Published in final edited form as:

Harv Rev Psychiatry. 2015 ; 23(3): 212–222. doi:10.1097/HRP.0000000000000048.

Epigenetic Mechanisms in the Pathophysiology of Psychotic Disorders

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Abstract

Epigenetics is the study of chromatin—the physical material that forms chromosomes, composed of DNA wound around specialized histone proteins—and of how the modification of chromatin acts to establish stable states of gene expression in a cell-specific manner. Chromatin is regulated through three mechanisms: DNA methylation, histone modification, and RNA interference. These basic biological processes form the molecular interface between the genome and the environment, contributing to the regulation of gene expression in health and disease. Investigation of epigenetic mechanisms is yielding exciting insights in many areas of medicine, and a large and rapidly growing literature describes epigenetics as central to many aspects of the pathophysiology of psychotic disorders. This article first discusses speculative points as to why the mechanisms of epigenetics may be satisfying explanatory mechanisms in the etiology of psychotic disorders, then details emerging experimental evidence of roles for the three types of epigenetic mechanisms in these illnesses, and finally discusses these mechanisms as potentially compelling areas of research for the development of future treatments.

Keywords

bipolar disorder; DNA methylation; epigenetics; histone modification; psychosis; schizophrenia

Psychiatric disorders are not simple Mendelian diseases, and the complexity of their patterns of inheritance and the variability of their presentations have hindered attempts to understand their etiology. In twin studies, schizophrenia and bipolar disorder show concordance rates of 50% and 40%–70%, respectively,^{1,2} with discordance attributed to the influence of nonshared environmental factors. These findings raise important and exciting questions. What are these mitigating factors? What are the biological mechanisms that mediate their effects? Are these mechanisms possible points of intervention to ameliorate or even prevent psychopathology? The emerging field of psychiatric epigenetics offers hope of answering questions such as these in the study of mental illnesses. This article discusses the application of epigenetics to the study of psychotic disorders and will highlight recent advances in our understanding of the interaction between an individual's genome and environment in the pathogenesis of these illnesses.

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Declaration of interest: The author reports no conflicts of interest. The author alone is responsible for the content and writing of this article.

EPIGENETICS

While almost every cell in the human body shares an identical genome, those cells form distinct tissues composed of diverse cell types that are differentiated to perform specialized tasks. And although identical twins share an identical genome in every neuron within their brains, their separate experiences throughout life result in the development of distinct individuals. The influence of the dynamic environment on what has traditionally been thought of as a static genome has long been of interest to medicine, and in recent years our understanding of the molecular mechanisms that mediate this interplay has greatly advanced through the study of epigenetics. Epigenetic mechanisms are basic biological processes that have been studied extensively in cancer and in many areas of behavioral neuroscience, and a growing body of evidence suggests that epigenetics plays an important role in the complex pathophysiology of psychotic disorders.

Epigenetics is a relatively young field that is focused on understanding the role of changes to chromatin (the physical material that forms chromosomes, composed of DNA wound around specialized histone proteins; see Figure 1) in the regulation of gene expression. Changes to chromatin structure result in stable and heritable (across cell division within an individual organism, and in some cases from individuals to their offspring)³ states of gene expression, without changes to the underlying DNA sequence. Three main divisions of epigenetic mechanisms serve to regulate chromatin structure and function: DNA methylation, post-translational histone modification, and RNA interference.

While epigenetics is thought of as the molecular interface between the genome and the environment, the specific environmental inputs that regulate these processes are just now being elucidated. Numerous environmental toxins, including metals,⁴ air pollutants,⁵ and persistent organic pollutants,⁶ exert their effects by modifying epigenetic marks. An individual's unique experience has also been shown to regulate chromatin structure; for example, early-life maternal care regulates DNA methylation at the promoter of the *GAD1* gene (*GAD1* encodes glutamic acid decarboxylase 67, one of two enzymes that catalyze the synthesis of GABA from glutamate) throughout life in rat hippocampus.⁷ A role for chromatin dynamics in each individual neuron's genomic response to that cell's unique environment within the brain (e.g., pattern of synaptic input and output, exposure to neurotrophic factors, and extracellular matrix) is beginning to emerge.⁸ Neuronal activation has long been associated with increased expression of c-fos^{9,10} and brain-derived neurotrophic factor (BDNF).¹¹ Contextual fear conditioning is a common experimental paradigm in the study of learning and memory, where animals learn to associate an otherwise benign event or environment with an aversive stimulus. Learning in this context leads to the induction of BDNF exons I, IV, VI, and DC and decreased DNA methylation of these regions through an NMDA receptor-dependent mechanism.¹² Animal models of addiction have been used to demonstrate neuronal activity-dependent phosphorylation of methyl-cytosine binding protein 2 (MeCP2), a "reader" of DNA methylation that recruits additional chromatin modifiers to a locus, mediated by calcium influx.¹³ A detailed understanding of how the complex system of chromatin regulation functions in the healthy brain to produce neuronal plasticity, and how it is dysfunctional in the emergence of psychiatric disease, is a grand challenge in the field.

As epigenetic mechanisms are basic biochemical pathways essential to the function of every cell and cell type, this rapidly expanding field offers great hope for improved understanding and interventions in virtually all areas of medicine. The field of psychiatry, which has long searched for a rigorous scientific understanding of the interplay between genome and environment, or nature and nurture, stands to benefit especially from the study of epigenetics. Epigenetics is currently being applied to a great number of psychiatric illnesses across the entire breadth of the field. One area that lends itself especially well to epigenetic investigation is addiction research, which at its core is the study of how a specific group of environmental exposures (drugs of abuse) leads to lasting changes in the structure and function of the brain, ultimately resulting in persistent behavioral pathology.¹⁴ While investigation of epigenetics is bearing fruit in multiple areas of psychopathology, the current discussion will focus on recent work applying the concepts of epigenetics to the study of psychotic disorders.

Molecular analysis of psychotic disorders has documented a great many diagnosis, neuronal circuitry, and cell type-specific changes in gene expression within the brains of affected individuals. Evidence for genetic mutations causative of these disorders has remained elusive, however, and the growing list of observed diagnosis-associated changes in DNA sequences contributes to disease pathogenesis in a non-straightforward manner.¹⁵ For these reasons, there is intense and growing interest in the role of epigenetics in the molecular pathophysiology of psychotic illness. This article will first describe speculative evidence as to why the mechanisms of epigenetics would be satisfying explanatory mechanisms in the etiology of psychotic disorders, then detail emerging experimental evidence of roles for the three types of epigenetic mechanisms in these illnesses, and finally discuss these mechanisms as potentially compelling areas of research for the development of future treatments.

CIRCUMSTANTIAL EVIDENCE

A heritable component is clearly at work in the pathogenesis of schizophrenia, as there is a 1% prevalence in the general population and a 50% concordance rate in monozygotic twins, yet over many years, linkage studies have failed to identify susceptibility genes of large effect. The downregulation of several genes such as *GADI* and *RELN* in the absence of mutations in these genes^{16,17} supports the role of epigenetic mechanisms in this disease. An important question is whether these modifications are causative of schizophrenia or are occurring secondarily. An epigenetic hypothesis of the origin of psychotic illnesses—and perhaps of psychiatric disease in general—has many advantages over hypotheses based on classic Mendelian genetics.

The first quality of epigenetic mechanisms that make them an attractive candidate in schizophrenia neuropathology is their metastable nature. Schizophrenia is characterized by a relapsing and remitting course, which is difficult to explain with a hypothesis based on any number of susceptibility genes. An inherited mutation or combination of susceptibility genes is stable, constant throughout the lifetime, and present in every cell of an individual. But that leaves the external environment as the only source of variability to dictate the very different

courses the disease may take—a hypothesis that is undermined by studies of monozygotic twins.¹⁸

By contrast, an epigenetic etiology offers more flexible outcomes than a hypothesis based on any number of DNA mutations. Genes may be inactivated by changes in chromatin structure, but this inactivation is reversible.^{19,20} In an epigenetic hypothesis of psychotic illness, dysfunction in a particular population of cells could fluctuate, crossing the threshold of disease repeatedly to account for remission and relapse.

Another quality of epigenetics that makes it distinct from more traditional genetic theories is the cell-specific nature of epigenetic mechanisms. As mentioned above, a DNA mutation is present in every cell of the body. The fidelity of epigenetic information is much lower than that of DNA replication,²¹ making it possible that during development, different cell lineages will adopt different epigenetic states, populating tissues and organs with these different states. This is, again, a possible explanation for the observed discordance for schizophrenia between monozygotic twins. Although the twins begin with identical DNA sequence and epigenetic profiles, epigenetic differences—ones that arise, during development, due to metastability induced by internal and external signals—may be partitioned differently between tissues, leading in the end to epigenetic inequalities between the brains of the twins.²² This partitioning of epigenetic dysfunction could also explain the widely varying subtypes of schizophrenia, where the particular array of cells and brain regions affected negatively by epigenetic events dictates a patient's particular experience with the disease.

Finally, epigenetic mechanisms are, by nature, stochastic events. This stochasticity, which is evident from the metastable and cell-specific aspects of epigenetics, is another feature of epigenetics that could help resolve the dauntingly inconsistent heritability of schizophrenia. Schizophrenia vulnerability is clearly a familial trait, but the mechanisms that allow the disease to “skip a generation” and to affect only one of a pair of monozygotic twins cannot be adequately explained with a static genetic model. Perhaps the inherited risk factor is a disposition to an epigenetic mechanism predisposing an individual to the disease, but what allows the disease to manifest is a stochastic epigenetic event, occurring randomly at a particular frequency in the development of the brain or of a section or sections of the brain.

DNA METHYLATION

In mammalian genomes, DNA may be methylated at carbon 5 of cytosine residues found in cytosine-guanine dinucleotides (CpGs; see Figure 2). This reaction is catalyzed by a family of DNA methyltransferase enzymes (DNMTs) composed of DNMT1, DNMT3a, and DNMT3b in humans. The human genome contains ~28 million CpGs, and 70%–80% are methylated in any given cell type.²³ The classic conception is that DNA methylation acts to inhibit gene activity, such that methylation of CpGs in a region of the genome acts to decrease transcription of genes in that region. This effect occurs through two mechanisms. First, when cytosine residues within the binding sequence of a transcription factor are methylated, that transcription factor may be blocked from binding. This effect varies from one transcription factor to another; for example, transcription factors CREB, E2F, and NF-

kappaB have been shown to be sensitive to methylation of cytosine residues within their binding sites,^{24–26} whereas the factors CTF, Sp1, and TCR-AFF are not affected by DNA methylation.^{27–29} Second, when cytosine residues in a region of the genome become methylated, they are recognized within the major groove of the DNA helix and bound by members of the methyl-binding domain protein family. Bound proteins in this family then act to recruit additional protein factors to the region to further modify the epigenetic state of that genomic locus. An inverse correlation between DNA methylation and gene expression holds true at promoter regions of genes and was thought to be universal, as early studies focused largely on promoter regions. With advancing technology, more recent work has assessed DNA methylation at a broader array of DNA elements, and more complex outcomes of DNA methylation have been observed. For example, gene body DNA methylation is positively correlated with gene expression, appears to support transcriptional elongation, and may play a role in regulation of mRNA splicing, and DNA methylation of enhancer and insulator elements has complex and often idiosyncratic outcomes.³⁰

Over evolutionary timescales, CpG dinucleotides have been depleted from the genome, as methylated cytosine is a hypermutable base. Cytosine residues can undergo spontaneous deamination, creating uracil that is recognized as foreign to the DNA and repaired. When methylcytosine undergoes deamination, however, the resulting base is thymine, which is not foreign to DNA, with the consequence that this mutation is repaired with lower efficiency. Within the genome there are regions called CpG islands, where CpG dinucleotides have been preserved through unknown mechanisms, and DNA methylation levels are ordinarily low. CpG islands are associated with the promoter regions of ~70% of known genes,³¹ and they play an important role in the regulation of gene activity.

Animal studies have shown DNA methylation to be a potent regulator of behavior—in some cases mediating a lifelong effect of early experience on temperament. Michael Meaney's group at McGill University has investigated a natural variation in rats, as some rat mothers are highly attentive to their offspring (highly attentive mothers), exhibiting high levels of a so-called licking-grooming behavior, whereas other mothers display low levels of this activity (lowly attentive mothers).³² This behavior is heritable, and in this paradigm, being raised by a lowly attentive mother is equated to experience of neglect. Dr. Meaney's group has found that offspring of lowly attentive mothers express low levels of the glucocorticoid receptor in the hippocampus and that this process is mediated by increased DNA methylation at this genomic locus. The outcome of this change is less effective negative feedback within the HPA axis, resulting in greater levels of circulating glucocorticoids and higher stress-reactivity throughout the lifespan. This phenomenon is reversible by cross-fostering; that is, offspring of lowly attentive mothers who are raised by highly attentive mothers develop into adults with low stress-reactivity, and vice versa.³² Interestingly, Dr. Meaney's group has extended this research into the effects of early life experience in humans who develop depression and suicidally;³³ they found increased DNA methylation at a neuron-specific glucocorticoid receptor gene and decreased gene expression in suicide victims with a history of childhood abuse.

Changes in DNA methylation at multiple genomic loci have been associated with the diagnoses of schizophrenia or bipolar disorder. In 2008, Mill and colleagues³⁴ used a CpG

island microarray approach to interrogate methylation-depleted genomic DNA samples obtained from postmortem frontal cortex of schizophrenia, bipolar disorder, and control subjects. This study observed DNA methylation changes at approximately 100 sites across the genome, including hypomethylation of two glutamate receptor-related genes (*WDR18* and *GRIA2*) and of two genes related to GABA signaling (*MARLIN-1*, encoding an RNA-binding protein involved in GABA_B-receptor production, and *KCNJ6*, encoding a G protein-coupled potassium channel). This study also found DNA methylation changes at multiple genes involved in neurodevelopment that were specific to the female component of the cohort (hypermethylation of *WNT1*, *NR4A2*, and *LHX5*, and hypomethylation of *LMX1B*).³⁴

Many studies have used site-specific techniques to investigate DNA methylation changes in psychotic disorders at predetermined regions of the genome. The *RELN* gene encodes reelin, an extracellular matrix protein secreted by GABAergic neurons that is downregulated in schizophrenia and is important for both cortical development and synaptic function throughout life. DNA methylation of the *RELN* promoter has been closely studied by multiple groups, and in 2005, two groups using complimentary methods^{35,36} found it to be increased in schizophrenia. The work of a third group failed to replicate these findings.³⁷ As mentioned above, the *GADI* gene encodes glutamic acid decarboxylase 67, the rate-limiting enzyme in the synthesis of GABA, and this gene has been consistently shown to be downregulated in the brains of schizophrenia and bipolar disorder patients.^{38–41} Cell culture experiments investigating the *GADI* gene have demonstrated a potent effect of DNA methylation on *GADI* transcription,⁴² and analysis of postmortem brain tissue has shown decreased methylation of the *GADI* promoter in schizophrenia patients.⁴³

The cause(s) of these changes remains poorly understood and is an area of active research. Transcription of the *DNA methyltransferase 1* gene is increased in specific populations of GABAergic interneurons in schizophrenia and bipolar disorder,^{44–46} and schizophrenia and bipolar disorder postmortem brain tissue contains a twofold increase of S-adenosyl methionine, the methyl donor in the DNA methylation reaction (see Figure 2). Multiple studies have observed increased levels of S-adenosyl homocysteine, the by-product of DNA methylation, in the circulation of male schizophrenia patients.^{47–50} These data support the concept of hyperactivity of the DNA methylation pathway in these disorders, but this inference is likely an oversimplification, as both hypermethylation and hypomethylation of various genes are observed in psychotic disorders, and global DNA methylation levels are unchanged.⁵¹

Due to the highly stable carbon-carbon bond linking the methyl group to the modified cytosine residue, DNA methylation was originally thought to be irreversible, and the possibility of active DNA demethylation was a long-lived controversy in the field. Recently, active demethylation of DNA has been shown to occur through the intermediary step of 5-hydroxymethylcytosine (5hmC)¹⁹ (Figure 2). 5-methylcytosine (5mC) is converted to 5hmC by oxidation catalyzed by the family of ten-eleven translocation methylcytosine dioxygenase enzymes (TET1, TET2, and TET3 in humans). Further oxidation reactions convert 5hmC to 5-formylcytosine (5fC) and 5fC to 5-carboxylcytosine (5-caC). The cytosine variants 5hmC, 5fC, and 5-caC can be removed by thymine DNA glycosylase, resulting in an abasic

site, which is then repaired through the base excision repair pathway.²⁰ One member of the TET family of enzymes, TET1, is overexpressed in the parietal cortex of psychotic patients,⁵² and this change was associated with increased global levels of 5hmC. The role of 5hmC and active DNA demethylation in the pathophysiology of psychotic disorders is an area of active research.

Perhaps even more exciting than the elucidation of the mechanisms of reversal of the stable mark of cytosine methylation is the recent discovery that specifically in the brain, DNA methylation occurs on cytosine residues outside of CpG dinucleotides. This methylation on non-CpG cytosines, whose frequency is similar to that within CpG dinucleotides, is specific to neurons, where it may actually be the dominant form of cytosine methylation.⁵³ The developmental timing of the accumulation of non-CpG cytosine methylation in the neuronal genome parallels increased expression of multiple “readers” of DNA methylation— notably MeCP2, which is disrupted in the neurodevelopmental disorder Rett syndrome.⁵⁴ Neuron-specific DNA methylation is speculated to regulate either experience-dependent patterns of synaptic development or the elaboration of the many distinct neuronal subtypes in the brain, or both.⁵⁵ The discovery of the prevalence of non-CpG cytosine methylation specifically in neurons reframes one of the central tenants of this young field, demonstrates how much is left to be learned, and strongly supports a central role for DNA methylation in regulating the many complex cellular processes unique to neurons.

HISTONE MODIFICATION

Chromosomes are not composed of naked DNA molecules but, instead, are formed of incredibly long DNA molecules wound around specialized proteins called histones to form chromatin. The smallest unit of chromatin is the nucleosome, composed of 146 base pairs of DNA wound around a group of eight histone proteins, two each of histones H2a, H2b, H3, and H4. Each histone protein contains a globular domain within the core of the nucleosome, as well as an aminoterminal tail that protrudes out from the core (Figure 1). Amino acids within these tails are specifically recognized and chemically modified by a vast number of enzymes, resulting in a large array of modifications, including acetylation, methylation, phosphorylation, ubiquitination, and sumoylation; the list is long and continues to grow (Figure 3).

Like DNA methylation, histone modification controls local gene activity through two mechanisms. First, the modification of histone tails acts to modify the charge of the histone proteins, strengthening or weakening the electrostatic interaction between the positively charged histone proteins and the negatively charged DNA. The strength of this interaction affects how tightly condensed the chromatin in a region is and therefore how accessible the DNA in that region is to the transcriptional machinery. Tightly condensed regions repressive of transcriptional activity are termed “heterochromatin,” whereas loosely condensed regions accessible for transcription are termed “euchromatin.” Second, specific histone modifications or combinations of modifications are recognized and bound by proteins that then act to recruit additional protein factors to further modify the chromatin in the region.

While DNA modification occurs exclusively through cytosine methylation (with important exceptions, e.g., cytosine hydroxymethylation), histone proteins can be modified in a large and varied number of ways. A recent study reported greater than 4000 distinct combinations of histone modifications⁵⁶ within individual nucleosomes. This incredibly complex system has been proposed to be the basis of a “histone code” that encodes a wealth of genomic regulatory information in the complex pattern of histone modifications present across the genome.⁵⁷ This high level of diversity may one day allow for greater genomic or neural-circuit specificity of therapeutic agents targeting histone modification than is likely possible for therapeutic agents targeting the relatively limited system of DNA methylation.

Among these many histone modifications, the two that have been best characterized to date are histone acetylation and histone methylation. Histone acetylation is controlled by the opposing actions of histone acetyltransferase (HAT) and histone deacetylase (HDAC) enzymes, and this modification occurs on the epsilon-amino groups of conserved lysine residues. Histone acetylation has long been associated with increased transcriptional activity through the mechanisms described above.⁵⁸ Acetylated lysine residues are recognized and bound by many proteins through bromodomains, including multiple components of the core transcriptional machinery. One of the most direct links between histone acetylation and transcriptional activation is the binding of TAFII250 (a component of the Transcription Factor IID complex, which is itself an active HAT) to acetylated histones through its two bromodomains.⁵⁹

Multiple lines of evidence point to a role for histone acetylation in the pathogenesis of psychotic disorders. The administration of high doses of methionine to mice has been used to model molecular and behavioral aspects of schizophrenia,⁶⁰ including hypermethylation and decreased expression of the *RELN* and *GADI* genes. Molecular and behavioral abnormalities in this model are rescued by the coadministration of HDAC inhibitor compounds (HDACi's). Overexpression of HDAC1 and HDAC2 in mouse brain results in decreased prepulse inhibition and other behaviors reminiscent of schizophrenia.^{61,62} In cell culture experiments, HDACi's induce increased expression of the *GADI* gene.⁶³ More directly, postmortem brain tissue from patients with schizophrenia express increased levels of HDAC1, and this increase is correlated with decreased expression of *GADI*.^{64,65}

Histone methylation is more complex than acetylation, occurring on both lysine and arginine residues in multiple configurations. Arginine can be monomethylated or dimethylated, and in the case of dimethylation, the methylation may be either symmetric or asymmetric. Lysine can be mono-, di-, or trimethylated. The different methylation levels of these residues are produced by the action of many distinct enzymes, including histone methyltransferases and histone demethylases. In addition, different levels of methylation have distinct effects, affording a greater diversity of outcomes to the methylation of histone tails with respect to other modifications.⁶⁶

Unlike histone acetylation, which predominantly serves to increase transcriptional activity, histone methylation can act either to increase or to repress transcriptional activity, depending on the specific residue that is methylated and the other modifications that are present in the region. For example, methylation of lysine 9 of histone H3 is associated with

transcriptionally silent chromatin regions,⁶⁷ whereas methylation of lysine 4 of histone H3 is associated with transcriptionally active regions.⁶⁸ Histone methylation can even act through the single protein HP1 to cause either gene activation or gene silencing, depending on the chromosomal context in which it is found.⁶⁹

Autolysis of histone modifications is potentially problematic for their being studied in postmortem human brain tissue, but histone methylation is more stable and less susceptible to autolysis than other modifications, making it especially appealing for postmortem analysis. Histone H3 lysine 4 trimethylation (H3K4me3) is a modification associated with transcriptional start sites and active euchromatic regions, whereas histone H3 lysine 27 trimethylation (H3K27me3) is a modification associated with heterochromatic regions repressive of gene activity.⁷⁰ In the prefrontal cortex of schizophrenia patients, decreased H3K4me3, accompanied by increased H3K27me3, has been observed at the promoter region of the *GAD1* gene,⁷⁰ which as described above is known to be downregulated in this disorder. A separate study by the same group found a genome-wide 30% increase in histone H3 arginine 17 methylation, a modification associated with transcriptional inhibition, in a subset of the schizophrenia patient cases within a cohort of postmortem human prefrontal cortex tissue samples.⁷¹ This change was associated with altered expression of metabolism-related genes in this brain region. Finally, in a recent study analyzing genome-wide association data from more than 60,000 individuals, the Psychiatric Genomics Consortium⁷² has identified methylation of H3K4 as the biological pathway most significantly associated with the grouped diagnoses of schizophrenia, bipolar disorder, and major depression.

RNA INTERFERENCE

RNA interference describes a complex set of mechanisms that regulate gene expression both pre- (RNA-induced transcriptional silencing) and post-transcriptionally (post-transcriptional gene silencing). Post-transcriptional gene silencing through targeted degradation of RNA transcripts in the cytoplasm is the more established route of RNA interference and can be triggered by both exogenous double-stranded RNA (dsRNA, as in the case of viral infection) or by endogenous micro-RNA (miRNA) encoded by the cell's own genomic DNA. These pathways converge when the processed dsRNA or miRNA is bound by the RNA-induced silencing complex (RISC). The bound RNA molecule, or "guide strand," allows RISC to specifically recognize and bind a target RNA molecule complementary to the guide strand, resulting in degradation of the target.

More recently, mechanisms of RNA interference have been shown to regulate gene expression at the level of transcription. This regulation occurs through the interaction of either a processed dsRNA or miRNA with the RNA-induced transcriptional silencing (RITS) complex. The RITS complex is then involved in the establishment, maintenance, and spread of a heterochromatic state at the genomic locus targeted by its bound guide strand. As most of the work describing the RITS complex has been performed in yeast, the extent of conservation of these mechanisms across species, as well as their role in epigenetic regulation of gene expression in humans, is an area of active research.

Although RNA interference is more poorly understood than other epigenetic mechanisms, mounting evidence suggests its involvement in the pathology of psychotic disorders. Abnormal expression of several miRNAs has been observed in the cortex of schizophrenia patients (increased expression of miR-16, -30b, and -181b, and decreased expression of miR-132).^{73–76} In a recent genome-wide association study of ~25,000 schizophrenia patients and 30,000 control subjects, miR-137 was associated with the most statistically significant disease-associated locus. In this same study, four of the other genome-wide significant locations were associated with genes predicted to be targets of regulation by miR-137.⁷⁷

TREATMENT IMPLICATIONS

In addition to the explanatory power of epigenetics in the pathophysiology of psychotic disorders, much of the allure of applying these concepts to the study of psychosis is the possibility of novel and improved treatment options for patients. Current drugs targeting DNA methylation are used as chemotherapy agents but are likely too toxic for psychiatric applications. Furthermore, most DNA methyltransferase inhibitors do not cross the blood-brain barrier and are active only during DNA replication, precluding their use in post-mitotic neurons.⁷⁸ As discussed above, the more diverse system of histone modification is likely more amenable to pharmacologic intervention.

Histone acetylation is important in cognitive functions, including learning and memory, and is of great interest in treating the cognitive symptoms of schizophrenia. In animal models, HDACi's have been shown to modulate fear conditioning; they enhance consolidation of fear memories when administered in rat amygdala⁷⁹ and facilitate extinction when infused into mouse hippocampus.⁸⁰ An extensive literature has investigated the use of valproic acid in treating schizophrenia, and this long-used drug has recently been shown to function as a nonselective inhibitor of class I HDAC enzymes.^{81,82} The augmentation of atypical antipsychotic medications with valproate in treating schizophrenia did not prove beneficial in a clinical trial,⁸³ but this outcome may have resulted from issues of valproate dosing.

Pharmacologic manipulation of the complex system of histone methylation is an exciting possibility in treating schizophrenia. Multiple histone methyltransferase inhibitors have recently been described, but their safety and efficacy in humans is still being studied.^{84,85} Histone demethylase inhibitors are also in development, and polyamine analogues have been developed that specifically increase levels of histone H3 lysine 4 dimethylation (H3K4me2), a marker of open euchromatic regions, leading to increased gene expression.⁷⁰

In addition to valproate, as discussed above, multiple existing drugs have recently been found to act at least in part through epigenetic mechanisms. In animal and cell culture experiments, lithium has been shown to induce decreased DNA methylation at multiple imprinted genes through a mechanism mediated by phosphatidylinositol 3-kinase and glycogen synthase kinase-3.⁸⁶ Dibenzodiazepine-type atypical antipsychotic medications, including clozapine, olanzapine, and quetiapine, have been shown to induce chromatin remodeling, including active DNA demethylation at GABAergic gene promoters in a dopamine-independent mechanism.^{87,88} These effects were not seen with other

antipsychotic agents such as haloperidol or risperidone. Finally, monoamine oxidase inhibitors have been suggested to be beneficial in treating schizophrenia,⁸⁹ and phenelzine and tranylcypromine are active as inhibitors of H3K4 demethylation.⁹⁰

Along with pharmacologic intervention, enhanced understanding of the epigenetics of psychotic disorders may one day produce less invasive interventions for these disorders in areas such as nutrition. The pathway of “one-carbon metabolism” provides methyl groups for a wide variety of methylation reactions in the cell, including DNA and histone methylation. Dietary folate is used to regenerate S-adenosyl methionine, the universal methyl donor, from S-adenosyl homocysteine, the by-product of methylation reactions, through a pathway involving methylene tetrahydrofolate reductase (MTHFR) and methionine synthase⁹¹ (Figure 2). In a multi-center clinical trial, folate supplementation was found to benefit negative symptoms of schizophrenia in a subset of the study population bearing a single nucleotide polymorphism (484C>T) in the *FOLH1* gene.⁹² *FOLH1* encodes glutamate carboxypeptidase, which is found in the intestinal brush border, where it facilitates absorption of dietary folate.

REMAINING CHALLENGES

Despite the explosive progress in our understanding of the epigenetics of behavioral neuroscience, a number of significant challenges remain. The brain is an amazingly complex organ composed of a large number of distinct classes of neurons, glia, and vascular and other supportive cells arranged in an intricate and highly orchestrated cytoarchitecture. The distinct neuronal subtypes play discrete roles in brain function in health, and pathological changes relevant to disease processes are not distributed equally across all neuronal subtypes. The vast majority of biochemical studies of brain tissue to date have been performed in homogenized cortex, which is problematic for at least two reasons. First, homogenizing the sample tissue discards a wealth of information in regard to the cellular and subcellular distribution of the molecules of interest, making it impossible to determine which subpopulations of cells are host to the findings of the study. Second, when the hypothesized change is present in only a fraction of the cells within the sampled tissue,⁴⁴ the signal will be lost or at least attenuated by including extraneous cell types in the measurement, leading to increased probability of type II statistical error. For these reasons, improved technology capable of sampling homogenous subpopulations of neurons from brain tissue for biochemical analysis is needed. Exciting work using fluorescence-activated cell sorting (FACS)⁹³ and microfluidic cell sorting is currently under way in pursuit of this goal. Another approach to this problem is analysis of isolated single neurons, but this approach will need to overcome significant limitations of throughput and also the limited sensitivity of downstream analytic techniques. In addition, psychopathology is produced not by altered function of single cells but by pathologic changes in the behavior of groups of neurons specialized to perform specific tasks at discrete locations within neuronal circuits.

Perhaps more problematic is the inaccessibility of human brain tissue for direct study. Epigenetic mechanisms are metastable and reversible, and having access to our organ of interest at only a single, postmortem time point makes it difficult or impossible to fully understand how these mechanisms are involved in disease progression, relapse, remission,

treatment response, and so on. Exciting work at the Lieber Institute for Brain Development has produced BrainCloud, a still-growing publicly available database of gene expression levels and DNA methylation measurements from postmortem human prefrontal cortex across the lifespan,⁹⁴ which demonstrates the dynamic nature of these phenomena.

Many groups are attempting to circumvent this problem by identifying other tissues that can be sampled from a living subject to offer insight into epigenetic processes relevant to brain function. Lymphocytes isolated from peripheral blood are the most commonly sampled tissue for this purpose. As mentioned above, epigenetic mechanisms act in a highly tissue-specific manner, and while changes in DNA methylation patterns are significantly correlated between the cortex and peripheral blood of an individual, differences in DNA methylation patterns between cortical tissue of separate subjects is greatly exceeded by the variation observed between cortex and blood within a single subject.⁹⁵ Even within the complex cytoarchitecture of the cortex, it has been shown that many aspects of the human neuronal epigenome are unique to neurons (i.e., not present in glia) and are unique to humans.⁹⁶ For these reasons, examination of human brain tissue, limited as it is, will be essential to further our understanding of the role of epigenetic mechanisms in the pathophysiology of psychosis. At the same time, investigations of peripheral human tissues and animal models are necessary and will hopefully lead to identification of biomarkers for disease susceptibility, progression, and response to pharmacotherapy. The combined efforts of multiple groups investigating postmortem human brain, peripheral human tissues and cultured cells, and animal models will hopefully be successful in forging a path forward.

CONCLUSIONS

Although the concepts of epigenetics were first described many decades ago, it is only within recent years that these ideas were embraced in the fields of neuroscience and psychiatry. Mounting evidence suggests that epigenetics may have a central role in the pathophysiology of psychotic disorders. Research in this relatively young field has already begun to yield results along the entire continuum, from biochemistry and basic neuroscience to potential therapies. Despite significant obstacles, research will continue to pursue the epigenetics of psychosis for this area's great promise of improved therapeutics for these devastating disorders. Regardless of how fruitful this line of investigation turns out to be in terms of treatment development for psychosis, epigenetic mechanisms will certainly continue to enhance our understanding of the neural underpinnings of behavior and how they are influenced by the experiences of each individual as they interact with their unique environment.

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Figure 1.

The physical structure of chromatin. The figure follows chromatin from the largest-scale structure, an entire chromosome, through progressive levels of structural organization and physical compaction, to the smallest-scale structures of individual DNA-associated nucleosomes and naked DNA. In reality, each nucleosome possesses eight amino-terminal tails (one from each histone protein), and each tail may be modified at multiple sites simultaneously. Lettered beads represent methylated cytosine residues within DNA and modifications present on amino acids within histone tails. A, acetylation; M, methylation; P, phosphorylation.

Figure 2.

Biochemical pathways of DNA methylation and demethylation and regeneration of the methyl-donor S-adenosyl methionine. 5caC, 5-carboxylcytosine; 5fC, 5-formylcytosine; 5hmC, 5-hydroxymethylcytosine; 5mC, 5-methylcytosine; BER, base excision repair; DNMT, DNA methyltransferase; MTHFR, methylene-tetrahydrofolate reductase; SAH, S-adenosyl homocysteine; SAM, S-adenosyl methionine; TDG, thymine DNA glycosylase; TET, ten-eleven translocation methylcytosine dioxygenase; THF, tetrahydrofolate.

Figure 3.

Simplified depiction of histone protein amino-terminal tails and sites of known post-translational modification relevant to regulation of chromatin function. A. A listing of the major post-translational modifications and the amino acids that have been observed to receive each modification within histone tails. B. Histone-tail amino acid sequences and known sites of modification for each of the four major histone proteins. Amino acids are numbered starting at the amino terminus. Specific modifications observed at each residue are depicted above or below the amino acid sequence. Ac, acetylation; Bi, biotinylation; Ci, citrullination; Me, methylation; Ph, phosphorylation; Su, sumoylation.