Epigenetic Basis of Mental Illness

Eric J. Nestler[1], **Catherine J. Peña**[1], **Marija Kundakovic**[1], **Amanda Mitchell**[1], and **Schahram Akbarian**[1]

[1]Departments of Neuroscience and Psychiatry, The Friedman Brain Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA

Abstract

Psychiatric disorders are complex multifactorial illnesses involving chronic alterations in neural circuit structure and function as well as likely abnormalities in glial cells. While genetic factors are important in the etiology of most mental disorders, the relatively high rates of discordance among identical twins, particularly for depression and other stress-related syndromes, clearly indicate the importance of additional mechanisms. Environmental factors such as stress are known to play a role in the onset of these illnesses. Exposure to such environmental insults induces stable changes in gene expression, neural circuit function, and ultimately behavior, and these maladaptations appear distinct between developmental versus adult exposures. Increasing evidence indicates that these sustained abnormalities are maintained by epigenetic modifications in specific brain regions. Indeed, transcriptional dysregulation and the aberrant epigenetic regulation that underlies this dysregulation is a unifying theme in psychiatric disorders. Here, we provide a progress report of epigenetic studies of the three major psychiatric syndromes, depression, schizophrenia, and bipolar disorder. We review the literature derived from animal models of these disorders as well as from studies of postmortem brain tissue from human patients. While epigenetic studies of mental illness remain at early stages, understanding how environmental factors recruit the epigenetic machinery within specific brain regions to cause lasting changes in disease susceptibility and pathophysiology is revealing new insight into the etiology and treatment of these conditions.

Keywords

depression; schizophrenia; bipolar disorder; histone acetylation; histone methylation; DNA methylation; microRNA; 3D chromatin structure

Introduction

Psychiatric disorders, which are among the leading causes of disease burden worldwide, impose an ever-increasing burden on humanity. All major psychiatric syndromes are

complex, heterogeneous conditions resulting from the interaction of several factors including genetic, neurobiological, cultural, and life experiences. Moreover, each of these syndromes is characterized by functional and transcriptional alterations in several limbic brain regions implicated in regulating stress responses, reward, and cognition (Box 1).

Increasing evidence over the last decade has identified epigenetic mechanisms as important effectors in psychiatric conditions (Adachi and Monteggia 2014; Akbarian 2014; Guidotti and Grayson 2014; Nestler 2014; Peña and others 2014). Indeed, being at the foundation of gene regulation, epigenetic mechanisms are ideal candidates for the study of psychiatric syndromes that are caused by the interactions between genetic factors and environmental exposures. Epigenetic mechanisms refer to the highly complex organization of DNA in a cell nucleus and include many types of histone and DNA modifications as well as alterations in many types of non-histone proteins and noncoding RNAs (Jaenisch and Bird 2003; Jenuwein and Allis 2001). Work to date implicating epigenetic regulation in the context of psychiatric syndromes has come from both animal models and postmortem human brains, although to a different degree for various psychiatric disorders as will be seen.

Early development marks a time of dramatic changes in the brain as well as enhanced susceptibility to many environmental insults. Epigenetic mechanisms of gene regulation are a particularly attractive explanation for how early life exposures to stress, toxins, and other stimuli exert life-long effects on neuropsychiatric phenomena (Kundakovic and Champagne 2015; Meaney 2001; Peña and others 2014). Indeed, developmental exposures may have broader impact on epigenetic states and brain circuits than similar exposures later in life. It is therefore important, in characterizing the epigenetic contributions to mental illness, to perform studies across the life cycle.

The present review brings together findings relating to epigenetic mechanisms in the three major psychiatric syndromes: depression, schizophrenia (SCZ), and bipolar disorder. We begin with a brief summary of epigenetic regulation in general (Nestler, 2014) and then present a progress report of how epigenetic studies of mental illnesses, from both adult and developmental perspectives, are providing new insight into the biological basis of these complex disorders and their treatment (Akbarian 2014; Guidotti and others 2005; Peña and others 2014). Due to space limitations, we focus on histone and DNA modifications and do not include discussion of noncoding RNAs, which are also proving to be important in epigenetic regulation in psychiatric disorders (Issler and Chen 2015). We also focus on epigenetic regulation in brain, and make only passing mention of investigations of blood and peripheral tissues. It is possible that peripheral measures, even if the specific changes are different from those in brain, could reflect brain regulation, an important empirical question for future research.

Overview of Epigenetic Mechanisms

The three billion nucleotides of DNA in a mammalian genome would be ~2 meters long if stretched out linearly, yet fit within a microscopic cell nucleus due to an extraordinary degree of organization and compaction in chromatin—nuclear material composed of DNA, histones, and non-histone proteins (Jaenisch and Bird 2003). The fundamental unit of

chromatin is the nucleosome, which consists of ~147 base pairs of DNA wrapped around a core histone octamer (~1.65 turns). Each octamer contains two copies each of the histones H2A, H2B, H3, and H4 (Fig. 1A). Epigenetic mechanisms control the spacing of nucleosomes and the degree to which they are condensed, which thereby determines gene activity. In simplified terms, chromatin exists across a continuum between an inactivated, condensed state (heterochromatin), which does not allow transcription of genes, and an activated, open state (euchromatin), which allows individual genes to be transcribed (Fig. 2). Regulation of the state of chromatin around specific genes, as well as in non-genic regions, is controlled by complex biochemical processes, which are described briefly here.

Histones

The best characterized chromatin regulatory mechanism in brain is the posttranslational, covalent modification of histones at distinct amino acid residues on their N-terminal tails (Jenuwein and Allis 2001). Such modifications include acetylation, ubiquitination, or SUMOylation at lysine (K) residues, methylation at lysine or arginine residues, and phosphorylation at serine or threonine residues (e.g., Fig. 1B), among many others. Acetylation generally promotes decondensation of chromatin and increases gene activity by negating the positive charge of K residues in histone tails and increases spacing between nucleosomes. In contrast, histone methylation can either promote or repress gene activity, depending on the residue undergoing methylation. Phosphorylation of histones is also associated with chromatin inhibition or activation. The roles of other histone modifications are less well understood. The diversity of histone modifications supports the "histone code hypothesis," which posits that the sum of modifications at a particular gene defines a specific epigenetic state of gene activation or silencing (Jenuwein and Allis 2001). However, as will be seen, such codes are likely to be highly complex and have yet to be identified.

The enzymes that add or remove these various covalent modifications of histones can be understood as "writers" and "erasers," respectively. For example, histone acetyltransferases (HATs) catalyze acetylation and histone deacetylases (HDACs) catalyze deacetylation, while histone methyltransferases (HMTs) catalyze methylation and histone demethylases (HDMs) catalyze demethylation. The specificity of numerous HATs and HDACs for specific K residues remains incompletely understood. In contrast, distinct HMTs and HDMs control the methylation of specific K and arginine residues and even the valence of methylation, that is, mono-, di-, or tri-methylated states. The functional consequences of histone modifications are mediated in part through "readers"—proteins that bind to specific modified residues and effect transcriptional change (Jaenisch and Bird 2003; Jenuwein and Allis 2001). For example, different families of *chromatin remodeling proteins*, which use ATP-derived energy to alter nucleosome spacing and condensation, recognize specific forms of modified histones and enhance or repress the activity of nearby genes. The involvement of this diverse family of proteins is just now being studied in the nervous system (Sun and others 2015; Vogel-Ciernia and Wood 2014). Ultimately, hundreds of proteins are thought to be recruited to a gene in concert with its activation or repression, again emphasizing the extraordinary complexity of epigenetic mechanisms.

DNA Methylation

DNA methylation occurs with the addition of a methyl group to the C5 position of cytosine (5mC) predominantly at cytosine-guanine dyads (CpG sites) (Adachi and Monteggia 2014; Klose and Bird 2006). It plays a pivotal role in cell differentiation, imprinting, and X chromosome inactivation. DNA methylation within gene promoters generally exerts a repressive effect on gene transcription. It can either prevent the association of DNA-binding factors with their target sequence or bind to methyl-CpG-binding proteins to recruit transcriptional co-repressors to modify the surrounding chromatin into a silenced state. More recent findings have indicated that a significant portion of DNA methylation occurs at non-CpG sites and that DNA methylation can either induce or suppress gene expression depending on other factors (Lister and others 2013). Finally, as discussed in the next paragraph, additional forms of cytosine modifications have been described.

Compared with histone tail modifications, most of which are considered readily reversible, DNA methylation is viewed as a more stable epigenetic change. DNA methylation is catalyzed by DNA methyltransferases (DNMTs). Despite evidence for the dynamic control of DNA methylation in adult brain, including its reversibility, the enzymatic basis of demethylation remains incompletely understood. Putative demethylases are enzymes best studied for their role in DNA repair, such as the growth arrest and DNA damage (GADD45) family of proteins. Similarly, members of ten-eleven translocation (TET) proteins oxidize 5mC into 5-hydroxymethylcytosine (5hmC), and subsequently into 5-formylcytosine and 5-carboxylcytosine (Guo and others 2011; Kriaucionis and Heintz 2009; Moore and others 2013). Through deamination, glycosylation, or base excision repair, these newly discovered forms of cytosine modification can then be converted back into an unmethylated state. Interestingly, 5mC oxidation derivatives are expressed at highest levels in neurons, and in contrast to the generally repressive effect of 5mC on gene expression, 5hmC is more correlated with transactivation (Szulwach and others 2011). Most studies of DNA methylation in psychiatric disorders to date have not distinguished between 5mC and 5hmC, which is clearly a major need in the field.

Epigenetic Mechanisms of Depression

Depression is a complex and heterogeneous disorder, although stressful life events are a major factor in depression vulnerability. Indeed, depression is only ~40% heritable, which emphasizes the involvement of nongenetic factors. Most of what we know about the epigenetic basis of depression comes primarily from studies of animals exposed to stress, with several chronic stress paradigms having the most construct and face validity with respect to the human syndrome (Fig. 3). These studies can be divided into two major approaches: those that expose rodents to chronic stress during adulthood and those that expose rodents to chronic stress during development. Both likely recapitulate key features of the human syndrome, and it will be important in future studies to bridge the two approaches as well as validate them with analyses of postmortem human brain tissue.

Histone Modifications in Adult Depression Models

The potential importance of epigenetic mechanisms in depression was suggested initially by observations that HDAC inhibition exerts antidepressant-like effects in adult rodent stress models (Covington and others 2009; Covington and others 2011b; Covington and others 2015; Schroeder and others 2007; Tsankova and others 2006). Systemic administration of highly nonspecific HDAC inhibitors (e.g., sodium butyrate), or direct injection of more selective inhibitors (e.g., MS275) into any of several brain regions, including nucleus accumbens (NAc), hippocampus, amygdala, or prefrontal cortex (PFC), alleviates depression-like symptoms in the chronic social defeat stress model in mice. Genome-wide studies of NAc gene expression in defeated mice treated systemically with fluoxetine or intra-NAc with MS275 demonstrated that both treatments reverse a large proportion of defeat-induced differential gene expression. Although each treatment also regulated subsets of unique genes, there was also significant overlap, suggesting that antidepressant effects of fluoxetine may in part be mediated by affecting histone acetylation (Covington and others 2009).

A major need of current research is to define the precise mechanisms by which histone acetylation controls depression- and antidepressant-related behavioral responses. There are numerous reports of stress or antidepressant regulation of specific HDAC isoforms; however, the mechanisms underlying this regulation and the specific target genes affected by each enzyme subtype remain largely unknown. Another need is to obtain genome-wide maps of any of several histone acetylation sites in several brain regions in depression models to define the genes and molecular pathways that mediate these responses.

Histone methylation is also implicated in depression. Chronic social defeat stress downregulates the histone methyltransferases G9a and G9a-like protein, which catalyze H3K9me2 (a major repressive mark) in NAc (Covington and others 2011a). Overexpression of G9a in this region is antidepressant and increased H3K9me2 at specific gene promoters is implicated in the antidepressant effect of fluoxetine (Robison and others 2013). Indeed, chronic exposure to fluoxetine reduces *Camkii* expression in NAc by reducing histone acetylation and increasing H3K3me2 levels at the *Camkiia* promoter in NAc. Interestingly, these effects are found in NAc of depressed humans exposed to antidepressants, suggesting that the stress-induced loss of repressive methylation is maladaptive and that the therapeutic effects of antidepressant drugs may act in part via the reinstatement of these marks at specific gene loci. Another gene that illustrates this mode of regulation is *Ras*. Reduced H3K9me2 at this gene in NAc of susceptible mice results in increased *Ras* expression, induction of ERK signaling, and, ultimately, CREB activation, which induces depression-like behavior (Covington and others 2011a).

Another repressive histone mark, H3K27me3, is increased upstream to the promoter of the *Rac1* gene in susceptible mice and this is associated with a sustained reduction in *Rac1* expression that influences characteristic dendritic spine changes in defeated mice (Golden and others 2013). These findings have been corroborated in depressed humans. H3K27me3 is implicated as well in the ability of chronic stress to suppress *Bdnf* expression in hippocampus (Tsankova and others 2006).

ChIP-chip analysis (chromatin immunoprecipitation followed by genome-wide promoter microarrays) examined stress-induced redistribution of H3K9me2 and H3K27me2 in NAc of mice subjected to chronic social defeat or protracted social isolation. Significant and dynamic changes in repressive histone methylation were observed in upstream regulatory regions in both models, with ~20% overlap (Wilkinson and others 2009). ChIP-seq was used to map H3K9me3, still another repressive histone mark, in hippocampus and found dramatic induction of the mark by restraint stress at repetitive elements (Hunter and others 2009; Hunter and others 2012), non-transcribed regions of the genome. Such an effect may influence genomic instability. Finally, whole forebrain overexpression of *Setdb1*, a histone methyltransferase that catalyzes H3K9me3, reduced depression-like behavior (Jiang and others 2008), suggesting that the increase in H3K9me3 after acute stress may represent an adaptive response.

Aside from the few examples cited above, human postmortem studies examining histone modifications in depression are sparse. Elevated levels of H3K4me3—a mark of gene activation—were reported at the synapsin gene family in PFC of depressed humans (Cruceanu and others 2013). There are also reports of altered H3K4me3 or H3K27me3 in promoter regions of several candidate genes (e.g., *OAZ, SYN2, BDNF, TRKB*) in postmortem PFC (Chen and others 2011; Fiori and others 2012), but thus far no genome-wide analysis of histone modifications in depressed human brain. This is a high priority for future research.

Chromatin Remodeling in Adult Depression Models

Very little is known concerning the role of chromatin remodeling complexes in depression or any other psychiatric syndrome. However, a recent study demonstrated that chronic social defeat stress induces a repressive chromatin remodeling complex in NAc, which by ChIP-seq was shown to mediate stress-repression of a set of genes important for mediating stress susceptibility (Sun and others 2015). Induction of the same complex was found in NAc of depressed humans, providing translational validation. Induction of this repressive complex at suppressed genes correlates with lower levels of activating histone marks (e.g., H3M4me3 and H4K16ac) and increased levels of certain repressive histone marks (e.g., H3K9me2), thus emphasizing the coordinated nature of epigenetic regulation (Fig. 4). These findings underscore the importance of mapping numerous epigenetic mechanisms genome-wide in order to define the combinatorial code of epigenetic changes associated with depression or antidepressant responses.

DNA Methylation in Adult Depression Models

In addition to the chromatin modifications described above, a growing body of evidence supports a role for DNA methylation in mediating the impact of stress on the brain. Chronic social defeat stress increases expression of *Dnmt3a* in NAc (LaPlant and others 2010). Overexpressing *Dnmt3a* in this region increases depression-like behavior while intra-NAc infusion of a DNMT inhibitor, RG108, exerts antidepressant-like effects. DNMT3a activity is generally associated with transcriptional repression suggesting that susceptibility may associate with down-regulation of transcriptional expression in NAc. Expression of DNMTs is altered in limbic and brain stem regions in depressed suicide completers (Poulter and

others 2008). Genome-wide analysis of DNA methylation will be important in establishing the precise mechanisms of this epigenetic modification in defeat-induced susceptibility.

DNA methylation of several candidate genes, within NAc and several other brain regions, has been studied in stress models. Examples include glial cell-derived neurotrophic factor (*Gdnf*) (Uchida and others 2011) in NAc and corticotropin releasing factor (CRF) in paraventricular nucleus of hypothalamus (PVN) (Elliott and others 2010; Sterrenburg and others 2011). CRF, a critical regulator of hypothalamic-pituitary-adrenal (HPA)-axis activation and other stress actions in brain, is increased in PVN of mice that are susceptible to social defeat and this is accompanied by decreased DNA methylation at the *Crf* promoter. Both effects are reversed by chronic imipramine treatment (Elliott and others 2010). DNA methylation is also increased at the *Crf* promoter in PVN of female rats subjected to chronic unpredictable stress, suggesting that DNA methylation may play a role in determining sex-specific regulation of HPA-axis function (Sterrenburg and others 2011). An imperative for the field is to generate genome-wide maps, not only of 5mC, but also 5hmC, in several brain regions in chronic stress models in animals as well as in human brain.

Epigenetics and Developmental Vulnerability to Depression

Early life adversity, which can have lifelong effects on behavioral outcomes, has been modeled in rodents using prenatal stress (where pregnant dams are stressed) or separation of pups from their mothers (Turecki and Meaney 2014). Natural variations in maternal care, with the extremes classified as either low or high grooming, likewise associate with differential stress responses among adult offspring (Meaney 2001). Research over the last decade has implicated epigenetic alterations, a subset of which are likely permanent, in the enduring effects of such early life stress. Such epigenetic alterations presumably affect depression vulnerability both by mediating sustained alterations in the steady state expression levels of certain genes and by altering other genes' inducibility in response to subsequent challenges later in life.

Several studies have examined the epigenetic consequences of prenatal stress on brain. In one series of experiments, investigators showed that maternal stress suppresses placental expression of 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2), which normally protects the developing fetus from maternal glucocorticoids. Such 11β-HSD2 down-regulation is associated with hypermethylation of its gene promoter and with increased stress vulnerability of offspring animals (Jensen-Peña and others 2012; O'Donnell and others 2012). In other studies, early prenatal stress elevated DNA methylation at the NGF1-A binding region of the glucocorticoid receptor (GR; *Nr3c1*) promoter exon 1_7 in offspring hypothalamus, and decreased methylation of the *Crf* promoter, with no changes at *Bdnf* (Mueller and Bale 2008). Hypermethylation at *NR3C1* was found similarly in infant cord blood from depressed mothers or those physically abused during pregnancy (Hompes and others 2013; Oberlander and others 2008; Radtke and others 2011). Finally, mice exposed to prenatal stress had elevated levels of *Dnmt3a* and *Dnmt1* mRNA in PFC and hippocampus at birth, changes that persisted into adulthood and were associated with hypermethylation of the *Reelin* and *Gad1* promoters, both implicated in SCZ (see below) (Matrisciano and others 2013).

There is also a large literature on the influence of maternal behavior on epigenetic endpoints in offspring brain. Maternal separation has been shown to alter levels of expression of several HDACs in PFC and other brain regions, although the genes influenced by such modifications remain unknown (Blaze and Roth 2013; Levine and others 2012). As in adults, treatment with nonselective HDAC inhibitors reverses the effects of maternal separation, while treatment with theophylline—which can activate HDACs in addition to its better described action as a phosphodiesterase inhibitor—had the opposite effect (Levine and others 2012).

Early maternal separation reduces DNA methylation in a known enhancer region for *Avp* expression in PVN, which is associated with increased *Avp* expression (Murgatroyd and others 2009). Maternal separation also alters DNA methylation and expression of *Nr3c1* and *Bdnf* in PFC and hippocampus, changes which could contribute to depression-like behavior observed later in life (Kundakovic and others 2013). As well, DNA methylation is altered by extreme adversity (e.g., abuse) during early life. Maternal maltreatment of rat pups (tramping, dragging, rough handling) leads to lasting hypomethylation at the *Bdnf* promoter in PFC (Roth and others 2009). These effects are partially rescued by ICV treatment with zebularine, a DNMT inhibitor. A recent human study assessed the impact of child abuse on genome-wide DNA methylation signatures in gene promoters in hippocampus (Labonté and others 2012a). DNA methylation patterns were compared between suicide completers with a severe history of child abuse and healthy controls, and hundreds of differentially methylated sites were identified. Importantly, DNA methylation levels in gene promoters were inversely correlated with gene expression at a genome-wide level, supporting the globally repressive role of DNA methylation. Similar observations have been made in suicide completers (Labonté and others 2012b).

Likewise, low versus high maternal care alters the epigenetic status of numerous genes, the best studied of which is *Nr3c1.* Low maternal care decreases histone acetylation at the *Nr3c1* exon 1_7 promoter in hippocampus (Weaver and others 2004), the same locus affected by prenatal stress and maternal separation (see above). This modification occurs coincidently with increased DNA methylation (McGowan and others 2011). The changes are associated with reduced GR expression and increased stress vulnerability. Treatment with the nonselective HDAC inhibitor, trichostatin A, infused either ICV or intra-hippocampus, reversed the effects of low maternal care on histone acetylation at *Nr3c1* and stress behavior (Weaver and others 2004; Weaver and others 2006). The impact of maternal care on the establishment of DNA methylation profiles spreads across large genomic regions: microarray analysis of a 6.5 mb region centered on *Nr3c1* showed that low maternal care induces hundreds of parallel DNA methylation changes colocalized with other chromatin modifications (Suderman and others 2012). These adaptations cluster in particular at protocadherin genes.

Similar alterations have been reported in hippocampus of suicide completers with a history of child abuse. Abused suicide completers exhibit lower expression levels of *Nr3c1* compared to nonabused suicides and controls (Labonté and others 2012c; McGowan and others 2009). This regulation is associated with altered DNA methylation within respective promoters. Importantly, these alterations appear to be specific to early-life adversity as

Nr3c1 transcriptional modifications found in brains of depressed patients without a history of child abuse do not associate with changes in DNA methylation (Alt and others 2010).

Maternal care in rats is reported to affect several additional genes, such as *Gad1* and *Grm1* in hippocampus (Bagot and others 2012; Zhang and others 2010) and *Esr1* in the hypothalamic medial preoptic area (Peña and others 2013). Pups raised with low maternal care show lower expression levels of these genes, which are associated with reciprocal changes in promoter methylation and in some cases with altered levels of histone acetylation or methylation. Importantly, these epigenetic events have been related to behavioral outcomes.

Stress beyond the early neonatal period also leaves an epigenetic mark. Three weeks of adolescent isolation stress in a *Disc1* mutant mouse induced mood-related behavioral alterations accompanied by hypermethylation of the tyrosine hydroxylase (*Th*) gene promoter in mouse VTA (Niwa and others 2013). Such hypermethylation at *Th* was sustained into adulthood and rescued by treatment with the GR antagonist RU38486.

This discussion shows robust effects of early life experiences on epigenetic endpoints and directly implicates epigenetic regulation of several candidate genes in stress-related phenomena. The next crucial step is the generation of genome-wide maps of histone and DNA modifications to obtain a complete view of the range of genes affected under these conditions.

Epigenetic Mechanisms of Schizophrenia

SCZ is a severely disabling disorder defined by positive symptoms such as delusions, hallucinations and disorganized thought, negative symptoms such as social withdrawal and apathy, and cognitive impairments. All antipsychotic medications, which produce symptomatic improvement for positive symptoms but far less efficacy for the other behavioral domains, antagonize (or are weak partial agonists at) the D2 dopamine receptor, with varied activity at a range of serotonergic and other receptors. While they have remained the primary therapeutic intervention for over half a century, most patients show an incomplete response. Rational drug development in SCZ remains challenging, given the absence of a unifying pathophysiology and a highly complex genetic risk architecture (Andreassen and others 2014; Neale and Sklar 2015; Rodriguez-Murillo and others 2012). However, SCZ is characterized by gene expression alterations in cerebral cortex and other brain regions and, given that transcriptional mechanisms depend on dynamic chromatin remodeling, genes with dysregulated expression in SCZ brain would be expected to show epigenetic alterations of their regulatory regions.

This view is supported primarily by postmortem human brain work, which has focused to date mainly on changes in DNA methylation at candidate gene promoters. One prominent example is *RELN*, which encodes reelin, whose promoter shows increased methylation in PFC and certain other brain regions of humans with SCZ (Abdolmaleky and others 2005; Grayson and others 2005). This hypermethylation is associated with reduced *Reln* expression and could be mediated by increased DNMT1 levels observed under these

conditions (Veldic and others 2004). Importantly, these changes in reelin are unrelated to a history of antipsychotic drug exposure (Guidotti and others 2000). Given that reelin, a secreted protein, controls neuronal migration during development, these data support a scheme whereby deficiencies in reelin drive some of the developmental abnormalities associated with SCZ.

Another gene that exhibits increased DNA methylation in PFC in SCZ is *SOX10*, which encodes a transcription factor important in development (Iwamoto and others 2005; Kato and Iwamoto 2014). This hypermethylation is associated with reduced *Sox10* expression and with altered expression levels of several genes associated with oligodendrocyte function (Iwamoto and others 2005). These findings are interesting in light of evidence that polymorphisms in *SOX10* are reported to influence the age of onset of SCZ (Yuan and others 2014), and with considerable data implicating myelin abnormalities in this syndrome (Roussos and Haroutunian 2014). Still another example is the finding that several human leukocyte antigen (HLA) genes show altered DNA methylation in PFC in SCZ, which is noteworthy given the purported role of inflammation in disease pathogenesis (Pal and others 2015). A smaller number of studies have examined histone modifications in postmortem human brain samples, such as increased levels of several HMTs in the brains of SCZ patients (Chase and others 2013). In addition, altered levels of H3K9K14 acetylation in PFC in SCZ correlate with altered expression levels of the affected genes, which include *GAD1* (glutamic acid decarboxylase-1, a key enzyme for GABA synthesis), *HTR2C* (serotonin 2C receptor), and *PPM1E* (protein phosphatase 1E) (Tang and others 2011).

Recent studies, when taken together, indicate robust epigenetic dysregulation of *GAD1* in PFC in SCZ, including excessive levels of repressive DNA and histone methylation (Akbarian and Huang 2006; Dong and others 2009; Guidotti and Grayson 2014; Huang and Akbarian 2007; Ruzicka and others 2015), at the expense of certain activating histone marks such as H3K4me3 (Huang and others 2007). Superimposed on these highly localized molecular alterations of nucleosomal histones at *GAD1* is a still poorly understood defect in the 3D architecture of the chromatin fiber containing this locus (see Box 2). This conclusion is based on the weakening of long range enhancer loopings that normally bypass many kilobases of linear genome to activate the *GAD1* promoter in PFC in SCZ (Bharadwaj and others 2013) (Fig. 5A). Such changes in 3D chromatin architecture are not limited to *GAD1*, since they have been found for other SCZ risk genes, such as *CACNA1C* (Fig. 5B). We expect the field to continue to gain further insight into these and other epigenetic mechanisms governing dysregulated GABAergic gene expression, given that a very recent in vivo neuroimaging study provided the first empirical evidence for impairments in GABAergic transmission in PFC of patients with SCZ—a hypothesis that until now was primarily driven by molecular and cellular studies in diseased brain tissue (Frankle and others 2015).

There is a much larger literature on altered DNA methylation, and in some cases histone modifications, of gene promoters in blood or other peripheral tissues of individuals with SCZ. These studies are too numerous to describe here in detail. However, it is interesting that several genes that show alterations in brain—for example, *RELN* and *GAD1*—are also reported to differ in peripheral tissues (Aberg and others 2014; Auta and others 2013; Gavin

and others 2009). Abnormal methylation status has been reported in blood for several additional candidate genes, such as *BDNF* (brain-derived neurotrophic factor), *5HTR1A* (serotonin 1A receptor), and *COMT* (catechol-O-methyltransferase) (Carrard and others 2011; Ikegame and others 2013; Kundakovic and others 2015; Walton and others 2014). Given that the epigenetic status of genes is highly cell type-dependent, with such patterns differing dramatically even across distinct neuronal cell types, it would be surprising if shared abnormalities in brain and blood were common. On the other hand, this is ultimately an empirical question and it is conceivable that aspects of SCZ might be reflected across several tissues.

To date only very few studies have pursued DNA methylation or histone modification changes in SCZ on a genome-wide scale in brain tissue or peripheral cells (Aberg and others 2014; Dempster and others 2011; Kano and others 2013; Melas and others 2012; Mill and others 2008; Wockner and others 2014), and none has harnessed the full power of modern next-generation sequencing technology, which offers the most unbiased view of the distribution of an epigenetic mark across the entire genome. Federally funded consortia, including *PsychENCODE* (http://psychencode.org), are currently underway, with the mission to characterize the epigenome of healthy and diseased brain cells in hundreds of SCZ and control specimens using next-generation sequencing technology.

Importantly, the aforementioned genome-wide (published) studies and ongoing consortia virtually all focus on areas of PFC, with only few studies exploring other brain regions such as the cerebellar cortex (Wockner and others 2014). Such near-exclusive focus on a single brain region is unfortunate, as the neural circuits of psychosis most certainly include many subcortical areas too. However, it is encouraging that some of the candidate genes explored thus far, such as *SOX10* or *GAD1*, resurfaced on the list of epigenetically dysregulated genes in genome-wide studies of human brain tissue (Wockner and others 2014) or of animal models (Connor and others 2012).

Finally, it should be mentioned that mutations in perhaps up to 50 genes, each encoding a different chromatin regulator, have been linked to a wide range of neurodevelopmental syndromes, including rare monogenic forms of SCZ (Ronan and others 2013). Chromatin defects in brain were traditionally considered static lesions of early development, but it is now clear that mutations and maladaptations of the epigenetic machinery cover a much wider continuum, including adult-onset neurodegenerative disease (Jakovcevski and Akbarian 2012; Klein and others 2011; Winkelmann and others 2012). For example, gene duplication of the HMTs, *KMT1D* and *KMT2F*, or the *MYTL1* and *ZNF804A* transcription factors, have been linked to some cases of SCZ (Hess and Glatt 2014; Kirov and others 2012; Lee and others 2012; Takata and others 2014).

It is striking that, in contrast to depression, where most epigenetic investigations have focused on rodent models, the situation is reversed for SCZ for which there have been relatively few animal studies. This could relate to the far more challenging prospect of generating rodent models of SCZ. There are reports that prenatal stress alters cortical expression levels of several enzymes involved in DNA methylation, mirroring findings in SCZ brain (Guidotti and others 2014). Genome-wide DNA methylation maps were obtained

for rat hippocampus in response to chronic olanzapine treatment (Melka and others 2014). As well, HDAC2 (Kurita and others 2012) and the transcription factor FosB (Dietz and others 2014) have been implicated in antipsychotic drug action in rodents. A challenge for the future is to better utilize animal models, and perhaps cell models such as patient-derived neurons, in epigenetic studies of SCZ.

Epigenetic Mechanisms of Bipolar disorder

Bipolar illness shows considerable overlap with SCZ in terms of genetic risk architecture, neurobiology, and, to a certain degree, treatment (such as the use of antipsychotic drugs) (Neale and Sklar 2015). In this context, it is therefore not surprising that both genome-wide and candidate gene DNA methylation mapping in postmortem brains of individuals with SCZ or bipolar disorder with psychosis revealed, for both diagnostic categories, a similar degree of subtle (but significant) changes at many gene promoters (Dong and others 2014; Mill and others 2008; Tang and others 2011; Xin and others 2012).

One particularly interesting locus is *HLA9*, which showed aberrant DNA methylation patterns in multiple postmortem brain cohorts and in peripheral blood and, surprisingly, also in sperm of subjects diagnosed with bipolar disorder (Kaminsky and others 2012). The molecular mechanisms driving this multi-tissue epigenetic dysregulation remain unclear. Furthermore, the aforementioned *GAD1* gene, which shows dysregulated expression and epigenetic regulation in SCZ, shows similar altered DNA methylation in hippocampus in bipolar disorder (Ruzicka and others 2015). While these findings collectively point to an emerging epigenetic risk architecture of bipolar disorder, a more definite assessment of the role of epigenetic dysregulation in bipolar disorder will have to await more comprehensive genome-wide maps not only of DNA methylation but also numerous histone modifications in larger cohorts of brains from subjects diagnosed with this syndrome. Careful transcriptome analyses of bipolar disorder (e.g., Cruceanu and others 2015) will also assist this effort as will more biologically driven diagnostic stratification of SCZ and bipolar patients.

Unexpectedly, regulators of H3K4 methylation recently emerged as a functional gene category showing one of the strongest links to the genetic risk architecture of bipolar disorder and related conditions such as SCZ, based on genome-wide association studies (Psychiatric Genetics Consortium 2015). This finding further underscores the critical role of epigenetic regulation in the biological basis of psychosis spectrum disorders.

Future Outlook

Although still in relatively early stages, work to date has demonstrated that many forms of epigenetic regulation are altered in limbic brain regions both in animal models of psychiatric disease and in postmortem tissue of humans with these disorders. These initial studies have identified several key challenges that will need to be addressed moving forward. A high priority for current research is to complete genome-wide assays for numerous chromatin mechanisms. Another high priority is to validate rodent findings in human tissue and, conversely, to recapitulate human findings in animal models so that causal data can be

obtained. Such efforts are needed for a large number of histone modifications and chromatin remodeling complexes, only a handful of which have been examined to date by genome-wide methods: DNA methylation—both 5mC and 5hmC, which have not yet been separately mapped genome-wide in most disease models, as well as a host of noncoding RNAs. We do not review the latter here, but numerous reports of alterations in microRNAs have appeared for psychiatric syndromes in animal models and human tissue, and these too require further investigation (Issler and Chen 2015).

Another important question is how epigenetic regulation is translated into transcriptional change, not only steady state alterations in expression but also altered inducibility in response to a subsequent challenge as well as changes in alternative splicing, which are thought to be under the control of epigenetic regulation. As noted earlier, no single modification examined to date is deterministic for a change in gene activity. In fact, modifications that are most clearly associated with a functional change, for example, H3K4me3 in promoting transcription, are associated with no change or even opposite changes in transcription at many genes. Such findings are consistent with the required involvement of numerous modifications that work in concert. Deciphering such a code, or chromatin signatures, will be a very difficult, yet also highly important, goal for future research.

A technical challenge in this effort is the heterogeneous cell population of even brain micronuclei, which makes it impossible to derive data as clear-cut as for cell culture systems. Methodologies are underway to isolate specific cell types from brain (Jiang and others 2008) and to perform genome-wide ChIP-seq, RNA-seq, and DNA methylation assays on much less starting material (e.g., Adli and Bernstein 2011). In the meantime, nearly all bioinformatics tools for genome-wide analysis have been developed based on simpler cell culture data, which are not optimal to detect the more subtle signals from terminally differentiated neurons, particularly with the high background noise unavoidable with in vivo studies. Improved analytical tools will require creative collaborations between biologists and bioinformaticians (Maze and others 2014). A further technical challenge, but scientific imperative, is to relate chromatin modifications to a host of transcription factors (e.g., GR, CREB, FosB, NFκB, β-catenin, and others) with which they act in concert to control disease-related behavioral abnormalities.

Beyond modifications of histones, chromatin remodeling, and DNA modifications, an important new effort in neuroepigenetics concerns the 3D organization of the genome in neurons and glia, mentioned briefly above. For example, chromosomal loop formations—which often require CCCTC-binding factor (CTCF)-binding factor, cohesins, and other proteins assembled into scaffolds and anchors—potentially bypass many kilobases, even mega-bases, of linear genome, thereby repositioning promoter-distal regulatory elements next to their target promoters. Exploration of regulatory DNA elements in the context of chromosomal loopings and higher order chromatin is beginning to assign regulation of genes and complex behavior for some of the noncoding sequences in the human genome (Bharadwaj and others 2013). Given that the majority of risk-associated polymorphisms in psychiatric disease, such as SCZ and bipolar disorder, are positioned in intergenic and intronic DNA, chromosome conformation capture assays, and other techniques that measure

interaction and spatial proximity of noncontiguous DNA elements in brain cells (see Box 2) are likely to gain increasing traction in studies of human and animal brain tissue (Mitchell and others 2014).

A major limitation in the field is the difficulty in relating chromatin modifications at a given gene to a functional outcome. Studies to date have relied by necessity on overexpressing or knocking out, or pharmacologically inhibiting, a chromatin modifying enzyme (e.g., an HDAC, HMT, or DNMT) within a given brain region like the NAc and studying the behavioral consequences. However, such manipulations regulate the targeted chromatin modification at hundreds or thousands of genes. One approach to overcome this limitation is to use engineered zinc finger proteins (ZFPs), sequence-specific transcription activator-like effectors (TALEs), or CAS9/CRISPR, coupled to an enzymatic moiety, to target a particular chromatin modification to a given gene of interest within a region of adult brain. While still early in development, such approaches would represent an enormous advance in the field, to test, for example, whether a single histone modification at a particular gene truly regulates that gene and resulting behaviors. We recently demonstrated that this is possible using an engineered zinc finger protein: we targeted increased H3K9me2 to the *FosB* gene in mouse NAc in vivo, thus mimicking a defect seen in this region of depressed humans, and this action reduced FosB expression and increased depression-like behavior (Heller and others 2014). Importantly, we provided evidence that the synthetic zinc finger increased H3K9me2 at the *FosB* locus selectively, with no effect on homologous loci. Further studies utilizing this and other locus-specific tools will dramatically advance studies in neuroepigenomics.

Note that there is virtually no mention of sex differences in epigenetic regulation in this review, because few if any animal studies have compared males and females, and few if any human studies have had sufficient power to compare the sexes. This is an urgent deficiency in the field given large sex differences in some psychiatric syndromes (e.g., depression is twofold more common in females). A focus on shared and different mechanisms of epigenetic regulation in males versus females is thus an extremely high priority for current research.

Conclusions

The ultimate goal of epigenetic studies of mental illness is to understand how genetic vulnerabilities interact with an individual's life experiences to establish stable changes at precise genomic loci, which then control the levels of gene expression or inducibility. Together, this linking of genes and environment through epigenetic mechanisms determines that individual's vulnerability to psychiatric syndromes over a lifetime. It is our expectation that these studies will reveal a host of genes whose products could serve as templates in future drug discovery efforts. It would also be interesting to determine whether drug effects on epigenetic endpoints in peripheral tissues (e.g., blood) might serve as useful biomarkers for clinical features of a given disorder, even if those changes in blood are different from those in brain. In these ways, epigenetic approaches promise unprecedented advances in our understanding, diagnosis, and treatment of psychiatric illness.

Acknowledgments

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Box 1

Limbic Brain Circuitry Implicated in Mental Illness

A majority of research on altered epigenetic regulation in psychiatric disorders has focused on changes within the brain's limbic circuitry, depicted here in rodent brain. This circuitry involves highly interconnected brain structures important for interpreting and responding to rewarding and aversive stimuli and for several domains of cognitive function (e.g., attention, working memory, and declarative memory). Depicted are dopaminergic neurons (*green*), which project from the ventral tegmental area (VTA) of the midbrain to NAc, PFC, amygdala (AMY), and hippocampus (HPC), among other regions. The NAc receives excitatory glutamatergic innervation (*red*) from the HPC, PFC, and AMY, while the HPC, PFC, and AMY exhibit reciprocal glutamatergic projections. Not shown are regions of the hypothalamus, and serotoninergic and noradrenergic nuclei, which are also inter-connected with the limbic brain and influence psychiatric disorders. From Peña and others (2014) with permission.
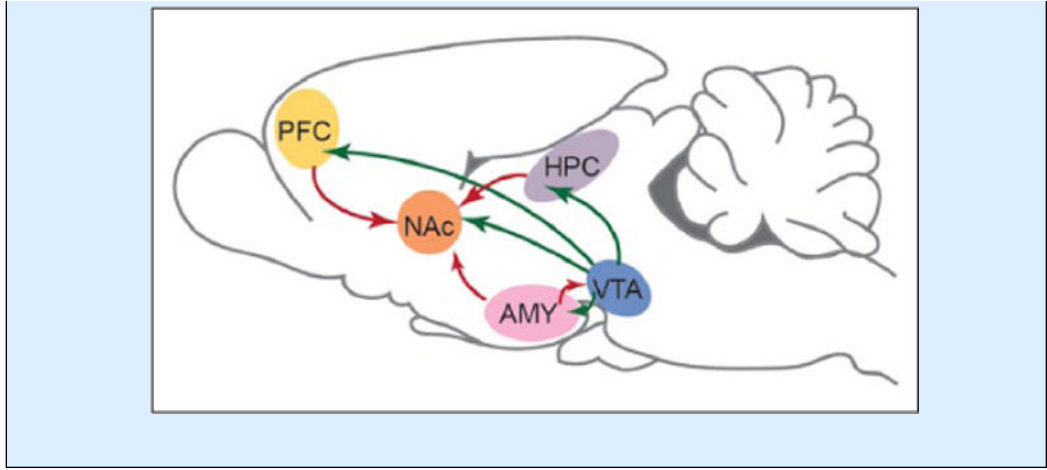
Box 2

### Three-Dimensional Studies of Chromatin Structure

Interphase chromosomes are thought to be arranged in a probabilistic, nonrandom fashion inside the nucleus (far left). To examine chromosome loop formations, chromosome conformation capture (termed 3C) and its derivatives are employed. 3C libraries are generated from cross-linked chromatin that first is digested with high doses of a restriction enzyme followed by religation, then removal of protein and quantification of ligation products generated from noncontiguous sequences. Conventional 3C measures the looping between two specific candidate sequences ("one vs. one") (top). Circular chromosome conformation capture (4C) libraries are creating from the 3C libraries by subjecting the 3C libraries to a second round of restriction digestion with a different enzyme. 4C captures the total set (genome-scale) of chromosomal loop formations for one specific locus ("one vs. all") (middle). Finally, HiC libraries are created by restriction digestion of cross-linked DNA, filling in using biotin-CTP and re-ligating the genome (bottom). Biotin labeled interactions are precipitated and sequenced. Global interactions are agnostically interrogated across the genome ("all vs. all") (bottom). Examples of the regulation of the 3D structure of genes in mental illness are given in Figure 5.
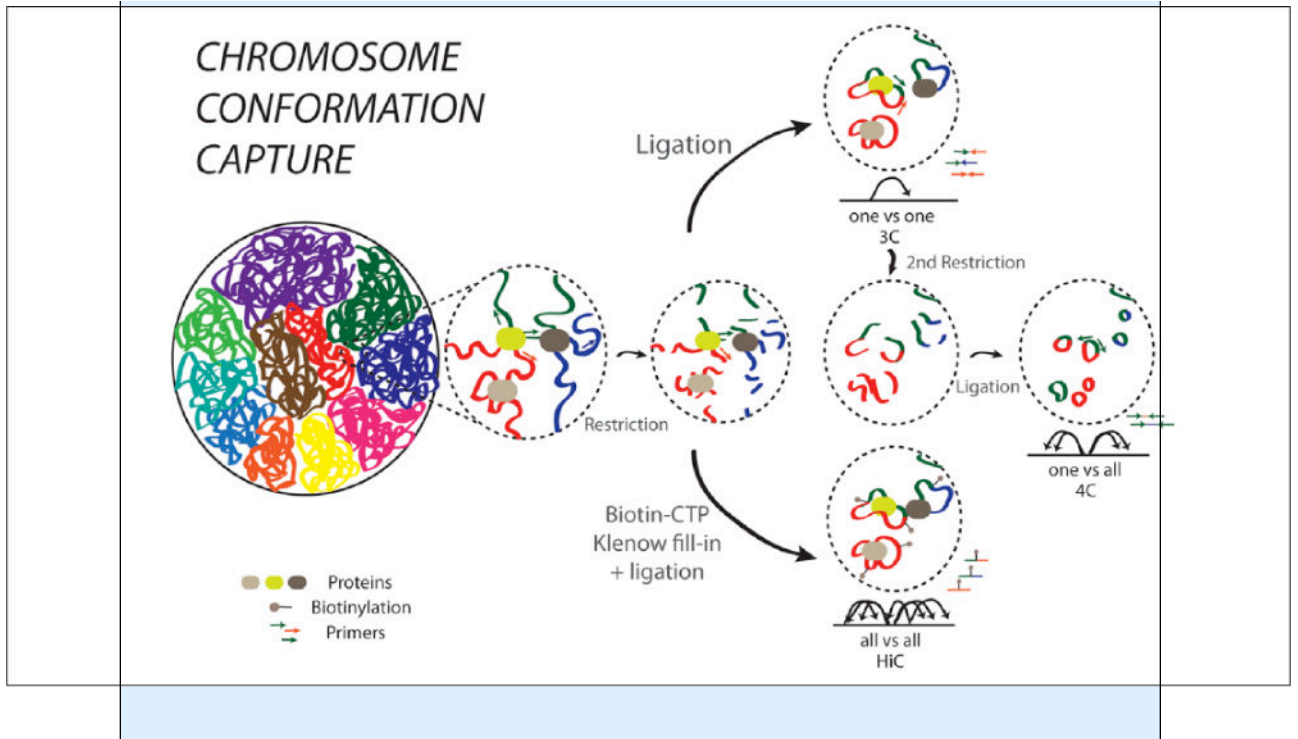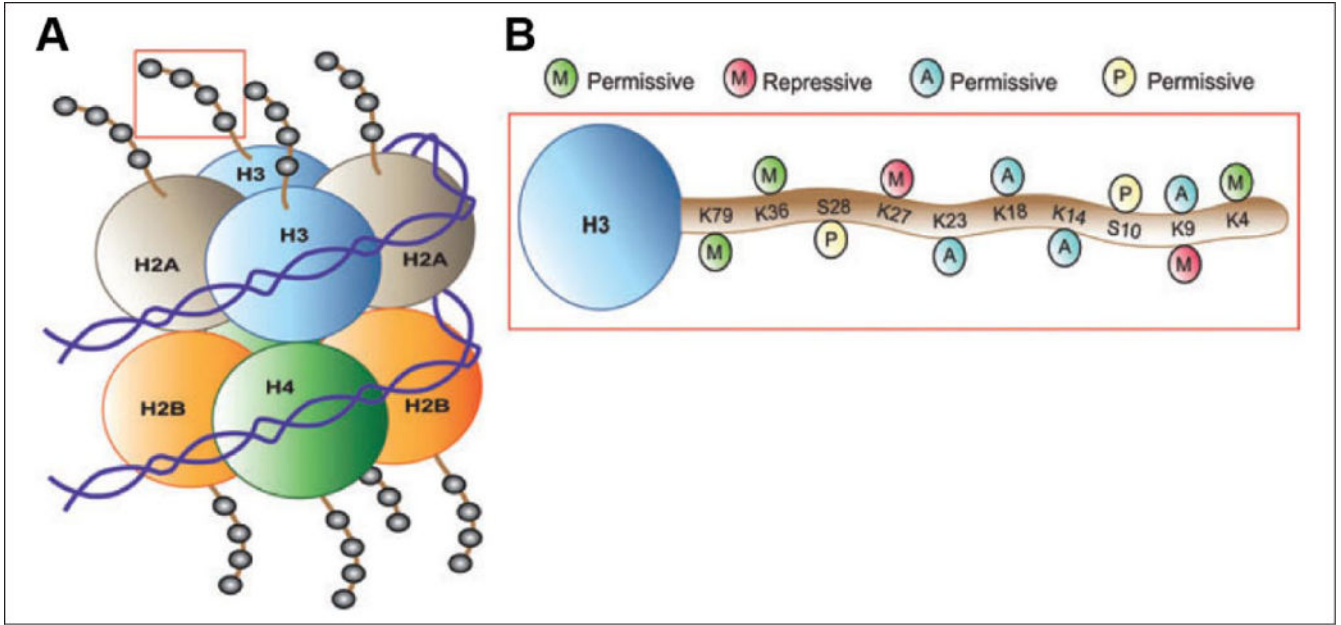
Figure 1.
Scheme of posttranslational modifications of histones. (A) The nucleosome is the functional unit of chromatin, composed of 147 bp of DNA wrapped around a core octamer of histone proteins (two copies each of H2A, H2B, H3, and H4). The N-terminal tails of these histones face outward from the nucleosome. (B) Combinations of acetylation, phosphorylation, methylation, and so on, on histone tails (here, H3 is depicted) alter chromatin compaction and regulate gene expression. Histone modifications that weaken the interaction between histones and DNA or that promote the recruitment of transcriptional activating complexes (e.g., H3 acetylation at K23, K18, K14, and K9, as well as methylation at K79, K36, and K4 or phosphorylation at S28 and S10) correlate with permissive gene expression. Histone deacetylation, which strengthens histone–DNA contacts, or histone methylation on K27 or K9, which recruits repressive complexes to chromatin, promote a state of transcriptional repression. Adapted from Tsankova and others (2007) (permission not required).
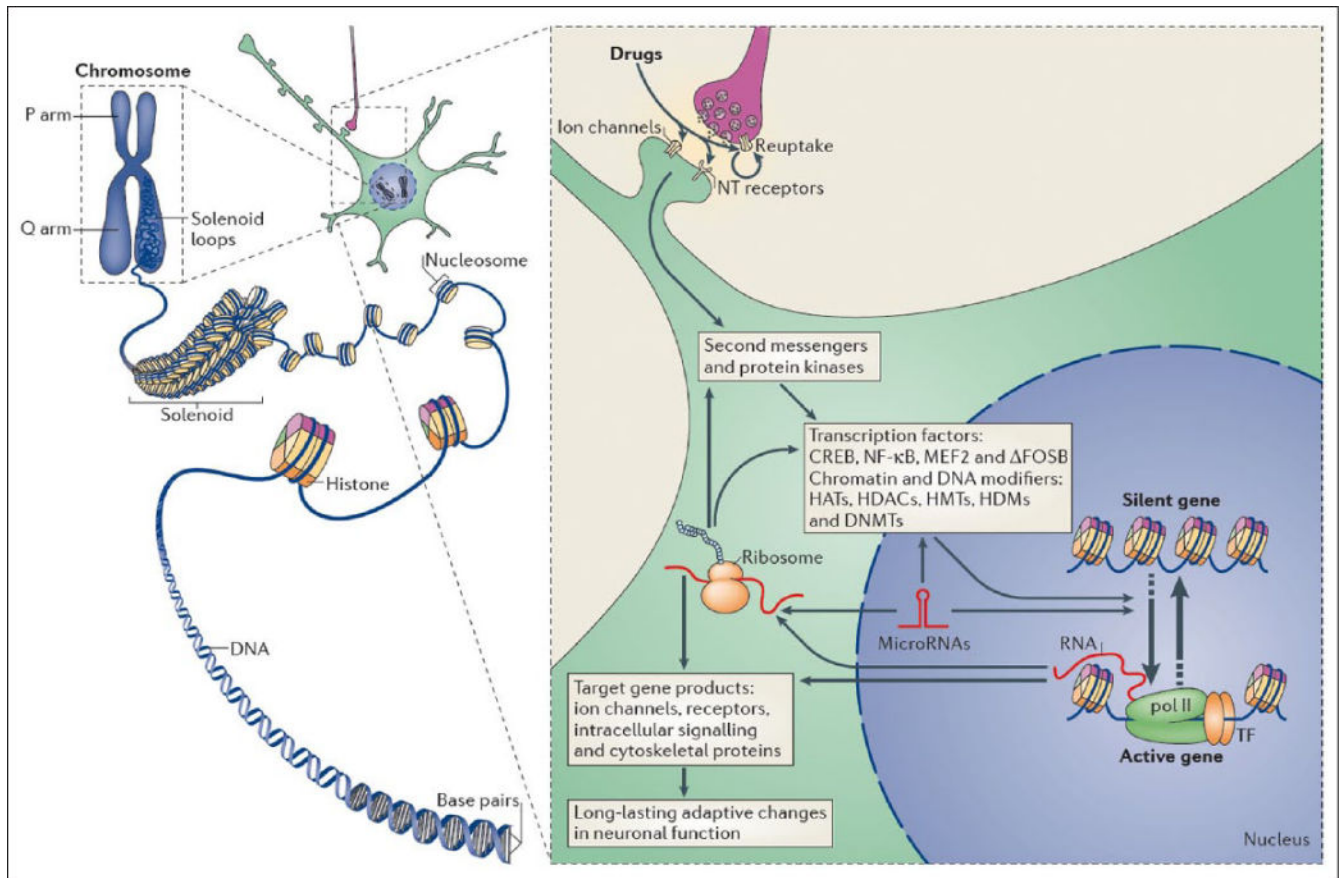
Figure 2.
Epigenetic regulation in brain. Left: In eukaryotic cells, DNA wraps around histone octomers to form nucleosomes, which are then further organized and condensed to form chromosomes. Unraveling compacted chromatin makes the DNA of a specific gene accessible to the transcriptional machinery. Right: Stress and other environmental stimuli act in large part by altering synaptic function to alter intracellular signaling cascades, which leads to the activation or inhibition of transcription factors and of many other nuclear proteins; the detailed mechanisms involved in the latter remain poorly understood. This leads to the induction or repression of particular genes, including those for noncoding RNAs; altered expression of some of these genes can in turn further regulate gene transcription. It is hypothesized that some of these changes at the chromatin level are extremely stable and thereby underlie lifelong susceptibility to mental illness. CREB = cAMP response element binding protein; DNMTs = DNA methyltransferases; HATs = histone acetyltransferases; HDACs = histone deacetylases; HDMs = histone demethylases; HMTs = histone methyltransferases; MEF2 = myocyte enhancing factor-2; NFκB = nuclear factor κB; pol II = RNA polymerase II. From Robison and Nestler (2011) (permission not required).

Repressed

Active

HDAC

HAT

Transcription factor

HMT

pol II

Heterochromatin

Euchromatin

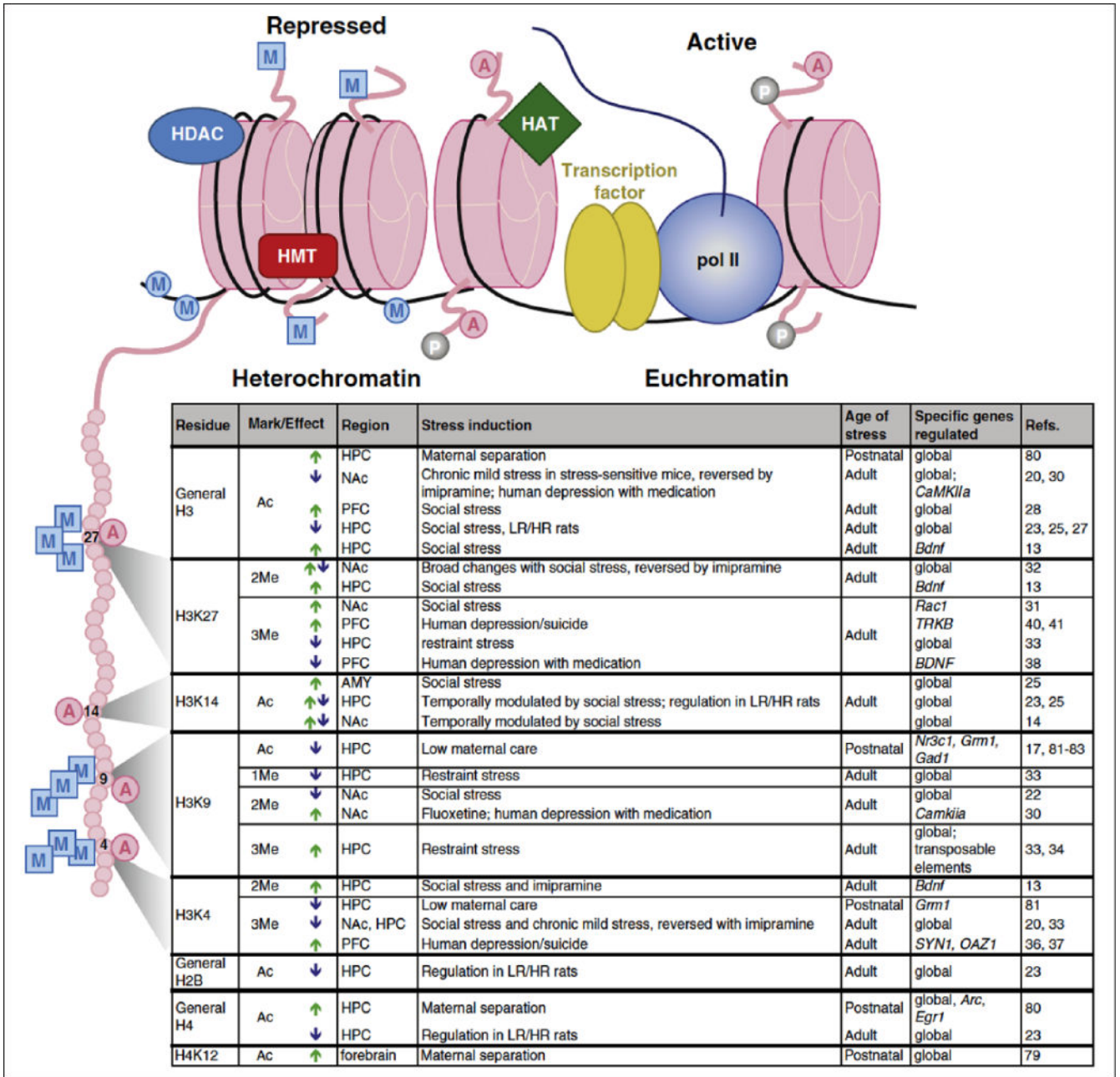| Residue	Mark/Effect		Region	Stress induction	Age of stress	Specific genes regulated	Refs.
General H3	Ac	↑	HPC	Maternal separation	Postnatal	global	80
		↓	NAc	Chronic mild stress in stress-sensitive mice, reversed by imipramine; human depression with medication	Adult	global; CaMKIIa	20, 30
		↑	PFC	Social stress	Adult	global	28
		↓	HPC	Social stress, LR/HR rats	Adult	global	23, 25, 27
		↑	HPC	Social stress	Adult	Bdnf	13
H3K27	2Me	↑↓	NAc	Broad changes with social stress, reversed by imipramine	Adult	global	32
		↑	HPC	Social stress		Bdnf	13
	3Me	↑	NAc	Social stress		Rac1	31
		↑	PFC	Human depression/suicide		TRKB	40, 41
		↓	HPC	restraint stress	Adult	global	33
		↓	PFC	Human depression with medication		BDNF	38
H3K14	Ac	↑	AMY	Social stress		global	25
		↑↓	HPC	Temporally modulated by social stress; regulation in LR/HR rats	Adult	global	23, 25
		↑↓	NAc	Temporally modulated by social stress		global	14
H3K9	Ac	↓	HPC	Low maternal care	Postnatal	Nr3c1, Grm1, Gad1	17, 81-83
	1Me	↓	HPC	Restraint stress	Adult	global	33
	2Me	↓	NAc	Social stress	Adult	global	22
		↑	NAc	Fluoxetine; human depression with medication		Camkiia	30
	3Me	↑	HPC	Restraint stress	Adult	global; transposable elements	33, 34
H3K4	2Me	↑	HPC	Social stress and imipramine	Adult	Bdnf	13
	3Me	↓	HPC	Low maternal care	Postnatal	Grm1	81
		↓	NAc, HPC	Social stress and chronic mild stress, reversed with imipramine	Adult	global	20, 33
		↑	PFC	Human depression/suicide	Adult	SYN1, OAZ1	36, 37
General H2B	Ac	↓	HPC	Regulation in LR/HR rats	Adult	global	23
General H4	Ac	↑	HPC	Maternal separation	Postnatal	global, Arc, Egr1	80
		↓	HPC	Regulation in LR/HR rats	Adult	global	23
H4K12	Ac	↑	forebrain	Maternal separation	Postnatal	global	79

Figure 3.

Examples of chromatin modifications regulated by stress or antidepressant treatment. Illustration (*top*) indicates histone octamers (*pink*) in heterochromatin (*left*) and euchromatin (*right*), along with associated proteins and histone tail/DNA modifications. Table (*bottom*) lists histone tail modifications of specific residues—depicted on the expanded histone tail illustration (*left*)—that are regulated by various stress paradigms or antidepressant treatments within the indicated brain regions. Arrows indicate an increase (*green*) or decrease (*blue*) in specific modifications. A = acetylation; P = phosphorylation; M (in a square) = histone methylation; M (in a circle) = DNA methylation; AMY = amygdala; HAT = histone acetyltransferase; HDAC = histone deacetylase; HPC = hippocampus; HMT =

histone methyltransferase; HR and LR = high responding and low responding, respectively (with respect to baseline locomotor activity); pol II =RNA polymerase II. Modified from Peña and others (2014) with permission.
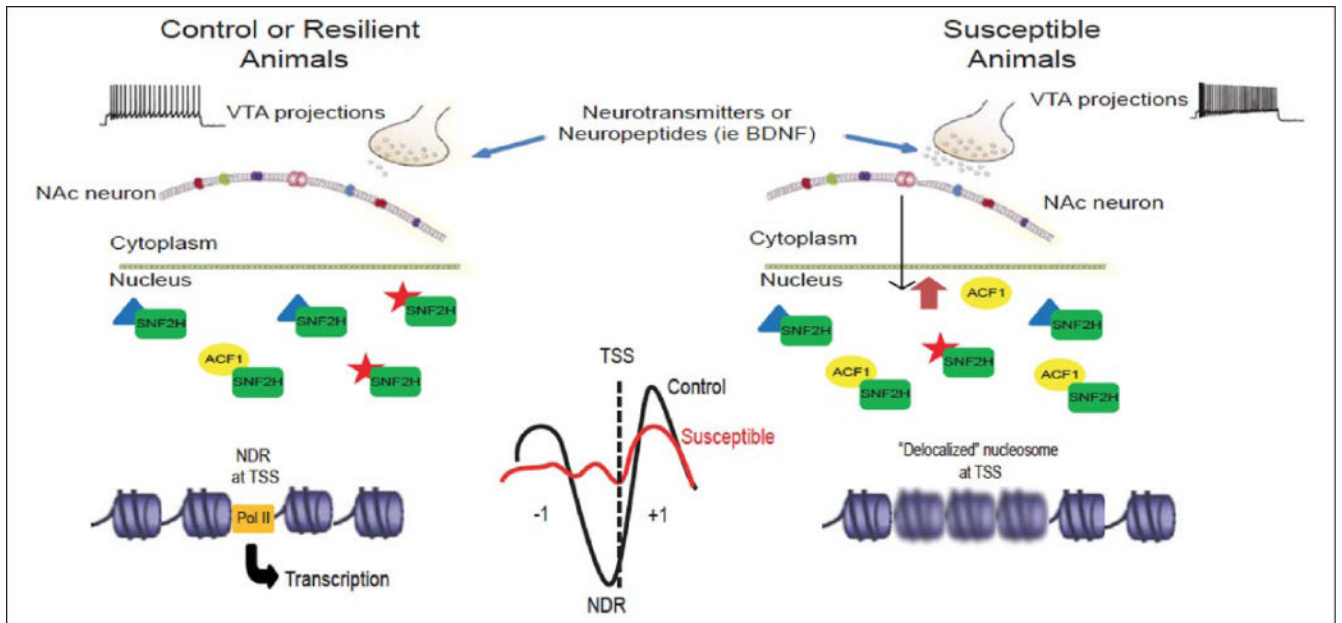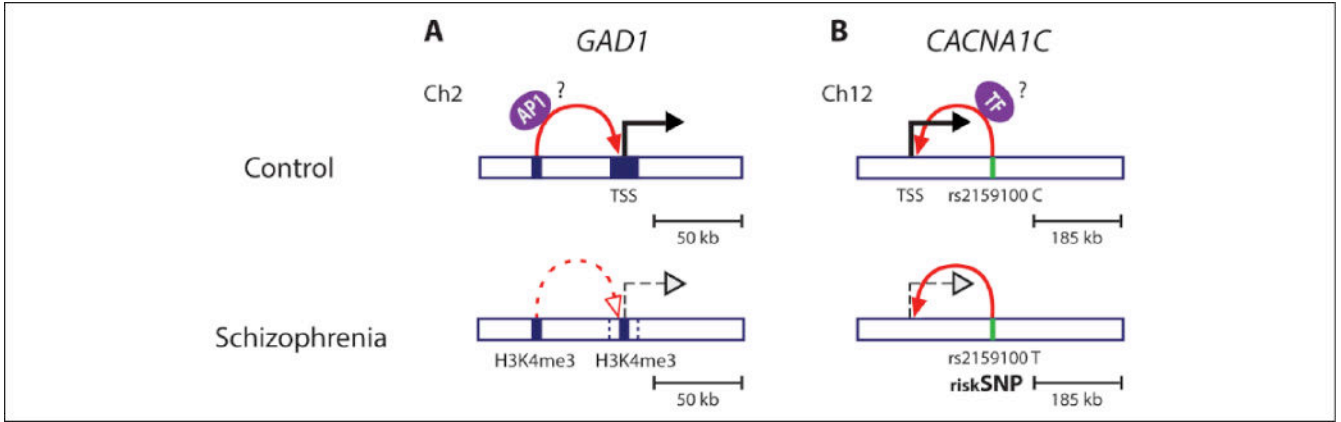
Figure 4.
Hypothesized role of chromatin remodeling ACF complex in NAc in stress susceptibility. Chronic social defeat stress CSDS, via increased burst firing of VTA neurons and BDNF release, induces ACF1 expression in NAc. The resulting upregulation of ACF complex activity, possibly through changes in TSS (transcription start site) nucleosome positioning, represses a set of genes in NAc, the reduced expression of which contributes to susceptibility. Blurry nucleosomes in the right figure represent weakly positioned or delocalized nucleosomes at TSSs. From Sun and others (2015) (permission not required).

Figure 5.
Higher order chromatin structure and schizophrenia. The role of higher order chromatin structure in transcriptional regulation of SCZ relevant genes has been shown for *GAD1* (A) and *CACNA1C* (B). (A) *GAD1*, encoding GABA synthesis enzyme, is located on chromosome 2 (Ch2) and frequently down-regulated in cerebral cortex of SCZ patients (dashed black arrow) and this is associated with lower levels of active histone marks, including H3K4me3 (blue square) at the *GAD1* transcription start site (TSS). The TSS region of *GAD1* has been shown to physically interact with an AP1 motif-enriched enhancer region located 50 kb further upstream (also enriched in H3K4me3 mark, blue square). Evidence has been presented that a chromatin loop (red arrow), that may carry a cargo such as AP1 transcription factors (purple oval) into close proximity to the core promoter region facilitating *GAD1* gene transcription, is weakened in brains of SCZ patients brain (dashed red arrow). This could contribute to lower *GAD1* expression. (B) Several single nucleotide polymorphisms (SNPs) residing in noncoding regions of the *CACNA1C* gene on chromosome 12 (Ch12) have been associated with lower *CACNA1C* expression and SCZ risk. The rs215100 T SCZ risk allele (green bar) resides in an intronic enhancer region, 185 kb downstream from the *CACNA1C* TSS, which has been shown to physically interact with the *CACNA1C* TSS (solid red arrow). The T allele confers lower transcriptional activity (dashed black arrow) as compared to C allele (solid black arrow), presumably by affecting the binding of transcription factors (TF, purple oval) and their interaction via chromosomal loops with the promoter *CACNA1C* region.