CHAPTER

# 1

# An Overview of the Molecular Basis of Epigenetics

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## INTRODUCTION

The role of epigenetic molecular mechanisms in regulation of CNS function is one of the most exciting areas of contemporary molecular neuroscience. This emerging field, variously referred to by neologisms such as *Behavioral Epigenetics* or *Neuroepigenetics*,[1,2] is being driven by shifts in our understanding of several of the fundamental concepts of traditional epigenetics and cognitive neurobiology. These changes in viewpoint can be categorized in a broad fashion into two domains: first, how does neuroepigenetics differ from traditionally defined developmental epigenetics; and second, what is the impact of epigenetics on the historical debate of "Nature versus Nurture"?

After a brief introduction to the basics of epigenetics at the molecular level in this chapter, this book overall will describe the current understanding of the roles of epigenetic processes at the molecular and cellular level, their impact on neural development and behavior, and the potential roles of these mechanisms in neurological and psychiatric disorders. Our goal is for the book to be the first unified synthesis of information concerning the role of epigenetic

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mechanisms in nervous system function. This chapter is an introduction to the overall contents of the book, which spans the range of topics including molecular epigenetics, development, cellular physiology and biochemistry, synaptic and neural plasticity, and behavioral models, and also incorporates chapters on epigenetically based disorders of the CNS.

One objective of the book is to begin to embrace the complexity of epigenetic mechanisms in the context of behavioral change. This book represents a critical first step toward synthesizing the complex puzzle of the molecular basis of behavioral plasticity and neural epigenetics.

## What is Epigenetics?

Epigenetics and its associated terminology have several different connotations, and specific terms need to be defined before we can discuss them in detail. We will start by defining the *genome* as DNA and the nucleotide sequence that it encodes. In contrast, the *epigenome* is the sum of both histone-associated chromatin assembly and the pattern of DNA methylation, thereby defining the moldings and three-dimensional structure of the genomic material inside the cell nucleus and providing a "molecular bridge" between genes and the environment. Despite these precise structural definitions for genome and epigenome, three definitions for the term "epigenetic" are currently in use in the literature.

The broadest definition includes the transmission and perpetuation of information that is not based on the sequence of DNA, for example, perpetuation of cellular phenotype through meiosis or mitosis. This process is not restricted to DNA-based transmission and can also be protein-based. This definition is broadly used in the yeast literature, as one example, wherein phenotypes that can be inherited by daughter cells are perpetuated past cell division using protein-based (e.g. prion-like) mechanisms.[3–5] Whether such mechanisms operate in mammalian neurons is a subject of current investigation.

Developmental biologists and cancer researchers tend to utilize a second definition for epigenetic: meiotically and mitotically heritable changes in gene expression that are not coded in the DNA sequence itself. The altered patterns of gene expression can occur through the impact on gene transcription of several mechanisms that are based on DNA, RNA, or proteins[6] (see below). The principal criterion for this definition of epigenetic is heritability. It is worth noting that the issue of heritability is fundamental to developmental biology where a major issue is the fidelity of cellular phenotype across proliferation that is critical for tissue differentiation.

A third definition posits that epigenetics is the mechanism for stable maintenance of gene expression changes that involves physically "marking" DNA or its associated proteins, which allow genotypically identical cells (such as all cells in an individual human) to be phenotypically distinct (e.g. a neuron is phenotypically distinct from a liver cell). The molecular basis for this type of change in DNA or chromatin structure in the nervous system is the focus of this chapter.[7–9] By this definition, the regulation of chromatin structure and attendant DNA chemical modification is equivalent to epigenetic regulation.

The common theme that is shared across all of the definitions is that epigenetics is a mechanism for storing and perpetuating a "memory" at the cellular level. The catalyzing phenomenon that has focused attention on these mechanisms is cell division. It is clear from

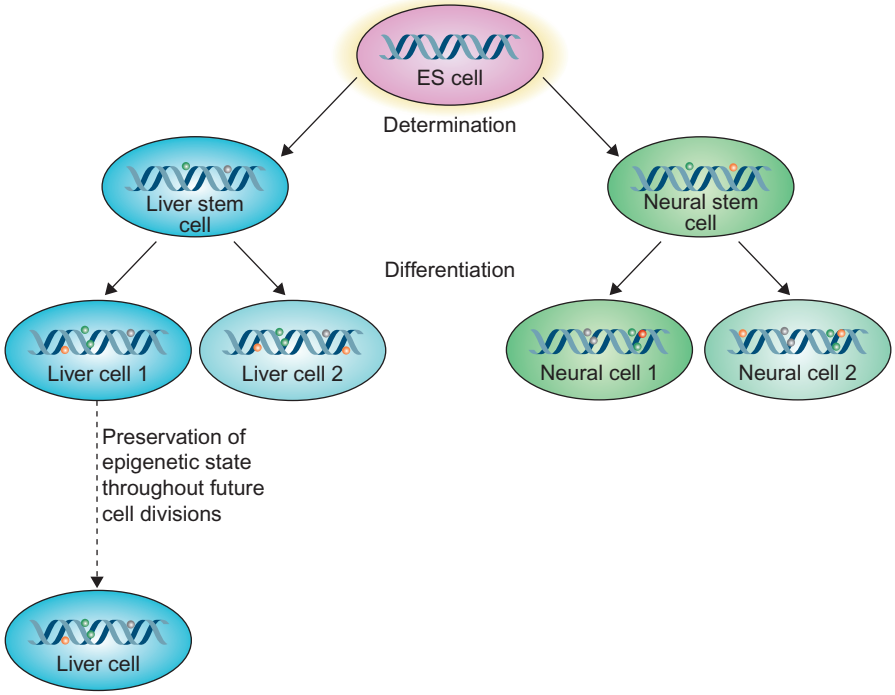


FIGURE 1.1 **Memory at the cellular level.** All embryonic cells begin with identical genotypes and phenotypes. External signals trigger developmental events that lead to the differentiation of cells. Mature cells become phenotypically distinct, but remain genotypically identical. The differences in gene expression persist in the face of numerous cell divisions, which indicates that they are self-sustaining. These developmentally induced changes in gene expression in mature cells are mediated by epigenetic regulation of gene expression. *Adapted from Levenson and Sweatt, 2005.*[7]

developmental studies that a mechanism is necessary for transferring information that concerns the differentiated state of the cell from mother cell to daughter cell; the phenotype must be perpetuated through many subsequent cell divisions which dilute any non self-perpetuating chemical marks. The mechanism for cellular memory does not rely on alterations in the sequence of DNA. This point is remarkable: a neuron and a liver cell, for example, differentiate from the same primordial embryonic stem cell and have the same DNA sequence within an individual (Figure 1.1). Therefore, the self-perpetuating mechanism for differentiation, which originates when the common parent cell divides, cannot be a change in DNA sequence. The distinct phenotypes of each cell are maintained by epigenetic mechanisms that can be detected in the pattern of expression of mRNA and protein in each cell.

## How *Neuroepigenetics* Differs from Traditional Epigenetics

The term *epigenetics* is derived from the theoretical and experimental work of Waddington.[10] Waddington coined the term to describe a conceptual solution to a conundrum that arises as a fundamental consideration of developmental biology. All of the different cells

in the body of one individual have exactly the same genome, that is, exactly the same DNA nucleotide sequence, with only a few exceptions in the reproductive, immune and nervous systems. Thus, in the vast majority of instances, one's liver cells have exactly the same DNA as the neurons. However, those two types of cells clearly are vastly different in terms of the gene products that they produce. How can two cells have exactly the same DNA but be so different? Especially when what *makes* them different is that they produce different gene transcripts that are read directly from the identical DNA. Waddington coined the term *epigenesis* to describe the conceptual solution to this problem. Some level of mechanism must exist, he reasoned, that was "above" the level of the genes encoded by the DNA sequence, that controlled the DNA readout. The mechanisms that allow this to happen are what we now refer to as *epigenetic* mechanisms. These epigenetic mechanisms specify in a neuron that genes A, C, D, L… are turned into functional products and, in a liver cell, that genes A, B, C, E… are turned into functional products. Epigenetic marks are put in place (or remodeled) during cell fate determination and serve as a cellular information storage system perpetuating cellular phenotype over the lifespan (see Figure 1.1).

A central tenet in the epigenetics field historically has been that epigenetic marks, once laid down as part of development, are immutable within a single cell and are subsequently inheritable across cell divisions. This concept has served developmental biologists well, and explains the permanence of, for example, cellular phenotype over the lifespan of an animal. Indeed, as will be described in more detail in Chapters 3 and 10, epigenetically driven programming of cells in the *nervous system* is a critical mechanism for cell fate determination and perpetuation of cellular phenotype. Moreover, disruption of these processes in humans contributes to a wide variety of neurodevelopmental disorders of behavior and cognition.

However, while stability of cell type is critical for any multicell organism, so too is the ability of cells to adapt phenotype to circumstance at various phases of the life cycle. Thus, recent studies of the CNS have indicated that while the permanence of epigenetic marks is a good general rule, there are some exceptions to that generalization in play in the nervous system and potentially other tissues as well. Thus, in some instances, epigenetic molecular mechanisms appear to be recruited to help drive acquired experience-dependent modifications in cognition and behavior. Numerous examples of these emerging discoveries, and the resulting change in viewpoint of the epigenome as being actively regulated as opposed to static, will be the topics of most of the chapters of this book.

When thinking about how to define epigenetics, consideration of the non-dividing nature of mature neurons in the nervous system leads immediately to a violation for these cells of the traditional defining aspect of epigenetics: heritability. By definition anything that happens in a non-dividing neuron cannot be *epigenetic*, if epigenetic connotes heritability. Nevertheless, it is clear that epigenetic *molecular mechanisms* are in action in non-dividing neurons in the nervous system, driven by organismal experience and cellular signaling. It seems that neurons have co-opted the classic epigenetic machinery that underlies cell-type specification to establish mechanisms for more subtle phenotypic variation. For this reason, the term epigenetic is undergoing a redefinition to accommodate the fact that epigenetic molecular changes can occur in cells but not necessarily be heritable in the traditional sense. Thus, our increased understanding of the role of epigenetic molecular mechanisms in experience-dependent transcriptional regulation in the nervous system is driving a re-formulation of how we should define epigenetics.[11]

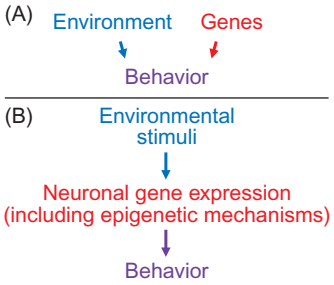


FIGURE 1.2 **The historical model with separate and distinct influences of genes and environment on behavior** (A) is now known to be incorrect. The historical model with separate and distinct influences of genes and environment on behavior has long been held to be biologically implausible as has been discussed by authors such as Lewontin, Gottelieb, Hebb, and Lehrman. Instead, contemporary studies have illustrated that environment and experience act in part through altering gene readout in the CNS in order to achieve their effects on behavior (B). One component of the processes by which the environment and experience alter individual behavior includes epigenetic molecular mechanisms such as regulation of chromatin structure and DNA methylation. The historical dichotomy between "nature" (genes) and "nurture" (environment and experience) is a false one – genes and experience are mechanistically intertwined. Epigenetic molecular mechanisms contribute to this intertwining.

## Epigenetics and the Historical Debate of Nature vs Nurture

In the broader context of cognitive neurobiology, the emerging field referred to as *behavioral epigenetics* has deep implications for the historical debate of the role of genetics versus environment in controlling behavior, a debate colloquially referred to as "Nature vs Nurture". The historical model with separate and distinct influences of genes and environment on behavior (Figure 1.2A) is now known to be incorrect. Instead, contemporary studies have illustrated that environment and experience act in part through altering gene readout in the CNS in order to achieve their effects on behavior (Figure 1.2B). A major component of the processes by which the environment and experience alter individual behavior includes epigenetic molecular mechanisms such as regulation of chromatin structure and DNA methylation. The historical dichotomy between "nature" (genes) and "nurture" (environment and experience) is a false one – genes and experience are mechanistically intertwined. The emerging discovery is that epigenetic molecular mechanisms contribute importantly to this intertwining. Epigenetic molecular mechanisms represent a previously hidden mechanistic layer that sits at the interface of genes and environmental experience. In a literal sense, epigenetic mechanisms in the nervous system are the site where experience modifies the genome.

Thus, a new aspect of epigenetic control of gene expression is now emerging from recent studies of epigenetic molecular mechanisms in the nervous system. Convincing evidence has accumulated that epigenetic mechanisms do not just contribute to phenotypic hardwiring at the cellular level. Rather, in the nervous system, with its abundance of terminally differentiated, non-dividing cells, epigenetic mechanisms also play a role in acute regulation of gene expression in response to a wide range of environmental signals, such as behavioral experience, stress, drugs of abuse, and many others. In addition, epigenetic mechanisms appear to contribute to both psychiatric and neurological disorders. In retrospect, these roles for epigenetic molecular mechanisms are not surprising. Even in their role in development,

epigenetic mechanisms sit at the interface of the environment and the genome. However, discoveries of active regulation of the epigenome in the adult CNS illustrate the unified, as opposed to dichotomous, relationship of genes and environment.

## What are the Epigenetic Marks and What do they do?

There are two basic *molecular* epigenetic mechanisms that are widely studied at present – regulation of chromatin structure through histone post-translational modifications, and covalent modification of DNA principally through DNA methylation. These two mechanisms will be discussed in the next sections of this chapter. Other epigenetic molecular mechanisms such as regulation of gene expression through non-coding RNAs, and recombination of non-genic DNA, are also known to exist and will be briefly discussed. Finally, for the last part of this chapter we will highlight a number of emerging *functional* roles for epigenetic mechanisms in the nervous system as an introduction to the rest of this book.

# DNA MODIFICATIONS

## Covalent Modification of DNA – Cytosine Methylation

A major mechanism whereby the genome can be epigenetically marked is DNA methylation. Methylation of DNA is a *direct chemical modification* of a cytosine C5 side-chain that adds a -$CH_3$ group through a covalent bond (Figure 1.3). Methylation of DNA is catalyzed by a class of enzymes known as *DNA methyltransferases* (DNMTs).[12] DNMTs transfer methyl groups to cytosine nucleotides within a continuous stretch of DNA, specifically at the 5-position of the pyrimidine ring.[13,14] Not all cytosines can be methylated; usually (but

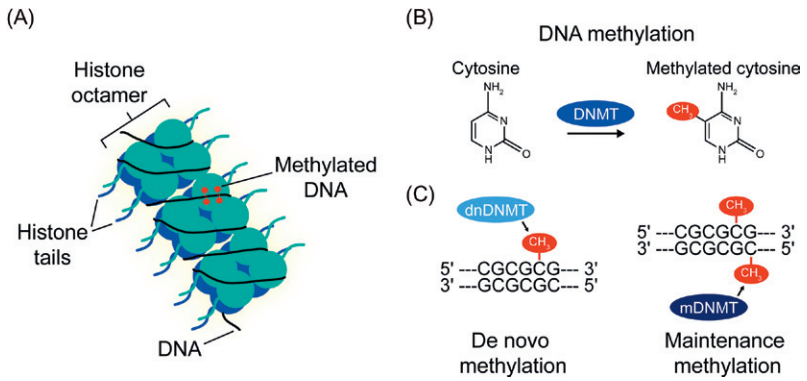


FIGURE 1.3 **DNA methylation and DNMTs.** DNA methylation. (A) Inside a cell nucleus, DNA is wrapped tightly around an octamer of highly basic histone proteins to form chromatin. Epigenetic modifications can occur at histone tails or directly at DNA via DNA methylation. (B) DNA methylation occurs at cytosine bases when a methyl group is added at the 5′ position on the pyrimidine ring by a DNMT. (C) Two types of DNMTs initiate DNA methylation. De novo DNMTs methylate previously non-methylated cytosines, whereas maintenance DNMTs methylate hemi-methylated DNA at the complementary strand. *Adapted from Day and Sweatt, 2010.*[2]

not always) cytosines must be immediately followed by a guanine in order to be methylated.[15,16] These "*CpG*" dinucleotide sequences are highly underrepresented in the genome relative to what would be predicted by random chance; however, about 70% of the CpG dinucleotides that are present are methylated.[17] The rest of the normally unmethylated CpG dinucleotides occur in small clusters, known as "*CpG islands*" that can occur both near gene transcription start sites and intragenically.[18,19] Among the methylated cytosines, only a minute portion (<3%) are located at the 5′ end of genes, with the remaining 97% of methylated cytosines found in intra- and intergenic sequences and within DNA repeats.[20] Thus, CpGs in regions of the genome that actively regulate gene transcription, such as promoters, are largely unmethylated.

There are two variants of DNMTs: *maintenance* DNMTs and *de novo* DNMTs. DNMT1 is the maintenance DNMT, DNMTs 3a and 3b are the de novo DNMT isoforms. Both maintenance and de novo DNMTs are expressed in most cells in the body including brain, although DNMT3b expression tends to be low in the adult CNS.[21] The two variants of DNMTs differ in one important respect, related to the conditions under which they will methylate DNA. De novo DNMTs methylate previously unmethylated CpG sites in DNA – sites which have no methyl-cytosine on either DNA strand. The maintenance DNMT isoform methylates *hemimethylated DNA* – DNA which has a methylated CpG already present on one strand but no methyl-cytosine on the complementary strand. These two different isoforms thereby serve distinct roles in the cell (see Figure 1.3). De novo DNMTs place new methylation marks on DNA, for example, when specific genes are first silenced as part of cell fate determination. Maintenance DNMTs perpetuate methylation marks after cell division. They regenerate the methyl-cytosine marks on the newly synthesized complementary DNA strand that arises from DNA replication. Thus, in summary: DNMT1, the maintenance DNMT, propagates epigenetic marks through cell generations in dividing cells, while DNMTs 3a and 3b, the de novo DNMTs, are responsible for laying down the initial patterns of DNA methylation when cell fate is determined.

What are the functional consequences of DNA methylation? In most cases that have been studied so far, methylation of DNA is associated with suppression of gene transcription and, in many cases, extensive DNA methylation triggers complete silencing of the associated gene. In other words, methylation is a process whereby a gene can be shut off functionally. It is important to note that the effect of cytosine methylation is highly dependent on the location of the methylated CpGs. The classic relation between DNA methylation and gene transcription holds, but only when the methylated sites are located in promoter regions (i.e. non-coding regions upstream from transcriptional start sites). Methylation of CpGs located within gene bodies is associated with the opposite effect – an increase in transcriptional activity. The precise molecular processes through which this occurs are complex and an area of intense investigation at present. The repressive effect of DNA methylation in gene promoters is better understood. Several proteins recognize and bind to methylated CpG residues independent of DNA sequence. The five proteins that are known to bind to methylated CpGs are MeCP2, MBD1, MBD2, MBD4 and Kaiso.[22,23]

One simplified model for how methyl-DNA binding proteins might suppress transcription is shown in Figure 1.4. In essence, the concept is that methylation of cytosines at CpG dinucleotides recruits methyl-DNA binding proteins at specific sites in the genome. Proteins that bind to methylated DNA have both a *methyl-DNA binding domain* (MBD) and a

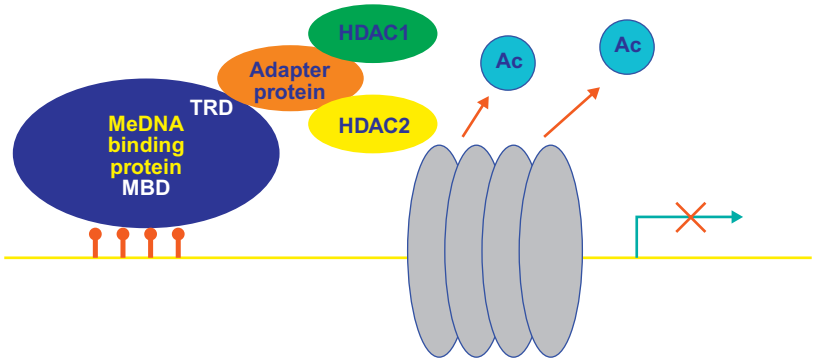


FIGURE 1.4 **A simplified scheme for DNA methylation-dependent gene silencing.** Methylation of cytosines at CpG dinucleotides (red lollipops) recruits methyl-DNA binding proteins, of which MeCP2 is a specific example. All proteins that bind to methylated DNA have both a methyl-DNA binding domain (MBD) and a transcription-regulatory domain (TRD). The TRD recruits adapter proteins such as Sin3A which, in turn, recruit histone deacetylases (HDACs). The HDACs alter chromatin structure locally through removing acetyl groups (Ac) from histone core proteins (gray spheres), leading to compaction of chromatin and transcriptional suppression. *Reproduced with permission from Elsevier.*

*transcription-regulatory domain* (TRD). The TRD recruits adapter/scaffolding proteins which, in turn, recruit histone deacetylases (HDACs) to the site. HDACs appear critical as they are the major common component to the two MBD repressive complexes, the Sin3a and NurF complexes. The HDACs alter *chromatin structure* locally – "chromatin" is the term describing nuclear DNA/protein complexes (Figure 1.5).[24,25] HDACs alter chromatin structure through removing acetyl groups from histone core proteins, leading to compaction of chromatin and transcriptional suppression. Thus, through this complex and highly regulated biochemical machinery, methylation of DNA triggers localized regulation of the three-dimensional structure of DNA and its associated histone proteins, resulting in a higher-affinity interaction between DNA and the histone core, and transcriptional repression by allosteric means. Consideration of this mechanism thus leads us to the second major category of epigenetic marks, histone post-translational modifications. However, before proceeding to histones, we will address a few additional aspects of regulation of DNA methylation that warrant our attention.

## Methylation Regulates Transcription through Multiple Mechanisms

It is important to note that while DNA methylation is usually (and historically) associated with transcriptional suppression, recent studies have indicated that DNA methylation can also be associated with transcriptional activation, by mechanisms that have not yet been determined.[26,27] Also, while DNA methylation leads to marked changes in the structure of chromatin that ultimately result in significant downregulation of transcription, it also can directly interfere with the ability of transcription factors to bind to DNA regulatory elements at specific nucleotide sequences. For example, the transcription factor Ets-1 and the boundary element CCCTC binding factor (CTCF) can efficiently bind to non-methylated, but not methylated, DNA.[28]

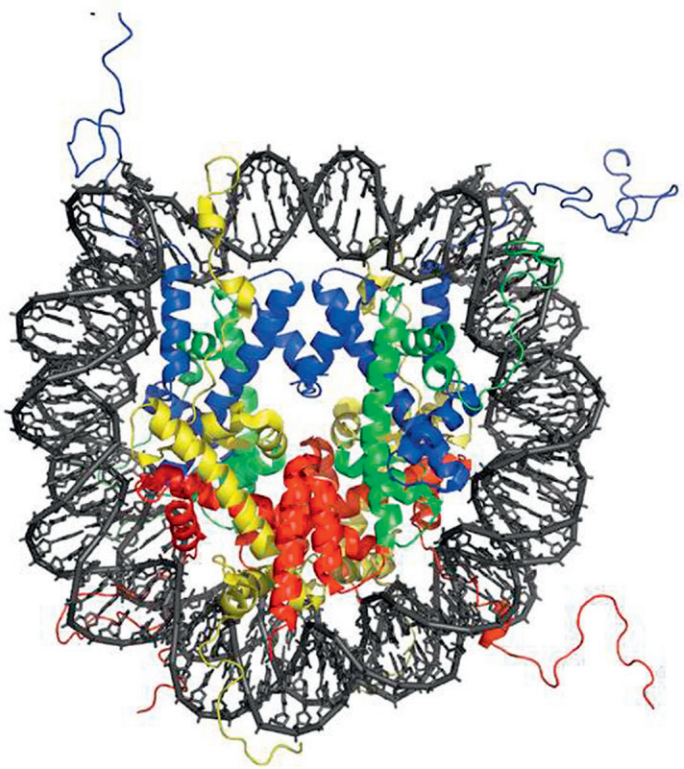

FIGURE 1.5 **The nucleosome.** Each nucleosome is comprised of an octamer of histone molecules, which consists of an $H3_2$-$H4_2$ tetramer and two H2A-H2B dimers. The N-termini of histones project out of the nucleosome core and interact with DNA. These histone tails can be epigenetically modified, and act as signal integration platforms.

## Active Regulation of DNA Demethylation

The idea of the occurrence of *active* DNA demethylation has been contentious.[29,30] Traditional epigenetic studies have posited only passive DNA demethylation as a result of cell division and failure to replicate DNA methylation marks when DNA daughter strands are synthesized post-mitotically. However, active demethylation through direct chemical removal of methyl groups on cytosines (or methylcytosines themselves) has been proposed by several groups including those of Szyf and Meaney and Sweatt and colleagues based on their early findings in this area.[31–36] Moreover, replication-independent active demethylation is a defining feature of embryonic development since the DNA methylation of the parental genomes is erased in early development followed by a *re*methylation in later fetal development.[37]

Thus, several pieces of recent information motivate investigating a potential role for active DNA demethylation in non-dividing cells in the mature CNS. First, indirect evidence exists for active DNA demethylation in the adult CNS in response to DNMT inhibitor application or behavioral training (fear conditioning) based on non-quantitative methods, such as PCR-based methods and methylation-dependent immunoprecipitation.[2,31,33,38] Second, two recent publications[39,40] have demonstrated rapid DNA demethylation and remethylation, referred to as "cycling" of methyl-cytosine (hereafter abbreviated mC) in cultured cells.

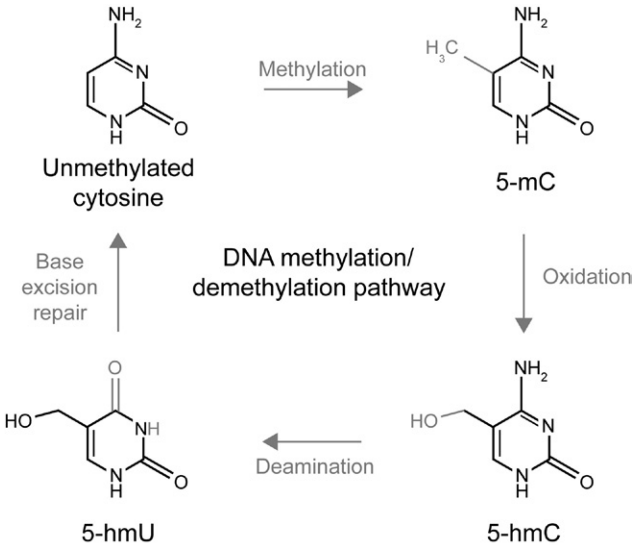


**FIGURE 1.6** **Proposed role for hydroxymethylcytosine in active DNA cytosine demethylation.** 5-mC is 5-methyl-cytosine; 5-hmC is 5-hydroxymethyl-cytosine, and 5-hmU is 5-hydroxymethyl-uracil.

This demethylation occurs too rapidly to be explained by passive demethylation through cell division and must therefore be due to an active demethylation process.

This has led to the hypothesis of the existence of rapid, active DNA demethylation in the adult CNS.[41–44] Investigators working in this area have proposed a specific demethylation mechanism: C-to-T conversion of mC, followed by base-excision repair of the resulting nucleotide mismatch. Most recently, exciting work from Hongjun Song and colleagues supported this idea[45–48] – these investigators demonstrated that DNA repair mechanisms are utilized to demethylate DNA in non-dividing neurons, specifically through base-excision repair mechanisms controlled by the Growth Arrest and DNA Damage 45 (GADD45-beta) regulatory system. This finding demonstrates that demethylation can occur independent of DNA replication, and in a terminally differentiated neuron.

This now substantial body of evidence supports the idea that active DNA demethylation can occur in non-dividing neurons, findings which make viable the idea that active control of DNA methylation may play a role in activity-dependent processes in the CNS throughout the life cycle.

## Other Forms of DNA Methylation

Two new studies[49,50] have shown that a novel DNA base, 5-hydroxymethyl-cytosine (hmC), may uniquely occur in the CNS and may serve as a precursor nucleoside for active demethylation. According to this idea, hmC is converted into 5-formyl-cytosine (5fC) and 5-carboxyl-cytosine (5caC) and, finally, restored to cytosine. The existence of this new sixth base (Figure 1.6) was only recently demonstrated convincingly.[49,50] HmC is most abundant in two categories of cells: the totipotent fertilized zygote and the CNS neuron. This observation is highly suggestive, as these are the two main cell types in which active DNA demethylation has been most convincingly demonstrated. One intriguing possibility is that

hyperplastic cells such as neurons and stem cells may have unique roles for this DNA base. Using a seizure model, Song's lab made the exciting discovery that TET-family (TET = Ten-Eleven Translocation) oxidases are necessary for neuronal activity to trigger rapid active DNA demethylation in non-dividing neurons in the CNS.[45,46] They hypothesize that these effects are due to TET1 increasing mC hydroxylation and precipitating active DNA demethylation by this mechanism. The implication of this idea is that hydroxylation of mC is a gateway for regulating active DNA demethylation in the CNS. These exciting ideas will be discussed in more detail in Chapter 3.

While most attention on DNA methylation has focused on mC and hmC, the several intermediate steps between hmC and mC, noted above, may be functionally significant. Moreover, methylation of other DNA bases, such as adenine, which has been characterized in prokaryotic and lower eukaryotic cells, may also be important in mammalian systems. This remains an area of active research.

## HISTONE MODIFICATIONS

Histones are highly basic proteins whose function is to organize DNA within the nucleus. As mentioned above, in the nucleus, DNA is tightly packaged into chromatin, a DNA-protein complex that consists of DNA in a double helix, histone proteins, and many associated regulatory proteins. Modification of histones is a crucial mechanism for epigenetic tagging of the genome.[25,51] Histone modification can occur as a consequence of DNA methylation, or can be mediated by mechanisms that are independent of DNA methylation and controlled by intracellular signaling.

The basic unit of chromatin is the *nucleosome*, which is composed of an octomer of histone proteins (containing two copies each of histones 2A, 2B, 3, and 4) around which is wrapped – like a rope on a windlass – the DNA double helix. The degree to which nucleosomes are condensed or packed is a critical determinant of the transcriptional activity of the associated DNA and this is mediated in part by chemical modifications of the N-terminal tails of histone proteins (Figure 1.7). Structural studies indicate that these N-terminal tails protrude from the nucleosome and are extensively modified post-translationally.[24]

Currently, four distinct post-translational modifications of histone tails have been well characterized: acetylation, methylation, ubiquitination and phosphorylation. All of these modifications serve as epigenetic tags or marks.[25] We will discuss each of these briefly in the following four sections; a more extensive discussion is included in Chapter 2.

### Acetylation

Acetylation of histones occurs at lysine residues, specifically on their side-chain amino group, which effectively neutralizes their positive charge. Histone acetyltransferases (HATs) catalyze the direct transfer of an acetyl group from acetyl-CoA to the ε-$NH^+$ group of the lysine residues within a histone.[52,53] Histone acetylation is a reversible process, and the enzymes that catalyze the reversal of histone acetylation are known as HDACs.

Classical isoforms of HDACs catalyze the removal of acetyl groups from lysine residues through a $Zn^{2+}$-dependent charge-relay system.[54,55] By way of background, there are a total

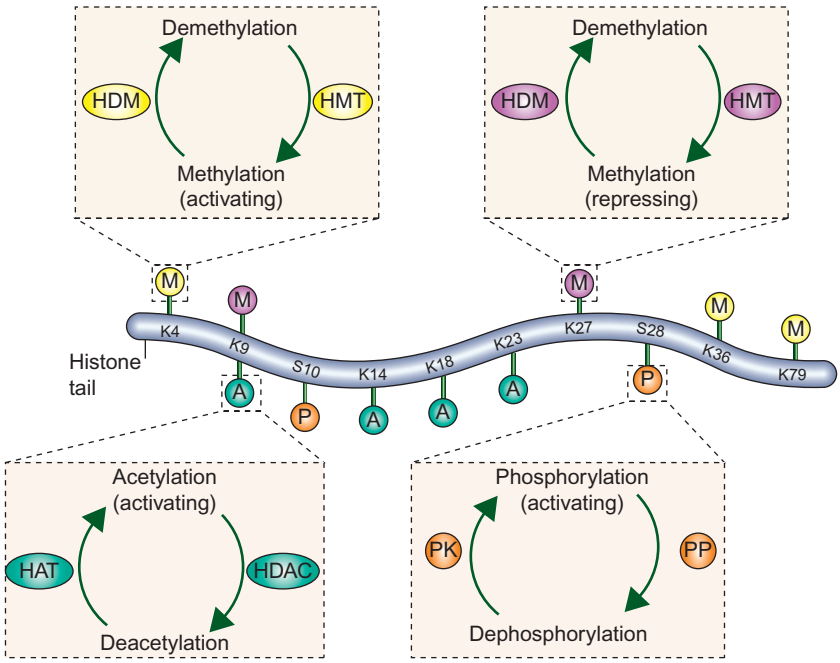


**FIGURE 1.7** **Post-translational modifications of histones.** The first 30 amino acids in the N-terminus of the human histone H3 are illustrated. Many sites in the N-terminus can be targets for epigenetic tagging such as acetylation (A), phosphorylation (P) and methylation (M). Regulation of each site is independent, and the integration of epigenetic tags elicits a finely tuned transcriptional response. The integration of signaling at the level of epigenetics is commonly referred to as the histone code. *Adapted from Levenson and Sweatt, 2005*[7] *and Tsankova et al., 2007.*[9]

of eleven HDAC isoforms broadly divided into two classes. HDACs 1, 2, 3, and 8 are Class I HDACs, while Class II encompasses HDAC isoforms 4, 5, 6, 7, 9, 10, and 11. The newly characterized SIR2 family of HDACs (the "Sirtuins"), termed Class III, operate through an $NAD^+$-dependent mechanism, but are not discussed further here.[56]

As will be discussed in Chapter 8, HDAC inhibitors are undergoing a period of rapid development in the pharmaceutical industry because of their potential applicability in cancer treatment and the emerging possibility of their utility in neurological and psychiatric disorders. HDAC inhibitors are the principal way to manipulate the epigenome pharmacologically at present. Trichostatin A (TsA) inhibits HDACs broadly across both Class I and Class II, while sodium butyrate, suberoylanylide hydroxamic acid (SAHA, aka Vorinostat or Zolinza), and MS275 (aka Entinostat) are more selective for Class I HDACs. Tubacin selectively inhibits HDAC6, for which tubulin is an established substrate. Valproate is also an HDAC inhibitor, but this drug also has several additional targets and the role of HDAC inhibition in valproate's clinical efficacy is unclear at this time.

The principal caveat to interpreting all studies utilizing HDAC inhibitors is the fact that "histone deacetylase" is actually a misnomer. HDACs should be more accurately described as "lysine deacetylases". Lysine amino-acid side chains are acetylated in a wide variety

of cellular proteins besides just histones. The list of known lysine-acetylated proteins is quite long, including transcription factors, cytoskeletal proteins, and numerous metabolic enzymes. Certain HDACs act on all of these proteins, not just their prototype histone substrate. Therefore, any behavioral effect of HDAC inhibitors might be due to alterations in acetylation of any of many intracellular targets.

The signal transduction processes controlling histone acetylation in the mature CNS are just beginning to be understood. One major control point is through activity-dependent transcription factors, which recruit HATs to particular genes in concert with their activation. This was first established for cAMP-response element binding protein (CREB).[57–61] The phosphorylation and activation of CREB by any of several protein kinases leads to its recruitment of CREB binding protein (CBP), which then acetylates the N-terminal tails of nearby histones to promote nucleosome separation and active gene transcription. Numerous transcription factors act in an equivalent manner to recruit CBP or related HATs to their target genes. Mechanisms controlling HDAC activity are less well established, however, several Class II HDACs are known to be regulated through their phosphorylation by several protein kinases, including $Ca^{2+}$-dependent protein kinase II (CaMKII) and cyclin-dependent protein kinase-5 (CDK5), which determines their nuclear versus cytoplasmic localization.[62–66]

## Methylation

Histone methylation is another major histone-directed epigenetic tag.[67] Similar to acetylation, methylation of histones occurs on ε-$NH^+$ groups of lysine residues, and is mediated by lysine methyltransferases (KMTs). Unlike acetylation, methylation of lysines preserves their positive charge. In addition, lysines can accept up to three methyl groups and thus exist in mono-, di- or trimethylated states. The effect of histone lysine methylation on gene regulation is highly complex, with the various valences of methylation on several distinct lysine residues mediating either transcription repression, activation, or elongation (Table 1.1). Arginine residues within histones can also be mono- or dimethylated on their guanidine nitrogen. This reaction is catalyzed by protein arginine methyltransferases (PRMTs). An overview of the major histone methylating and demethylating enzymes is given in Table 1.1.

## Ubiquitination

Ubiquitination of histones was identified 29 years ago[68] but has only recently begun to be characterized in detail. Ubiquitin, a protein with 76 amino acids that is named for its ubiquitous distribution in all cell types and high degree of conservation across species, is usually, but not always, attached to proteins as a signal for degradation by the proteasome.[69] Like other proteins, histones are ubiquitinated through attachment of a ubiquitin to the ε-$NH^+$ group of a lysine.[70] Ubiquitination of histones H2A, H2B, H3 and H1 has been observed.[71–73] Most histones appear to be mono-ubiquitinated, although there is evidence for poly-ubiquitination. The role of histone ubiquitination in the control of gene transcription in the nervous system remains poorly understood.

**TABLE 1.1** Examples of Mechanisms for Regulation of Histone Acetylation and Methylation

| Modification | Function | Writers | Erasers |
|---|---|---|---|
| Acetylation | Activates (H3, H4) | CBP, p300 | HDACs 1–11 |
| Methylation | Activates (H3K4) | MLL1, SetD1a | JARID/SMCX |
| | Elongates (H3K36) | Set2 | JHDM1 |
| | Represses | | |
| | (H3K9) | G9a, SUV39H1 | JMJD2a, LSD1 |
| | (H3K27) | EZH2 | JMJD3, UTX |
| | (H4K20) | SetD8 | PHF8 |

*Even more complicated, because each methylated residue can be mono-, di- or trimethylated, with distinct functional consequences.*
*JMJD2A also is a H3K4me3 eraser; and LSD1 is also active at K3me sites.*

## Phosphorylation

Phosphorylation of histones H1 and H3 was first observed in the context of chromosome condensation during mitosis.[74,75] H3 was the first histone whose phosphorylation was characterized in response to activation of mitogenic signaling pathways.[76] Phosphorylation of serine 10 on H3 is mediated by at least three kinases in the CNS: ribosomal S6 kinase-2 (Rsk2), which is downstream of extracellular signal-regulated kinase (ERK); mitogen- and stress-activated kinase-1 (Msk1), which is downstream of both ERK and p38 mitogen-activated kinases (MAPK); and the aurora kinase family member Ipl1.[77–80] Evidence also implicates aurora kinases in the phosphorylation of serine 28 in histone H3.[81] Phosphatases remove phosphate groups from histones.[82] To date, the phosphatases PP1 and PP2A, and the PP1 inhibitor DARPP32, have been shown to regulate H3 phosphorylation in the CNS.[78,83,84] In most cases, phosphorylation of histones is associated with gene activation, although much further work is needed to define the precise mechanisms involved.

## Histone Subunit Exchange

Besides direct chemical post-translational modification of histones, an additional mechanism for altering the function of the histone components of the nucleosome is exchange of histone isotypic variants into and out of the histone octamer (Figure 1.8). This energy-dependent *chromatin remodeling* process is one mechanism for persistently altering the transcriptional efficacy of a given chromatin particle. Certain histone isomers, such as histone H2A.Z are associated with the absence of DNA methylation and transcriptional activation. The individual histone variants appear to differ in the capacity to support specific modifications, with some likely more associated with increased epigenetic "plasticity" and others more closely allied to epigenetically stable genomic regions. Determining whether active histone subunit exchange of this sort is actively regulated in the CNS is an area of contemporary investigation.

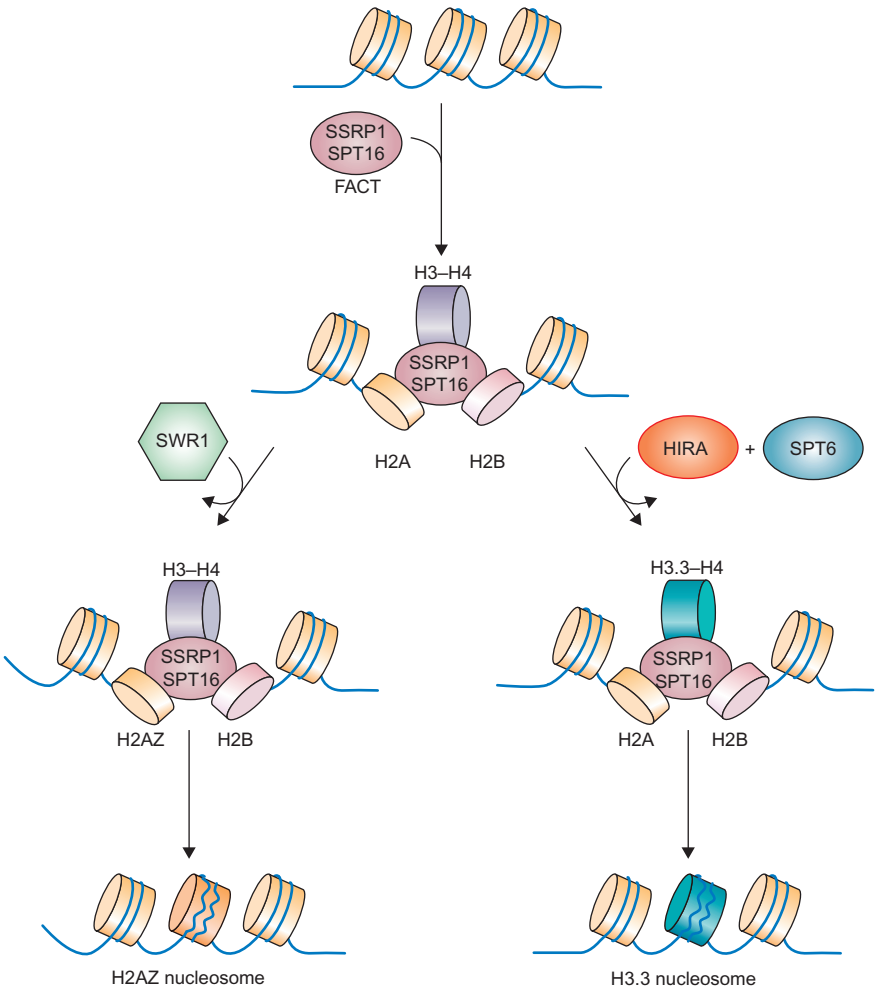


FIGURE 1.8 **Histone subunit exchange.** Under certain circumstances, histone subunits are replaced within existing nucleosomes, in concert with gene regulation. Examples of histone variants are: Histone H3: H3.1, H3.2, H3.3; Histone H2: H2 A.Z, MacroH2A. SSRP1, SPT16, SWR1, HIRA, and SPT6 are chromatin remodeling enzymes involved in subunit exchange. *Reproduced with permission from Nature Publishing Group.*

## A Histone Code for Regulating CNS Function?

The plethora of functional histone modifications capable of affecting gene transcription has led to the proposal that one purpose for the complex biochemical signaling at this locus is that a *histone code* might be involved in transcriptional regulation. The basic concept is that specific patterns of histone post-translational modifications might help encode the salience of cell-surface signals and their contingencies. This general hypothesis that there is an epigenetic "histone code" for regulating CNS function is new and still quite speculative (see Chapter 2), and derives from an earlier idea proposed by David Allis and colleagues.[25] The

overall concept is that multiple post-translational histone modifications may be integrated together, combinatorially driving neuronal gene expression patterns by recruiting signaling complexes and thereby remodeling the structure of chromatin. As different histone modifications may be driven by different upstream signaling pathways, multiple signals might thus converge on the nucleus, controlling gene readout through regulating chromatin structure. This would result in a mapping of multiple histone alteration states onto subsets of genes that are transcribed as a result of those changes. In principle, these specific patterns of histone (or more broadly speaking, chromatin) modifications might help encode a transient or lasting set of signals reflecting specific experience-dependent patterns of neuronal activity. This fascinating idea of a combinatorial histone/epigenetic code operating in CNS function is the topic of the following chapter in this book.

### Other Mechanisms of Epigenetic Tagging in the CNS

We have so far focused on epigenetic mechanisms that are DNA-centric, which result in modification of either the DNA itself or associated histone proteins. According to the broadest definition of epigenetics – which includes any non-DNA-sequence-based system for the perpetuation of information across time and across cell replication – any protein- or RNA-based system for storage of cellular memory is also epigenetic. Our approach is to focus on the actual function of epigenetic mechanisms, that of transcriptional regulation, as opposed to an emphasis on the assumed physiochemical nature of the modification or heritability. This more inclusive approach provides neurobiologists with a richer and more integrated set of candidate mechanisms. In the following sections, we will briefly comment on several additional molecular modalities for epigenetic perpetuation of cellular phenotype that operate in the CNS.

## NON-CODING RNAs

An additional set of epigenetic mechanisms operating in the CNS derives from the activity of several types of non-coding RNA molecules. The best characterized are small RNAs, which include microRNAs, small interfering RNAs (siRNAs), and small nuclear RNA (snRNAs). RNA interference (RNAi) is a mechanism whereby the expression of cognate genes is disrupted through the action of double-stranded RNA molecules.[85] Pioneering studies suggested that the RNAi machinery is used in the nucleus and is involved in the formation of heterochromatin and epigenetic tagging of histones in yeast. Genetic disruption of RNAi pathways leads to relaxation of heterochromatin around centromeres, which causes erroneous expression of normally silent genic regions and a decrease in the repressive methylation of histone H3.[86,87] Small RNAs that are produced by a specialized ribonuclease can associate with DNA and direct the formation of a protein complex that promotes the formation of heterochromatin.[88] Small RNAs have multiple functions within a cell, including activation, repression, or interference with gene expression, and have been implicated in a number of cognitive disorders. For example, microRNA binds to 3′ untranslated regions of messenger RNA and thereby either promotes the degradation of the messenger RNA or suppresses its expression through translational mechanisms. MicroRNAs may thereby control expression of the majority of genes within the genome, and represent a critical component of normal

physiology and function in the developing and adult nervous system. Moreover, microRNAs are often an integral force within the complexes that are attracted to the genome by specific DNA or histone marks, and thus link epigenetic signatures to transcriptional activity.

Less well studied is a series of long non-coding RNAs. These are typically longer than 200 nucleotides and can be spliced like messenger RNAs to form active biological molecules, including small RNAs. Long non-coding RNAs have been shown to regulate the recruitment of chromatin remodeling complexes to particular genes in simpler systems and likely play an important role in the controlling gene transcription.[89] The concept that activity-dependent regulation of neuronal gene expression can be mediated by small and large non-coding RNAs is discussed further in Chapter 5.

## NON-GENIC DNA

Most investigations to date concerning the role of epigenetic mechanisms in the nervous system have focused on promoter regulation, as we have been discussing thus far. However, recent work from the research groups of Adrian Bird, Rusty Gage, Eric Nestler, and Michael Meaney has begun to explore dynamic DNA/histone changes in association with the presence of DNA repeat sequences in CNS neurons.[90]

One example of these new studies involves L1 retrotransposition. L1 elements belong to the long interspersed element (LINE) class of repeat sequences, which are an active class of non-LTR (long terminal repeat) retro-elements in the human genome. Full-length, functional L1 elements are autonomous because, once expressed, they encode proteins (e.g. reverse transcriptase, endonucleases) necessary for their own retrotransposition (i.e. reincorporation into the genome). L1 elements are retrotransposons that insert extra copies of themselves throughout the genome using a copy-and-paste mechanism, and are thus able to influence chromosome integrity and gene expression upon reinsertion.

A particularly intriguing current hypothesis is that L1 elements are active and "jumping" during neuronal differentiation, potentially allowing L1 insertions to generate genomic plasticity in neurons by altering the transcriptome of individual cells. Among a number of mechanisms, L1 element insertion could alter neuronal gene transcription by affecting promoter location and efficacy, altering splice sites within genes, or triggering aberrant activation of a gene by local insertion of binding sites for transcription factors. In a mechanism specifically involving epigenetic mechanisms, an L1 element that has inserted upstream of another gene might become methylated, silencing transcription. By these mechanisms L1-induced variation could affect neuronal plasticity and behavior, broadening the spectrum of behavioral phenotypes that can originate from any single genome – a topic that is discussed in more detail in Chapter 11.

## PRION-BASED EPIGENETIC INHERITANCE

Prions are proteins encoded in the DNA of most eukaryotes that are capable of a conformationally dependent self-perpetuating biochemical reaction. The basis of this

reaction is that prion proteins are synthesized in an inactive form that, when triggered by an exogenous signal, convert into an active form that can alter a cellular phenotype. Moreover, the active form, once generated, can act upon other inactive prion molecules and render them activated. By this means, once activated in a cell, the prion proteins are able to establish a self-perpetuating biochemical reaction that is both persistent across time and might be heritable across cell division.

Thus, prions represent a viable, protein-based system for epigenetic memory. Once a protein has been converted into its prion form, that protein promotes the transition of other cognate proteins into the prion form. This epigenetic mechanism is broadly used in yeast, as noted above, wherein phenotypes that can be inherited by daughter cells are perpetuated past cell division using protein-based mechanisms.[3–5]

Recently, a provocative series of studies has suggested that, in *Aplysia*, the cytoplasmic polyadenylation element binding protein (ApCPEB) assumes a prion-like conformation after synapses are strengthened.[5] By assuming a prion-like conformation, it is hypothesized that ApCPEB can maintain a stable synaptic state in the face of protein turnover. This hypothesis, which requires further investigation, will be discussed in more detail in Chapter 5.

## EPIGENOME ORGANIZATION AND HIGHER ORDER CHROMATIN STRUCTURES

While wrapping of genomic DNA into nucleosomal structures results in a several-fold increase in packaging density, as compared to naked DNA, the actual level of compaction in the vertebrate nucleus is about three orders of magnitude higher.[91] These chromosomal arrangements in the interphase nucleus are not random and loci with active transcription are more likely to be clustered together and positioned towards a central position within the nucleus, while heterochromatin and silenced loci tend to locate towards the nuclear periphery.[92,93] Chromatin loopings, in particular, are among the most highly regulated "supranucleosomal" structures and pivotal for the orderly process of gene expression, by enabling distal regulatory enhancer or silencer elements positioned a few, or many hundred kilobases apart from a gene, to interact directly with that gene's promoter.[94,95] There is a growing realization of the importance of these and other higher order chromatin structures for transcriptional regulation, but very little is known about their role in the context of epigenetic regulation in the nervous system. To date, less than a handful of studies have explored loop formations in brain tissue.[96–98] Clearly, functional explorations of higher order chromatin structures in the context of learning and plasticity, or various brain disorders, will provide important new insights into this layer of regulation.

## ROLES FOR EPIGENETIC MECHANISMS IN THE NERVOUS SYSTEM

In the previous sections, we have presented a brief overview of the basic molecular and biochemical mechanisms governing epigenetic regulation. In the following sections, we explore the functional significance of epigenetics in several aspects of *neural* systems.

## EPIGENETIC MECHANISMS IN NERVOUS SYSTEM DEVELOPMENT

The role of epigenetic mechanisms in nervous system development is a focus of several chapters of this book. However, by way of brief introduction here, we will highlight one of the prototype examples of a role for epigenetic mechanisms in neural development, a core process which contributes to cell fate determination in neurons and impinges upon epigenetic molecular mechanisms for its readout.

Neurons express a complement of proteins that are important for their function, but would be detrimental to other cell types. These include proteins that are involved in excitability, transmitter release and the maintenance of transmembrane potential. Many genes that are to be expressed in neurons, but not in other cell types, have a *neuron-restrictive silencer element* (NRSE) in their promoters.[99–101] This regulatory element, which is approximately 21–24 base pairs long, can completely silence a gene in non-neuronal cells.[101]

The first step toward understanding how NRSEs confer tissue-specific regulation of gene expression was identification of the transcription factors that bind to this regulatory element (Figure 1.9). The *RE1-silencing transcription factor* (REST or NRSF for neuron-restrictive

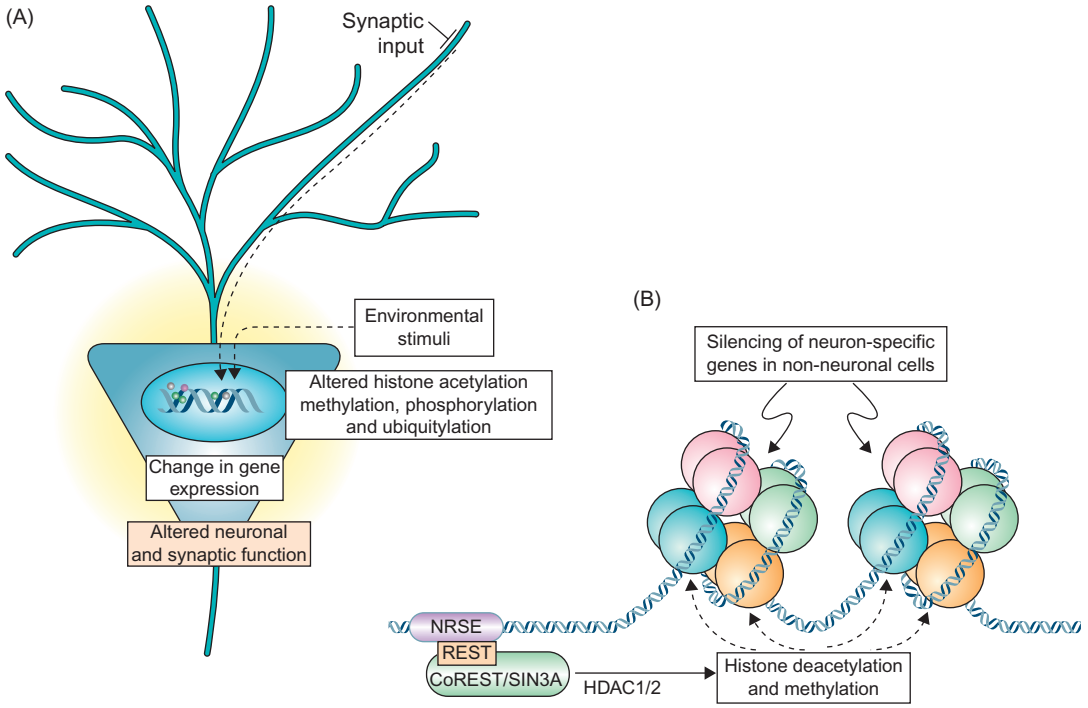


FIGURE 1.9 **Epigenetics in nervous system development.** The RE1-silencing transcription factor (REST)/REST co-repressor (CoREST) system. The NRSE upstream of genes to be silenced in non-neuronal cells recruits REST as a mediator of transcriptional repression. Sin3A, CoREST and REST, acting in concert with additional factors such as HDAC1 and 2, leads to chromatin condensation and gene silencing. *Adapted from Levenson and Sweatt, 2005.*[7]

silencer factor) was the first transcription factor that was shown to bind to NRSEs and repress gene expression.[102] The REST protein is ubiquitously expressed in cells outside the nervous system, where it acts to repress the expression of neuronal genes.[102] Deletion of the REST gene or functional inhibition of the protein in non-neuronal tissues leads to erroneous expression of neuronal genes and embryonic lethality, whereas ectopic expression of REST in the nervous system inhibits expression of neuronal genes, and results in developmental dysfunction.[102–104] Therefore, REST is important in determining whether a cell has a neuronal phenotype.

REST-dependent gene silencing requires the action of transcriptional co-repressors, two of which have been identified as the REST-binding proteins *Sin3A* and *CoREST*.[105–107] The cellular expression pattern of Sin3A is nearly identical to that of REST, which indicates that most REST-dependent gene repression might be co-mediated by Sin3A.[108] The expression of CoREST is more restricted, which indicates that it might be important in mediating specific gene expression patterns in subtypes of cells.

REST-mediated gene silencing requires modulation of chromatin structure. REST/Sin3A repressor complexes are associated with HDAC1, whereas REST/CoREST complexes with HDAC2.[20,106,107,109] Thus, REST-dependent gene silencing with either co-repressor seems to involve decreases in histone acetylation. CoREST has also been shown to associate with members of the hSWI–SNF complex, which is an ATP-dependent chromatin remodeling complex.[110] Interestingly, REST/CoREST-dependent chromatin remodeling, including decreases in histone acetylation and increases in DNA methylation, does not seem to be restricted to the immediate region around an NRSE silencer sequence; rather, the formation of heterochromatin extends across several genes that flank an NRSE.[111] These observations indicate that REST-dependent gene silencing, and thus cellular differentiation, involves the action of several proteins which, through decreases in histone acetylation and/or increases in DNA methylation, ultimately mark DNA epigenetically for repression. Interestingly, recent work has implicated REST in controlling dynamic, activity-dependent changes in gene expression within fully differentiated adult neurons.[112] This highlights a general theme that proteins that serve a particular function in development often play very different roles in adult tissues.

## NEUROGENESIS IN THE ADULT CNS

Not too long ago, the widely held dogma was that there is no new generation of neurons in the adult CNS. However, fascinating results have shown that neurogenesis does indeed continue into the adult in a small number of brain regions, including the hippocampal dentate gyrus, a phenomenon shown by Fred Gage and his collaborators to occur in the adult *human* hippocampus as well.[113] How was this established? Cancer patients sometimes receive treatment with the drug bromo-deoxy uridine (BrdU). It selectively affects dividing cells by being incorporated into their DNA upon de novo DNA synthesis. An ancillary aspect of this is that BrdU selectively labels freshly divided cells. Post-mortem analysis of the brains of cancer patients who had received BrdU as a chemotherapeutic treatment revealed that indeed new dentate granule cells are produced in an ongoing fashion in the adult human brain.

Investigation of how epigenetic mechanisms regulate this process is one of the most exciting areas of contemporary study concerning the roles of epigenetic molecular mechanisms in the functioning of the adult CNS. This topic is covered in detail in Chapter 12.

## CIRCADIAN RHYTHMS

The physiology of most organisms is modulated by time of day. These daily rhythms persist in the absence of external environmental cues, have a period of approximately 24 hours, and are commonly referred to as circadian rhythms. Circadian rhythms are generated endogenously by a biological timekeeping mechanism known as the circadian clock, which comprises intricate feedback loops of transcription and translation.[114,115] In addition, the mechanisms that are responsible for entrainment of the circadian clock to the environment (such as light) rely on signaling pathways that induce changes in transcription within particular brain regions. In mammals, the master circadian clock – which entrains the body's rhythm to light – resides in the suprachiasmatic nucleus (SCN), which is situated in the anterior hypothalamus.[116,117] However, most other brain regions and most peripheral tissues have been shown to have endogenous circadian clocks which presumably control rhythms in diverse physiological functions.[114,117,118]

The heart of any circadian clock lies in the transcription–translation feedback loop, which is known to be modulated by epigenetic mechanisms. Thus, the genome likely undergoes daily changes in its epigenetic state. The acetylation of histones H3 and H4 associated with the promoters of genes that form part of the core molecular clock mechanism are differentially regulated during a circadian cycle.[114,119] Moreover, infusion of the HDAC inhibitor trichostatin A into the SCN increases the expression of the clock genes mPer1 and mPer2, which indicates that epigenetic states directly affect the expression of the molecular components of the circadian clock.

Adjusting the phase of the circadian clock also requires transcription. The most salient phase-resetting environmental stimulus is light, and pulses of light induce changes in the transcription of several genes that comprise the molecular clock.[115,120] Epigenetic mechanisms seem to be associated with this regulation, as discrete pulses of light induce increases in acetylation of histones H3 and H4 associated with the promoters of mPer1 and mPer2.[119] Moreover, discrete light pulses induce significant increases in the phosphorylation of histone H3 in the SCN in vivo.[121] These observations indicate that regulation of the epigenetic state of the nucleus is a core molecular mechanism of the circadian clock, which is used to generate rhythmic gene expression and to establish a stable phase relationship between gene expression, an animal's behavior and physiology, and the environment.

## PERSISTING EFFECTS OF LIFE EXPERIENCE: NURTURING AND TRANSGENERATIONAL EFFECTS

Mother rats that exhibit strong nurturing behaviors toward their pups, for example, by frequently licking and grooming their offspring, produce lasting alterations in the patterns of DNA methylation in the central nervous systems (CNS) of their pups, which persist

throughout adulthood. Studies by Meaney and colleagues have presented evidence that these changes in DNA structure result in decreased anxiety-like behavior and a strong maternal nurturing instinct in the adult offspring as compared with offspring of mothers that show lower levels of grooming behavior.[34] These observations will be described in more detail in Chapter 4.

There are several interesting implications of these types of studies, which demonstrate persisting epigenetic marks and altered adult behavior in response to life experiences. First, this work indicates that experientially acquired alterations in DNA methylation affect behaviors in the adult. Second, the persistence of neonatally acquired patterns of DNA methylation in the mature CNS is consistent with the hypothesis that epigenetic mechanisms contribute to lasting cellular effects, that is, cellular memory in the CNS. Finally, and perhaps most importantly, studies of this sort suggest a specific epigenetic mechanism in the CNS for behaviorally perpetuating an acquired behavioral characteristic across generations – a particularly robust example of behavioral memory that is potentially subserved by epigenetics. The idea of transgenerational perpetuation of acquired epigenetic marks and the data supporting their existence will be described in Chapter 13.

## EPIGENETIC MECHANISMS AND CELLULAR INFORMATION STORAGE

As already alluded to previously, there are numerous examples that illustrate the importance of epigenetic mechanisms in information storage at the cellular level. They indicate that epigenetic mechanisms are widely used for the formation and storage of cellular information in response to transient environmental signals. Storage of information at the cellular level is in some ways analogous to behavioral memory storage in the adult nervous system. Moreover, the lasting cellular changes are triggered by a transient signal in each case, which is also a commonality between cellular memory and the formation of behavioral memory in the CNS.

A prototype example of the analogy between developmental memory and behavioral memory is mammalian cellular differentiation. Once an embryonic precursor cell is triggered to differentiate into a particular cell type (e.g. a liver cell), that cell and its subsequent daughter cells might be required to undergo thousands of cell divisions over the lifetime of the animal. How does a liver cell remember that it is a liver cell when, over the course of cell division, it must replicate de novo its entire genome? The information clearly cannot be contained in the DNA sequence itself. As mentioned above, the answer to this question involves epigenetic mechanisms, which allow the cell's identity to be manifest as the subset of genomic DNA that it expresses. The DNA is marked by, for example, DNA methylation at specific sites that are acquired as part of the differentiation process but are self-perpetuating during DNA replication and cell division. A role for non-coding RNAs in this process is also likely. Thus, a liver cell perpetuates its specific acquired pattern of gene expression across cellular generations and over time through these epigenetic marks – an example of memory at the cellular level.

The formation of epigenetic memory is not limited to mammalian cells. Plants are induced to flower by a process called vernalization that also involves epigenetic

mechanisms. For example, a biennial plant must experience a period of cold weather between its first and second years of existence for its flowering to be triggered. Exposure to cold in biennial plants results in activation of epigenetic mechanisms that involve methylation of DNA-binding proteins and acetylation of histones, and these processes trigger mitotically stable changes in the pattern of gene expression. In this way, plant cells "remember" their exposure to the winter cold and are prepared to allow the plant to flower during the next spring.

Another example involves T cells of the mammalian immune system. The commitment of T-lymphocyte precursors to a wide variety of differentiated states with different patterns of gene expression is triggered by numerous epigenetic mechanisms that involve DNA methylation and histone modifications. These processes are important in the formation of long-lasting immunological memory in response to a transient signal from the environment.

However, epigenetic mechanisms are also extant and operable in non-dividing, terminally differentiated neurons in the adult CNS. Adult neurons no longer have to deal with the problem of heritability, but the basic epigenetic mechanisms important for information storage during development are also important for storing memory that manifests itself behaviorally in the adult. Chapter 5 will discuss the idea that these mechanisms are conserved in the adult nervous system, where they have been co-opted to serve the formation of behavioral memories. Thus, current hypotheses posit that epigenetic mechanisms subserve changes in neuronal function in the adult that are components of memory at the behavioral level. Epigenetic processes may constitute a unified set of molecular mechanisms that allow information storage in systems as diverse as yeast, plants, and cellular differentiation and memory storage in the mammalian CNS.

## HUMAN COGNITION AND COGNITIVE DISORDERS

As a final comment we would be remiss if we did not highlight the fact that there is a considerable body of evidence, albeit indirect, implicating disruption of epigenetic mechanisms as a causal basis for *human* cognitive dysfunction. Here we will briefly describe several instances wherein derangements in molecular components of the epigenetic apparatus have been implicated in human cognitive disorders. These issues are discussed in much greater detail in Chapters 6–10. In broad overview, in interpreting these findings of a role for epigenetic molecular mechanisms in human behavior, an important caveat applies. When considering these cases it is important to distinguish between a developmental need for epigenetic mechanisms, to allow formation of a normal nervous system, versus an ongoing need for these mechanisms as part of cognitive processing per se in the adult. The majority of the attention to date has justifiably focused on developmental roles for epigenetics in establishing the capacity for cognitive function in the adult. However, the experimental results outlined above and in a number of chapters in this book implicate an ongoing and active role for epigenetic mechanisms in cognition and behavior in the adult. Thus, we believe it is timely and worthwhile to consider a possible component of cognitive disruption in those disorders outlined below to be due to a loss of active utilization of epigenetic mechanisms, necessary for normal cognition, in the mature post-developmental CNS.

**TABLE 1.2** Disorders of Human Cognition Partly Attributed to Dysfunction in the Mechanisms that Underlie Epigenetic Marking of the Genome

| Disease | Gene | Function | Epigenetic Affect | References |
|---|---|---|---|---|
| Rubinstein–Taybi syndrome | CREB-binding protein (CBP) | CBP is a histone acetyltransferase | ↑ histone acetylation | 8,109 |
| Rett syndrome | MecP2 | MeCP2 binds to CpG dinucleotides and recruits HDACs | ↓ histone acetylation | 14,43,79,88, 129,136 |
| Fragile X mental retardation | Trinucleotide expansions in FMR1 and FMR2 genes | Expansion of CGG or CCG repeats results in aberrant DNA methylation around FMR1 and FMR2 genes | ↑ DNA methylation. ↓ histone acetylation | 4,5,90,133 |
| Alzheimer's disease | Amyloid precursor protein | APP intracellular domain acts as a Notch-like transcription factor. Associated with the HAT Tip60 | ↑ histone acetylation | 25,50,53, 60,84,126 |
| Schizophrenia | Reelin | Reelin is an extracellular matrix protein, involved in synapse development | ↑ DNA methylation around reelin gene | 9,80 |

In this vein, several disorders of human cognition can be at least partly attributed to dysfunction in the mechanisms that underlie epigenetic marking of the genome (Table 1.2). Rubinstein–Taybi syndrome (RTS), an inherited autosomal dominant disease, is due to mutation of the gene encoding CBP, the transcriptional co-activator and HAT discussed earlier.[122,123] Several studies using animal models to investigate the molecular basis of RTS indicate that deficiency in CBP has severe consequences for long-term memory formation. Rett syndrome (RS) is an inherited, X-linked disease that appears to be due, in most cases, to loss-of-function mutations in the gene encoding MeCP2, the methyl-DNA binding protein.[124–126] Using genetic animal models, it was discovered that overexpression of MeCP2 enhanced long-term memory formation and the induction of hippocampal long-term potentiation (LTP), indicating that MeCP2 modulates memory formation and induction of synaptic plasticity.[127] Fragile X syndrome, the most commonly inherited form of mental retardation, is brought about by an abnormal expansion of repeated trinucleotide sequences within one of two different Fragile X genes: FMR1 and FMR2.[128,129] Both FMR1 and FMR2 contain a polymorphic trinucleotide repeat, CGG and CCG respectively, in their 5′ untranslated regions responsible for the loss of gene expression.[130,131] Expansion of these repeats results in hypermethylation of these regions and flanking CpG islands, leading to transcriptional silencing of the FMR and surrounding genes. The most widespread of senile dementias, Alzheimer's disease, appears to be due, in part, to an increase in soluble β-amyloid peptides in the brain.[132] These peptides are created by endo-proteolytic cleavage of the transmembrane amyloid precursor protein (APP) by β- and γ-secretases.[133] Interestingly, cleavage of APP results not only in production of an extracellular β-amyloid fragment, but also an intracellular fragment, the APP intracellular domain (AICD), that

regulates transcription through recruitment of the adapter protein Fe65 and the HAT Tip60, suggesting that some of the pathology of Alzheimer's disease might be due to dysregulation of histone acetylation.[134–137] Finally, schizophrenia is a serious disorder of cognition, rendering sufferers unable to function normally in social situations and in performing everyday cognitive tasks. An emerging body of evidence suggests that deficiencies in the extracellular matrix protein reelin may contribute to the pathophysiology of schizophrenia, at least in a subset of patients.[138] The promoter of reelin contains several sites for DNA methylation, and inhibitors of HDAC and DNMT activity increase expression of reelin, indicating that epigenetic mechanisms govern reelin expression.[139]

All of these observations indicate that dysfunction of the normal epigenetic status of the genome can have dramatic consequences on normal cognitive function.[7] These studies also suggest that drugs which target the epigenome might represent viable therapies in treating various diseases affecting cognition, as will be discussed in more detail in Chapters 6–8.

## SUMMARY – ACTIVE REGULATION OF EPIGENETIC MARKS IN THE NERVOUS SYSTEM

In this brief overview, we have presented an emerging view of the epigenome and its role in the adult CNS. New studies are being published at a rapid pace demonstrating that epigenetic mechanisms are involved in mediating diverse experience-driven changes in the CNS. These experience-driven changes in the adult CNS are manifest at the molecular, cellular, circuit, and behavioral levels. Overall, these diverse observations demonstrate that the epigenome resides at the interface of the environment and the genome. Furthermore, it is now becoming clear that epigenetic mechanisms exert a powerful influence over behavior. Future studies geared toward understanding the role of the epigenome in experience-dependent behavioral modification will clearly be important for, and relevant to, not only the memory field but studies of diverse types of psychiatric and neurological disorders as well.

Chromatin is a dynamic structure that integrates potentially hundreds of signals from the cell surface and effects a coordinated and appropriate transcriptional response. It is increasingly clear that epigenetic marking of chromatin and DNA itself is an important component of the signal integration that is performed by the genome as a whole. Moreover, changes in the epigenetic state of chromatin can have lasting effects on behavior. We hypothesize that the CNS has co-opted mechanisms of epigenetic tagging of the genome for use in the formation of long-term memory and many other forms of long-lasting neural plasticity seen in both health and disease. In our estimation, understanding the epigenetic regulation of neural and glial function will be vital for fully understanding the molecular processes that govern normal brain function as well as the range of brain abnormalities that underlie diverse disease states.

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