin or polycomb regions have been found (H3K9me3 and H3K27me3, respectively). Human orthologs of enhancers with increased H3K27ac marks are enriched for genetic variants associated with AD, suggesting a role for immune-related enhancer elements in AD predisposition [27]. A role for H3K4me3 in AD is further implicated by the lysine methyltransferase *Kmt2a.* Loss of *Kmt2a* in mouse forebrain neurons partially recapitulates the loss of H3K4me3 in the CK-p25 model, and interestingly, Kmt2a itself is downregulated in CK-p25 [28].

H4K16ac is a histone mark generally associated with active gene expresssion and is localized to both enhancers and promoters. By inhibiting the formation of the 30-nm-like fibers and inhibiting the ability of chromatin remodeling factor ACF to mobilize nucleosomes, H4K16ac alters chromatin structure [29]. H4K16ac has been linked to aging and DNA damage

processes, which are both associated with neurodegenerative diseases [30–32]. ChIP-seq profiling of H4K16ac in postmortem temporal lobe from AD and controls spanning a range of ages shows dramatic redistribution of H4K16ac in aging and disease. While both gains and losses are found, normal aging is associated predominantly with increases of H4K16ac peaks, with the number of H4K16ac peaks doubling in the healthy aged cortex. By contrast, H4K16ac is dramatically lost in the AD cortex, pointing to an inability to upregulate H4K16ac in the aged AD brain. H4K16ac peaks positively correlate with expression of nearby genes suggesting that the altered H4K16ac landscape could have functional implications. Importantly, disease-altered H4K16ac peaks are associated with AD-associated single nucleotide polymorphisms and with expression quantitative trait loci of AD, but not other diseases. H4K16ac peaks that are altered in AD appear, therefore, to represent critically important loci as many of them are identified as AD associated by genome-wide association studies [33].

HDAC2 levels are upregulated following neurotoxic insults in cultured cells, in the hippocampus and prefrontal cortex of AD mouse models, and in the hippocampus of postmortem samples from AD patients [34]. In the CK-p25 AD mouse model, increased binding of HDAC2 to promoter regions of genes with critical roles in learning and memory and synaptic plasticity is accompanied by reduced acetylation levels of H2BK5, H3K14, H4K5, and H4K12, reduced RNA polymerase II binding, and reduced gene expression [34]. Thus, increased HDAC2 levels may lead to impaired synaptic function, a well-characterized pathological feature of AD [35]. Pointing to a direct effect of chromatin alterations, acetylation of non-histone proteins such as P53 and Tau are not altered in this model. Strikingly, hippocampal HDAC2 knockdown rescues gene expression levels, enhances synaptic density, and mitigates memory impairments, but has no effect on neuronal survival [34]. Therefore, epigenetic blockade of memory functions in the surviving neurons might play critical roles in dementia, in addition to the impairments that are caused by loss of neurons.

In addition to reduced transcription of genes that are critical for proper neuronal function, aberrant upregulation of genes that are normally silenced may also occur in AD. In *Drosophila* models of tauopathies, which include AD, both wild-type and mutated Tau (pseudohyperphosphorylated Tau^{E14}) cause a reduction in H3K9me2 and HP1 [36]. Loss of these heterochromatin marks and proteins is associated with promiscuous expression of genes that are normally silenced or expressed at low levels in the fly head (e.g., *Nvd*, *Ir41a*, *Ago3*, and *CG15115*), while highly expressed genes are not affected [36]. Tau also causes reduced lamin protein levels in *Drosophila,* and abnormal lamin invaginations are present in nuclei from AD postmortem frontal cortex. Because of the interactions of heterochromatin with the lamin nucleoskeleton, it is expected that such alterations will impact chromatin structure. Indeed, lamin dysfunction leads to heterochromatin relaxation, neurodegeneration, and DNA damage, likely through stablization of F-actin and dysruption of the linker of nucleoskeleton and cytoskeleton, which bridges the actin cytoskeleton and the lamin nucleoskeleton [37]. It is hence possible that lamin dysfunction mediates several toxic effects of hyperphosphorylated Tau in multiple different tauopathies (Figure 1).

H3K9 methylation might also be relevant in Parkinson's disease, as α-synuclein, a major aggregated protein in the disease, increases global mono- and dimethylation of H3K9 in *Drosophila* and cultured neuroblastoma cell models [38] (see [39] for additional details on the effect of α-synuclein on epigenetic regulation in Parkinson's disease).

HD

HD was one of the first neurodegenerative diseases to be studied in the context of epigenetic regulation. Excellent reviews summarize these data [40–42], and here we highlight more recent findings. HD impacts multiple abilities in patients and can cause movement, cognitive, and

psychiatric impairments. An autosomal dominant disease, HD is caused by polyglutamine expansion in the first exon of the huntingtin (*HTT*) gene. Chromatin alterations found in HD are therefore likely downstream effects of these repeat expansions. HD models and HD human brain tissue show alterations in gene expression, increased H3K9me3 heterochromatin domains [43], and importantly, the histone acetyl transferase CBP is mislocalized to polyglutamine aggregates in HD cultured cells, mouse models, and HD postmortem brain [44]. Increased H3K9me3 levels and heterochromatin condensation could be mediated in part by the chromatin remodeling factor ATRX [45]. Additionally, multiple other transcriptional regulators are impaired by mutant *HTT* [46]. Work with *Drosophila* HD models initially identified a beneficial effect of HDAC inhibitors to dramatically mitigate neurodegeneration [47]; these findings were corroborated by mammalian models [48–51] and suggested that decreased histone acetylation might be involved in HD mechanisms (Figure 1). It is striking that HDAC inhibitors may protect against neurodegeneration in both HD and AD models, in spite of different underlying pathogenic mechanisms. These effects may reflect the sequestration of the histone acetyl transferase CBP in HD, while in AD, as noted above, upregulation of HDACs is found. Alternatively, it is also possible that neurons react to various stressors by limiting the transcriptional output and that mitigation of this chronic effect using HDAC inhibitors reinstates the transcriptional profile and thus could be beneficial for a number of brain diseases. Surprisingly, in the HD82Q mouse model, which expresses a mutated and truncated version of *HTT* bearing 82 polyglutamine repeats, histone hypoacetylation of hundreds of H3K9 H3K14 and H4K12 loci is not correlated with severe hippocampal and cerebellar transcriptional dysregulation [52]. These results suggest that histone deacetylation and transcriptional impairments might be independent. Even though HDAC inhibitors showed some initial promise in clinical trials for HD, unwanted side effects led to efforts to develop more selective inhibitors [41].

Recent efforts to target histone methylation in HD show that reduced H3K9me3 levels with the chromatin remodeling drug nogalamycin slows disease progression in R6/2 HD transgenic mice, which express *HTT* exon 1 with 150 CAG repeats [53]. It is noteworthy that levels of heterochromatin associated marks vary considerably in different animal models of neurodegenerative diseases, with little to no changes observed in mouse models of AD [27], reduced levels in *Drosophila* models of tauopathies [36], and increased levels in mouse models of HD [53]. While these changes may reflect specific outcomes of the disease-associated proteins, they may also involve genome-wide redistribution of such marks rather than global increases or decreases. Accordingly, the levels of H3K4me3, a mark associated with gene activation, are lower at promoters of downregulated genes in HD postmortem brain and in HD mouse models. Increasing the levels of H3K4me3 by targeting H3K4me3 demethylase is protective in mouse and *Drosophila* HD models [54]. ChIP-seq analysis of neuronal nuclei isolated by sorting postmortem human prefrontal cortex of HD cases and controls identified neuron-specific alterations in H3K4me3, with many of the altered H3K4me3 peaks located near genes with synaptic functions [55,56].

Another approach to characterize chromatin alterations associated with brain diseases is the use of cultured neurons that are differentiated from induced pluripotent stem cells (iPSCs). Such neurons derived from juvenile-onset HD patients show altered expression of nearly 2000 genes, compared to neurons derived from controls. More than a quarter of these genes are centered on neurodevelopment, with *NEUROD1* and transforming growth factor (TGF)-β genes (*TGFB2, TGFB3*, and *TGFB3R*) identified as critical hubs [57]. Chromatin profiling of H3K4me3, H3K27ac, and H3K36me3 has revealed that genes near altered H3K4me3 peaks are associated with cell adhesion, and genes near altered H3K27ac are associated with synaptic

transmission, neuron differentiation, and actin cytoskeleton [57]. These and other studies suggest that alterations in histone methylation and acetylation occur in HD and that a full understanding of these changes could allow the development of more directed and hopefully selective therapeutics.

It is noteworthy that correlating histone modifications with the cellular transcriptional state at the genome-wide level is not a straightforward task. This is likely due to several reasons. First, the functional outcomes of many histone modifications are not fully understood or may depend on the specific context in which the specific mark resides. Second, the majority of studies to date have focused on a few selected and well-studied marks, while the transcriptional outcome, as it might be coded by a histone code, may depend on additional modifications. Third, additional mechanisms clearly converge with the chromatin state to dictate gene expression and the overall effect of these factors will be difficult to assay. Finally, the chromatin state regulates additional processes including DNA replication and DNA damage response, further complicating attempts to directly correlate such marks with the transcriptional profile.

Further alterations to the epigenome in HD include changes in expression of histone variants. In a study aimed at defining biomarkers for HD, *H2AFY*, which encodes the histone variant macroH2A1, was identified as upregulated in blood cells of patients with HD, compared to healthy controls or patients with other neurodegenerative diseases [58]. H2AFY protein levels were reduced in blood from HD patients treated with HDAC inhibitor, but not placebo [58]. Genes related to chromatin structure may thus serve as peripheral biomarkers and assist in monitoring the pharmacodynamic responses to treatment.

Additional Insight into the Disease State

Neurons as postmitotic cells rely on various mechanisms to maintain their identity thoughout life. Epigenetic mechanisms are required, but are not sufficient, to maintain the gene expression state of differentiated neurons [59]. For example, a combination of transcription factors (*die-1* and *che-1*) as well as a MYST-type histone acetyltransferase are required to induce and maintain left/right laterality in *Caenorhabditis elegans* ASE sensory neurons [60].

Alterations in histone modifications as they occur in aging or disease may contribute to neuronal reentry to the cell cycle, a unifying theme in many neurodegenerative diseases [61]. H3K27me2/3 is deposited by the polycomb repressive complex 2 (PRC2), which contains the enzymatic components EZH1 or EZH2, as well as nonenzymatic proteins SUZ12 and JARID2. Supporting a role for repressive histone methylation to maintain neuron-specific transcriptional states, reduced H3K27me3 in medium spiny neurons (MSNs) of the striatum in mice resulted in perturbed expression of genes, both upregulated and downregulated. However, genes whose expression was reduced were not identified as H3K27me3 targets in wild-type MSNs, suggesting that their decrease was a secondary effect [62]. While most H3K27me3 target genes in MSNs were insensitive to PRC2 deficiency, transcriptional regulators that were upregulated generated positive feedback loops that maintained high expression of their targets [62]. Interestingly, most upregulated genes show bivalent H3K27me3 and H3K4me3 marks in MSNs, indicating that the loss of H3K27me3 results in release of a barrier to their transcription. Several genes with cell-death-promoting functions were among those upregulated, and mice with neuronal PRC2 deletion developed progressive neurodegeneration, impaired motor performance and died early [62].

Increased H3K27me3 levels, however, can also be deleterious, as loss of A-T mutated (ATM) in ataxia–telangiectasia leads to increased stabilization of EZH2 and increased H3K27me3 levels

in human and mouse cerebellum [63]. Knockdown of *EZH2* in an ATM-deficient mouse model of ATM mitigates neurodegeneration and behavioral impairments, indicating that the increased H3K27me3 levels contribute to toxicity [63]. Strikingly, increased H3K4me3 in ATM-deficient neurons was also associated with cell cycle re-entry, suggesting that proper balance of H3K27me3 is critical for maintaining healthy neurons, with reduced or increased levels associated with aberrant gene expression, impaired cell cycle control, and neurodegeneration.

While many of the examples above involve global alterations in histone modifications, changes in the nuclear localization or recruitment of chromatin remodeling factors also play important roles to maintain neuronal integrity. ALS and frontotemporal dementia (FTD) are neurodegenerative diseases that share both pathological hallmarks and genetic causes. ALS is characterized by progressive demise of motor neurons that leads to muscle weakness and paralysis, whereas FTD variants present with behavior, personality, and/or language deficits. However, significant clinical and genetic overlap suggests that ALS and FTD form in fact a disease spectrum. TDP-43, an RNA- and DNA-binding protein that forms insoluble aggregates in ALS and FTD subtypes (FTD-TDP), limits the recruitment of the chromatin remodeling factor Chd1 to stress response genes in *Drosophila* [64]. This in turn limits nucleosome clearance from the gene body of heat shock protein genes, reduces their expression, and impairs the cellular capacity to cope with various toxic insults. While mass spectrometry analysis has revealed no alterations in global histone post-translational modifications in postmortem temporal cortex of FTD cases, the protein levels of CHD2, the human ortholog of fly Chd1, are dramatically reduced in this brain region [64]. In addition, mutant TDP-43 or FUS, an additional ALS-associated protein, reduce the protein levels of Brg1 [65] a component of the chromatin remodeling complex nBAF. nBAF is a critically important complex in neuronal differentiation and function [66]. Therefore, an inability to maintain dynamic epigenetic responses due to altered levels and recruitment of remodeling factors may also contribute to age-dependent neuronal vulnerability.

Implications for Therapy and Outstanding Open Questions

In cellular and animal models and in the postmortem human brain, disease-associated alterations of chromatin point to specific pathways that might be perturbed in disease. Critical questions include whether these changes are causally involved with disease initiation, progression, or severity, and whether their discovery can direct the development of novel therapeutics.

As we consider the global changes in the epigenetic landscape and gene expression that occur in neurodegenerative diseases, it will be important to understand if disease modulation can be achieved by epigenetic editing of specific genes via precision medicine. Alternatively, if disease etiology involves the deregulation of multiple different genes, global strategies to collectively correct global expression levels might be preferable or necessary.

Locus-specific epigenetic changes can be achieved by fusing chromatin modifying enzymes to DNA-binding platforms, including zinc finger proteins, transcription activation-like effectors, or the clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 system [67]. These systems offer methods by which to alter chromatin structure without systemic administration of inhibitors (such as HDAC inhibitors) that could have pleiotropic side effects or impact non-histone proteins. The applicability of such techniques in the adult brain remains to be tested, and will likely involve major delivery, safety, specificity, and efficacy hurdles. However, some recent findings suggest that epigenetic editing might offer future therapeutic approaches. In one such study, the expression of *Dlg4,* which encodes PSD95, a major component of the postsynaptic density with critical scaffolding functions, was manipulated by zinc finger nuclease

(ZFN) targeting the *Dlg4* promoter [68]. PSD95 levels are reduced in mouse models of AD, suggesting that restoring PSD95 expression could ameliorate synaptic deficits [69]. ZFN was fused to G9a and SUV39-H1 to promote the repressive marks H3K9me2 and H3K9me3, respectively, or to the activation domain of the viral protein VP16 (tetrameric VP64) [68]. Increased expression of *Dlg4* by targeting VP16 to the *Dlg4* promoter rescued memory impairments in AD mouse models, while reduced expression with SUV39-H1 led to strong reduction in NMDA excitatory postsynaptic currents [68].

In several neurodegenerative diseases, repetitive sequences result in heterochromatization of nearby chromatin followed by reduced gene expression. In Friedreich's ataxia, GAA repeats cause reduced elongation and impede transcription of the *frataxin* (*FXN*) gene. Conjugating JQ1, the bromodomain extraterminal domain (BET) inhibitor, and bromodomain-containing protein 4 (BRD4) ligand, to polyamides that target GAA microsatellite repeats (termed synthetic transcription elongation factors), specifically recruits BRD4 to *FXN* and promotes the switch from paused RNA polymerase II to productive elongation [70]. These findings support a future role of epigenome editing to alter specific genomic locations and restore the normal balance of gene expression in the brain. Interestingly, conflicting data exist on the effects of JQ1 itself to regulate memory. Korb *et al.* [71] found that JQ1 reduces the expression of immediate early genes (IEGs) following BDNF stimulation in cultured neurons, and disrupts long-term memory formation in mice. Benito *et al.* [72], however, found that JQ1 enhances long-term potentiation, promotes long-term memory in controls and models of AD, and predominantly increased IEGs. Adding another complication, a third study [73] found no effect of a BET inhibitor (I-BET858) to alter BDNF-stimulated IEGs, and identified a group of secondary and late response genes as targets of BET inhibition. Due to these data and because BET inhibition causes autism-like behavior in mice [73], BET inhibition as a possible therapeutic strategy requires more studies to delineate mechanisms and possible complications. Indeed, the brain imposes a unique challenge to genomic and transcriptomic profiling due to the multitude of cell types in the single tissue (neurons, astrocytes, microglia, and oligodendrocytes) and subtypes (excitatory and inhibitory neurons, unique anatomical location, cortical layer identity, neurotransmitter, etc.). Novel techniques now allow cell-type-specific analysis of transcription [74] and DNA methylation in the brain [75]. Single cell analysis of DNA–protein interactions [76] and chromatin 3D structure [77] might be applied in future studies to uncover neuronal cell-type-specific chromatin structure and interneuronal variations (see [78] for a review of epigenetic control on gene expression in the brain).

Concluding Remarks

We are only beginning to understand the changes in chromatin structure and function that occur in neurodegenerative diseases and how they contribute to disease pathogenesis [79,80]. Emerging insights, however, highlight the critical importance of maintaining chromatin dynamics and proper levels of DNA methylation and histone modifications, with imbalances leading to possibly catastrophic degenerative outcomes. The age dependence of all neurodegenerative diseases suggests that such imbalances may accumulate over time until repair and stress-response pathways finally collapse leading to irreversible neuronal damage. Technologies aimed at restoring chromatin dynamics and proper gene expression may provide novel therapeutic strategies, if applied sufficiently early, and could be combined with therapies addressing other aspects of these diseases, for example protein misfolding and aggregation. See Outstanding Questions for additional open questions. The continuous development of novel techniques to examine chromatin structure and function [74–77,81], some of which are already applied to the study of the nervous system, will provide exciting advances in our understanding of epigenetic regulation in neurodegenerative diseases.

Outstanding Questions

It is becoming clear that robust changes in the levels of histone modifications are accompanying degenerative processes in the brain. Key issues are whether changes to the epigenetic landscape are causal in neurodegenerative disease progression or initiation, or a consequence, and if so, in which specific ways.

What are the cell-type-specific changes (in neurons as well as associated glia) that occur in the chromatin landscape of histone and DNA marks during brain degeneration? When do these changes start? When do they become nonreversible?

Are there common changes across different degenerative diseases, or are diseases distinct with respect to associated chromatin changes that accompany neural deterioration?

How do surviving neurons differ from neurons that succumb to disease with respect to their chromatin profile? Could these changes allow us to understand epigenetic mechanisms that contribute to maintaining neuronal viability? Do different neural types use similar or distinct epigenetic mechanisms of survival?

Can small molecules that target epigenomic modifiers be applied to protect the brain from age and disease associated chromatin changes?

Can epigenomic editing be used in the adult brain to alter locus-specific chromatin structure? Can these techniques be safely used to stably reinstate normal gene expression patterns in brain cells?

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