Neuropsychopharmacology REVIEWS (2013) 38, 3–22
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Histone Regulation in the CNS: Basic Principles of Epigenetic Plasticity

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Postmitotic neurons are subject to a vast array of environmental influences that require the nuclear integration of intracellular signaling events to promote a wide variety of neuroplastic states associated with synaptic function, circuit formation, and behavioral memory. Over the last decade, much attention has been paid to the roles of transcription and chromatin regulation in guiding fundamental aspects of neuronal function. A great deal of this work has centered on neurodevelopmental and adulthood plasticity, with increased focus in the areas of neuropharmacology and molecular psychiatry. Here, we attempt to provide a broad overview of chromatin regulation, as it relates to central nervous system (CNS) function, with specific emphasis on the modes of histone posttranslational modifications, chromatin remodeling, and histone variant exchange. Understanding the functions of chromatin in the context of the CNS will aid in the future development of pharmacological therapeutics aimed at alleviating devastating neurological disorders.

Neuropsychopharmacology Reviews (2013) **38,** 3–22; doi:10.1038/npp.2012.124; published online 25 July 2012

Keywords: histone PTMs; chromatin remodeling; histone variants; neuronal plasticity

INTRODUCTION

Transcriptional Control of Plasticity in the Central Nervous System (CNS)

Neuroplasticity is a multifaceted and dynamic process involving gene–environment interactions that result in both short- and long-term changes in gene expression, cellular function, circuit formation, neuronal morphology, and behavior. Elevated plasticity marks the early stages of mammalian neurodevelopment as the brain rapidly increases in size with the expansion of neuronal subtypes. Following terminal differentiation, neurons undergo periods of axonal and dendritic branching that allow for fine-tuning of synaptic contacts and the generation of elaborate circuits, many of which can persist throughout the lifetime of an individual. As the brain matures, such connections remain malleable permitting alterations in the synaptic strength of specific circuits required for various forms of experience-dependent plasticity. Throughout adulthood, environmental stimuli are continuously encoded at the level of the synapse in a process unparalleled in other tissues.

A substantial body of literature indicates that environmental stimuli experienced during early stages of neurodevelopment result in altered patterns of transcription in the brain that are essential for the establishment and maintenance of synaptic connections (Greer and Greenberg, 2008). The mammalian brain depends on numerous complex and highly regulated mechanisms to appropriately activate or silence gene programs in response to environmental input and developmental cues. At the molecular level, these events are controlled by activity-dependent signaling pathways that mediate gene expression by modifying the activity, localization, and/or expression of transcriptional-regulatory enzymes in combination with alterations in chromatin structure in the nucleus (McClung and Nestler, 2008). A large body of literature indicates that alterations in chromatin state and transcriptional programs in adult neurons are important for mediating various aspects of experience-dependent plasticity, such as learning and memory, stress responsivity, and cognition.

Chromatin Overview

Chromatin, the intimate association of genomic DNA with histone proteins, is the physiological form of our genome and the substrate for processes that regulate cellular gene expression. The fundamental repeating unit of chromatin is the nucleosome, which consists of approximately 147 bp of superhelical DNA wrapped around the radial surface of an octamer of highly conserved core histone proteins (two copies each of H2A, H2B, H3, and H4). Epigenetic

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Received 27 February 2012; revised 7 May 2012; accepted 8 May 2012

mechanisms mediate distinct cellular gene expression profiles *in vivo* without directly affecting DNA sequence, thereby influencing transcription with far-reaching implications for human biology, health, and disease (Egger *et al*, 2004; Feinberg, 2007; Borrelli *et al*, 2008). As disruptions in chromatin are increasingly linked to human cancer, the next frontier of epigenetics, as well as human health, is to identify the role that epigenetic mechanisms might have in the potentiation of non-neoplastic diseases, including neuronal disorders.

Epigenetic mechanisms regulate normal brain function, and histone modifications, along with other forms of chromatin remodeling, have been linked to neural plasticity and multiple forms of behavioral memory (Levenson and Sweatt, 2005; Borrelli *et al*, 2008; Dulac, 2010; Nestler, 2011). Therefore, it is not surprising that several neurological disorders involve mutations in genes that encode chromatin-binding and/or -modifying enzymes. Examples include mutations in the gene encoding the chromatin-remodeling factor, ATRX, that causes α-thalassemia and X-linked mental retardation syndrome, as well as in genes encoding the histone H3K4me3-demethylase, JARID1C, that causes epilepsy X-linked mental retardation, the histone H3K9me1/2-methyltransferase complex, G9a/GLP (EHMT2/1), that results in a human mental retardation syndrome, and the histone acetyltransferase, CREB-binding protein, that causes Rubinstein-Taybi syndrome (Gibbons *et al*, 1995; Alarcon *et al*, 2004; Kleefstra *et al*, 2006; Tahiliani *et al*, 2007; Schaefer *et al*, 2009). A common theme of these disorders is that mutations in epigenetic regulators can alter chromatin structure and induce a broad spectrum of neurological and behavioral deficits. Furthermore, environmentally induced alterations in chromatin structure in the absence of mutations have been shown to be critically important for a variety of neuronal functions, including those related to synaptic activity, cognition, and reward (Day and Sweatt, 2011; Robison and Nestler, 2011; West and Greenberg, 2011). In this review, we will attempt to describe the general principals of chromatin regulation as they relate to histone biology (ie, chemical modifications, chromatin remodeling, and histone variant exchange), with specific emphasis on the role that such processes may have in guiding various aspects of neural plasticity (Figure 1, left panel).

HISTONE MODIFICATIONS

Basic Principles of a Combinatorial Code

Histone proteins are subject to a diverse array of covalent modifications that occur primarily, but not exclusively, at amino (N-) and carboxy (C-) termini (ie, tails). The tail regions of core histones contain flexible and highly basic amino-acid sequences that are generally conserved across eukaryotic organisms, and it is well established that histone tails act as substrates for several types of posttranslational modifications, including acetylation, methylation, ADP-ribosylation, ubiquitylation, and phosphorylation (Figure 1, right panel). Such modifications have long been correlated with various nuclear functions including replication, chromatin assembly, and transcription.

Our laboratory, along with others, originally articulated the concept that distinct patterns of histone modifications act in concert with DNA methylation, noncoding RNAs (ncRNAs), and transcription factors to generate 'histone-epigenetic codes' that are read by effector proteins (ie, regulatory molecules that bind to unmodified or modified histones, as well as specific DNA-binding complexes, to modulate chromatin function and transcriptional outputs) to elicit specific downstream transcriptional events (Strahl and Allis, 2000; Turner, 2000; Jenuwein and Allis, 2001; Jaenisch and Bird, 2003; Spitale *et al*, 2011). In particular, histone modifications have been demonstrated to function in a combinatorial manner, thereby increasing their indexing potentials and capacities for information storage. Roles for histone posttranslational modifications in the regulation of chromatin structure have now been extensively studied, and many groups have clearly demonstrated the validity of mapping these modifications across mammalian epigenomes. Studies of histone modifications have led to the discovery of a large number of 'marks' (ie, histone modifications), 'writers' (eg, enzymes such as kinases, histone acetyltransferases (HATs), and histone methyltransferases (HMTs) that modify specific substrates by adding phosphate, acetyl, or methyl groups, respectively), 'erasers' (enzymes such as histone deacetylases (HDACs) and histone demethylases (HDMs) that catalyze the removal of specific histone modifications), and 'readers' (these include a large number of regulatory proteins that contain unique domains that recognize acetyl, methyl, phosphate groups, *etc*), some of which have been linked to the aspects of neural plasticity and behavioral adaptation (Borrelli *et al*, 2008). Over the last decade, these concepts have gained increased attention in the field of neuroscience, as researchers attempt to identify how molecular signaling cascades encode phenotypic outputs in the nucleus to form long-lasting environmentally influenced neural circuits.

'Active' *vs* 'Repressive' Histone Modifications

Considerable research points to the critical involvement of histone modifications in transcriptional output. Among histone modifications, acetylation is by far the most extensively studied, including in the nervous system, and has been shown to directly modulate gene transcription (Brownell *et al*, 1996; Kundu *et al*, 2000). Histone methylation has also received significant attention with respect to its indexing potential, as it can exist in multiple valence states (eg, mono- (me1), di- (me2), and trimethylated (me3) forms) and exhibits slow turnover kinetics under normal cellular conditions, potentially allowing for the long-term propagation of information throughout the lifetime of an animal (ie, 'epigenetic memory'), or even to their progeny (see Rice and Allis (2001) for a review of general differences between histone acetylation and methylation). Although

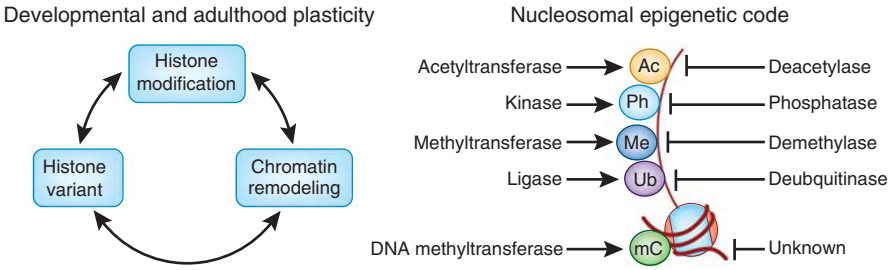

Figure 1. Neuronal plasticity involves three major mechanisms of chromatin regulation (histone posttranslational modifications, histone variant exchange, and nucleosomal remodeling) (left panel). Nucleosomes are subject to a diverse array of covalent modifications including, but not limited to, acetylation, phosphorylation, methylation, ubiquitylation, as well as direct methylation of DNA (right panel). Note that although a bona fide DNA demethylase has yet to be described, the growth arrest and DNA damage enzyme, Gadd45, as well as oxidation reactions of 5-methylcytosine to 5-hydroxymethylcytosine, have been implicated in DNA demethylase-like activities in cells.

evidence exists to support such propagation events throughout periods of cell division (ie, during mitosis), it is difficult to imagine how histone-based mechanisms could contribute to transgenerational inheritance of acquired parental characteristics, especially as most behavioral traits are exclusively neuronally encoded (see Future Outlooks: *Histone Regulation as a Means of Generating Heritable Plasticity*).

Acetylation at several lysine residues throughout the N-terminal tails of core histone proteins is generally associated with gene activation and is regarded as an epigenetic mark associated with dynamic chromatin. Acetylation acts *in cis* to effectively neutralize the positive charge of histone proteins, thereby decreasing the electrostatic affinity between histone tails and negatively charged DNA (Allis *et al*, 2007). These events ultimately function to relax chromatin structure allowing for the recruitment of various effector proteins, transcriptional coactivators (some of which contain specific acetyl–lysine recognition motifs, referred to as bromodomains), and members of the general transcriptional machinery. The enzymatic systems responsible for the addition and removal of acetyl groups have been fully characterized and consist of multiple classes of relatively promiscuous HATs, which catalyze the direct transfer of acetyl groups to histone lysine residues, and HDACs that function to remove these modifications.

Methylation of lysine and arginine residues, some of which overlap with sites of histone acetylation, can be associated with either gene activation or repression depending on the residues being modified (see Kouzarides, 2007). For example, methylation of lysine 4 or 36 on H3 is associated with transcriptional initiation/pausing and transcriptional elongation, respectively, whereas methylation of lysine 9 or 27 on H3 is more strongly correlated with transcriptional repression/silencing. As previously alluded to, categorizing methylation states as active *vs* repressive is further complicated because multiple methylation valences are possible, with each state being controlled by distinct 'writers,' 'erasers,' and 'readers.' For example, methylation of H3K9 occurs in a seemingly non-processive manner, with the euchromatic heteromeric G9a/GLP HMT complex contributing to H3K9me1 and H3K9me2, and the heterochromatic HMT, Suv39H1, catalyzing H3K9me3. These different valence states are similarly demethylated by distinct HDMs, mainly consisting of Jumonji C (JmjC) domain-containing enzymes (eg, Jmjd2a) and are 'read' by specific effector proteins that determine transcriptional and physiological outputs (Shinkai and Tachibana, 2011). Similar to that of acetylation, the enzymes responsible for adding methyl groups to histone tails (HMTs) have been extensively characterized. Interestingly, histone methylation was once thought to represent a stable chromatin 'mark' that might act to control chromatin structure and the potentially related patterns of gene expression, indefinitely; however, much data now exists to refute this assumption, as numerous site and valence state-specific HDMs have been discovered (Tsukada *et al*, 2006).

Both histone acetylation and methylation, occurring at multiple sites throughout different histone proteins, have been the focus of an ever-growing number of studies in the field of neuroscience and appear to be especially relevant to investigations of neuropsychiatric disease (many of which will likely be discussed in subsequent reviews). It is important to consider, however, that many studies have attempted to examine the functions of histone marks in isolation, without regard to the multivalent nucleosomal landscape of the cells being examined. Such approaches require caution, as it is the combination of intra- and internucleosomal modifications that likely dictate functional outputs of chromatin. For example, a number of chromatin-associated proteins contain multiple 'reader' domain-binding modules that are able to engage in multivalent interactions with numerous histone marks simultaneously (Ruthenburg *et al*, 2011). Furthermore, specific histone modifications are known to directly influence the deposition of others, as observed with H2B ubiquitination and H3K4me3 (Sun and Allis, 2002). Such interactions likely promote distinct functional outputs that cannot be predicted by a focused analysis of a single modification in isolation. Therefore, in the future, it will be necessary to employ more modern proteomic techniques (eg, large scale mass spectrometry analysis of histone modifications) to better address this issue of stimulus-driven encoding of combinatorial histone marks in neurons. Given the vast amount of literature currently available pertaining to the

regulation of histone acetylation and methylation in the nervous system, we will now focus our attention on histone phosphorylation, a modification that is also likely sensitive to patterns of activity-dependent nuclear signaling and yet has received limited attention from the neuroscience community. Although not further discussed here, recent findings linking alterations in histone acetylation and methylation, as well as associated chromatin modifiers, to synaptic function, learning and memory, and psychiatric disease should inspire future studies in the field of neuroplasticity, as roles for chromatin regulation in these processes are becoming increasingly clear (Schaefer *et al*, 2009; Gupta *et al*, 2010; Maze and Nestler, 2011; Peter and Akbarian, 2011).

Histone Phosphorylation and Cross Talk Interactions in the CNS

Much less is known concerning the function of histone phosphorylation in mammalian cells. Despite its long-standing history (Stevely and Stocken, 1966; Gutierrez and Hnilica, 1967) and the presence of numerous serine, threonine, and tyrosine residues speckled throughout the primary sequences of histone proteins. Early work in this area focused primarily on the phosphorylation of linker histones (eg, H1, Sweet and Allis, 1993) and of the core histone H3 (eg, H3S10 phosphorylation (H3S10p)) as universal markers of mitotic and meiotic chromatin condensation (Wei *et al*, 1998, 1999). More recently, studies have begun to examine the role of phosphorylation on other core histone proteins, such as H2B, which was only recently demonstrated to function during apoptotic chromatin compaction (Ajiro, 2000; Cheung *et al*, 2003). To date, genome-wide analyses of histone phosphorylation marks have not been reported, making it difficult to fully appreciate the actions of these modifications *in vivo*.

Although H3S10p was first characterized as a mitotic signature in proliferating cells (potentially indicating a repressive function, as gene expression is significantly reduced during cell division), it is now known to exist at gene promoters in postmitotic cells and has been linked to transcriptional activation. Specifically, stimulation of growth factors and neurotransmitter-mediated kinase pathways induce rapid and transient phosphorylation of H3S10 at promoters and throughout coding regions of immediate-early genes (IEGs, Thomson *et al*, 1999; Clayton *et al*, 2000; Crosio *et al*, 2003). In cultured striatal neurons, for example, cAMP or glutamate stimulations have been shown to increase H3S10p enrichment at the *c-fos* promoter (Li *et al*, 2004; Brami-Cherrier *et al*, 2007). In the adult brain, neuronal stimulation resulting in the activated release of neurotransmitters, such as dopamine, acetylcholine, and glutamate, induce H3S10p in the dentate gyrus of the mouse hippocampus (Crosio *et al*, 2003). Furthermore, numerous other environmental stimuli have been found to induce H3S10p in brain, including administration of drugs of abuse (Brami-Cherrier *et al*, 2005) and kainic acid-induced seizures (Sng *et al*, 2006). At IEG promoters, H3S10p is often associated with acetylation at H3K14 (Lo *et al*, 2000), a modification that has been shown to be regulated by a variety of stimuli in the adult brain (eg, social stress, Covington *et al*, 2009) and is coupled to transcriptional activation. Combinations of specific modifications functioning together to activate gene transcription suggest the existence of common protein binders and/or concerted protein complexes that recognize multivalent histone marks.

Numerous nuclear-localized protein phosphatases and kinases have been shown to regulate histone phosphorylation across various cell types and tissues. For example, Aurora kinases A and B have been demonstrated to catalyze the addition of phosphate groups at H3S10, whereas mitogen-activated protein (MAP) kinase phosphatase-1 has previously been shown to promote the enzymatic removal of phosphate groups at this residue (Pascreau *et al*, 2003; Kinney *et al*, 2009). In brain, the dopamine and cAMP regulated protein phosphatase inhibitor, DARPP-32, as well as the MAP kinase, MSK1, provide excellent examples of enzymes regulating H3S10p in response to environmental stimuli, indicating histone phosphorylation as an important regulator of adult neuronal function (Brami-Cherrier *et al*, 2005; Stipanovich *et al*, 2008).

Similar to H3S10p, H3S28 phosphorylation has been implicated in mitotic regulation and IEG activation, with recent data indicating a direct relationship between H3S28p and transcription. Specifically, increased H3S28p, in the absence of H3S10p, was found to be sufficient to trigger the transcriptional activity of a subset of IEGs and does not appear to engage in multivalency with H3S10p within the same nucleosome (Dunn and Davie, 2005). H3S28p is believed to disrupt polycomb-mediated silencing by reducing H3K27me3, therefore allowing for the increased enrichment of H3K27ac at gene promoters and the subsequent upregulation of gene expression (Lau and Cheung, 2011). To date, this phosphomodification, along with numerous others (eg, H3T3p, H3T6p, H3T11p, H3Y41p, *etc*), is yet to be investigated in the nervous system. Given that neurons are highly responsive to environmental cues and are capable of rapidly responding to cellular activity through the initiation of numerous phospho-dependent signaling cascades, it is likely that histone phosphorylation states will have important roles in the integration of receptor-mediated signaling and influence transcriptional outputs necessary for proper neuronal function, either by *cis*- or *trans*-acting mechanisms (see below).

Histone Readers

Another important issue in chromatin biology and epigenetics relates to the question of how patterns of histone modifications, such as phosphorylation and acetylation, are ultimately translated into meaningful biological phenomena, both at the levels of transcription and overall chromatin structure. The identification of histone 'reader' proteins that specifically recognize and bind histone

modifications via specific histone mark-recognition motifs has greatly contributed to our mechanistic understanding of chromatin biology. For example, as H3S10p appears to be involved in both mitotic chromosomal condensation and transcriptional activation, processes that are seemingly unrelated, it stands to reason that each process might require distinct chromatin 'readers' to appropriately execute these independent functions (ie, scenarios of broad chromatin compaction *vs* localized decompaction, respectively). Although the specific H3S10p readers functioning during periods of mitotic condensation have yet to be identified, it is possible that an increase in the genomic prevalence of this mark during mitosis functions to promote a binary methyl-phospho switch, which leads to the loss of heterochromatic protein 1 (HP1, an H3K9me3 reader) (Bannister *et al*, 2001; Lachner *et al*, 2001) binding to allow for proper chromosomal segregation during cell division. On the other hand, during situations of transcriptional activation, specific isoforms of the chaperone 14-3-3 have been demonstrated to directly bind H3S10p and this interaction has often been shown to increase in strength with the addition of a neighboring acetyl group at K14 (Macdonald *et al*, 2005). This specific phospho-acetyl combination, as well as its recognition by 14-3-3, appears to be necessary for the initiation of transcriptional elongation (Karam *et al*, 2010). These data further illustrate the importance of synergistic readings of distinct combinations of histone modifications, and indicate the need for future research aimed at identifying these interactions *in vivo* in postreplicative neurons, which likely have evolved novel mechanisms to facilitate these chromatin effector functions to meet the demands of a non-regenerative and highly plastic cellular environment.

CHROMATIN REMODELING

Basic Properties

One of the most unique properties of mammalian cells is their ability to package and sufficiently organize large amounts of DNA (~1.7 m) into extraordinarily compact nuclei (~5 m in diameter), thereby allowing for stable patterns of replication and transcription, which can vary greatly from tissue to tissue. Along with posttranslational modifications of histones (described above), ATP-dependent chromatin remodeling appears to be essential for both the establishment and dissolution of appropriate patterns of chromatin structural organization throughout the nucleus (Ho and Crabtree, 2010). It has been recognized for many years that nucleosomes are organized as regularly spaced, nonrandom repeating arrays, with patterns of nucleosomal spacing and occupancy varying significantly between different cell types and across organisms (Van Holde, 1989). Appropriate nucleosomal positioning and spacing patterns, as well as the ability of the cell to establish proper modes of nuclear compartmentalization and to coordinate 'long-range' intrachromosomal interactions, are essential to all aspects of nuclear function (see Sadeh and Allis (2011) for a review of nucleosome positioning/occupancy).

Families of ATP-Dependent Chromatin-Remodeling Proteins

A large number of studies have suggested that during the transition from unicellular eukaryotes to vertebrate organisms, ATP-dependent chromatin-remodeling proteins/complexes evolved to meet the demands of a dramatically altered, and seemingly more complex, chromatin landscape. These evolutionary processes have resulted in an increased number of genes (~30) encoding these remodeling subunits (although the increased numbers of ATPase gene products likely do not explain the full extent of the complexity observed with vertebrate remodeling complexes) and the use of combinatorial assembly, which together allow for the existence of hundreds of remodeling complexes in higher order eukaryotes. Specifically, SWI-like ATP-dependent chromatin-remodeling complexes can be categorically divided into four major subfamilies based on the structure and sequence of the ATPase subunits contained within: SWI/SNF, CHD, ISWI, and INO80 complexes. Each subfamily consists of at least one to six similar ATPases, many of which have been shown to remodel nucleosomes, transfer histone octamers in *trans*, and generate superhelical torsion in DNA. The assembly of ATPases into large multiprotein complexes further increases the number of specific chromatin-remodeling activities in the cell, where the same ATPase can be shared within different remodeling complexes.

Although it is true that chromatin-remodeling complexes have a vital role in guiding aspects of transcriptional regulation, it seems that these ATPase subunits do not behave in a consistent manner, making it difficult to classify these proteins simply as transcriptional activators or repressors. For example, Brahma-associated factor (BAF) complexes, which belong to the SWI/SNF family of ATP-dependent remodeling proteins, have been demonstrated to function as both repressors and activators of transcription and have further been shown to alternate these functions at a single locus (Chi, 2003). These data suggest that the relationship between chromatin-remodeling activity and transcription may be more complex than originally conceived and allow dynamic patterns of regulation through differential recruitment of effector proteins, transcription factors, and components of the general transcriptional machinery. Accordingly, BAF complexes have been demonstrated to directly interact with numerous transcription factors, oftentimes in a tissue-specific manner, thereby allowing complex subunits to acquire context-dependent, and cell type-specific, functions that are ultimately dictated by their specific interacting proteins (see Trotter and Archer (2008) for a review). Such findings indicate that although these remodeling complexes function globally in their regulation of chromatin, they may also have key roles in the precise regulation of gene programs necessary to establish distinct cellular functions. With respect to CNS

development, cellular functions of BAF complexes will be described in further detail throughout this review. In addition, similar to SWI/SNF, other remodeling complexes are also necessary for many aspects of gene expression and cellular function such that ISWI remodeling complexes are important for establishing and maintaining higher order X-chromosomal structural integrity (Deuring *et al*, 2000), and the INO80 family of proteins appear to be necessary for telomere function and DNA replication during mitosis (see Morrison and Shen (2009) for a review).

Impact of Chromatin Remodeling on Neural Development

Recently, with respect to the development and function of the CNS, it has been argued that no other tissue within the mammalian system rivals the ability of the brain to diversify transcriptional programs to allow for such complex patterns of postmitotic gene regulation (Ho and Crabtree, 2010). These transcriptional profiles are, in turn, functionally related to the aspects of neuronal cellular identity, synapse development, circuit formation, and ultimately behavior; therefore, it is not surprising that a large number of neural-related phenotypes are beginning to emerge from genetic studies of chromatin-remodeling proteins (see Yoo and Crabtree (2009) for a review). Numerous studies now have reported the BAF family of remodeling complexes, which are based on the Brm and Brg1 core ATPases, as being essential for the development of the nervous system.

In mammals, BAF complexes are thought to be polymorphic, in that distinct subunit members, each of which are encoded by homologous gene families, assume mutually exclusive occupancy in the complex (eg, all mammalian BAF complexes contain a BAF45 subunit; however, this subunit is encoded as four distinct BAF45 isoforms (A, B, C, and D), where only one isoform can be incorporated into a single multimeric BAF complex at any given time), with the core ATPase subunit encoded by Brg1 and Brm in vertebrates (Wang *et al*, 1996a, 1996b; Olave *et al*, 2002). Distinct BAF subunits are encoded by varying number of genes allowing for numerous patterns of complex assembly; however, the core constituents of mammalian BAF complexes remain consistent. Although Brm inactivation was not shown to result in any obvious neural phenotypes (Reyes *et al*, 1998), Brg1 did appear to be necessary for numerous aspects of neurodevelopment. In fact, mice lacking Brg1 display pre-implantation or peri-implantation lethality, most likely due to deficits in neural tube formation based on studies performed in heterozygous mutants (Bultman *et al*, 2000; Lessard *et al*, 2007). Furthermore, mutations in BAF155, a subunit that has been proposed to shield BAF complexes from proteosomal degradation and promote the nuclear localization of the complex, similarly result in peri-implantation lethality and neural tube defects (Kim *et al*, 2001; Sohn *et al*, 2007). Unlike Brg1 inactivation in *Xenopus*, which promotes the expansion of neural progenitors (Seo *et al*, 2005), Brg1 inactivation in vertebrates was shown to interfere with neural progenitor self-renewal and results in the eventual loss of neural progenitor populations (Matsumoto *et al*, 2006; Lessard *et al*, 2007). Therefore, it can be inferred that BAF complexes function during early neurodevelopment to promote maintenance and differentiation of neural progenitors, thereby allowing for proper neural tube formation. To date, genome-wide mapping studies of BAF complex subunits in neurons have not been reported, so it remains unclear as to the exact role that BAF complexes might have in guiding chromatin-regulated transcriptional programs necessary for early neurodevelopmental processes; however, studies performed in embryonic stem (ES) cells indicate that thousands ($\sim$10 000) of BAF-binding sites exist throughout the mammalian genome, and that nearly half of these sites localize to transcriptional start sites (Ho *et al*, 2009). Such patterns of BAF complex enrichment in ES cells indicate that chromatin-remodeling complexes may indeed be critical for aspects of developmental transcriptional plasticity, yet the exact roles of these genome-wide chromatin associations remain unclear.

As alluded to earlier, in vertebrates, chromatin-remodeling complexes undergo a process of combinatorial assembly, whereby distinct subunit compositions are observed between different cell types, allowing for a variety of unique protein–protein interactions between the remodeling complex and associated binders (Ho and Crabtree, 2010). These unique interactions seem to be tailored to fit the needs of specific cell types, and data indicate that such patterns of combinatorial assembly are important to neurodevelopmental processes. For example, BAF family complex members Brg1, BAF57, and BAF170 have been shown to associate as a larger complex with the neural restrictive silencing factor (NRSF/REST) and its co-repressors (eg, Sin3A, CoREST, and MeCP2) in non-neuronal cells (Ooi *et al*, 2006). NRSF/REST expression is restricted to non-neuronal cells, and as a zinc finger domain containing transcription factor, binds to target motifs (RE1) and represses transcription of neuronal specific genes through association with its co-repressors (Chong *et al*, 1995; Chen *et al*, 1998; Lunyak *et al*, 2002; Ballas *et al*, 2005). Recent evidence demonstrates that the repressive activity of NRSF/REST requires a functional bromodomain interaction with Brg1, whereby Brg1 is recruited to RE1 sites to synergistically control the expression of neuronal gene programs in non-neuronal cells (Ooi *et al*, 2006). The existence of a functional BAF complex seems to be required for NRSF/REST-mediated repression, as inactivation of Brg1 results in the unsilencing of neuronal specific genes in non-neuronal tissues. Furthermore, these events appear to concomitantly enhance histone H4 deacetylation at RE1 target loci (Watanabe *et al*, 2006), indicating a mechanistic link between BAF-mediated chromatin remodeling, histone posttranslational modifications, transcription factor recruitment, and gene regulation.

One can imagine a situation in neuronal cells where alterations in complex composition could result in drastically

different protein–protein interactions, thereby allowing for the activation/repression of distinct transcriptional programs required for the maintenance of basal and activity-dependent neuronal function (see Yoo and Crabtree (2009) for a review). In line with this reasoning, landmark discoveries from the laboratory of Gerald Crabtree demonstrated that BAF complexes undergo a process of subunit switching during neurodevelopment, which appears to be necessary for the establishment of postmitotic cellular identity. In this work, it was discovered that during neuronal differentiation, two subunits of the neural progenitor BAF complex (npBAF), BAF45a and BAF53a, were diminished in their expression and were replaced by BAF45b and BAF53b (nBAF) specifically in postmitotic neurons (Lessard *et al*, 2007). Whereas the npBAF complex is functionally specialized to promote the self-renewal properties of neural stem cells, the nBAF complex seems to be dedicated to functions of fully differentiated neurons. Knockout of the nBAF complex subunit BAF53b was demonstrated to promote deficits in activity-dependent dendritic outgrowth in neurons, a phenotype that could not be rescued by add back/rescue experiments with BAF53a (Wu *et al*, 2007). These data suggest that neurodevelopmental patterns of combinatorial assembly are required to maintain specific biological functions in the nervous system, and that these events do not simply represent a situation of molecular redundancy. It should be noted, however, that it remains unclear how these complexes differ at the level of chromatin regulation (ie, where they associate throughout the genome, what they bind to/recruit, *etc*), thereby making it difficult to assess the direct *vs* indirect consequences of disrupting these modes of combinatorial assembly in the developing nervous system. More work is needed to mechanistically link the molecular activity of these distinct subunits to the phenotypic outcomes associated with altered neuronal function.

ATP-Dependent Chromatin Remodeling: Relevance to Neurological Disease

Although a wealth of data indicate that chromatin-remodeling activities are integral to the proper development of the nervous system, the potential impact of such activities in adult brain remain unclear. Current findings suggest that specific chromatin-remodeling enzymes/subunits have important roles in the regulation of activity-dependent transcription in the nervous system, a process important for numerous aspects of synapse development, both developmentally and in adulthood.

Genetic association studies attempting to identify putative risk alleles for schizophrenia and trisomy 21 (Down syndrome) have recently identified single-nucleotide polymorphisms (SNPs) in, or near, coding regions for specific chromatin-remodeling complex subunits. Such studies aimed at associating particular genes with neurodevelopmental disorders require identification of mutations that do not result in spontaneous abortion or other severe developmental perturbations unrelated to brain development and physiology. Specifically, SNPs in the coding region of *SMARCA2*, the gene that encodes BRM in the SWI/SNF-remodeling complex, were identified in association with schizophrenia, whereby risk alleles of missense *SMARCA2* polymorphisms resulted in lower nuclear localization efficiencies, and risk alleles of intronic polymorphisms correlated with significantly reduced expression of *SMARCA2* in adult prefrontal cortex (Sengupta *et al*, 2006; Koga *et al*, 2009). Although intriguing, these studies are presented with explicit caveats, including a lack of a genome-wide significant *p*-value for genetic associations, as well as the absence of an observable genetic linkage between *SMARCA2* polymorphisms and schizophrenia in Caucasian individuals. Independent studies aimed at investigating the molecular mechanisms contributing to cognitive deficits characteristic of Down syndrome, have uncovered potential associations with the dual specificity tyrosine-phosphorylation-regulated kinase 1A (DYRK1A), whose gene is localized in the critical region of chromosome 21 affected in Down syndrome patients. In a transgenic mouse model of Down syndrome (152F7 line), it was found that a gene dosage imbalance of *Dyrk1a* resulted in the deregulation of chromosomal gene clusters located in proximity to NRSF/REST target binding sites (Lepagnol-Bestel *et al*, 2009). It was further demonstrated that Dyrk1a, under normal circumstances, binds to the SWI/SNF BAF complex associated with NRSF/REST, and that such associations are necessary for the proper transcriptional maintenance of NRSF/REST target genes. In trisomy 21, however, DYRK1A is dysregulated, thereby leading to increased expression of NRSF/REST, subsequent disturbances in NRSF/REST-SWI/SNF complex assembly, and deficits in dendritic growth/complexity in the adult brain. Taken together, these data, along with findings demonstrating the existence of brain-specific/-restricted chromatin-remodeling proteins (eg, CHD5, a remodeling ATPase known to interact with HDAC2, which has been linked to gene expression patterns associated with aging and Alzheimer's disease) (Potts *et al*, 2011), demonstrate that regulatory control over chromatin-remodeling activities/enzymes in the adult CNS is important to numerous aspects of adult neurological function; however, dissecting the developmental *vs* adulthood contributions of such complexes to neuropsychiatric disease remains difficult.

ATRX: A Critical Regulator of Chromatin State and Histone Dynamics

Another member of the SWI/SNF family of DNA-dependent ATPases is ATRX, a protein encoded by a large X-chromosomal gene spanning greater than 300 kb of genomic DNA (Picketts *et al*, 1996). ATRX was originally identified because of mutations located within its coding sequence that lead to a rare mental retardation syndrome characterized by severe cognitive deficits, as well as other developmentally linked disabilities and α-thalassemia (Gibbons *et al*, 1995).

The ATRX protein consists of a number of conserved domains that are important for its function, including a N-terminal globular domain (Argentaro *et al*, 2007), which is homologous to the DNMT3 family of DNA methyltransferases, known as the ATRX-DNMT3-DNMT3L (ADD) domain (the ADD domain is further composed of subdomains including GATA-1-like and plant homeodomain (PHD) zinc fingers) (Xie *et al*, 1999; Aapola *et al*, 2000). The C-terminus of ATRX contains seven conserved collinear domains conferring its ATPase activity, and other regions are important for mediating its protein–protein interactions and subnuclear localization (Bérubé, 2011) (see Figure 2 for detailed ATRX domain structure). ATRX is generally associated with repressive chromatin and has been shown to directly interact with the polycomb group protein EZH2 (Cardoso *et al*, 1998), the methyl-CpG-binding protein MeCP2 (Nan *et al*, 2007), and the heterochromatin-associated protein HP1alpha (Bérubé *et al*, 2000; Lechner *et al*, 2005). In most cell types, ATRX exclusively resides within the nucleus and tends to localize to highly repetitive sequences throughout the genome (eg, pericentromeric satellite sequences, ribosomal DNA, telomeres, *etc*) (McDowell *et al*, 1999; Law *et al*, 2010). In recent years, we have begun to gain better insight into the specific mechanisms controlling the tethering of ATRX to heterochromatic loci. Specifically, evidence now exists that ATRX might target chromatin directly through GATA-1-like domain interactions with the DNA template (Cardoso *et al*, 2000; Law *et al*, 2010). Alternatively, ATRX might associate with target chromatin through binding interactions with core/canonical and/or variant histone proteins, or through interactions with specific histone posttranslational modifications, either directly, or in combination with its interacting proteins HP1 (Kourmouli *et al*, 2005) or MeCP2 (Nan *et al*, 2007). It was recently demonstrated that the ADD domain of ATRX is able to efficiently bind H3K9me3 (a known heterochromatic mark) specific peptides in the absence of H3K4me3/2, due to an inability of the ATRX PHD finger domain to recognize and bind H3K4me3 (Dhayalan *et al*, 2011; Eustermann *et al*, 2011; Iwase *et al*, 2011). Currently, it is unclear if other adjacent chemical modifications of nucleosomes, such as H3S10p, a mark that is dramatically induced in the adult CNS in response to environmental stimulation, can impact ATRX localization in chromatin; however, given the combinatorial nature of histone modifications *in vivo*, it is likely that other mechanisms of ATRX recruitment/displacement will be identified.

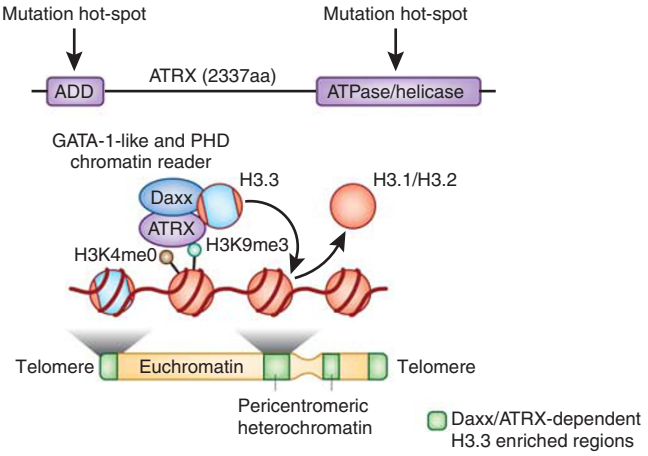

Figure 2. The chromatin remodeler ATRX recognizes histone-modification states and modulates chromatin dynamics *in vivo*. The ATRX protein contains a N-terminal ADD and C-terminal ATPase domain, which function in the regulation of its binding and enzymatic activities, respectively. The ATRX ADD recognizes multiple histone marks, including H3K4me0 and H3K9me3. Daxx, an H3.3 specificity factor, and ATRX complex together as a chaperone system specifically deposit the replication-independent histone variant H3.3 at telomeric and pericentromeric heterochromatic regions of the genome. Note: mutations in ATRX patients map to both the ATRX-DNMT3-DNMT3L (ADD) and catalytic ATPase/helicase domains.

Detailed examinations of the ATRX protein domain structure have also suggested an alternative function for this ATP-dependent remodeling protein at heterochromatin. In addition to its interactions with MeCP2 and HP1, ATRX has also been shown to directly interact with the death-associated domain protein Daxx (Xue *et al*, 2003; Tang *et al*, 2004), which is known to have an important role in apoptotic signaling in a variety of cell types, including neurons (Raoul *et al*, 2002; Junn *et al*, 2005). ATRX and Daxx are known to co-localize to pericentromeric heterochromatin and at promyelocytic leukemia bodies *in vivo* (Xue *et al*, 2003), and ATRX/Daxx complex associations have been demonstrated to exhibit ATPase-dependent mononucleosomal remodeling activity (Tang *et al*, 2004). Nucleosomal dynamics, specifically histone deposition and eviction, are tightly regulated by the actions of specific chaperone proteins and via the enzymatic activity of ATP-dependent remodelers. Recently, Daxx was identified as a chaperone protein for the replication-independent deposition of the histone H3 variant, H3.3, a paralog of the canonical H3 proteins, H3.1 and H3.2 (Drane *et al*, 2010; Lewis *et al*, 2010). Daxx was demonstrated to directly bind to the core AAIG motif of H3.3 (the AAIG motif partly distinguishes H3.3 from H3.1 and H3.2, as the canonical H3 variants contain a SAVM motif at amino acids 87–90), and the ATRX/Daxx complex was shown to be required for the assembly of H3.3-containing nucleosomes, specifically at pericentromeric heterochromatin, rDNA repeats, and telomeres in mouse ES cells and in mouse embryonic fibroblasts (Drane *et al*, 2010; Goldberg *et al*, 2010; Lewis *et al*, 2010; Wong *et al*, 2010) (Figure 2). Although it appears that the formation of functional ATRX/Daxx complexes is necessary for the proper establishment of specialized chromatin domains, the specific role of ATRX in this process remains unclear. ATRX seems to function primarily to maintain heterochromatic stability, in that loss of ATRX expression, at least in the case of mouse ES cells, results in the increased expression of telomeric transcripts, consistent with its role in chromatin silencing (Goldberg *et al*, 2010).

Alterations in the expression and/or activity of ATRX, both in rodents and humans, result in severe developmental

defects, characterized by a number of negative clinical outcomes including psychomotor deficits, cognitive disability, congenital abnormalities, and α-thalassemia (Weatherall *et al*, 1981). Recent studies have also identified ATRX mutations in a number of cancers, including pancreatic neuroendocrine tumors and glioblastoma multiforme, suggesting that ATRX function is critically important to numerous aspect of cellular homeostasis (Heaphy *et al*, 2011; Schwartzentruber *et al*, 2012). Interestingly, Rett syndrome, a neurodevelopmental autism spectrum disorder caused by mutations in the *MECP2* gene (Amir *et al*, 1999), has been indirectly linked to ATRX syndrome, although the direct functional consequences of disrupting ATRX/MeCP2 interactions in brain remain unclear. It seems plausible, however, that these two proteins function synergistically during neurodevelopment to regulate heterochromatic/euchromatic gene expression, as discussed above. Consistent with the notion that disruptions in heterochromatic stability are the result of ATRX dysfunction in ATR-X patients, it was discovered that many of the mutations resulting in disease phenotypes mapped to the ADD and ATPase/helicase domains of ATRX. Such mutations result in reduced expression of the protein and loss of its enzymatic activity (total null mutations in the gene encoding ATRX result in embryonic lethality) (Gibbons *et al*, 1995; Picketts *et al*, 1996). Loss-of-function and gain-of-function studies of ATRX have provided valuable insight into the consequences of ATRX dysfunction during neurodevelopment. For example, mice lacking exon 2 of ATRX, which mimics human patient mutations associated with milder forms of mental retardation, exhibit memory impairments and loss of cognitive functions, as determined through novel object recognition and contextual fear-conditioning tasks (Shioda *et al*, 2011). Such impairments were demonstrated to result from alterations in dendritic spine formation in the prefrontal cortex, an outcome that could be traced back to aberrant increases in the expression of CamKII, and the subsequent promulgation of CamKII-dependent phosphorylation events affecting downstream GTPase signaling in this brain region.

Broad Implications for Environmentally Influenced Chromatin Remodeling in Adult Brain

At this point, it should be apparent that neurodevelopmental disruptions in ATP-dependent remodeling activity, ATPase subunit expression and modes of complex combinatorial assembly often result in severe neurological phenotypes. However, open questions remain concerning the roles of these enzymes in the adult CNS, in the absence of mutations. For example, an expansive body of literature now indicates that a large variety of environmental stimuli (eg, stress, exposure to drugs of abuse, social interactions, sensory learning, *etc*) can promote alterations in the expression and activity of histone-modifying enzymes and the re-distribution of nucleosomal histone modifications throughout the genome. Given that histone-modification states and the subsequent recruitment of effector proteins are known to heavily impact chromatin-remodeling events, it seems plausible that environmentally induced alterations in the nucleosomal landscape of adult animals will result in, or will be the result of, changes in the activity of chromatin-remodeling complexes in brain. These events might occur at the level of expression or through attenuation/potentiation of distinct modes of combinatorial assembly. For example, recent studies exploring rodent models of drug abuse and stress have demonstrated dynamic alterations in the expression of H3K9me3, the heterochromatic mark recognized by the remodeling protein ATRX, throughout the brain (Hunter *et al*, 2009; Maze *et al*, 2011). These changes, although surprising, were later examined using chromatin immunoprecipitation coupled to next generation massively parallel DNA sequencing (ChIP-seq; this technique is used to map global protein/histone modification binding sites throughout the genome) to address the function of these dynamics in the adult CNS. In the case of chronic exposure to drugs of abuse, H3K9me3 was found to be significantly depleted throughout highly repetitive regions of the genome, specifically at loci encoding retrotransposable elements (eg, LINEs, SINEs) and endogenous retroviruses (Maze *et al*, 2011). Previously, it was demonstrated that loss of H3K9me3 enrichment at these loci correlated with increased expression of LINE elements in brain, an event that has recently been suggested to contribute to epigenetic and behavioral heterogeneity in animal populations (Singer *et al*, 2010). Although currently unexamined, it will be interesting to further investigate the impact of dynamic patterns of environmentally regulated H3K9me3 depletion on ATRX function in adult brain.

Given the heterogeneity of cell types throughout the CNS and the level of circuit specificity required to generate complex behaviors, it will also be necessary to further examine patterns of combinatorial assembly in adult animals exposed to a wide variety stimuli. This work will require a more detailed biochemical characterization of associations between chromatin-remodeling proteins, histone-modifying enzymes, and the histone marks, themselves. As remodeling events intrinsically result in dynamic patterns of histone exchange (ie, deposition *vs* eviction), more attention will need to be paid to such events, as recent studies suggest that histone turnover, under non-replicating conditions, can occur very rapidly within cells (ie, on the order of hours) (Deal *et al*, 2010), potentially negating the role for histone-modification dynamics at specific loci. Taken together, appropriate maintenance of neural plasticity likely requires a combination of many of these mechanistic scenarios, and caution needs to be taken in the interpretation of these events in isolation. At this point, we are arguably in the infancy of our understanding of chromatin remodeling in the adult CNS, yet to fully comprehend the contribution of histone regulation to neuropsychiatric phenomena, a more complete understanding of these processes will be required.

HISTONE VARIANTS

Background

Exciting new research indicates that in addition to nucleosomal remodeling and covalent histone modifications, eukaryotic cells generate variation in chromatin structure through the introduction of variant histone proteins. Histone variants (Allis *et al*, 1980) provide a means for introducing primary sequence differences that might function, at least in part, by altering the covalent modification status of these variants independently of canonical histones to expand the regulatory repertoire of chromatin (Rando and Ahmad, 2007; Henikoff, 2008). Distinct, specialized core histone proteins have been shown to differentially reside throughout specific genomic loci, including at centromeres (eg, CENP-A, a variant of H3) (Smith, 2002), within regions of 'active' chromatin (eg, H3.3) (Ahmad and Henikoff, 2002), throughout inactivated X chromosomes in females (eg, macroH2A, a variant of H2A) (Costanzi and Pehrson, 1998), and at damage foci in chromatin following DNA double-stranded breaks (eg, γ-H2A.X) (Redon *et al*, 2002).

The majority of highly conserved canonical core histones (H3, H4, H2A, and H2B) are transcribed and translated in a replication/cell cycle-dependent manner in dividing metazoan cells. Many of these histone proteins are encoded from multi-copy, intronless gene clusters and are deposited in chromatin in a replication-coupled manner to allow for appropriate packaging of genomic DNA (Albig and Doenecke, 1997). All core histone proteins in mammals, with the exception of H4, have numerous sequence variants, and although much research has focused on the role of histone posttranslational modifications in the process of epigenetic inheritance (eg, genetic imprinting, where one of the two parental (maternal or paternal) alleles for a given gene is silenced in the offspring), it is becoming clear that incorporation of histone variants may provide an alternative means to encode and transmit information from one generation to the next (see Banaszynski *et al* (2010) for a review). Histone variants often contain minor sequence variations (eg, H3.1 *vs* H3.2 *vs* H3.3) or exhibit significantly dissimilar structures (eg, macroH2A, CENP-A) from their canonical counterparts (Rogakou and Sekeri-Pataryas, 1999). Furthermore, histone variants have been suggested to exhibit cell type-specific expression patterns, which, given the heterogeneous nature of the brain, may prove to be important for their respective functions in the nervous system.

It appears that metazoan cells have evolved histone variants, some of which are highly conserved, to accommodate the dynamic structure of the chromatin template, which is continuously subjected to factors that assemble, disrupt, and remodel nucleosomes to allow access to underlying DNA for transcriptional regulation (Ho and Crabtree, 2010). The major histone variants in mammals are deposited in chromatin in a replication-independent manner during times in which newly synthesized canonical histones are not available. For example, in terminally differentiated postmitotic neurons, which are no longer undergoing DNA replication, it can be assumed that these cells continue to have access to replication-independent histone variants, often encoded by one or two genes that are synthesized throughout the cell cycle (Frank *et al*, 2003). This process not only allows cells to provide continuous pools of histones for nucleosome replacement outside of S phase but also enables the generation of biochemically distinct nucleosomes that promote different patterns of chromatin regulation in cell type-specific and temporally precise manners.

H3 Variants: Basic Mechanisms

Mammalian cells express multiple distinct genetically encoded variants of histone H3 proteins (H3.1, H3.2, and H3.3/primate-specific H3.X and H3.Y (Wiedemann *et al*, 2010)). H3.1 and H3.2 represent canonical histones and are found throughout the genome as clustered repeat arrays, with their transcription and deposition tightly coupled to DNA replication, and their stability controlled by 3′ stem looped structures (Pandey and Marzluff, 1987). H3.3, however, is a non-canonical histone variant that is encoded by two isolated genes (*H3F3A*, *H3F3B*) and is constitutively expressed in non-dividing cells in a polyadenylated and promoter-dependent manner (Wellman *et al*, 1987). H3.3 differs from canonical H3 species at one amino-acid residue in the histone tail (serine 31) and at a cluster of three residues in the core histone fold (alanine 87, isoleucine 89, and glycine 90). The three amino-acid variations in the histone fold have been shown to be necessary for H3.3 replication-independent incorporation in chromatin (Ahmad and Henikoff, 2002). Although the variant histone H3.3 differs from H3.2 and H3.1 at only four and five amino acids, respectively, H3.3 is specifically enriched at transcriptionally active genes, within gene promoters, at specific genomic repeats, such as telomeres and at regulatory elements in mammalian ES cells and in neuronal precursors (Goldberg *et al*, 2010) (Figure 3).

As mentioned earlier, higher eukaryotes have evolved separate chaperones and deposition pathways for the different histone H3 variants, with two major deposition pathways identified through previous work; replication-coupled deposition of H3.1/H3.2 by the chromatin assembly factor 1 (CAF1) complex, and replication-independent deposition of H3.3 by the HIRA complex (see Probst *et al* (2009) for a review; Figure 3). HIRA (homolog of yeast Hir1) has been demonstrated to interact with ASF1a/b as a multisubunit complex specific for H3.3 chromatin deposition and has been shown to be required for H3.3 incorporation at genic loci in ES cells (Ray-Gallet *et al*, 2002; Tagami *et al*, 2004). Interestingly, H3.3 has also been shown to enrich throughout specific repetitive sequences, such as telomeres, and at most regulatory elements in a HIRA-independent manner. It is now known that the SWI/SNF chromatin remodeler ATRX, in complex with Daxx, functions to promote H3.3 localization at telomeres (Goldberg *et al*, 2010; Wong *et al*, 2010); however, the exact

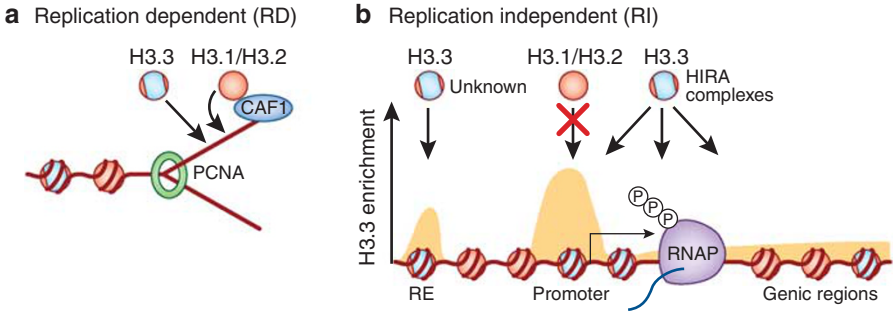

Figure 3. The deposition of distinct H3 variant proteins involves the activity of multiple distinct chaperone complexes. (a) The replication-coupled deposition of H3.1/H3.2 is mediated by the chromatin-assembly factor 1 (CAF1) complex in association with the proliferating cell nuclear antigen (PCNA-a processivity factor for DNA polymerase in eukaryotic cells). (b) Replication-independent deposition of H3.3, however, is mediated by several HIRA complexes acting throughout genic regions of the genome, as well as by other unidentified complexes associated with H3.3 deposition at specific regulatory element (RE)-binding sites. Pericentromeric and telomeric deposition of H3.3 (not shown here, but see Figure 2) are carried out by ATRX/Daxx complexes.

mechanisms controlling H3.3 enrichment at most regulatory sites throughout the genome remain unclear. Recently, HIRA was demonstrated to further interact with the SWI/SNF-remodeling protein CHD1, which has been implicated in the *in vivo* deposition of H3.3, and might contribute to a regulatory site-specific deposition pathway; however, H3.3 continues to be deposited in chromatin in the absence of either CHD1 or HIRA (Bonnefoy *et al*, 2007; Konev *et al*, 2007). Finally, HIRA has been shown to directly interact with Mef2 *in vivo*, resulting in the activation of Mef2 target genes during skeletal muscle differentiation (Yang *et al*, 2011). This process requires Asf1 complex association, and was demonstrated to be negatively mediated by Cabin1 binding to HIRA. Although it is unknown whether the transcriptional effects of these associations necessitate, or are responsible for, H3.3 deposition at putative target loci, one could postulate that in neurons, given the role of Mef2 in the mediation of numerous aspects of synaptic function (Flavell *et al*, 2006), H3.3 deposition might be tightly linked to transcriptional programs required for neural plasticity. To date, H3.3-specific deposition complexes have not been examined in postmitotic cells. Given the probable importance of the replication-independent chromatin-regulatory machinery in non-dividing cells, it is likely that future investigations in neurons will yield valuable insights into these processes.

H3 Variant Exchange: Markers of Dynamic *vs* Static Chromatin?

Following nucleosomal assembly and deposition, H3/H4 tetramers have been observed to display stable expression in chromatin in comparison to H2A/H2B dimers, as measured by levels of displacement during replication and transcription (Kimura, 2005). Recent cytological studies in *Drosophila* have demonstrated more rapid rates of H3/H4 exchange than originally predicted, indicating that nucleosomal turnover may be accelerated at specific euchromatic loci (Ahmad and Henikoff, 2002). Later studies using metabolic labeling of histones coupled to tiling arrays (CATCH-IT) provided evidence for extraordinarily rapid nucleosomal turnover kinetics and indicated H3.3 as a principal component of these dynamics (Deal *et al*, 2010). Genome-wide mapping studies also found that another histone variant, H2A.Z, which is similar to H3.3 in that it is encoded and deposited throughout the entirety of the cell cycle, co-localizes with H3.3 at specific promoters and regulatory elements that, due to active modes of transcriptional regulation, are oftentimes unstable and are found to be depleted of nucleosomes (termed 'nucleosome-free regions') (Henikoff, 2009). These findings further indicate a role for H3.3 in marking/coordinating dynamic chromatin at highly active genomic loci. Consistent with these studies, ChIP-seq data from ES cells identified distinct patterns of H3.3 *vs* H3.2 enrichment throughout genic loci, specifically within gene bodies and at regulatory sites (Goldberg *et al*, 2010). Such enrichment profiles were tightly correlated with highly expressed genes, corresponding 'active' histone modifications (eg, H3K4me1) and components of the transcriptional machinery (eg, RNA Pol II) (Ray-Gallet *et al*, 2011); however, enrichment at inactive genes was also observed, perhaps accounting for a 'poised' chromatin state at these loci (Mito *et al*, 2007; Tamura *et al*, 2009). H3.3 incorporation profiles were further demonstrated to be dynamic in response to differentiation from ES cells to neuronal progenitors (Goldberg *et al*, 2010). Although similar studies have yet to be carried out in neurons, it will be interesting to further investigate the role of H3.3-dependent nucleosomal dynamics in the regulation of activity-dependent transcription, as plasticity in the nervous system likely necessitates rapidly re-organized and remodeled chromatin landscapes. Furthermore, if alterations in H3.3 genomic enrichment represent states of dynamic chromatin, then what are we to assume concerning the functions of H3.1 and H3.2 in the regulation of chromatin architecture? Although a new study using heavy isotope metabolic labeling of proteins in rats suggests that both H3.3 and canonical H3 proteins, as well as other

variants including H2A.Z, exhibit extreme stability in rodent brain (ie, stable heavy peptides were observed up to 12 months following metabolic labeling) (Savas *et al*, 2012), it remains unclear as to what role, if any, H3.1 and H3.2 proteins might have in the adult CNS. On the basis of previous mechanistic studies (outlined above), one would assume that following terminal neuronal differentiation, canonical H3 proteins would no longer be able to be actively incorporated in chromatin. Therefore, if these proteins remain stable for long periods of time in brain, keeping in mind that an obvious caveat of this study was their inability to distinguish between cell types (ie, samples most likely contained a mixture of neurons, glia, *etc*), then one could assume that H3.1 and H3.2 act as markers of 'static' or 'inert' chromatin, potentially enriching throughout heterochromatic loci that are not prone to rapid turnover, unlike those expected to be occupied by H3.3. To date, these questions remain unanswered; however, data are beginning to be generated to support such a hypothesis. A quantitative mass spectrometry study aimed at examining global posttranslational-modification states between human H3.1, H3.2 and H3.3 identified differences in modification profiles, suggesting that they exhibit unique biological functions *in vivo* (Hake *et al*, 2006). Specifically, H3.3 was shown to enrich for marks associated with active chromatin (eg, lysine 9/14 acetylation, lysine 36 trimethylation, *etc*), whereas H3.2 contained mainly repression-associated modifications (eg, lysine 27 trimethylation). Interestingly, H3.1 was observed to enrich for both active and repressive modifications, although the repressive marks appeared to differ from those observed with H3.2. At this time, it is impossible to directly extrapolate these findings to brain, but these data indicate that H3 variants might function independently to maintain distinct chromatin states throughout the lifetime of an animal.

As described above, one interesting feature of H3.3 *vs* H3.1/H3.2 is the existence of a serine residue at position 31 in its N-terminal tail region (H3.3S31) that replaces an alanine found in canonical H3 proteins. H3.3S31 was demonstrated to be phosphorylated during mitosis in mammalian cells with temporal and spatial specificity in contrast to H3S10 and H3S28 phosphorylation (Hake *et al*, 2005). Although H3.3S31p likely contributes to the aspects of chromatin signaling during mitosis, a single replacement of the serine with an alanine at position 31 did not influence the deposition pathway of H3.3, suggesting that this phosphorylation event is not critical for H3.3 incorporation in chromatin. At this time, it is unknown whether this modification exists in neurons; however, given the potential importance of H3.3 in postmitotic cells, future attention should be paid to this possibility. Furthermore, another H3 mark, H3 threonine 32 phosphorylation (H3T32p), exists in all H3 variants; however, only in the H3.3 variant is H3T32 sequence identical to H3T11, phosphorylation of which, like H3S10p, is important for meiosis in proliferating cells (ie, only in this variant is H3T32 preceded by a serine residue at position 31, similar to Thr11, which is preceded by Serine 10) (Govin *et al*, 2010). Therefore, it is possible that this mark, in combination with H3.3S31p, represents a novel chromatin signature involved in cell-type-specific gene-regulatory patterns. It is becoming increasingly clear that more thorough analyses of histone-modification states in brain need to be performed. Mass spectrometric analysis has begun to identify potentially novel modification sites, as well as novel combinations of histone modifications in brain. To this effect, it will be important for future proteomic investigations to carefully examine H3-variant modification states specifically in neurons, both basally and in response to a variety of environmental stimuli.

Histone Variants in Neuroscience

Proteins involved in chromatin remodeling and regulation of histone modifications have been shown to have critical roles in processes contributing to neurodevelopment, neuronal physiology, synaptic plasticity, and behavioral memory (Borrelli *et al*, 2008). As chromatin maintenance in non-dividing cells relies on the incorporation of replication-independent histone variants, it is likely that histone variants will greatly impact neuronal biology. In fact, several lines of evidence support this hypothesis, with numerous histone variants (eg, H2AX, H3.3, macroH2A) likely contributing to the basic aspects of neuronal function. In line with H3.3's prominent role in postreplicative nucleosomal exchange, *H3f3a* RNA and H3.3 protein levels have been shown to increase in numerous models of cell differentiation (Wu and Bonner, 1982; Lord *et al*, 1990; Krimer *et al*, 1993), and high levels of H3.3 protein have been observed in rat brain (Pina and Suau, 1987). Specifically, the latter study observed a progressive increase in H3.3 protein expression, with concomitant reductions in H3.1/2, in rat cortex with age, suggesting that H3.3 is the dominant H3 protein in adult brain. However, as with the metabolic labeling study demonstrating the existence of long-lived H3.3 and H3.2 peptides in brain, no distinctions were made between cell types, so it is difficult to know whether such changes are neuronal specific. Furthermore, this study similarly demonstrated the presence of H3.1/2 expression in aged adult brain, albeit at lower levels than H3.3; therefore, future studies will be needed to fully dissect these expression pattern differences, as well as the genomic distributions and functions of H3 variants in the developing and adult CNS.

To date, very little is known concerning the biological functions of histone variants. In fact, only recently have patient mutations been identified to suggest that these variants, themselves, may be important for biological homeostasis (eg, mutations in *H3F3A*, specifically at lysine 27 and glycine 34, have been implicated in pediatric glioblastoma) (Schwartzentruber *et al*, 2012). Interestingly, in *Drosophila*, deletion of both H3.3 variant gene copies (A and B) results in partial but incomplete lethality, with ~42% viability accompanied by transcriptional defects and complete sterility (Hodl and Basler, 2009; Sakai *et al*, 2009).

Hypomorphic H3.3 mice, generated by retroviral gene trap insertion into the *H3f3a* gene, similarly exhibit partial neonatal lethality and deficits in fertility (Couldrey *et al*, 1999). In addition, H3.3 hypomorphs also display neuromuscular defects, suggesting an essential role for H3.3 in postmitotic neurons; however, the precise function of H3.3 in adult brain remains unknown, as no true knockout studies, conditional or otherwise, have been performed. Although a functional role for H3.3 in neurodevelopment has yet to be truly established, one can speculate that alterations in H3.3 deposition pathways may contribute to these processes, as mutations in ATRX, a bona fide H3.3 chaperone, result in an X-linked mental retardation phenotype. Additionally, a recent study found that calcineurin-mediated dephosphorylation of Daxx during periods of cellular activity results in increased loading of H3.3 at IEG regulatory elements in neurons (Michod *et al*, 2012). Although compelling, the precise mechanisms mediating Daxx localization at euchromatic loci, as well as the functional consequences of H3.3 deposition in neurons, remain unclear. Other histone variants, such as H2AX, H2A.Z, and macroH2A, are highly expressed in adult neurons (with H2AX also displaying progressive increases in expression with age in rat cortex) (Bosch and Suau, 1995; Akbarian *et al*, 2001); however, beyond a very basic understanding of histone variant expression in brain, little is known concerning the functions of these variants in the developing and adult nervous systems. When one considers the formal possibility that variants of one particular family can 'mix' with other histone variants from other families, both within single mononucleosomal units and throughout localized nucleosomal arrays, a staggering potential exists in distinct biological readouts. Compositional variation at the nucleosomal level has been described as a 'nucleosome code' (Bernstein and Hake, 2006), whose potential in neuronal functions has yet to be fully appreciated or explored.

FUTURE OUTLOOKS

Reconciling the Dangers of Hyper-Enthusiasm with Cautious Interpretations

Over the past decade, neuroscientists have made great strides in advancing our understanding of the role of chromatin biology/histone regulation throughout numerous aspects of neurodevelopment, neuroplasticity, neurophysiology, and behavior. During this time, the field has experienced an explosively renewed interest in the molecular mechanisms controlling complex behaviors associated with neuropsychiatric disease, and data are beginning to suggest that histone regulation/dysregulation might have an integral role in mediating these processes. Although these important discoveries certainly deserve celebration, it is our view that the field has only begun to scratch the surface of these complicated patterns of cellular/organismal regulation. Future attempts to define the precise chromatin-associated mechanisms involved in complex behavioral states will require a combination of more detailed biochemical and molecular approaches to appropriately integrate mechanistic analyses with descriptive output. Until such time that more mechanistic insights are ascertained, it is important that researchers approach their findings with a level of cautious optimism and interpret their data accordingly.

A growing trend in the field of 'neuroepigenetics' is the desire to assign chromatin-related functions to alterations in the expression and/or activity of known chromatin-modifying enzymes and remodeling proteins. This current focus is logical, as a system does not currently exist to directly manipulate histone marks in mammalian cells due to the large number of redundant/clustered histone genes encoded in vertebrates. Although many chromatin-modifying enzymes (eg, HATs, HDACs, HMTs, HDMs, *etc*) have been demonstrated to display dynamic, and sometimes persistent, patterns of regulation in brain, events that are often correlated with global changes in histone-modification states, it is becoming increasingly clear that many, if not all, of these enzymes are capable of modifying non-histone substrates (both nuclear and cytosolic), thereby complicating functional interpretations of knockout/knockdown and overexpression studies. Although it is likely that endogenous alterations in enzyme activity contribute to changing chromatin landscapes, such assertions are difficult to causally define. Numerous groups have begun to address this issue through mutational studies, where it is possible to directly control/manipulate the catalytic activity and/or binding properties of chromatin-modifying/-remodeling enzymes (eg, acetyl bromodomains, methyl chromodomains, *etc*) to assess chromatin-related enzymatic and recruitment functions *in vivo*; however, until all chromatin-associated enzymes, many of which are likely still to be identified, are fully biochemically characterized, it will be very difficult to assign direct chromatin functions to stimulus-dependent alterations in enzymatic activity.

Similarly, it is tempting to assume that environmentally induced alterations in global histone-modification states are directly involved in the regulation of activity-dependent gene transcription in brain; however, to date, such contributions have been difficult to demonstrate outside of indirect correlations. Although it has been known for years that specific histone modifications (keeping in mind that only select modifications have been examined) are capable of influencing basal transcription (eg, H3K4me3), it remains unclear as to the exact role that stimulus-induced changes in histone modifications might have in the regulation of neuronal transcription following periods of cellular activity. Also, caution should be taken in broadly assigning functionality to specific histone modifications (eg, H3K4me3 is activating *vs* H3K27me3 is repressive), as rarely do these modifications exist in isolation, but rather in combination with other marks to allow for a large variety of intra- and internucleosomal associations through recruitment of effector proteins. Moreover, individual lysine residues are well known to be acetylated, methylated, or most recently crotonylated (eg, H3K9, H3K27, *etc*), leading

to distinct biological readouts due to highly regulated 'switching' events that are only beginning to be appreciated. We wonder if every exposed lysine residue has multiple options depending upon its physiological context, and whether the brain might draw heavily upon an array of posttranslational modifications and variant choices to guide phenotypic outcomes.

Additionally, in eukaryotic genetic model organisms, like *Saccharomyces cerevisiae*, a model that allows for direct mutations of histones *in vivo*, the majority of histone N-terminal tail point mutants have been demonstrated to be completely viable despite apparent evolutionary pressures to conserve near-invariant histone amino-acid sequences (Hyland *et al*, 2005; Dai *et al*, 2008). Although specific core amino acids located at the H3/H4 histone-fold motif have been shown to be important for aspects of transcriptional silencing in yeast (this core is centered around lysine 79 of H3 and may be important to nucleosomal stability) (Park *et al*, 2002), these data indicate a high level of functional redundancy in histone modifications that must be considered. With the advent of ChIP-seq, we are now beginning to gain a clearer picture of the relative distribution of histone marks throughout the mammalian epigenome, both basally and following periods of cellular activity. These data will ultimately allow us to compile comprehensive maps of histone modifications, transcription factors, remodeling complexes, *etc* to accurately describe associations between transcriptional outputs and histone regulation in the nervous system. It is important to keep in mind, however, that these approaches, along with global analyses of histone marks, rely heavily on the use of commercially available modification-specific antibodies, which, according to recent reports, vary greatly in their efficiencies and epitope specificities (Egelhofer *et al*, 2011). In some cases, the existence of one mark can greatly influence the epitope accessibility of another, especially when modifications occur in close proximity. For example, a phosphorylation event at one residue might greatly impact the ability of an antibody to recognize a methylation state at an adjacent residue (eg, H3S10p *vs* H3K9me3) (Duan *et al*, 2008), even if these marks occur in combination as identified by mass spectrometry. As a consequence, great care should be taken to appropriately validate modification-specific antibodies, as the quality of an antibody can greatly affect one's results and subsequent interpretations. As novel modifications (eg, histone–lysine crotonylation) (Tan *et al*, 2011), or combinations thereof, are discovered (some of which may be brain specific), it will be important to integrate these findings into the existing neuroepigenetic framework to allow for a more comprehensive understanding of chromatin biology in the context of neurological function and disease. Furthermore, viewing patterns of histone regulation in relation to other important regulatory events in the nucleus (eg, DNA methylation, ncRNA function, *etc*) will be essential to future investigations.

Finally, due to the cellular heterogeneity of neuronal tissues, one of the major challenges of investigating histone regulation in the CNS is our current difficulty in defining individual chromatin states and remodeling activities within specific functional regions of the brain, in neuronal subtypes (eg, dopaminergic, cholinergic, glutamatergic, GABAergic, *etc*) and between neurons and other cell types of the CNS (eg, glia), all of which likely vary significantly in global chromatin profiles, which support unique transcriptional outputs and cellular functions. Recent advances in the field of neuroscience have made it possible to isolate pure neuronal cell populations using fluorescence-activated cell sorting, which relies on endogenous cellular markers or transgenic tags (eg, green fluorescent protein (GFP) expressed from neuronal subtype specific promoters), to appropriately distinguish and separate distinct cell types in adult brain (Jiang *et al*, 2008). One potential limitation of this approach, however, lies in the processing and dissociation of cells required to sort individual cell populations for future chromatin analysis. It remains unclear if such processing can influence the chromatin state of cells, especially in the absence of fixation, and whether this technique is ultimately amenable to analysis of subtle changes in chromatin landscapes following different environmental perturbations. Nonetheless, such approaches have greatly advanced the field by allowing neuronal cell type-specific analysis *in vivo* and should inspire confidence in future studies aimed at tackling these difficult questions.

Histone Regulation as a Means of Generating Heritable Plasticity

One question that still remains in the field of neuroepigentics is whether environmentally mediated alterations in chromatin modifications/structure can contribute to the transgenerational transmission of complex behaviors. Although it is tempting to think that such scenarios are possible, it is difficult to conceptualize how this would occur at the level of histones. Limited examples exist to indicate that this form of epigenetic inheritance occurs during mitosis, in which 'information' contained within focal nuclear microenvironments of parental cells can be accurately inherited by postreplicative daughter cells to allow for physiologically relevant gene expression profiles in progeny (see Zaidi *et al* (2010) for a review). Such sustained patterns of transcription have been shown to be accompanied by the retention of specific transcription factors, as well as associated histone modifications and patterns of DNA methylation, at promoters in a process known as mitotic bookmarking. Although these events have been suggested to represent a novel mechanism of inheritable epigenetic control, thereby allowing for the sustainability of cellular identities following mitosis, it remains unclear as to whether histone modifications themselves are important for this process, or if they simply exist as a result of the preservation of transcription factor binding at these sites following cell division.

It should be noted that reliable transmission of physiological and behavioral traits have now been observed through the paternal lineage (eg, (Ng *et al*, 2010; Dietz *et al*, 2011)), suggesting that mechanisms may indeed exist to allow for such instances of transgenerational inheritance.

Some early evidence indicates a role for DNA methylation in this process; however, the exact mechanisms controlling this transmission remain unknown. During spermatogenesis, the majority of canonical histones are exchanged for protamines, which are small, highly basic proteins that promote the formation of tightly packed DNA structures important for normal sperm function. Recent high-throughput sequencing experiments have demonstrated that only ~4% of the haploid genome is occupied by nucleosomes consisting of both canonical and variant histone proteins, some of which are testes specific (Hammoud *et al*, 2009). Although a number of histone modifications have been identified in sperm (eg, H3K4me2/3, H3K27me3), they tend to localize to very specific developmental loci, including *HOX* gene clusters, certain ncRNAs, imprinted genes, and bivalent promoters. Therefore, given that the majority of changes in environmentally regulated histone modifications in brain have been found to affect neuronal specific/enriched genes, it seems unlikely that such changes would be observed in, or would be transmitted by, sperm. Also, the heavily condensed state of sperm would appear to act as a barrier for the transmission of 'active' chromatin, which would likely be required to transmit many of the aberrant transcriptional states described in brain following periods of plasticity. As far as maternal germ-line transmission of behaviors is concerned, much more work is needed to appropriately control for gestational influences, as *in utero* experiences likely supersede mechanisms of strict epigenetic inheritance and are difficult to avoid in the absence of *in vitro* fertilization, which itself can affect phenotypic outcomes.

Alternatively, it seems plausible that the establishment or dissolution of histone marks in brain following periods of behavioral adaptation act to initially alter chromatin structure through modulation of chromatin-remodeling events, which may subsequently lead to persistent changes in chromatin architecture and transcriptional activity. Such events ultimately result in what can be referred to as a state of chromatin metaplasticity, and it is our view that these global changes in chromatin function, even in the absence of long-lived modification changes, likely contribute to lifelong patterns of behavioral plasticity. Our review has focused primarily on chromatin-remodeling pathways with an emphasis on histone-centric events. However, we acknowledge that a complete understanding of brain functions will require integration of what we have described here with other well-established epigenetic events, such as DNA methylation (see Deaton and Bird (2011) for a review), targeting of these machineries by ncRNAs (see Wang and Chang (2011) for a review) and, most certainly, other activities that have yet to be described that collectively influence the complexities of a chromatin environment. Finally, we note that non-histone proteins themselves are known to undergo extensive posttranslational modifications, sometimes reflecting closely what is seen in histone proteins. The existence of 'histone mimicry' reminds us that the general paradigm of using this covalent 'language' of marks, writers, readers, and erasers points to a protein 'code' that is not limited to histones alone (Sampath *et al*, 2007). A future challenge will be to determine the likely key target substrates of these activities, many of which may lie beyond histone proteins.

The Holy Grail of Neuroepigenetic Research: Identification of Druggable Targets for Future Neuropsychiatric Pharmacological Intervention

It comes as no surprise that such rapid advances in neuropsychiatric/neuroepigenetic research has led to heightened enthusiasm for the development and clinical implementation of pharmacotherapies aimed at targeting chromatin-related enzymes. For example, HDAC inhibitors (HDACi) are now being used routinely in the clinic to treat a variety of human illnesses, including numerous cancers, parasitic infections, and inflammatory diseases. Although much less is known concerning the actions of HDACi in the alleviation of neuropsychiatric and neurodegenerative disorders, some deacetylase inhibitors, such as valproic acid, are commonly used to treat symptoms related to bipolar disorder (Emrich *et al*, 1981), and rodent work has indicated that other HDACi may prove effective in treating a variety of neurological maladies including depression-related illnesses (Covington *et al*, 2009), drug addiction (Romieu *et al*, 2008), Huntington's disease, and Parkinson's disease (see Abel and Zukin (2008) for review). These inhibitor therapies range significantly in terms of their specificity and efficacy, with numerous drugs now designed to target individual chromatin-modifying enzymes (HDACs, HMTs, *etc*) with extremely high-binding efficiencies. Although potentially promising as future treatments for psychiatric disorders, to date, all inhibitors are designed to target chromatin-modifying enzymes, as opposed to attributes of the histone modifications themselves. As indicated previously, chromatin-regulatory proteins are highly promiscuous in function and substrate specificity. Therefore, by inhibiting the activity of these enzymes, we are indirectly affecting more global aspects of nuclear function (ie, off-target substrate effects of histone and non-histone proteins), which may ultimately result in aberrant clinical outcomes. Indeed, the use of HDACi to treat cancer can result in severe side effects and variable efficacies (see Venugopal and Evans (2011) for review), many of which cannot be rationalized in the treatment of neuropsychiatric disorders. Another point of interest in attempting to identify druggable targets concerns the question of where we should be focusing the majority of our attention? For a number of years, it has been the goal of molecular neurobiology to identify specific genes/proteins that are dysregulated in disease, and then to use this information to develop pharmacological therapeutics; however, it is becoming clear that neuropsychiatric disorders result from, or can be characterized by, more global alterations in chromatin structure, as numerous associated remodeling and/or modifying enzymes have been demonstrated to be important for phenotypic outcomes. Therefore, it is likely

that treating neurological diseases will require large-scale manipulations of chromatin regulation; however, it will be important to allow for aspects of targeted genomic specificity, a property that currently available inhibitors do not offer. Recently, inhibitors have been generated to specifically target chromatin-binding modules in regulatory proteins, which do not directly affect catalytic activity and often display higher substrate specificity, providing potentially more suitable alternatives for future neuropharmacological interventions. In 2010, a cell-permeable small molecule (JQ1) that competitively binds to acetyl–lysine recognition motifs (a.k.a. bromodomains) was characterized and shown to act as a highly potent and specific inhibitor of the bromodomain and extra-terminal (BET) family member BRD4 (Filippakopoulos *et al*, 2010). Such inhibition by JQ1 was later demonstrated to promote antiproliferative effects in a model of human squamous carcinoma, validating the possibility that such drugs can be used in a biologically relevant manner. Although very few of these binding-module inhibitors currently exist, it seems highly probable that similar targeting strategies may prove beneficial to the alleviation of numerous neurological diseases. Much more work is needed, however, to identify chromatin-binding proteins that may serve as suitable targets.

Conclusion

In conclusion, chromatin/histone regulation appears to have important roles in both the developing and adult CNS, and this regulation seems to be critical to many aspects of neural plasticity that directly influence the establishment of complex behavioral phenotypes. Although alterations in chromatin stability seem to contribute greatly to disease, including neuropsychiatric phenomena, the precise mechanisms underlying these clinically relevant outcomes remain unclear. As we learn more about these processes, we are beginning to truly appreciate the biological complexities of human psychiatric illness and are starting to gain a better understanding of how to clinically treat these disorders based upon their molecular and biochemical attributes. Although much more work is needed, advances in neuroepigenetics research are now occurring at a rapid pace and promise to greatly contribute to the future of molecular medicine.

DISCLOSURE

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We express our apologies to colleagues whose work could not be included in this review. We thank Drs Rosemary Bagot and Herbert E Covington III, as well as anonymous reviewers for critical reading of this manuscript and the members of the Allis laboratory for their helpful discussions. K-MN is supported by a *Women and Science* postdoctoral fellowship. IM, K-MN, and CDA are supported, in part, by grants from the NIMH (RO1 MH094698-01, P50 MH096890-01).

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