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Epigenetic mechanisms in neurodevelopmental and neurodegenerative disease

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Abstract

The exploration of brain epigenomes, which consist of various types of DNA methylation and covalent histone modifications, is providing new and unprecedented insights into the mechanisms of normal neural development, neurological disease and aging. Traditionally, chromatin defects in brain were considered static lesions of early development that occurred in the context of rare genetic syndromes but it is now clear that mutations and maladaptations of the epigenetic machinery cover a much wider continuum, including adult-onset neurodegenerative disease. Here, we describe how recent advances in neuroepigenetics have contributed to an improved mechanistic understanding of developmental and degenerative brain disorders, as well as how they could influence the development of future therapies for these conditions.

Introduction

In the living cell, the functional definition of the human genome goes far beyond its linear sequence of 6 (or when haploid, 3) billion basepairs. The 'epi-(*greek for 'over', 'above'*)genome', with its rich cache of highly regulated, structural modifications of DNA cytosine and histone residues and variants, defines the moldings and three-dimensional structure of the genomic material inside the cell nucleus, thereby providing a molecular bridge between genes and the environment. The epigenome is also responsible for orchestrating the myriads of transcriptional units, condensed chromatin clusters and many other features that distinguish between various cell types and development- or disease-states that share the same genome within the same subject (see also Box 1, 2 and Fig. 1).

In this review, we describe how the field of epigenetics is dramatically reshaping the current thinking about neurological and neurodegenerative diseases. Remarkably, only a few years ago, this field's primary focus was on a single mark, DNA methylation, in the context of cell division and early development. At first glance, these topics seemed to bear little relevance to the postnatal and adult brain that has a large proportion of postmitotic and highly differentiated cells, as in humans, the majority of neurons develop, differentiate and permanently exit from the cell cycle many weeks prior to birth. However, three recent major developments—each of which will be highlighted in this review—have the potential importance of epigenetics in brain development and disease to be reconsidered. First, human studies have indicated that the epigenetic landscape remains 'plastic' throughout all periods of brain development and aging, and ongoing dynamic regulation occurs even in neurons and other postmitotic constituents of the brain[1–4]. Second, disordered chromatin organization and function have been ascribed key pathogenic roles not only in several

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neurodevelopmental syndromes of early childhood, but also in a subset of adult onset hereditary neurodegenerative disorders[5,6]. Third, a rapidly expanding repertoire of chromatin modifying drugs has shown an unexpected therapeutic potential for a wide range of degenerative and functional disorders of the nervous system [7–11]. These three lines of discoveries have now coalesced, thereby igniting an enormous interest in the chromatin-associated mechanisms of neurological disease and providing the foundations for a new discipline, 'neuroepigenetics'[12].

Epigenetics and the mature brain

While the focus of this review is on epigenetic changes in various neurological diseases, the first issue to consider is whether the *normal* course of maturation and aging is associated with changes in the brain's epigenome (which is defined by the combined set of DNA methylation markings and histone modifications and variants, see also Box 1). On the one hand, this hypothesis that aging is associated with brain epigenetic changes is attractive, given that there are widespread age-related changes in gene expression in the cerebral cortex, including the downregulation of many neuronal genes[13,14]. However, in contrast to the accumulation of somatic mutations and other structural brain DNA changes that affect promoter function during aging (which are likely to be irreversible)[15], most or perhaps all epigenetic markings studied to date (Box 1) are now thought to be reversible, and there is no *a priori* reason why there would be the unidirectional accumulation of a specific epigenetic mark in aging brain chromatin. Nonetheless, an increasing body of literature indicates that a substantial reorganization of the brain epigenome occurs during postnatal development and aging. Human cerebral cortex, for example, shows complex and gene-specific changes in the amounts of methylcytosine (mC5; cytosines are methylated at the carbon 5 position), and there is a steady rise in mC5 at many promoters that continues into old age in conjunction with subtle changes (mostly a decline) in expression of transcripts originating from these promoters [2,3]. Such age-related epigenetic drifts could impact vulnerability to neurodegenerative disease. For example, in mouse cerebellum the levels of the mC5 derivative, hydroxymethyl-cytosine (5hmC; see also Box 1), are subject to a 10-fold increase from postnatal week 1 to adulthood[16]. Notably, among the genes that are affected by increasing 5hmC amounts at their promoters during cerebellar maturation, pathways for aging-related neurodegenerative diseases and angiogenesis were overrepresented and included at least 15 genes linked to hereditary forms of spinocerebellar ataxia, a neurological syndrome defined by severe motor dysfunction with the degeneration of cerebellar Purkinje neurons and other systems[16]. Also, of relevance, ten-eleven translocation (TET) proteins are responsible for converting mC5 to hmC5, and the active domains of these proteins belong to the same dioxygenase superfamily as hypoxia-inducible factor (HIF), an oxygen sensor that has been ascribed with a key role in angiogenesis and oxidative stress responses[16]. Perhaps TET proteins also function as oxygen sensors, thereby linking the epigenetic status of brain cells to (mal)adaptive processes related to impaired tissue perfusion and oxygen supply.

As well as changes in DNA methylation during development and aging, the epigenetic landscapes of histone post-translational modifications (PTMs) are also subject to dynamic changes. An age-dependent regulation of histone methylation markings that can differentiate between open and repressive chromatin has been documented for the human prefrontal and cerebellar cortex[17], and hundreds of loci undergo substantial chromatin remodeling in cortical neurons during the transition from infancy to advanced age[4]. Furthermore, the brains from mice that are prone to accelerated senescence (the SAMP8 line) and have learning and memory deficits show age-related drifts in histone PTMs. These epigenetic drifts are defined by a loss of the markings associated with active gene expression, such as histone H4 lysine 20 monomethyl (H4-K20me1) and H3-K36me3 (Figure 1 and Box 1), in

conjunction with a robust rise in the repressive mark, H3-K27me3[18]. Likewise, in the hippocampus of 16 month old wild-type mice, genomic regions associated with actively expressed genes show a robust decline in acetylated H4-K12[19], a histone PTM that like H3-K36me3 is linked to the transcriptional elongation process[20]. In addition, histone deacetylase inhibitor-induced upregulation of H4-K12ac dramatically improves hippocampal-dependent learning and memory in aged mice[19]. It is possible that age-related drifts in brain epigenomes negatively affect neuronal [10,15] and oligodendroglial[21] transcriptomes, thereby contributing to a decline in the signaling capacity of nerve cells, defects in axon myelination and other molecular defects that have been linked to cognitive disorders of the adult brain with[22] or without neurodegeneration[14]. Thus, together, these findings, leave little doubt that brain epigenomes are indeed subject to dynamic changes throughout all periods of maturation and aging, and this may have important implications for the neurobiology of disease.

MONOGENETIC NEUROLOGICAL DISEASE ASSOCIATED WITH EPIGENETIC CHANGES

Chromatin remodeling and the proper assignment of epigenetic marks on the genome are of fundamental importance for brain ontogenesis. These processes are also key control points in the stepwise transition from pluripotency to neural precursors to terminally differentiated neurons and glia[23], and are involved in developmental events such as neuronal migration and connectivity formation[24]. Perhaps unsurprisingly, to date more than a dozen neurological syndromes have been linked to single gene mutations in DNA methyltransferase and histone modifying enzymes, or their ‘reader’ proteins (Table 1). Interestingly, however, this list includes not only embryonic defects and multi-organ syndromes, but also specific neurological disorders that shown an onset of symptoms in early childhood (for example, Rett syndrome), or neurodegeneration and regression starting after adolescence (some cases with Kleefstra syndrome) or as late as the third or fourth decade of life (for example, hereditary sensory and autonomic neuropathy with early onset dementia, type 1, HSAN1) (Table 1). Using the molecular defects in these rare monogenic causes of intellectual disability and adult onset dementia as a starting point, the unraveling of the underlying pathophysiology of these diseases could then open up hitherto unexplored therapeutic avenues both for these specific conditions and, eventually, perhaps for a broader spectrum of neurological disease.

Mutations affecting DNA methylation and neurological disease

Hypomorphic (partial loss-of-function) mutations in the DNA methyltransferase *DNMT3B* are responsible for a multiorgan syndrome - Immunodeficiency, Centromere Instability, Facial anomalies (ICF 1) - which includes mental retardation and defective brain development[25,26]. Cultured cells from subjects diagnosed with ICF show imbalances in repressive (mC5 and H3K27me3) and facilitative (H3K4me3 and H3K9ac) markings at pericentric (the ‘middle’ portions of the chromosome that surround the centromere, or the contact point for mitotic spindle attachment during cell division) and DNA repeats[26] and at hundreds of promoters throughout the genome. This is thought to contribute to the dysregulated expression of genes important for brain development, immune defense and many other key functions[27]. A near identical syndrome, *ICF 2*, which is also defined by DNA hypomethylation defects but at a different set of pericentric repeat sequences (Fig. 3), is caused by mutations in *Zinc Finger and BTB domain containing 24* (*ZBTB24*), which encodes a putative transcriptional repressor[28,29].

Mutations and structural variants in the X-linked gene *MECP2*, which encodes a methyl-CpG-binding protein, have been linked to Rett syndrome (RTT), a disorder of early

childhood with an incidence of 1 in 10,000 that is associated with developmental and cognitive regression and a broad range of neurological symptoms[29,30]. Although males with RTT typically succumb in the perinatal period, females survive as they are somatic mosaics for the *MECP2* gene due to X-inactivation, and often appear grossly normal at birth before typically developing symptoms before the age of 2 years. Since the initial discovery of *MECP2* mutations as a cause of RTT[30], both loss-of-function mutations and copy number increases of *MECP2* have been linked to various neurodevelopmental diseases, including individuals diagnosed with childhood onset schizophrenia and autism[31]. Studies in *Mecp2* mutant mouse lines have provided insights into an unexpectedly complex molecular pathophysiology and linked specific neurological RTT phenotypes to transcriptional regulation at specific promoter sequences (Box 3). However, as for ICF syndrome, the chromatin pathology in RTT goes far beyond dysregulated promoter activity and is likely to involve pericentromeric repeats and other heterochromatic domains. For example, many RTT cases show decreased clustering of the chromocenters (which represent clusters of highly condensed chromatin) inside the nucleus and *Mecp2* deficient neurons do not show the expected chromocenter expansions after induction of neuronal activity[32,33]. Neuronal chromatin, which contains much higher amounts of Mecp2 compared to glia[34,35], shows a global disorganization in *Mecp2* deficient brains, with supranormal amounts of the linker histone H1, hyperacetylation of nucleosome core histones, and de-repression of transcriptional activity at pericentromeric repeats, retrotransposons and other normally silent DNA repeats[36]. Alterations in the amounts of Mecp2, which could compete with H1 for occupancy of linker DNA[36], could also affect nucleosome repeat length and the general architecture of chromatin fibers (Figure 1 and Box 1).

It was recently discovered that monogenetic brain disorders associated with defective DNA methylation are not limited to neurodevelopmental diseases but also include some cases who have been diagnosed with HSAN1[5], a rare neurodegenerative condition characterized by various neuropathies and early onset dementia in the third or fourth decade of life. The mutations found in this condition are positioned in the coding sequence of the DNA methyltransferase 1 (*DNMT1*) gene, specifically within the targeting sequence domain important for the nuclear localization and preferential enrichment of this enzyme at pericentric and other repeat DNA, as well as for its activity and stability[5]. Indeed, peripheral blood cells from individuals with HSAN1 showed hypomethylated at DNA repeat sequences and subtle methylation imbalances (mostly hypo-) at hundreds of gene promoters[5]. Furthermore, additional mutations in close proximity to the targeting sequence domain of *DNMT1* are found in some kindreds with autosomal dominant cerebellar ataxia, deafness and narcolepsy (ADCA-DN), which is also associated with early onset dementia[6]. At first glance, it is surprising that these *DNMT1* loss-of-function mutations selectively affect postmitotic neurons. DNMT1 functions as part of the DNA replication machinery during the S phase of the cell cycle, and tracks the replication fork to remethylate the newly synthesized DNA strands[37], which are mechanisms considered relevant only for dividing cells. However, DNMT1 may also play a part in maintaining the amount of DNA methylation in quiescent and even postmitotic cells, because this protein remains enriched at the pericentric repeats and other condensed portions of the genome after S-phase is completed[38]. This cell cycle-independent DNMT1 function is dependent on it having an intact targeting sequence domain[38], and therefore one could speculate that the deleterious *DNMT1* mutations in the affected individuals with HSAN1 result in faulty chromatin remodeling at repeat sequences of differentiated cells, including neurons. Of note, mutant mice that are mosaic for a prenatal *Dnmt1* gene deletion in neural precursors lose virtually all of their brain cells with DNA hypomethylation within one month after birth[39], and it will be interesting to find out whether similar phenotypes would emerge in animals in which *Dnmt1* mutations are limited to the portions of the targeting sequence domain that are mutated in HSAN1 pedigrees.

It is remarkable that each of these three neurological conditions involving dysregulated DNA methylation—ICF, RETT and HSAN1—is associated with widespread chromatin defects across the genome of differentiated CNS cells, including pericentric and other repeat-rich domains that until now have been barely explored in the neurosciences. It will be extremely interesting to follow future developments in this area, which are likely to shed more light on the role of epigenetic regulation of these repeat DNAs in healthy and diseased brains.

Heritable brain disorders with histone defects

Loss-of-function mutations in the *ATRX* (α-thalassemia, mental retardation, X-linked) gene, which encodes a multifunctional chromatin regulator, are responsible for various X-linked mental retardation syndromes[40]. ATRX protein expression progressively increases during brain development and, in mice, *Atrx* ablation results in excessive apoptosis and defective migration of young neurons[40,41]. Another *Atrx* mutant mouse line, which hasa deletion in the *Atrx* zinc-finger motif, is a genetic model for similar human cases who are afflicted with a milder form of X-linked mental retardation[42]. This mouse model shows impaired learning behaviors, elongated, structurally abnormal dendritic spines (a classical neuropathological abnormality found in various neurodevelopmental conditions[43]) and defective synaptic signaling in the frontal cortex [42]. However, the molecular mechanisms that link *ATRX* mutations to these neurological phenotypes remain unclear. One possible mechanism involves H3.3, a histone H3 variant that was originally associated with active transcription but was subsequently linked to ribosomal[44], telomeric and pericentric repeats[45–47]. ATRX, together with its binding partner and histone chaperone, the death domain associated protein DAXX, is essential for replication-independent H3.3 incorporation into nucleosomes [45–47]. It has been suggested that ATRX preferentially targets G-rich tandem repeats which tend to form four-stranded aberrant DNA structures (called G-quadruplexes) and refolds them back into doublestranded DNA and a regular nucleosomal organization[44].

Whether any of these mechanisms are indeed relevant in the context of neurological disease remains to be investigated. However, notably there are at least there monogenetic disorders that show prominent epigenetic dysregulation of repeat DNA, including the aforementioned ICF1 and ICF2 syndromes and ATRX (Figure 3). Further work will be required to clarify whether the observed chromatin defects at the sites of repeat DNA are indeed a major factor for the disordered neurodevelopment common to these conditions. Furthermore, numerous deleterious mutations in histone modifying enzymes have been identified as monogenetic cause for neurodevelopmental or neurodegenerative disease (Table 1).

DISORDERED CHROMATIN IN NEURODEGENERATIVE DISEASE

Historically, studies on chromatin structure and function, and epigenetically driven concepts in general received little attention by researchers exploring the pathophysiology of slowly progressing neurodegenerative disorders such as Alzheimer's, Parkinson's or Huntington's disease. Two independent lines of discovery have now moved epigenetics towards the center stage in these fields. First, initial reports of the therapeutic benefits of histone deacetylase inhibitor drugs in the nervous system utilized preclinical models for Huntington's disease, a hereditary condition caused by the excessive expansion of a CAG repeat in the huntingtin gene[48,49]. Second, the genetic findings described earlier in the review link rare types of adult onset dementia associated with hereditary neuropathy[5] or cerebellar ataxia[6] to deleterious mutations in proteins that requlate DNA or histone methylation. These findings may imply that other, more common types of adult-onset neurodegenerative disease could also be related to the defective regulation of brain chromatin.

Indeed, 'synucleinpathies' such as Parkinson's disease and dementia with Lewy bodies (DLB) are associated with loss of DNMT1 protein from the cell nuclei in brains from patients with these conditions and brains from transgenic mice that overexpress synuclein[50]. This in turn leads to deregulation of DNA methylation at the promoters of several disease-associated genes, including that of alpha-synuclein itself [50]. There is additional, indirect, evidence that the delicate balance in the amounts of DNMT1 and other DNA methyltransferases, including DNMT3a, inside the nuclei of brain cells plays an important part in neuronal health and function. For example, the *in vivo* induction of motor neuron apoptosis by toxic drugs or peripheral nerve lesions is associated with a pre-apoptotic rise in the amounts of DNMT1 and DNMT3a in the nuclei of motor neurons[51]. Furthermore, the amounts of methyl-cytosine and the expression of *DNMT* are reportedly increased in the pyramidal neurons in the cerebral cortex of subjects diagnosed with the sporadic motor neuron disease amyotrophic lateral sclerosis,(ALS)[51]. Adding further complexity, there is evidence that DNMT1 and DNMT3a are not only targeted to the nucleus but are also abundant in mitochondria, including those that are localized in distal neuronal processes and synapses[51]. Whether or not these DNA methyltransferase enzymes regulate mitochondrial functions in brain cells remains to be investigated, but preliminary evidence suggests that mitochondrial DNA, like nuclear DNA, is subject to the methylation and hydroxymethylation of cytosine residues[52].

Likewise, histone modifying enzymes could play a part in neurodegenerative disease. Because much of the evidence so far has been correlative, the neuroepigenetic field eagerly awaits further work that can confirm (or refute) that histone modifying enzumes have a key role in the etiology or progress of neurodegenerative disorders. For example, the pathological sequestration of transcription factors vital for neuronal health, such as the cAMP response binding protein CREB and its partner, the histone acetyl-transferase CREB-binding protein[48,53] (CBP, and mutations in the gene encoding CBP are responsible for Rubinstein-Taybi syndrome, Table 1), has also been associated with the beta amyloid plaques in brains from individuals with Alzheimer's disease[54] and the polyglutamine aggregates and nuclear inclusions in Huntington's chorea[55]. Furthermore, a hyperactivity of transglutaminase TG2 in Huntington's disease leads to excess nuclear amounts of lysine ε-amino - glutamine γ-carboxamide bonds, which are resistant to proteolysis[56]. This results in an abnormal configuration of nuclear actin-cofilin complexes[57] and potentially abnormal histone polyamination, which leads to defective expression of a transcription factor with an essential role in mitochondrial biogenesis, peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1-α)[58]. Furthermore, excessive histone H3K9 methylation[59] and increased amounts of macroH2A1 (a variant histone broadly associated with repressive chromatin remodeling) expression[60] have been observed in blood and brain tissues from individuals with Huntington's disease, including the striatum and frontal cortex that are heavily affected by the underlying neurodegenerative process in this disease. Such changes, in conjunction with additional defects in nucleosomal structure and function, may contribute to the decreased expression of the neurotrophic factor BDNF, dopamine receptors, MAP kinase signaling components and other transcriptional changes that have been observed in the striatum from people with Huntington's disease[61]. Therefore, the hypothesis that histone modifying enzymes are involved in the pathophysiology of neurodegenerative diseases is clearly an interesting one that warrants further investigation.

Epigenetic targets in the treatment of neurological disease

Therapeutic targeting of histone acetylation

There is great enthusiasm in academia and industry to develop new epigenetic therapies, and this has been further boosted by the recent *U.S. Food and Drug Administration* approvals for the potent histone deacetylase inhibitors (HDACi) suberoylanilide hydroxamic acid (SAHA,

trade name vorinostat) and romidepsin (trade name Istodax) in the treatment of hematologic malignancies. Indeed, a large body of preclinical work has suggested that HDACi might have therapeutic potential in a surprisingly wide range of neurological conditions. These include acute brain injury and stroke paradigms, various neurodegenerative conditions such as Parkinson's, Alzheimer's, triplet Repeat disease (including Huntington's and spinocerebellar ataxias),motor neuron disease[8–11], as well as depression and other psychiatric illnesses[62–64](Table 2). However, it is still unclear whether HDACi would indeed benefit the patients affected by any of these conditions.

Moreover, like their counterparts - the histone acetyltransferases (HATs) -both the class I/II HDACs (commonly defined by their zinc-containing catalytic domain) and the (nicotinamide NAD+ dependent) class III HDACs, or sirtuins, regulate lysine acetylation of non-histone proteins[65,66]. Indeed, non-histone targets of sirtuin 1 are thought to mediate the molecule's neuroprotective and cognitive phenotypes[67,68]. Such promiscuity of HDACs for their target proteins adds further complexity to the interpretation of their therapeutic potential that has emerged in models of neurological disease. Consider the example of fronto-temporal dementia (FTD), a genetically heterogeneous neurodegenerative condition that primarily affects the frontal lobes and is associated with at least three different types of inclusion bodies in brain tissue [69]. Haploinsufficiency for *PROGRANULIN* (*GRN*) underlies one type of FTD that is defined by brain inclusions containing TAR-DNA binding protein 43 (TDP-43) [69]. Importantly, *GRN* expression is robustly upregulated by the HDAC SAHA, and it has been suggested that SAHA could become a promising treatment for FTD[70]. In contrast, FTDP-17, or fronto-temporal dementia with parkinsonism, is defined by the abnormal accumulation of the microtubule binding protein tau in the brain due to excessive tau acetylation[71]. Thus, blocking tau acetylation by inhibiting the HAT CBP and promoting tau deacetylation via activation of the HDAC SIRT1 has been proposed as a new therapeutic avenue to treat FTDP-17 and other 'taupathies' such as Alzheimer's disease[71]. Notably, upregulation of SIRT1 also suppresses β-amyloid production in the brains of mice modeling Alzheimer's disease[72]. Interestingly, however, the pan-sirtuin inhibitor nicotinamide also has therapeutic benefit in an Alzheimer's model consisting of transgenic mice that overexpress tau[73]. Although nicotinamide is a fairly broadly acting drug that has many effects beyond sirtuin inhibition, a subset of SIRT2-specific inhibitors elicits therapeutic benefits in some models of Parkinson's disease[74]. Thus, depending on the type of neurodegenerative disease and the specific HDACs and HATs involved, modulation of HDAC and HAT activity could have very different and potentially opposing clinical implications. Furthermore, HDACi are generally considered to promote neuronal growth and differentiation, but there is also evidence that they could have potentially detrimental effects on the orderly maturation of astro- and oligodendrocytes[75–77]. Therefore, caution is warranted when evaluating the potential benefits of these drugs in multiple sclerosis[76], white matter ischemia[9] and other neurological conditions that involve the damage or injury of the brain's non-neuronal cellular constituents. In addition, the activation of normally epigenetically silenced retrotransposons could be another potential HDACi side effect[78] (Box 4).

In addition to HAT and HDAC activators or inhibitors, other types of drugs that interfere with histone acetylation pathways have recently emerged. For example, the bromodomains of the BET (bromodomain and extraterminal) protein family, which are classical 'readers' of acetylated chromatin and facilitate transcriptional activation[79], can be targeted by highly specific small-molecule inhibitors that have been shown to correct transcriptional dysregulation in hematopoetic and solid malignancies[80,81], inflammation and other medical conditions previously shown to be HDACi-sensitive[82]. Because multiple BET reader proteins, including Brd (bromodomain-containing proteins) 1–3 show robust and widespread expression in the mature mouse brain (www.brain-map.org)[83], it will be interesting to

explore bromodomain inhibitors in the context of the some of the neurological disease models discussed above. As for the HAT and HDAC drugs, the specificity and side effect profiles of bromodomain inhibitors will also requires additional investigation.

Therapeutic targeting of histone methylation

The clinical potential of drugs that can interfere with the regulation of histone methylation is also a promising but as yet largely unexplored field. There are probably up to 100 histone methyltransferases (KMTs) and demethylases (KDMs) encoded in the human genome, and many of these enzymes are defined by functional domains outside of the catalytic site that are thought to contribute to target specificity and genomic occupancy patterns[7]. Like for the HDACi, a subset of histone methyltransferase inhibitors are in clinical trials for cancer treatment, and are likely to be explored in the context of neurological disease in the not too distant future[7]. An interesting candidate is the small molecule BIX-01294, which is an inhibitor for the histone H3K9-specific methyltransferases G9a/Glp[84]. This drug de-represses neuronal gene expression[84] and, when administered directly into the ventral striatum (a key structure in the brain's addiction circuitry), strongly enhances the development of reward behaviors in mice exposed to the stimulant drug cocaine[85]. It remains to be determined if BIX-01294 elicits learning and memory-enhancing effects outside of the stimulant addiction paradigm. However, it is interesting to note that the drug's mechanism of action could, at least in part, involve the inhibition of G9a/Glp-mediated repressive chromatin remodeling at the promoters of *Bdnf, Cdk5, Arc* and other genes that function as key regulators for spine density and synaptic connectivity in the brain[85].

Therapeutic targeting of DNA methylation

Several DNA methylation inhibitors, including the cytidine analogues 5-azacytidine (5-Aza-CR), zebularine and nucleoside analogs that sequester DNMT enzymes after being incorporated into DNA[86], have been well characterized and are approved or are in preclinical and clinical trials for the treatment of cancer[86]. When administered directly into brain tissue of mice and rats, these and other types of DNMT inhibitors disrupt synaptic plasticity and hippocampal learning and memory, and are powerful modulators of reward and addiction behaviors[87–90]. However, nucleoside analogs are thought to act primarily at sites of DNA synthesis and replication during the cell cycle, and thus at first glance, they seem to be of little relevance for postmitotic neurons and glia. Notably, though drugs such as N-Phthalyl-L-tryptophan/RG 108, which interfere with sites at which DNMT is active independently of DNA replication, still elicit a robust impairment of hippocampal learning and memory[91,92]. The potential of drugs interfering with DNMT activity may go beyond these examples of synaptic and behavioral plasticity. For example, treatment with DNMT inhibitors can confer stroke protection after mild ischemia **in mice** and, furthermore, haploinsufficiency for the *Dnmt1* gene in mice is associated with smaller infarction volumes after acute ischemia and stroke [93,94]. Furthermore, a recent study reported a strong, anti-apoptotic effect of RG 108, which inhibits the active site of DNMT, in an injury-based mouse model of ALS[51].

Topoisomerase inhibitors as potential neurotherapeutics

Topoisomerases (topos) are DNA cleaving enzymes that are important in the processes of replication and recombination, transcription and chromatin remodeling[95]. A recent study using an unbiased high-content screening approach in mouse primary cortical neurons discovered that a diverse group of molecules that function as topoisomerase I or II inhibitors can unlock the expression of the normally epigenetically silenced paternal allele of the gene encoding ubiquitin protein ligase E3A (*Ube3a*). These topo inhibitors [mediate their effect by reducing the expression of the imprinted *Ube3a* antisense RNA (*Ube3a-ATS*)[96]. The

expression of this antisense RNA is normally repressed on the maternal chromosome in conjunction with the allele-specific DNA methylation of an imprinting center (which isa DNA or chromatin structure that carries epigenetic information about parental origin)[97]. Like hundreds of other loci defined by parent-of-origin-specific gene expression, *Ube3a* was considered epigenetically stable throughout life[98]. This hypothesis now needs to be revised, however, given that even a single intrathecal infusion of the FDA-approved topoisomerase inhibitor topotecan was sufficient to relieve silencing of the paternal *Ube3a* (sense) transcript in lumbar spinal neurons for an extended period of at least 3 months[96]. The most obvious explanation for topotecan's mechanism of action - altered DNA methylation of the *Ube3a* imprinting center - has been ruled out, thus the underlying mechanism(s) remain a mystery.

The reactivation of paternal *UBE3A* expression via topoisomerase inhibition could provide a starting point to investigate potential therapies for the neurodevelopmental disorder Angelman syndrome, which is caused by loss of function mutations and deletions at the maternal *UBE3A* locus[99–101], and for which there are currently no effective treatments. Although it is presently not known whether topoisomerase-mediated reversal of imprinting-related gene expression is specific to the *UBE3A* locus, this issue could be addressed by, for example, fusing topoisomerase enzymes to customized motifs for sequence-specific binding at *UBE3A*. For example, chi zinc finger nucleases or transcription activator-like (TAL) effectors of plant pathogenic bacteria have been fused to the FokI restriction enzyme, allowingthe induction of 'custom-made' DNA strand breaks ' at specific and even at unique loci in the genome[102,103]. It is certainly worth exploring whether similar strategies can be used tocreate a chimeric protein comprised of a topoisomerase enzyme fused to a custom-made zinc finger or TAL effector sequence that can specifically target the *UBE3A* imprinting center.

Outlook

Neuroepigenetics, as a field, is evolving at a rapid pace. We are beginning to understand more about the neural mechanisms that mediate the reversible and bidirectional regulation of DNA methylation and various histone modifications. Furthermore, as discussed in this review, the epigenetic dysregulation of gene expression and chromatin architecture could play a prominent part in the pathophysiology of various neurological disorders. However, in many of these disease conditions the mutant epigenetic regulator seemingly operates in a highly complex and multifunctional manner at a large number of genomic loci. For example, Rett, ICF1 and 2 and HSAN1 syndromes are a heterogeneous group of neurodevelopmental and neurodegenerative disorders caused by mutations in DNA methyl-reader and methyl-writer proteins, and each disease exhibits disordered DNA methylation at numerous single copy genes as well as in specific repeat DNA sequences in subdomains of constitutive heterochromatin. It will be important to clarify, for each disorder, which genomic loci affected are important for disease manifestation, progression and the potential of eventual cure, and which are mere bystanders and irrelevant to the disease process.

For the numerous types of chromatin modifying drugs that have shown some promise in preclinical studies, it will be important to pinpoint their key mechanism of action. For example, are the beneficial effects of HDAC inhibitors in various acute and chronic neurodegenerative and cognitive disorders due to their broad effects on histone modifications or, instead, due to changes in the acetylation of non-histone proteins (such as the presynaptic molecule and regulator of vesicle release Bruchpilot[104]) or a combination thereof? Whatever the underlying mechanism of action, we predict that, as in the fields of oncology and general medicine, which are currently pursuing hundreds of clinical trials with epigenetic drug targets, the treatment options for neurodevelopmental and

neurodegenerative diseases will soon be enriched by an array of chromatin compounds that are emerging from preclinical and translational research (Table 2).

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Box 1: The organization and composition of the epigenome

The basic unit of chromatin is the nucleosome, which consists of 146 bp of genomic DNA wrapped around an octamer composed of the core histones, H2A, H2B, H3 and H4. Chromatin fibers are defined as arrays of these nucleosomes, which are connected by linker DNA and linker histones (Figure 1). The combined set of DNA and histone modifications and histone variants provide the major building blocks for the epigenome[117,118].

DNA methylation

Two related but functionally very different types of DNA modifications, methylation (m) and hydroxymethylation (hm) of cytosine carbon 5 (C5) mostly in CpG dinucleotides, occur primarily within CpG-enriched islands (which areoften defined by a GC percentage >50% across a minimum of 200 bp)[119]. The mC5 and hmC5 markings show a strikingly different distribution, with hmC5 mostly confined to the 5' ends of genes, at amounts that overall correlate with gene expression activity[120,121]. In contrast, only a minute portion (<3%) of mC5 locates to CpG islands at the 5' end of genes, where it is thought to function as a repressive mark, while the remaining 97% of mC5 is found in intra- and intergenic sequences and within DNA repeats[122].

Post-translational histone modifications (PTMs)

According to recent studies, the number of amino acid residue-specific PTMs in a typical vertebrate cell may be as high as 130[123]. PTMs include mono (me1), di (me2)- and tri (me3) methylation, acetylation and crotonylation, polyADP-ribosylation and small protein (for example, ubiquitin and SUMO) modification of specific lysine residues, as well as arginine methylation and citrullination, serine phosphorylation, tyrosine hydroxylation, and several others[123–125]. Various combinations and interrelations of these site- and residue-specific PTMs show close association with the functional architecture of chromatin, and specific epigenetic signatures have been identified for proximal promoters and gene bodies at sites of actual or potential transcription, for enhancer and other regulatory sequences and for condensed and silenced chromatin[126].

Histone variants

Apart from the core histones H2A,H2B,H3 and H4, metazoan genomes encode a number of histone variants that provide another layer of epigenetic regulation. Some of the well known variants include H3.3, H2A.Z and H2A.X which, in contrast to the canonical histones, are subject to replication-independent expression and assembly[127], and have strong effects on nucleosome stability and compaction[128].

Linker histones

These histones, such as H1, are crucial for the three-dimensional architecture of chromatin and the 'zigzag' arrangement of nucleosomes by regulating linker DNA folding, and the amounts of linker DNA strongly correlate with nucleosome repeat length [129] (Figure 1). Importantly, the amounts of H1 in neurons are much lower than in most other cell types[129], which explains the observation that the average nucleosome repeat length in rat neurons is 40bp shorter than in glia (162 versus 201 bp)[130]. These neuron-specific features could have implications for disease vulnerability, because the Rett syndrome protein MeCP2 competes with H1 for linker DNA binding sites[131] and, furthermore, H1 levels are markedly increased in Mecp2 deficient mouse brains[36].

Box 2: Readers, writers and erasers of chromatin marks

It is still under debate whether the epigenome's constituents play a causal part in establishing functional chromatin states. For example, the presence of 'open chromatin'-associated histone modifications and variants at a specific locus in the genome is thought to reflect a process driven by transcriptional regulation and dynamic repositioning of nucleosomes. From this perspective, at least some DNA and histone modifications function merely as a 'cog' in the chromatin remodeling machinery but are not necessarily key drivers[132]. Whatever the importance of DNA and histone modifications in driving the chromatin remodeling process, an increasing number of molecules that attach ('writers'), or that erase DNA or histone modifications, or that bind ('readers') to a specific epigenetically modified site, have emerged as key players in the pathophysiology and potential treatment of neurological disease. Most, or virtually all, epigenetic markings that have been studied to date in brain are subject to a bidirectional and potentially highly dynamic regulation in the context of neuronal activity and various other paradigms[133,134].

The underlying molecular machineries that contribute to the epigenome are often complex; for example, three DNA methyltransferases (*DNMT1, DNMT3a and DNMT3b*) establish and maintain DNA methylation marks. The actions of these enzymes are counterbalanced by active demethylation pathways involving mC5 hydroxylation and oxidation via ten-eleven translocation (TET) dioxygenases, or activation-induced deaminase (AID) and APOBEC-mediated deamination of mC5 or hmC5, followed by base excision repair-mediated replacement with (unmethylated) cytosine[135,136]. Other systems show a surprising degree of diversity, or perhaps redundancy, at the genetic level. For example, the various families of histone methyltransferases and demethylases together could easily account for > 100 genes in a mammalian genome[137,138]. Proteins that bind to a specific epigenetic mark are defined by their characteristic reader module; well studied examples include the methyl CpG binding domain (MBD) for mC5-DNA, the bromodomain for lysine acetylation, and the 'chromo', 'Tudor', 'MBT', 'WD40repeat' and 'PHD finger' domains that target methylated lysines or arginines in a residuespecific manner[125]. Conversely, specific methyl-lysine marks could become the target of 50–100 reader proteins. For the 'open chromatin' mark histone H3-trimethyl-lysine 4 (H3K4me3), the reader proteins include many components of the RNA polymerase II-associated transcriptional initiation complex, while other marks such as H3K9me3 are primarily targeted by transcriptional repressors and regulators of chromatin condensation[139].

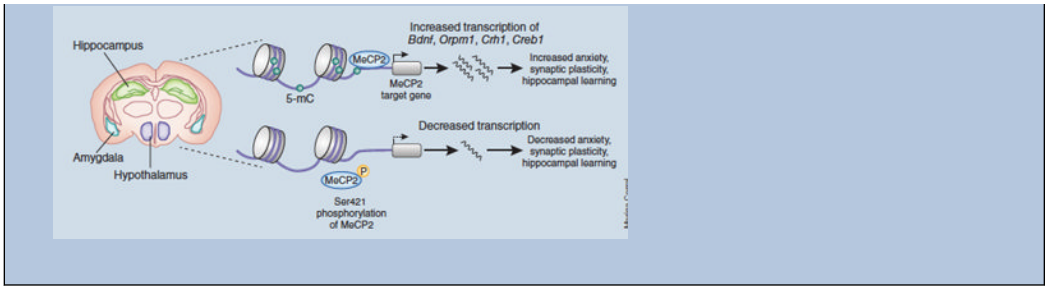
Box 3: The complex pathophysiology of MeCP2 [Au: this box title will probably be too long, so I've suggested shortening it]

Among the growing list of genes associated with a brain-specific chromatin disorder, the largest share of the literature relates to the Rett syndrome-associated gene *MECP2*. The following discussion on emerging views of *MECP2*-associated transcriptional (dys)regulation exemplifies the challenges and complexities in the endeavor to identify and pinpoint the molecular pathology even in a monogenetic disease.

Studies in both humans and micehave shown that phenotypic consequences arise when the amounts of functional MeCP2 protein drop below or rise above its normal amounts in brain[140]. Recent comparisons of the transcriptomes from gain- and loss-of-function *Mecp2* mutant mice to those from wildtype mice have provided some insights into the complexities of MeCP2-mediated transcriptional regulation at specific promoter sequences and have alsoimplicated specific transcriptional changes in some of the emotional and behavioral changes that have been observed across the clinical spectrum of *MECP2* mutations. For example, in basal forebrain including the amygdala, more than 32 genes with an established role in the regulation of anxiety and social behaviors, including the neuropeptide corticotropin-releasing hormone (*Crh*) and the G-protein coupled mu-opioid receptor (*Oprm1*), were highly sensitive to *Mecp2* gene dosage[141,142]. In particular, elevated levels of anxiety in mice that overexpress *Mecp2* are thought to be directly related to increased MeCP2-binding and upregulated transcription at the *Crh* and *Oprm1* genes[141]. Conversely, decreased anxiety in some Mecp2 loss of function mutants has been linked to decreased expression of *Crh* and other gene expression changes[142].

These results, taken together, seem counterintuitive to the originally described role of MeCP2 as a transcriptional repressor and, adding to the complexity, neuronal activity induces MeCP2 phosphorylation at multiple serine sites, thereby altering the protein's affinity to its target gene promoters[143]. *In vivo*, ablation of a phospho-serine site downstream of MeCP2's transcriptional repression domain (S421) enhanced binding of MeCP2 to the brain-derived neurotrophic factor (*Bdnf*) promoter, resulting in increased neurotrophin expression and improved hippocampal learning and plasticity[144](see figure below). Thus, a highly complicated, multi-layered process is involved in fine-tuning of MeCP2 expression, which in turn controls many genes that have a key regulatory role in higher order behaviors.

Figure for Box 3 Fine-tuning of MeCP2 function and higher order behavior. (top) A schematic presentation of MeCP2 protein is shown, including methyl-CpG binding (MBD) and transcriptional repressor (TRD) domains, and neuronal activity-sensitive phosphorylation sites. (bottom). In adult mouse forebrain, promoter-bound MeCP2 upregulates the expression of key regulatory factors that controll emotional and affective states, learning and memory. *In vivo*, phosphorylation of MeCP2-serine 421 decreases promoter binding and gene expression, with corresponding changes in behavioral states. These observations were established by work in the amygdala, hypothalamus and hippocampus of *Mecp2* mutant and wildtype mice (in schematic mouse brain section, amygdala, blue, hippocampus, green, hypothalamus, purple).



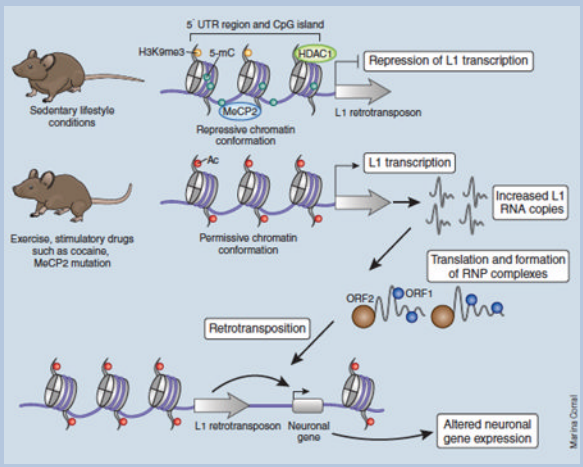
Box 4: Epigenetic control of the brain's 'jumping genes'

Retrotransposons, or 'jumping genes', are commonly defined as mobile elements that can proliferate in the germline of plants and mammals, thus contributing to the potential for massive genome expansion during the course of evolution[145]. Approximately 40–50% of the human genome is derived from mobile elements and, more importantly, several classes of retrotransposons, which duplicate via RNA intermediates and then insert into a different site of the genome, remain active even in present day humans[146]. Some of the best known examples of retrotransposons include LINE1 (L1), Alu and SVA elements[146], and it likely that new insertions by these elements contributes to genome plasticity in individual brain cells[147]. In particular, the hippocampus and striatum are defined by much higher rates of *de novo* retrotransposition compared to other somatic tissues such as blood, heart and liver[147,148]. It has been estimated that each hippocampal cell could harbor up to 800 new insertions, indicating a hitherto unexpected degree of genomic plasticity[148]. Interestingly, both L1 and Alu-based insertion events into human brain genomes disproportionally affect actively expressed genes, including many that are pivotal for synaptic transmission such as those encodingthe dopamine receptor DRD3, the amino acid transporters SLC6A5, SLC6A6 and SLC6A9 and the RAI1 transcription factor that has been previously implicated in schizophrenia and the 17p11.2 (Smith Magenis) deletion syndrome[147]. Therefore, retrotransposon activity could contribute to neuronal diversity[149] and even drug- or experience-dependent plasticity[150,151] (Box Figure).

Insertional mutagenesis could also have potentially detrimental consequences, for example, theaberrant activation or repression of neighboring genes, exon shuffling and gene deletions [149]. Importantly, epigenetic regulators are vital for a cell's ability to suppress retrotransposon activity and L1, Alu and other elements that utilize the L1 replication machinery are epigenetically silenced via repressive DNA methylation and histone modifications[152]. Therefore, when repressive chromatin remodeling at sites of mobile elements is 'exchanged' with transcriptional activation complexes such as when the WNT3a/β-catenin complex binds to the L1 promoter[153,154], excessive amounts of insertional mutagenesis could cause cell damage. For example, loss of the repressive DNA methyl-reader protein MECP2 in neural cultures or in the brains of mice with MECP2 mutation results in a modest excess of L1 activity[36,155] in conjunction with changes in global chromatin states (Box Figure). Increased L1 retrotransposition was also recently detected in cells from people with ataxia telangiectasia, an autosomal recessive neurodegenerative condition caused by mutations of the DNA damage sensor and serine/threonine kinase ATM[78]. Loss of ATM results in increased reverse transcriptase efficiency, thereby fostering new L1 insertions in the genome[78]. It is conceivable that Rett syndrome and ataxia telangiectasia are merely the 'tip of the iceberg' and that numerous other neurological conditions with links to transposon dysregulation may emerge in the near future.

Figure for Box 4: Epigenetic regulation of retrotransposon activity in the nervous system. A schematic presentation of the LINE-1 (L1) retrotransposon is shown, including the CpG island upstream of two open reading frames (ORFs) for transcriptional control. Epigenetic 'brakes' preventing excess L1 transcription in the brain include DNA methylation and repressive chromatin remodeling, involving the actions of the histone deacetylase HDAC1 and the methyl-CpG-binding protein MECP2. Physiological activities are associated with mild relaxation of L1 repression, while stimulant drugs-of-abuse or loss of MECP2 could substantially upregulate the number of L1 RNA copies. The L1 RNAs are then translated, assembled into complexes for active

retrotranspositions, thus resulting in genome toxicity through excess amounts of *de novo* genome integrations



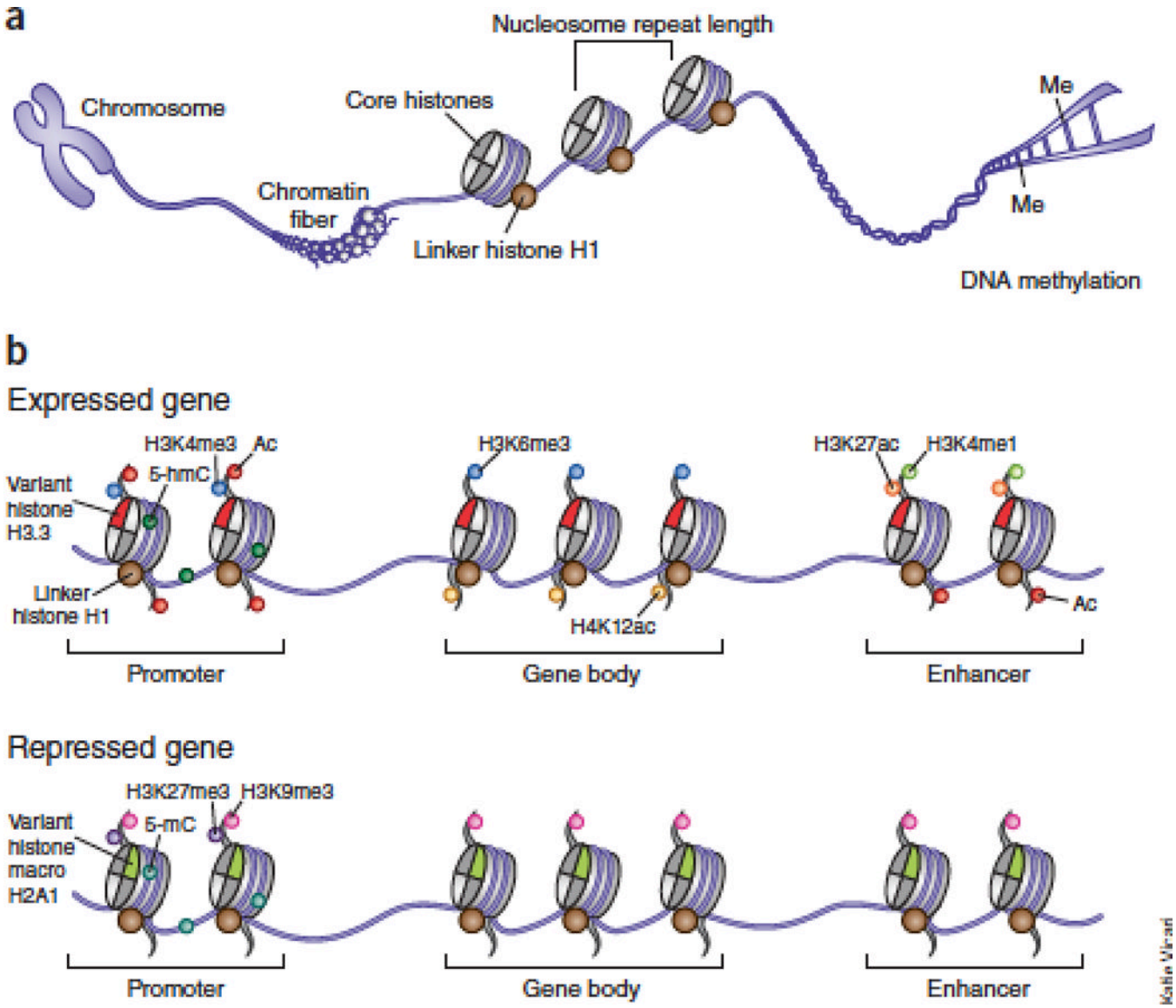


Figure 1. The epigenome and chromatin organization

The top part shows that chromosomes are organized into domains of loose (euchromatin) or highly condensed (heterochromatin) chromatin and other loosely defined higher order structures (such as ‘globules’), some of which are tethered to the nuclear membrane. The bottom part shows 11 nm ‘beads-on-a-string’ chromatin fiber comprised of nucleosomal arrays connected by linker DNA, and linker histones as major regulators of nucleosomal repeat length. The distribution of DNA methylation and a small subset of > 100 posttranslational histone markings, linker histones and core histone variants represents differential regulation at (left) active promoters and gene bodies as opposed to enhancers, or (right) silenced and repressed chromatin, as indicated.

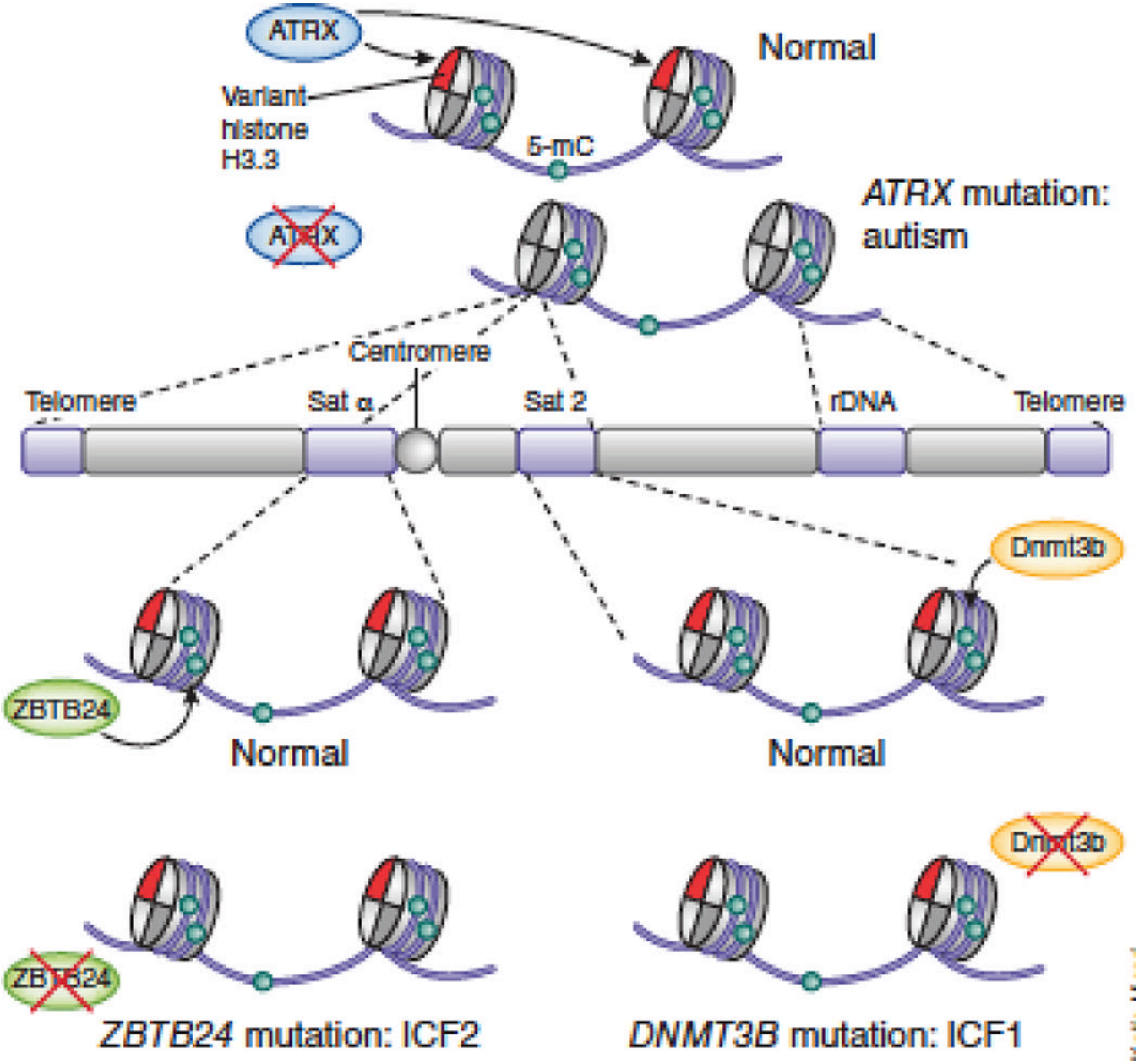


Figure 2. Example monogenetic brain disorders with a heterochromatin defect

Three examples of monogenetic brain disorders associated with defects in heterochromatin are shown. Mutations in genes encoding the histone demethylase DNMT3B (associated with the disorder ICF1) or the transcriptional repressor ZBTB24 (associated with the disorder ICF2) result in hypo(DNA mC5) methylation of different types of pericentric satellite repeats[28,106]. The multifunctional chromatin regulator ATRX controls the incorporation of the variant histone H3.3 not only just into nucleosomes surrounding the transcription start sites of active genes but also at various repeat sequences, as indicated. Loss of function mutations in *ATRX* have been associated with various X-linked mental retardation syndromes.

Table 1

Monogenetic brain disorders associated with DNA methylation and histone modification defects

Gene (OMIN*)	Function	Syndrome(s)	OMIM # (disease)
ATRX (Xq21.1) *300032	Replication-independent nucleosome remodeling and histone H3.3 incorporation	Alpha-thalassaemia, X-linked with mental retardation (ATRX), autism[40]	#301040
CREBBP (16p13.3) *600140 *EP300* (22q13.2) *602700	Transcriptional co-activator, histone acetyl-transferase	Rubinstein-Taybi syndrome (RSTS) 1 and 2[105]	RSTS1/#180849 RSTS2/#613684
DNMT1 (19p13.2) *126375	DNA methyltransferase. Disease mutations are associated with hypomethylated repeats and promoters	Hereditary hensory and autonomic neuropathy type 1 with adult-onset dementia (HSAN1E)[5], autosomal dominant cerebellar ataxia, deafness and narcolepsy (ADCA-DN)[6].	#614116
DNMT3B (20q11.21) *602900	DNA methyltransferase. Disease mutations are associated with hypomethylation of pericentric repeats	Immunodeficiency, centromere instability, facial anomalies (ICF1) mental retardation syndrome[28,106]	#242860
ZBTB24 (6q21) *614064	Transcriptional repressor and regulator of DNA methylation at pericentric repeats	Immunodeficiency, centromere Instability, facial anomalies (ICF2) mental retardation syndrome[28,29]	#614069
KDM5C (Xp11.22)/JARID1C *314690	Histone H3-lysine 4 demethylase	X-linked mental retardation[107], autism[108]	#300534
KMT1D (9q34.3) (also:EHMT1) *607001	Histone H3-lysine 9 methyltransferase	Kleefstra (mental retardation) syndrome[109], schizophrenia[110], non-specific psychiatric phenotypes and neurodegenerative disease in post-adolescence period[111]	#610253
KMT3B (5q35.2-q35.3) (also NSD1) *606681	Histone H3-lysine 36 and H4-lysine 20 methyltransferase	Sotos (mental retardation) syndrome[112]	#117550
PHF8 (Xp11.22) *300560	Histone H3-lysine 9 demethylase and transcriptional activator	X-linked mental retardation withoutcleft lip and/or palate (Siderius-Hamel)[113,114]	#300263
RSK2 (Xp22.12) *300075	Serine/threonine kinase (of both histones and non-histone proteins)	Coffin-Lowry X-linked mental retardation syndrome[115]	#303600
MECP2 (Xq28) *300005	Methyl CpG binding protein	Rett and other neurodevelopmental syndromes, autism[116]	#312750

Table 2

Chromatin modifying drugs as potential therapies for neurological disease

Drug type and mechanism	Representative compound(s)	CNS effects in preclinical models	FDA approval for non-neurological condition
Sequesters DNMT at DNA replication fork	5-aza-cytidine, zebularine (nucleoside analogues)	Disrupted hippocampal learning and also reward behavior[87–90] in wild-type mice and rats. Conferred stroke protection in mice [93,94]	Yes
DNMT active site inhibitor	RG108	Prevented cell death in a mouse model of motor neuron disease model [51]. Impaired hippocampal learning[92] in rats.	
Class I/II HDAC inhibitor	phenylbutyrate, SAHA, TSA	Had a broad neuroprotective profile in acute and chronic injury and neurodegeneration models, including ischemia, Huntington's and other polyglutamine andtriplet diseases, and a Parkinson's disease model (MPTP toxicity)[8–11] Improved hippocampus-dependent learning and cognition and exerted antidepressant-like effects [62,63]	Yes
Class III HDAC inhibitor	nicotinamide AGK2	Reduced amounts of phospho-tau and improved cognition in hippocampal learning tasks in a mouse model of Alzheimer's disease[73] Reduced α-synuclein toxicity and dopaminergic cell death in a *Drosophila* model of Parkinson's disease [74]	
Histone methyltransferase Glp/ G9a inhibitor	BIX-01294	Enhanced reward behavior in mice after exposure to a stimulant drug (cocaine)[85]	
p300 histone acetyl transferase inhibitor	C646	Reduced amounts of tau l and neurotoxicity in cultured rat neurons and cells from people with Alzheimer's disease[71].	
Topoisomerase I inhibitor	Topotecan, irinotecan	Reversed imprinting-mediated silencing of *Ube3a*(the Angelman's syndrome gene) in mouse brain [96]	Yes
Topoisomerase II inhibitor	Etoposide, dexrazoxane	Reversed imprinting-mediated silencing of *Ube3a* in mouse brain[96]	Yes