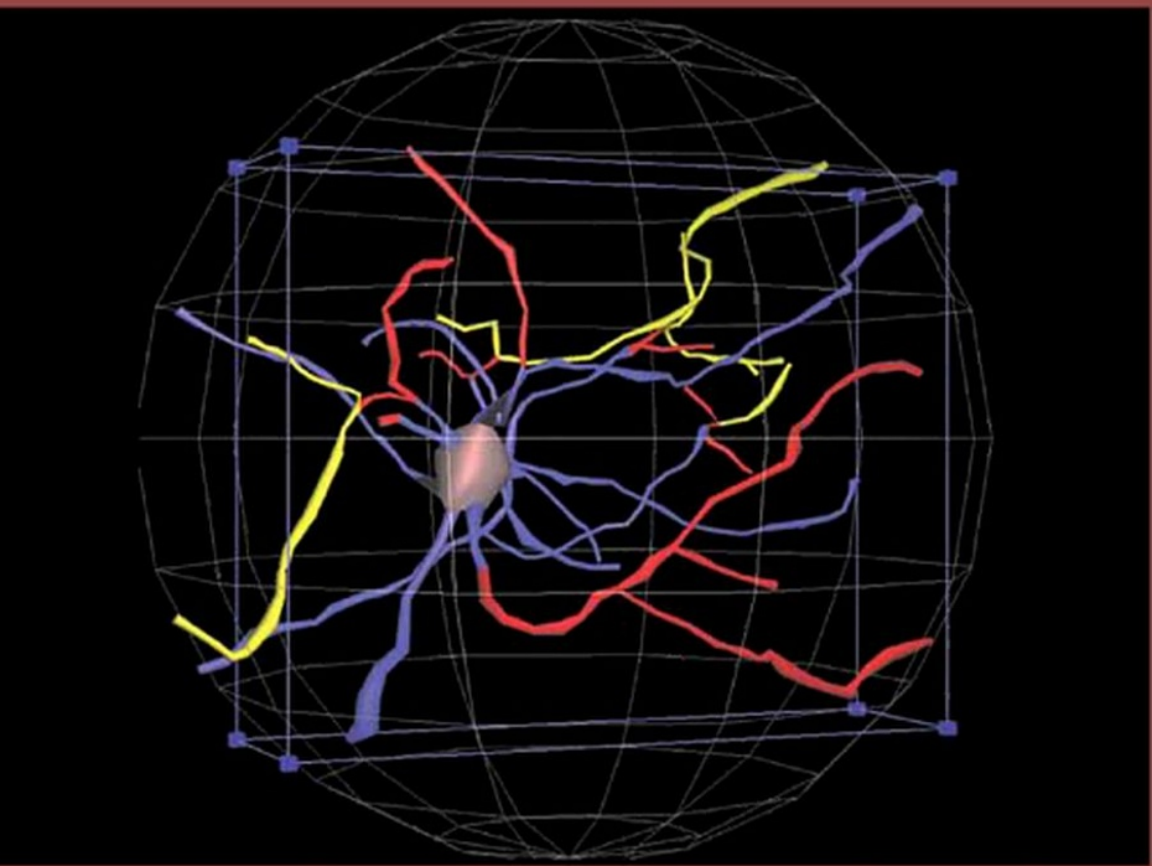
INTERNATIONAL REVIEW OF NEUROBIOLOGY

Novel Approaches to Studying Basal Ganglia and Related Neuropsychiatric Disorder

Volume 89



Edited by
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THE ROLE OF DNA METHYLATION IN THE CENTRAL NERVOUS SYSTEM AND NEUROPSYCHIATRIC DISORDERS

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I. Introduction
II. DNA Methylation and DNA Methyltransferase
III. DNA Methylation Mediates Gene Silencing
IV. Role of DNA Methylation in the CNS and Neuropsychiatric Disorders
V. Conclusion Remarks
References

DNA methylation is an epigenetic mechanism in which the methyl group is covalently coupled to the C5 position of the cytosine residue of CpG dinucleotides. DNA methylation generally leads to gene silencing and is catalyzed by a group of enzymes known as DNA methyltransferases (Dnmt). During development, the epigenome undergoes waves of demethylation and methylation changes. As a result, there are cell type/tissue-specific DNA methylation patterns. Since DNA methylation changes only happen during DNA replication to maintain methylation patterns on hemimethylated DNA or establish new methylation, Dnmt expression generally decreases greatly after cell division. However, significant levels of Dnmts were noticed specifically in postmitotic neurons, suggesting a functional importance of Dnmt in the nervous system. Accumulating evidence showed that DNA methylation correlates with certain neuropsychiatric disorders such as schizophrenia, Rett syndrome, and ICF syndrome. Studies of methyl-CpG-binding proteins, Dnmt inhibitors, and Dnmt knockout mice also explored the key role of DNA methylation in neural development, plasticity, learning, and memory. Though an enzyme exhibiting DNA demethylation capability in vertebrates still remains to be identified, DNA methylation status in the CNS appeared to be reversible at certain genomic loci. This supports a maintenance role of Dnmt to prevent active demethylation in postmitotic neurons. Taken together, DNA methylation provides an epigenetic mechanism of gene regulation in neural development, function, and disorders.

INTERNATIONAL REVIEW OF NEUROBIOLOGY, VOL. 89
DOI: 10.1016/S0074-7742(09)89004-1

0074-7742/09 $35.00

I. Introduction

The word "epigenetics" is normally defined as "the study of mitotically and/or meiotically heritable changes in gene function that cannot be explained by changes in DNA sequence" (Bird, 2007; Jaenisch and Bird, 2003). However, given the fact that many chromatin marks are short-lived and not transmissible between generations and that DNA methylation pattern can also be rapidly removed during development, arguing for the emphasis on heritability for epigenetics may not be necessary (Bird, 2007).

DNA methylation and histone modification are the two major epigenetic mechanisms. As the fundamental unit of chromatin, the nucleosome consists of DNA wrapping around an octamer histone core. This enables DNA to be tightly packaged into the nucleus. The epigenetic mechanisms adjust gene activity by altering accessibility of DNA to the transcription machinery without changing the genetic code. While modification of DNA by methylation generally leads to gene silencing, posttranslational modifications of histone proteins including acetylation, methylation, phosphorylation, ubiquitination, or sumoylation can lead to both gene activation and repression (Jenuwein and Allis, 2001). One of the best-studied histone modifications is the acetylation status of lysine residues, a reversible process that is catalyzed by either histone acetylase (HAT) or histone deacetylase (HDAC). The addition of an acetyl group decreases the interaction between the negatively charged DNA backbone and the positively charged histone tail. This interaction can lead to a less compact nucleosome, and open access to transcription factor complexes. Conversely, HDAC removes the acetyl group, potentially leading to gene transcription repression. Methylation of histones is more complex since each distinct (mono-, di-, or tri-) methylation of different lysine residue can have opposite effects on transcription. For instance, H3 at lysine 4 (K4) is associated with transcriptional activation whereas methylations on histone H3 at K9 or K27 are usually indicative of transcriptional inhibition (Kouzarides, 2007). Moreover, methylation of histones can be reversed as well (Klose *et al.*, 2006; Shi *et al.*, 2004). This newly discovered mechanism for histone demethylation adds another wrinkle into the understanding of how histones regulate gene expression. It has been demonstrated that epigenetic mechanisms play pivotal roles in translating environmental stimuli into long-lasting gene expression changes in the nervous system which is required under both physiological (such as learning and memory) (Feng *et al.*, 2007) and pathological conditions (such as psychiatric disorders) (Tsankova *et al.*, 2007). However, most of these studies focused on histone modifications. For example, in Aplysia, histone acetylation plays a major role in excitatory transmitter activated gene expression which is needed for long-term synaptic plasticity (Guan *et al.*, 2002). Also, the mutation of a histone acetyltransferase gene is believed to be the cause of a mental

retardation disease Rubinstein–Taybi syndrome (RTS) (Petrij *et al.*, 1995). In a histone acetyltransferase mutant RTS mouse model, either suppression of transgene expression or HDAC inhibitor administration could rescue the impairment on long-term memory stabilization (Korzus *et al.*, 2004). More strikingly, in a mouse model of neurodegeneration, increased histone acetylation by inhibitors of HDAC could induce recovery of learning and memory (Fischer *et al.*, 2007; Guan *et al.*, 2009). It is believed that different histone modifications, in combination or alone, define a specific epigenetic mark (histone code) that will lead to different gene expression scenarios. For example, acute and chronic cocaine addiction induced expression of different genes which are associated with different epigenetic regulatory mechanisms (Colvis *et al.*, 2005). The epigenetic mechanism's effect on higher neural functioning appears to be universal since some other epigenetic factors' modulating roles were also found. For instance, KAP1, a vertebrate-specific epigenetic repressor, was found to control gene expression in the hippocampus and modulate the behavioral response to stress (Jakobsson *et al.*, 2008). A further understanding of epigenetic mechanisms in neural functioning may advance our approaches to neuropsychiatric disorder therapies.

Not as dynamic as histone tail modification, covalent 5-cytosine methylation is deemed to be a more static mark. DNA methylation pattern maintenance or establishment only happens during DNA replication (Cameron *et al.*, 1999; Holliday, 1999). DNA methylation normally will not change in nondividing cells which makes DNA methylation a less prominent candidate for dynamic gene expression regulation within postmitotic neurons. However, recent findings support important functional roles of DNA methylation in the nervous system. Since the function of histone modification in the nervous system has been well reviewed (Colvis *et al.*, 2005; Tsankova *et al.*, 2007), we will focus on the role of DNA methylation in this chapter.

II. DNA Methylation and DNA Methyltransferase

Methylation of DNA at the fifth carbon of the cytosine ring exists in all vertebrates, fungi, flowering plants, and some invertebrate insects as well as certain bacterial species. The biological functions of cytosine methylation are quite different in prokaryotes and eukaryotes. In prokaryotes, DNA methylation occurs at adenine as well as cytosine bases, playing a central role in the host restriction system. In eukaryotes, DNA methylation is restricted to cytosine bases and is coupled with a repressed chromatin state and inhibition of gene expression. In mammals, 60–90% of CpG sites are methylated, and the majority of the remaining unmethylated residues exist in CpG islands within gene promoters (Bird, 2002; Jaenisch and Bird, 2003).

DNA methylation is catalyzed by a group of DNA methyltransferases (Dnmts). The eukaryotic Dnmt family has four members: Dnmt1, Dnmt3a, Dnmt3b, and Dnmt3l.

The first murine Dnmt was cloned in 1988 and was later named Dnmt1 (Bestor *et al.*, 1988). It is the most abundant Dnmt in mammalian cells. The enzyme is approximately 1620 amino acid long. The N-terminal 1100 amino acids constitute the regulatory domain and the C-terminal residues constitute the catalytic domain. Dnmt1 predominantly methylates hemimethylated CpG dinucleotides. In a kinetic analysis with unmethylated and hemimethylated 36- and 75-mer oligonucleotides, Dnmt1 showed a 7- to 21-fold preference for hemimethylated DNA (Pradhan *et al.*, 1999). During cell replication, the newly synthesized DNA, which is hemimethylated, must be methylated precisely to maintain the established DNA methylation pattern. Dnmt1 is located at the replication fork and methylates newly biosynthesized DNA strands directly (Hermann *et al.*, 2004). For this reason, Dnmt1 is coined as a maintenance Dnmt.

De novo Dnmts are another group of Dnmts, which can effectively methylate cytosine residues in unmethylated DNA. This event happens particularly during germ cell development and embryogenesis. Dnmt3a and Dnmt3b comprise the two major *de novo* Dnmts. Dnmt3a and Dnmt3b have a high degree of primary structural homology, but they are encoded by different genes mapped to different chromosomes. The Dnmt3 enzymes share similar architectural features to Dnmt1 with a large N-terminal regulatory region connected to a C-terminal catalytic domain (Pradhan and Esteve, 2003). But when functionally compared to Dnmt1, Dnmt3a exhibits a lower level of methyltransferase activity, suggesting that it may require small molecules or protein cofactors for optimal activity (Hsieh, 1999). Overexpression experiments showed that both Dnmt3a and Dnmt3b can methylate a stable episome *in vitro*, but Dnmt3b could not lead to the same degree of *de novo* methylation as Dnmt3a. Dnmt3a and Dnmt3b have differential expression patterns. Dnmt3a is more ubiquitous whereas Dnmt3b is usually expressed at low levels except in the testis, thyroid, and bone marrow (Xie *et al.*, 1999). Although the activities of Dnmt3a and Dnmt3b are reduced after differentiation, Dnmt3b level is greatly increased in tumor cell lines. This evidence suggests that Dnmt3a and Dnmt3b have different functional roles aside from redundancy in *de novo* methylation. Indeed, Dnmt3b is specialized for CpG methylation within repetitive sequences at pericentric satellite regions of chromosomes. Mutation of the Dnmt3b gene was found to be associated with immunodeficiency, centromere instability, and facial anomalies (ICF) syndrome, which is characterized by unmethylated DNA within pericentric regions of chromosomes (Hansen *et al.*, 1999; Xu *et al.*, 1999).

Dnmt3l (Dnmt3 *l*ike) is the third member of the Dnmt3 family and lacks independent methyltransferases activity. However, Dnmt3l may cooperate with other *de novo* Dnmts. For instance, Dnmt3l binds to the C-terminal of Dnmt3a and

Dnmt3b, increasing the activity of these enzymes up to threefolds (Suetake *et al.*, 2004). In contrast, Dnmt3l has never been found to affect the activity of Dnmt1. Dnmt3l might be important for genomic imprinting since targeted disruption of Dnmt3l resulted in biallelic expression of genes normally imprinted and expressed from one paternal origin (Bourc'his *et al.*, 2001).

Based on sequence homology to Dnmts, a molecule named Dnmt2 was cloned (Goll and Bestor, 2005). It is the most conserved and most widely distributed Dnmt. However, targeted deletion of Dnmt2 in ES cells caused no detectable effect on global DNA methylation, implying that Dnmt2 is not essential for DNA methylation (Okano *et al.*, 1998).

III. DNA Methylation Mediates Gene Silencing

During development, the epigenome undergoes waves of demethylation and methylation change. As a result, various cell type/tissue-specific DNA methylation patterns occur at specific times. Global *de novo* methylation happens specifically during germ cell development and early embryogenesis. How Dnmt enzymes are recruited to the DNA to pursue DNA methylation modification is still unclear. Recent studies suggest there are at least three possible means. First, Dnmt3 enzymes themselves may target DNA via specific domains. For instance, the PWWP domain in the N-terminal half of Dnmt is highly conserved and is important for protein–protein interaction. Mutation of the PWWP domain in *de novo* Dnmts completely abolished its chromatin targeting capacity and led to reduced DNA methylation (Ge *et al.*, 2004). Second, Dnmt may also interact with site-specific transcriptional repressors to target DNA methylation. This idea comes from research on DNA methylation of tumor suppressor genes, which frequently causes transcriptional silencing during cancer. It has been shown that a leukemia-promoting PML-RAR fusion protein induces gene hypermethylation and silencing by recruiting Dnmts to target promoters (Di Croce *et al.*, 2002). Other research also uncovered the mechanistic link between Dnmt and transcriptional repression (Brenner *et al.*, 2005; Robertson *et al.*, 2000). All these findings suggest that the coupling of Dnmt with transcription factors may be a general mechanism for the generation of specific DNA methylation patterns. The third novel mechanism is that DNA methylation might be targeted by transcriptional gene silencing pathways in response to RNA interference (RNAi) signals. In plants, RNAi-mediated transcriptional silencing always results in *de novo* methylation of the gene promoter. Even though the detailed molecular mechanisms have yet to be disclosed, studies of mammalian cell cultures have also suggested a similar mechanism (Morris *et al.*, 2004).

Another fundamental question aside from how DNA is targeted by Dnmt for methylation modification is how methylated DNA leads to silencing of gene expression. So far, four models have become apparent. In the first, DNA methylation can prevent the transcriptional activator binding to the target DNA sequence, leading to direct repression of transactivation (Watt and Molloy, 1988). In the second, the Dnmt protein itself might physically link to HDAC and histone methylase (HMT) proteins. For example, Dnmt1 is itself associated with HDAC activity *in vivo*. HDAC1 has been shown to bind Dnmt1 and can purify methyltransferase activity from nuclear extracts. The transcriptional repression domain of Dnmt1 can also recruit HDAC directly (Fuks *et al.*, 2000). Dnmt3b can also couple with other chromatin-associated enzymatic activities common to transcriptional repression such as histone methylation (Geiman *et al.*, 2004). These raise the possibility that Dnmts mediate nonenzymatic roles in transcriptional silencing which rely on chromatin modification by complexing with other transcriptional repressor proteins (Robertson *et al.*, 2000; Rountree *et al.*, 2000). Besides the role in epigenetic modification of cytosine, Dnmts also function to silence gene expression directly through transcriptional repression. The third repression model is that DNA methylation within the gene body exerts a repressive effect on transcriptional elongation. Even though transcriptional silencing is often associated with promoter methylation, a considerable number of methylated CpGs are found in gene bodies including both intronic and exonic regions. When a transgene, which is methylated exclusively in a region downstream of the promoter, is introduced into a specific genomic site, it yields a clear decrease in the transgene expression relative to the unmethylated control. At the same time, there is a reduction in RNA polymerase II occupancy and chromatin accessibility. It is, therefore, proposed that the dense intragenic DNA methylation initiates the formation of a chromatin structure that reduces the efficiency of transcription elongation (Lorincz *et al.*, 2004). However, this model may not apply universally since DNA methylation of the gene body was also found to directly correlate with transcription level (Hellman and Chess, 2007). The fourth model of DNA methylation-induced gene transcription silencing is that methyl-CpG-binding proteins directly recognize methylated DNA and recruit corepressor molecules to silence transcription and also modify surrounding chromatin (Nan *et al.*, 1997, 1998). The family of methyl-CpG-binding proteins includes six members named MBD1–MBD4, Kaiso, and methyl-CpG binding protein 2 (MeCP2) (Fan and Hutnick, 2005; Klose and Bird, 2006). They are characterized by the methyl-CpG-binding domain (MBD), the protein motif responsible for binding methylated CpG dinucleotides. MBD1 can also interact with histone 3 lysine 9 (H3K9) methyltransferases to modify the surrounding chromatin by histone modification. The absence of MBD2 remarkably reduced tumorigenesis, implying that MBD2 contributes to tumor formation (Sansom *et al.*, 2003). MBD2 also reportedly shows robust demethylation ability *in vitro* (Bhattacharya *et al.*, 1999), but attempts

to repeat the experiment failed in other laboratories (Bird, 2002). Though MBD3 contains a well-conserved MBD domain, it does not directly bind to methylated DNA because of a crucial amino acid substitution. As for MBD4, it is best known for its role in DNA repair (Hendrich *et al.*, 1999). It binds preferentially to G:T mismatches resulting from methyl-CpG deamination. Specifically, it may function to minimize mutations at methyl-CpG in particular. A novel methyl-CpG-binding protein named Kaiso lacks the MBD, but it can still recognize methylated DNA through zinc-finger domains (Prokhortchouk *et al.*, 2001). MeCP2 interacts specifically with methylated DNA and mediates transcriptional repression. MeCP2 binds tightly to chromosomes in a methylation-dependent manner. It in turn associates with a corepressor complex containing the transcriptional repressor mSin3A and HDAC. The relieved transcriptional repression by the deacetylase inhibitor TSA indicates that histone deacetylation is essential to this repression mechanism. In this way, MeCP2 links two global mechanisms of gene regulation, DNA methylation, and histone deacetylation in this transcriptional repression machinery.

IV. Role of DNA Methylation in the CNS and Neuropsychiatric Disorders

Epigenetic mechanisms, which include histone modification and DNA methylation, are believed to adjust the chromatin remodeling and accessibility of gene promoters (Jaenisch and Bird, 2003) to regulate neural adaptive gene expression. The essential roles of histone modification have been well demonstrated (Tsankova *et al.*, 2007). Different histone modifications, in combination or alone, define a specific epigenetic mark (histone code) that leads to different gene expression scenarios (Colvis *et al.*, 2005). Moreover, increased histone acetylation by HDAC inhibitors can even induce recovery of learning and memory (Fischer *et al.*, 2007; Guan *et al.*, 2009). However, the function of DNA methylation in the CNS is still unclear. Not as dynamic as reversible histone tail modifications, DNA methylation is a more static epigenetic mark, making it a less prominent candidate for dynamic neural gene expression regulation.

However, the importance of DNA methylation in the brain has been demonstrated by its association with some neurological disorders such as Rett syndrome (Amir *et al.*, 1999) and ICF syndrome (Hansen *et al.*, 1999). Mutations of MeCP2 have been found in about 80% of Rett syndrome patients (Amir *et al.*, 1999). Rett syndrome is one of the most common mental retardation diseases in females. Patients appear normal for the first 6–18 months of life, but gradually lose speech and motor skills. However, patients can still survive into adulthood, which suggests no progressive neurodegeneration. In the nervous system, MeCP2 is mainly expressed in neurons and shows an upregulation postnatally

(Jung *et al.*, 2003; Shahbazian *et al.*, 2002). Meanwhile, expression of MeCP2 is ubiquitous in rodents and humans, although protein levels vary between tissues. Mutations of MeCP2 only cause neurological defects, suggesting that there is a critical role for methyl-binding proteins/DNA methylation in the nervous system. By using transgenic mouse models (Chen *et al.*, 2001; Guy *et al.*, 2001), brain-specific deletion of MeCP2 at E12.5 resulted in an identical phenotype to that of the null mutation. Moreover, deletion of MeCP2 in only postnatal CNS neurons also led to a similar neuronal phenotype. These findings implicate that Rett syndrome is indeed caused by MeCP2 deficiency in the CNS as opposed to peripheral tissues, and the function of MeCP2 is more important in nervous system, especially in mature neurons (Kriaucionis and Bird, 2003). In addition, mutations of Dnmt3b gene have also been noticed in around 60% of ICF syndrome patients (Hansen *et al.*, 1999). Although the patients are characterized with immune defects, chromosomal instability as well as facial abnormalities, neurological defects including mental retardation is also common. Indeed, aberrant expression of genes regulating development and neurogenesis, which are relevant to the phenotypes, has been found in cell lines derived from ICF individuals (Jin *et al.*, 2008). Another case showing the importance of DNA methylation and/or methyl-CpG-binding proteins in brain function comes from MBD1 mutant mice. Besides reduced neuronal differentiation, adult MBD1 mutants have impaired spatial learning and significant reduction in long-term potentiation (LTP) of the hippocampus (Zhao *et al.*, 2003), potentially indicating the important role of DNA methylation in higher neural functioning. However, the search for methyl-CpG-binding protein regulated genes is still elusive (Tudor *et al.*, 2002). For instance, microarray study of MeCP2 null mice reveals only subtle transcriptional changes in the brain which could be explained as the sensitivity limitation of accurate detection of low-abundance transcripts or regional transcription differences. Alternatively, there may be some other DNA methylation or transcription-independent roles, which still require further investigation.

The studies of Dnmts can no doubt advance our understanding of the role of DNA methylation in the CNS. The expression of both the maintenance DNA methylation catalyzing enzyme Dnmt1 and the *de novo* DNA methyltransferases Dnmt3a and Dnmt3b have been characterized in the nervous system (Feng *et al.*, 2005; Goto *et al.*, 1994; Inano *et al.*, 2000; MacDonald *et al.*, 2005; Watanabe *et al.*, 2006). Dnmt1 is expressed at remarkably high levels in the embryonic nervous system. Additionally, almost all mature neurons in brains of adult mice express Dnmt1 at substantially high levels as compared with other organs. In the mouse brain, Dnmt3b is mainly expressed in early embryonic stages and neural progenitor cells before E15.5, whereas Dnmt3a is predominantly expressed in later embryonic stages through adulthood within neural precursor cells, maturing neurons, oligodendrocytes, and a subset of astrocytes (Feng *et al.*, 2005). As we discussed earlier, during cell replication, the newly synthesized DNA is

hemimethylated (methylated parent strand and unmethylated daughter strand) and has to be precisely methylated on the daughter strand to maintain the mitotic inheritance of the methylation pattern from the parent strand. The maintenance DNA methyltransferase Dnmt1 is located at the replication fork and methylates hemimethylated DNA directly. Meanwhile, during germ cell development and embryogenesis, some DNA strands will switch from the unmethylated to the methylated state. *De novo* DNA methyltransferases Dnmt3a and Dnmt3b catalyze this process by adding methyl group to the unmethylated DNA to establish a new methylation pattern. Both *de novo* methylation and maintenance methylation happen during DNA replication. Generally, the DNA methylation pattern will not change after cell division, and afterward the Dnmt expression level in somatic cells diminishes greatly. That nondividing neurons still hold substantial levels of Dnmt1 and Dnmt3a (but not Dnmt3b) postnatally is highly indicative of their functional importance in the nervous system.

Interestingly, it was found that in schizophrenic postmortem brains, Dnmt1 is selectively overexpressed in the GABAergic neurons together with a downregulation of reelin and glutamic acid decarboxylase (GAD) 67 (Veldic *et al.*, 2004). Similarly, decreased expression of the $GABA_A$ receptor $\alpha 1$ gene was hypermethylated at its promoter region in suicidal/major depression disorder brains (Poulter *et al.*, 2008). Although the causal relationship is hard to establish, Dnmt1 knockdown in mouse primary neuronal culture was accompanied by increased reelin mRNA expression (Noh *et al.*, 2005). These data support the hypothesis that the aberrant gene expression changes in neuropsychiatric disorder brains may be the consequence of a Dnmt-mediated hypermethylation of the corresponding promoters.

Recent Dnmt inhibitor studies demonstrated that hippocampal LTP can be blocked by Dnmt inhibitors within rodent hippocampal slices (Levenson *et al.*, 2006; Nelson *et al.*, 2008). Moreover, these Dnmt inhibitors could also prevent rat memory formation following contextual fear conditioning (Miller and Sweatt, 2007). These support the notion that DNA methylation may target-specific genes involved in synaptic plasticity, learning, and memory. However, the Dnmt inhibitors (5-aza 2′-deoxyazacytidine and Zebularine) are nucleoside analogues that must be incorporated into DNA during DNA synthesis before they become inhibitors of Dnmts (Constantinides *et al.*, 1978; Jones *et al.*, 1982; Lyko and Brown, 2005). How they functioned in nondividing neurons (Tsankova *et al.*, 2007) or whether they actually worked in dividing cells such as glial cells and neural blast cells are unknown. The cytotoxicity effect of Dnmt inhibitors is also a concern for a broad application (Juttermann *et al.*, 1994; Lyko and Brown, 2005). Meanwhile, deficiency of selective Dnmt inhibitors makes it difficult to differentiate individual effects among different Dnmts. The future availability of less toxic, subtype specific Dnmt inhibitors will definitely improve our understanding of Dnmt/DNA methylation's role in the CNS. Alternatively, Dnmt mutant research can provide more direct evidence of DNA methylation's role in the nervous system.

Targeted mutation of Dnmt1 or Dnmt3b results in embryonic lethality before midgestation or E15.5, respectively (Li *et al.*, 1992; Okano *et al.*, 1999). A conventional deletion of Dnmt3a also causes lethality between postnatal weeks 2–3 (Okano *et al.*, 1999). Nervous system-specific conditional Dnmt knockouts were generated to better study the function of DNA methylation in the nervous system (Fan *et al.*, 2001; Hutnick *et al.*, 2009; Nguyen *et al.*, 2007). By utilizing the Cre/loxP system to inactivate Dnmt1 in neuroblasts of developing embryos (Fan *et al.*, 2001), it was found that the Dnmt1 deficiency in mitotic neural precursor cells resulted in DNA hypomethylation in daughter cells. Mice showing a nearly complete loss of Dnmt1 in the brain die immediately after birth because of respiratory distress. Mice whose brains have a low fraction of hypomethylated cells are viable but show a rapid loss of these cells and complete elimination within 3 weeks postnatally. This clearly proves that DNA methylation is required for neural cell survival. Interestingly, a similar nervous system-specific Dnmt3a knockout leads to fewer motor neurons in the hypoglossal nucleus (Nguyen *et al.*, 2007). Mice are born healthy, but degenerate and die prematurely. Their neuromuscular function and motor coordination defects display the role of Dnmt3a in neuromuscular control of motor movement (Nguyen *et al.*, 2007). Consistent with the predication that normal DNA methylation is required for the development of synaptic plasticity, studies of Emx1Cre triggered Dnmt1 deletion exclusively in telencephalic precursors found that thalamocortical LTP could not be induced. Dnmt1 deletion also blocked the development of somatosensory projection in the sensory cortex and induced striking cortical and hippocampal degeneration (Golshani *et al.*, 2005; Hutnick *et al.*, 2009) which suggest DNA methylation's roles in neuronal maturation.

DNA methylation also appears to play a role in neural cell fate specification. During development, neurons are always generated before astrocytes in the developing brain. How glial genes are restricted from developing neurons has long been of interest. There is increasing evidence that the transduction of these signals is regulated by epigenetic mechanisms at the level of DNA methylation and histone modification (Feng *et al.*, 2007; Hsieh and Gage, 2004, 2005). It has been shown that promoter demethylation of the glial fibrillary acidic protein (GFAP) gene, a gene normally expressed in astrocytes, is correlated with the GFAP gene transcription during astrogliogenesis (Takizawa *et al.*, 2001; Teter *et al.*, 1994). Conditional deletion of Dnmt1 in neural progenitor cells resulted in DNA hypomethylation and precocious astroglial differentiation (Fan *et al.*, 2005). Moreover, demethylation of genes in the gliogenic JAK–STAT pathway leads to enhanced activation of STATs, which triggers astrocyte differentiation. This finding provides a mechanism by which DNA methylation controls astrogliogenesis through STAT activation and promoter activation of GFAP glial marker genes.

However, all these findings are derived from Dnmt conditional knockouts in neural progenitor cells, so it is hard to discern whether the observed phenotypes are due to a loss of Dnmt in dividing progenitor cells or postmitotic neurons. A preliminary experiment indicated that at least the majority of the phenotypes are attributed to Dnmt's deletions from progenitor cells. In another strain of CamKIICre Dnmt1 conditional knockout mice, in which Dnmt1 deletion only occurs in postmitotic neurons, neither genomic hypomethylation nor obvious cell death were detected (Fan *et al.*, 2001). This may not be surprising because normal methylation patterns were in place before Dnmt1 was removed. However, the question remains why these neurons keep so much Dnmt postmitotically. It still cannot be ruled out that the Dnmt may still have some DNA methylation-independent or complementary (between Dnmt1 and Dnmt3a) functions in adult neurons. More than a decade ago, it was already proposed that Dnmt may serve to maintain DNA methylation after base-excision repair of the G:T mismatch that occurs spontaneously upon deamination of the methylated cytosine (Brooks *et al.*, 1996). Meanwhile, Dnmts may also be required to keep the DNA methylation pattern if methylation turnover (DNA demethylation) exists in adult neurons. Maintaining the genetic and epigenetic integrity in neurons could be critical since, unlike other cell types, nonrenewable postmitotic neurons have to function properly for their entire lifespan.

Active DNA methylation turnover in postmitotic neurons provides a compelling evidence for the existence of DNA demethylase and provides another function for Dnmts in neuronal cells. In contrast to blocking methylation of newly replicated DNA to passively demethylate DNA, active demethylation is replication independent. Some factors must take action to remove the methylation from already methylated DNA without cell division. In this sense, postmitotic neurons provide a good model to study with. Though Dnmts were recognized a long time ago, evidence for an enzyme exhibiting DNA demethylation capability in vertebrates is still lacking. By far, the most definitive DNA demethylases come only from plants (Zhu, 2008) instead of mammals. Though a number of mammalian DNA demethylases have been reported (for instance: MBD2 (Bhattacharya *et al.*, 1999), Gadd45a (Barreto *et al.*, 2007), Gadd45b (Ma *et al.*, 2009)), the results either could not be reproduced by other labs or led to controversial findings (Ooi and Bestor, 2008). One explanation of this difficulty is that intergenerational transmission of DNA methylation patterns within plants and mammals are fundamentally different (Ooi and Bestor, 2008). Plants transmit DNA methylation pattern with accretion of additional methylation during each generation, which makes the DNA demethylase necessary to prevent excess DNA methylation interference of nearby gene expression. Whereas mammalian germ cells may not require active DNA demethylation since they go through DNA methylation erasure first, followed by a reestablishment of the

methylation pattern. Another possibility to explain the difficulty of identifying DNA demethylase is that DNA demethylation may actually be a coupling effect of several factors, as was recently noticed in the zebrafish, in which deaminase, glycosylase as well as Gadd45 work together to modulate DNA demethylation (Rai *et al.*, 2008).

Recently, there has been accumulating evidence proposes active demethylation in the CNS. For example, research of rodents' nurturing effect on offspring's stress response proved that DNA methylation in CNS could be quite dynamic (Francis *et al.*, 1999; Weaver *et al.*, 2004). As we know, early experiences can profoundly affect adult behavioral pattern and parenting style can influence a child's future personality and behavior. Interestingly, researchers found that offspring of high licking/grooming and arched-back nursing (LG-ABN) rat mothers are less fearful and show a more modest hypothalamic–pituitary–adrenal stress response in their adult lives (Liu *et al.*, 1997). How the maintenance of early environmental factors affects the phenotype is interesting. In a cross-fostering experiment, the biological offspring of a "low-LG-ABN" mother were raised by a "high-LG-ABN" mother. They showed a similar stress response to the normal offspring of a "high-LG-ABN" mother (Francis *et al.*, 1999). This implies that some nongenomic modulation may be responsible for this behavioral effect. It has been shown that there is a dynamic change of the epigenome around the glucocorticoid receptor (GR) gene promoter region, which leads to increased GR expression in adult offspring of "high-LG-ABN" mothers (Weaver *et al.*, 2004). A key transcription factor binding site, within the first exon of GR, is switched from the unmethylated state to the methylated state right after birth. Strikingly, exposure to "high-LG-ABN" mothering appears to demethylate this CpG site. Also histones surrounding the GR promoter became more acetylated. As a result, DNA methylation, together with histone acetylation, provides open access for GR transcriptional activation. The sustained GR expression is believed to maintain a long-term modest stress response. Additionally, this epigenetic regulation of GR expression could still be dynamic in the brain (Weaver *et al.*, 2005). A central administration of HDAC inhibitor in the adult rat reverses the stress response under "low-LG-ABN" care, whereas providing a methyl group donor for DNA methylation converts the stress response in "high-LG-ABN" offspring. This further proves the causal relationship between DNA methylation and long-term behavioral change. However, it is worthy noting that the patchy DNA demethylation evidence (Miller and Sweatt, 2007; Weaver *et al.*, 2004) identified so far in the CNS are still short of a cellular resolution. We are not aware whether they are from dividing neural progenitors and/or glia which is resulted from passive demethylation by blocking Dnmt access during DNA replication or from postmitotic neurons as a result of active demethylation by potential DNA demethylase.

V. Conclusion Remarks

DNA methylation provides an epigenetic means of neural gene expression regulation. In the past decade or so, the pivotal roles of DNA methylation as well as Dnmts in the CNS have been recognized in neural differentiation, cell survival, cell maturation, neural plasticity, as well as some neuropsychiatric disorders. However, a definitive function of Dnmt in the postmitotic neurons is still elusive. An active DNA methylation turnover in the neuronal cells also needs to be confirmed. In the future, genome-wide studies of both Dnmt targeting genes and neuronal DNA methylation change under both physiological and pathological conditions will further improve our understanding of the role of DNA methylation in neural function and potentially benefit neurological disorder therapies.

References

Amir, R. E., Van den Veyver, I. B., Wan, M., Tran, C. Q., Francke, U., and Zoghbi, H. Y. (1999). Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. *Nat. Genet.* **23,** 185–188.

Barreto, G., Schafer, A., Marhold, J., Stach, D., Swaminathan, S. K., Handa, V., Doderlein, G., Maltry, N., Wu, W., Lyko, F., and Niehrs, C. (2007). Gadd45a promotes epigenetic gene activation by repair-mediated DNA demethylation. *Nature* **445,** 671–675.

Bestor, T., Laudano, A., Mattaliano, R., and Ingram, V. (1988). Cloning and sequencing of a cDNA encoding DNA methyltransferase of mouse cells. The carboxyl-terminal domain of the mammalian enzymes is related to bacterial restriction methyltransferases. *J. Mol. Biol.* **203,** 971–983.

Bhattacharya, S. K., Ramchandani, S., Cervoni, N., and Szyf, M. (1999). A mammalian protein with specific demethylase activity for mCpG DNA. *Nature* **397,** 579–583.

Bird, A. (2002). DNA methylation patterns and epigenetic memory. *Genes Dev.* **16,** 6–21.

Bird, A. (2007). Perceptions of epigenetics. *Nature* **447,** 396–398.

Bourc'his, D., Xu, G. L., Lin, C. S., Bollman, B., and Bestor, T. H. (2001). Dnmt3L and the establishment of maternal genomic imprints. *Science* **294,** 2536–2539.

Brenner, C., Deplus, R., Didelot, C., Loriot, A., Vire, E., De Smet, C., Gutierrez, A., Danovi, D., Bernard, D., Boon, T., Pelicci, P. G., Amati, B., *et al.* (2005). Myc represses transcription through recruitment of DNA methyltransferase corepressor. *EMBO J.* **24,** 336–346.

Brooks, P. J., Marietta, C., and Goldman, D. (1996). DNA mismatch repair and DNA methylation in adult brain neurons. *J. Neurosci.* **16,** 939–945.

Cameron, E. E., Bachman, K. E., Myohanen, S., Herman, J. G., and Baylin, S. B. (1999). Synergy of demethylation and histone deacetylase inhibition in the re-expression of genes silenced in cancer. *Nat. Genet.* **21,** 103–107.

Chen, R. Z., Akbarian, S., Tudor, M., and Jaenisch, R. (2001). Deficiency of methyl-CpG binding protein-2 in CNS neurons results in a Rett-like phenotype in mice. *Nat. Genet.* **27,** 327–331.

Colvis, C. M., Pollock, J. D., Goodman, R. H., Impey, S., Dunn, J., Mandel, G., Champagne, F. A., Mayford, M., Korzus, E., Kumar, A., Renthal, W., Theobald, D. E., *et al.* (2005). Epigenetic mechanisms and gene networks in the nervous system. *J. Neurosci.* **25,** 10379–10389.

Constantinides, P. G., Taylor, S. M., and Jones, P. A. (1978). Phenotypic conversion of cultured mouse embryo cells by aza pyrimidine nucleosides. *Dev. Biol.* **66,** 57–71.

Di Croce, L., Raker, V. A., Corsaro, M., Fazi, F., Fanelli, M., Faretta, M., Fuks, F., Lo Coco, F., Kouzarides, T., Nervi, C., Minucci, S., and Pelicci, P. G. (2002). Methyltransferase recruitment and DNA hypermethylation of target promoters by an oncogenic transcription factor. *Science* **295,** 1079–1082.

Fan, G., and Hutnick, L. (2005). Methyl-CpG binding proteins in the nervous system. *Cell Res.* **15,** 255–261.

Fan, G., Beard, C., Chen, R. Z., Csankovszki, G., Sun, Y., Siniaia, M., Biniszkiewicz, D., Bates, B., Lee, P. P., Kuhn, R., Trumpp, A., Poon, C., *et al.* (2001). DNA hypomethylation perturbs the function and survival of CNS neurons in postnatal animals. *J. Neurosci.* **21,** 788–797.

Fan, G., Martinowich, K., Chin, M. H., He, F., Fouse, S. D., Hutnick, L., Hattori, D., Ge, W., Shen, Y., Wu, H., ten Hoeve, J., Shuai, K., *et al.* (2005). DNA methylation controls the timing of astrogliogenesis through regulation of JAK-STAT signaling. *Development* **132,** 3345–3356.

Feng, J., Chang, H., Li, E., and Fan, G. (2005). Dynamic expression of *de novo* DNA methyltransferases Dnmt3a and Dnmt3b in the central nervous system. *J. Neurosci. Res.* **79,** 734–746.

Feng, J., Fouse, S., and Fan, G. (2007). Epigenetic regulation of neural gene expression and neuronal function. *Pediatr. Res.* **61,** 58R–63R.

Fischer, A., Sananbenesi, F., Wang, X., Dobbin, M., and Tsai, L. H. (2007). Recovery of learning and memory is associated with chromatin remodelling. *Nature* **447,** 178–182.

Francis, D., Diorio, J., Liu, D., and Meaney, M. J. (1999). Nongenomic transmission across generations of maternal behavior and stress responses in the rat. *Science* **286,** 1155–1158.

Fuks, F., Burgers, W. A., Brehm, A., Hughes-Davies, L., and Kouzarides, T. (2000). DNA methyltransferase Dnmt1 associates with histone deacetylase activity. *Nat. Genet.* **24,** 88–91.

Ge, Y. Z., Pu, M. T., Gowher, H., Wu, H. P., Ding, J. P., Jeltsch, A., and Xu, G. L. (2004). Chromatin targeting of *de novo* DNA methyltransferases by the PWWP domain. *J. Biol. Chem.* **279,** 25447–25454.

Geiman, T. M., Sankpal, U. T., Robertson, A. K., Zhao, Y., Zhao, Y., and Robertson, K. D. (2004). DNMT3B interacts with hSNF2H chromatin remodeling enzyme, HDACs 1 and 2, and components of the histone methylation system. *Biochem. Biophys. Res. Commun.* **318,** 544–555.

Goll, M. G., and Bestor, T. H. (2005). Eukaryotic cytosine methyltransferases. *Annu. Rev. Biochem.* **74,** 481–514.

Golshani, P., Hutnick, L., Schweizer, F., and Fan, G. (2005). Conditional Dnmt1 deletion in dorsal forebrain disrupts development of somatosensory barrel cortex and thalamocortical long-term potentiation. *Thalamus Relat. Syst.* **3,** 227–233.

Goto, K., Numata, M., Komura, J. I., Ono, T., Bestor, T. H., and Kondo, H. (1994). Expression of DNA methyltransferase gene in mature and immature neurons as well as proliferating cells in mice. *Differentiation* **56,** 39–44.

Guan, Z., Giustetto, M., Lomvardas, S., Kim, J. H., Miniaci, M. C., Schwartz, J. H., Thanos, D., and Kandel, E. R. (2002). Integration of long-term-memory-related synaptic plasticity involves bidirectional regulation of gene expression and chromatin structure. *Cell* **111,** 483–493.

Guan, J. S., Haggarty, S. J., Giacometti, E., Dannenberg, J. H., Joseph, N., Gao, J., Nieland, T. J., Zhou, Y., Wang, X., Mazitschek, R., Bradner, J. E., DePinho, R. A., *et al.* (2009). HDAC2 negatively regulates memory formation and synaptic plasticity. *Nature* **459,** 55–60.

Guy, J., Hendrich, B., Holmes, M., Martin, J. E., and Bird, A. (2001). A mouse Mecp2-null mutation causes neurological symptoms that mimic Rett syndrome. *Nat. Genet.* **27,** 322–326.

Hansen, R. S., Wijmenga, C., Luo, P., Stanek, A. M., Canfield, T. K., Weemaes, C. M., and Gartler, S. M. (1999). The DNMT3B DNA methyltransferase gene is mutated in the ICF immunodeficiency syndrome. *Proc. Natl. Acad. Sci. USA* **96,** 14412–14417.

Hellman, A., and Chess, A. (2007). Gene body-specific methylation on the active X chromosome. *Science* **315,** 1141–1143.

Hendrich, B., Hardeland, U., Ng, H. H., Jiricny, J., and Bird, A. (1999). The thymine glycosylase MBD4 can bind to the product of deamination at methylated CpG sites. *Nature* **401,** 301–304.

Hermann, A., Goyal, R., and Jeltsch, A. (2004). The Dnmt1 DNA-(cytosine-C5)-methyltransferase methylates DNA processively with high preference for hemimethylated target sites. *J. Biol. Chem.* **279,** 48350–48359.

Holliday, R. (1999). Is there an epigenetic component in long-term memory? *J. Theor. Biol.* **200,** 339–341.

Hsieh, C. L. (1999). *In vivo* activity of murine *de novo* methyltransferases, Dnmt3a and Dnmt3b. *Mol. Cell. Biol.* **19,** 8211–8218.

Hsieh, J., and Gage, F. H. (2004). Epigenetic control of neural stem cell fate. *Curr. Opin. Genet. Dev.* **14,** 461–469.

Hsieh, J., and Gage, F. H. (2005). Chromatin remodeling in neural development and plasticity. *Curr. Opin. Cell Biol.* **17,** 664–671.

Hutnick, L. K., Golshani, P., Namihira, M., Xue, Z., Matynia, A., Yang, X. W., Silva, A. J., Schweizer, F. E., and Fan, G. (2009). DNA hypomethylation restricted to the murine forebrain induces cortical degeneration and impairs postnatal neuronal maturation. *Hum. Mol. Genet.* **18,** 2875–2888.

Inano, K., Suetake, I., Ueda, T., Miyake, Y., Nakamura, M., Okada, M., and Tajima, S. (2000). Maintenance-type DNA methyltransferase is highly expressed in post-mitotic neurons and localized in the cytoplasmic compartment. *J. Biochem.* **128,** 315–321.

Jaenisch, R., and Bird, A. (2003). Epigenetic regulation of gene expression: How the genome integrates intrinsic and environmental signals. *Nat. Genet.* **33**(Suppl.), 245–254.

Jakobsson, J., Cordero, M. I., Bisaz, R., Groner, A. C., Busskamp, V., Bensadoun, J. C., Cammas, F., Losson, R., Mansuy, I. M., Sandi, C., and Trono, D. (2008). KAP1-mediated epigenetic repression in the forebrain modulates behavioral vulnerability to stress. *Neuron* **60,** 818–831.

Jenuwein, T., and Allis, C. D. (2001). Translating the histone code. *Science* **293,** 1074–1080.

Jin, B., Tao, Q., Peng, J., Soo, H. M., Wu, W., Ying, J., Fields, C. R., Delmas, A. L., Liu, X., Qiu, J., and Robertson, K. D. (2008). DNA methyltransferase 3B (DNMT3B) mutations in ICF syndrome lead to altered epigenetic modifications and aberrant expression of genes regulating development, neurogenesis and immune function. *Hum. Mol. Genet.* **17,** 690–709.

Jones, P. A., Taylor, S. M., Mohandas, T., and Shapiro, L. J. (1982). Cell cycle-specific reactivation of an inactive X-chromosome locus by 5-azadeoxycytidine. *Proc. Natl. Acad. Sci. USA* **79,** 1215–1219.

Jung, B. P., Jugloff, D. G., Zhang, G., Logan, R., Brown, S., and Eubanks, J. H. (2003). The expression of methyl CpG binding factor MeCP2 correlates with cellular differentiation in the developing rat brain and in cultured cells. *J. Neurobiol.* **55,** 86–96.

Juttermann, R., Li, E., and Jaenisch, R. (1994). Toxicity of 5-aza-2′-deoxycytidine to mammalian cells is mediated primarily by covalent trapping of DNA methyltransferase rather than DNA demethylation. *Proc. Natl. Acad. Sci. USA* **91,** 11797–11801.

Klose, R. J., and Bird, A. P. (2006). Genomic DNA methylation: The mark and its mediators. *Trends Biochem. Sci.* **31,** 89–97.

Klose, R. J., Kallin, E. M., and Zhang, Y. (2006). JmjC-domain-containing proteins and histone demethylation. *Nat. Rev. Genet.* **7,** 715–727.

Korzus, E., Rosenfeld, M. G., and Mayford, M. (2004). CBP histone acetyltransferase activity is a critical component of memory consolidation. *Neuron* **42,** 961–972.

Kouzarides, T. (2007). Chromatin modifications and their function. *Cell* **128,** 693–705.

Kriaucionis, S., and Bird, A. (2003). DNA methylation and Rett syndrome. *Hum. Mol. Genet.* **12**(Spec. No. 2), R221–R227.

Levenson, J. M., Roth, T. L., Lubin, F. D., Miller, C. A., Huang, I. C., Desai, P., Malone, L. M., and Sweatt, J. D. (2006). Evidence that DNA (cytosine-5) methyltransferase regulates synaptic plasticity in the hippocampus. *J. Biol. Chem.* **281,** 15763–15773.

Li, E., Bestor, T. H., and Jaenisch, R. (1992). Targeted mutation of the DNA methyltransferase gene results in embryonic lethality. *Cell* **69,** 915–926.

Liu, D., Diorio, J., Tannenbaum, B., Caldji, C., Francis, D., Freedman, A., Sharma, S., Pearson, D., Plotsky, P. M., and Meaney, M. J. (1997). Maternal care, hippocampal glucocorticoid receptors, and hypothalamic-pituitary-adrenal responses to stress. *Science* **277,** 1659–1662.

Lorincz, M. C., Dickerson, D. R., Schmitt, M., and Groudine, M. (2004). Intragenic DNA methylation alters chromatin structure and elongation efficiency in mammalian cells. *Nat. Struct. Mol. Biol.* **11,** 1068–1075.

Lyko, F., and Brown, R. (2005). DNA methyltransferase inhibitors and the development of epigenetic cancer therapies. *J. Natl. Cancer Inst.* **97,** 1498–1506.

Ma, D. K., Jang, M. H., Guo, J. U., Kitabatake, Y., Chang, M. L., Pow-Anpongkul, N., Flavell, R. A., Lu, B., Ming, G. L., and Song, H. (2009). Neuronal activity-induced Gadd45b promotes epigenetic DNA demethylation and adult neurogenesis. *Science* **323,** 1074–1077.

MacDonald, J. L., Gin, C. S., and Roskams, A. J. (2005). Stage-specific induction of DNA methyltransferases in olfactory receptor neuron development. *Dev. Biol.* **288,** 461–473.

Miller, C. A., and Sweatt, J. D. (2007). Covalent modification of DNA regulates memory formation. *Neuron* **53,** 857–869.

Morris, K. V., Chan, S. W., Jacobsen, S. E., and Looney, D. J. (2004). Small interfering RNA-induced transcriptional gene silencing in human cells. *Science* **305,** 1289–1292.

Nan, X., Campoy, F. J., and Bird, A. (1997). MeCP2 is a transcriptional repressor with abundant binding sites in genomic chromatin. *Cell* **88,** 471–481.

Nan, X., Ng, H. H., Johnson, C. A., Laherty, C. D., Turner, B. M., Eisenman, R. N., and Bird, A. (1998). Transcriptional repression by the methyl-CpG-binding protein MeCP2 involves a histone deacetylase complex. *Nature* **393,** 386–389.

Nelson, E. D., Kavalali, E. T., and Monteggia, L. M. (2008). Activity-dependent suppression of miniature neurotransmission through the regulation of DNA methylation. *J. Neurosci.* **28,** 395–406.

Nguyen, S., Meletis, K., Fu, D., Jhaveri, S., and Jaenisch, R. (2007). Ablation of *de novo* DNA methyltransferase Dnmt3a in the nervous system leads to neuromuscular defects and shortened lifespan. *Dev. Dyn.* **236,** 1663–1676.

Noh, J. S., Sharma, R. P., Veldic, M., Salvacion, A. A., Jia, X., Chen, Y., Costa, E., Guidotti, A., and Grayson, D. R. (2005). DNA methyltransferase 1 regulates reelin mRNA expression in mouse primary cortical cultures. *Proc. Natl. Acad. Sci. USA* **102,** 1749–1754.

Okano, M., Xie, S., and Li, E. (1998). Dnmt2 is not required for *de novo* and maintenance methylation of viral DNA in embryonic stem cells. *Nucleic Acids Res.* **26,** 2536–2540.

Okano, M., Bell, D. W., Haber, D. A., and Li, E. (1999). DNA methyltransferases Dnmt3a and Dnmt3b are essential for *de novo* methylation and mammalian development. *Cell* **99,** 247–257.

Ooi, S. K., and Bestor, T. H. (2008). The colorful history of active DNA demethylation. *Cell* **133,** 1145–1148.

Petrij, F., Giles, R. H., Dauwerse, H. G., Saris, J. J., Hennekam, R. C., Masuno, M., Tommerup, N., van Ommen, G. J., Goodman, R. H., Peters, D. J., *et al.* (1995). Rubinstein-Taybi syndrome caused by mutations in the transcriptional co-activator CBP. *Nature* **376,** 348–351.

Poulter, M. O., Du, L., Weaver, I. C., Palkovits, M., Faludi, G., Merali, Z., Szyf, M., and Anisman, H. (2008). GABAA receptor promoter hypermethylation in suicide brain: Implications for the involvement of epigenetic processes. *Biol. Psychiatry* **64,** 645–652.

Pradhan, S., and Esteve, P. O. (2003). Mammalian DNA (cytosine-5) methyltransferases and their expression. *Clin. Immunol.* **109,** 6–16.

Pradhan, S., Bacolla, A., Wells, R. D., and Roberts, R. J. (1999). Recombinant human DNA (cytosine-5) methyltransferase. I. Expression, purification, and comparison of *de novo* and maintenance methylation. *J. Biol. Chem.* **274,** 33002–33010.

Prokhortchouk, A., Hendrich, B., Jorgensen, H., Ruzov, A., Wilm, M., Georgiev, G., Bird, A., and Prokhortchouk, E. (2001). The p120 catenin partner Kaiso is a DNA methylation-dependent transcriptional repressor. *Genes Dev.* **15,** 1613–1618.

Rai, K., Huggins, I. J., James, S. R., Karpf, A. R., Jones, D. A., and Cairns, B. R. (2008). DNA demethylation in zebrafish involves the coupling of a deaminase, a glycosylase, and gadd45. *Cell* **135,** 1201–1212.

Robertson, K. D., Ait-Si-Ali, S., Yokochi, T., Wade, P. A., Jones, P. L., and Wolffe, A. P. (2000). DNMT1 forms a complex with Rb, E2F1 and HDAC1 and represses transcription from E2F-responsive promoters. *Nat. Genet.* **25,** 338–342.

Rountree, M. R., Bachman, K. E., and Baylin, S. B. (2000). DNMT1 binds HDAC2 and a new co-repressor, DMAP1, to form a complex at replication foci. *Nat. Genet.* **25,** 269–277.

Sansom, O. J., Berger, J., Bishop, S. M., Hendrich, B., Bird, A., and Clarke, A. R. (2003). Deficiency of Mbd2 suppresses intestinal tumorigenesis. *Nat. Genet.* **34,** 145–147.

Shahbazian, M. D., Antalffy, B., Armstrong, D. L., and Zoghbi, H. Y. (2002). Insight into Rett syndrome: MeCP2 levels display tissue- and cell-specific differences and correlate with neuronal maturation. *Hum. Mol. Genet.* **11,** 115–124.

Shi, Y., Lan, F., Matson, C., Mulligan, P., Whetstine, J. R., Cole, P. A., Casero, R. A., and Shi, Y. (2004). Histone demethylation mediated by the nuclear amine oxidase homolog LSD1. *Cell* **119,** 941–953.

Suetake, I., Shinozaki, F., Miyagawa, J., Takeshima, H., and Tajima, S. (2004). DNMT3L stimulates the DNA methylation activity of Dnmt3a and Dnmt3b through a direct interaction. *J. Biol. Chem.* **279,** 27816–27823.

Takizawa, T., Nakashima, K., Namihira, M., Ochiai, W., Uemura, A., Yanagisawa, M., Fujita, N., Nakao, M., and Taga, T. (2001). DNA methylation is a critical cell-intrinsic determinant of astrocyte differentiation in the fetal brain. *Dev. Cell* **1,** 749–758.

Teter, B., Osterburg, H. H., Anderson, C. P., and Finch, C. E. (1994). Methylation of the rat glial fibrillary acidic protein gene shows tissue-specific domains. *J. Neurosci. Res.* **39,** 680–693.

Tsankova, N., Renthal, W., Kumar, A., and Nestler, E. J. (2007). Epigenetic regulation in psychiatric disorders. *Nat. Rev. Neurosci.* **8,** 355–367.

Tudor, M., Akbarian, S., Chen, R. Z., and Jaenisch, R. (2002). Transcriptional profiling of a mouse model for Rett syndrome reveals subtle transcriptional changes in the brain. *Proc. Natl. Acad. Sci. USA* **99,** 15536–15541.

Veldic, M., Caruncho, H. J., Liu, W. S., Davis, J., Satta, R., Grayson, D. R., Guidotti, A., and Costa, E. (2004). DNA-methyltransferase 1 mRNA is selectively overexpressed in telencephalic GABAergic interneurons of schizophrenia brains. *Proc. Natl. Acad. Sci. USA* **101,** 348–353.

Watanabe, D., Uchiyama, K., and Hanaoka, K. (2006). Transition of mouse *de novo* methyltransferases expression from Dnmt3b to Dnmt3a during neural progenitor cell development. *Neuroscience* **142,** 727–737.

Watt, F., and Molloy, P. L. (1988). Cytosine methylation prevents binding to DNA of a HeLa cell transcription factor required for optimal expression of the adenovirus major late promoter. *Genes Dev.* **2,** 1136–1143.

Weaver, I. C., Cervoni, N., Champagne, F. A., D'Alessio, A. C., Sharma, S., Seckl, J. R., Dymov, S., Szyf, M., and Meaney, M. J. (2004). Epigenetic programming by maternal behavior. *Nat. Neurosci.* **7,** 847–854.

Weaver, I. C., Champagne, F. A., Brown, S. E., Dymov, S., Sharma, S., Meaney, M. J., and Szyf, M. (2005). Reversal of maternal programming of stress responses in adult offspring through methyl supplementation: Altering epigenetic marking later in life. *J. Neurosci.* **25,** 11045–11054.

Xie, S., Wang, Z., Okano, M., Nogami, M., Li, Y., He, W. W., Okumura, K., and Li, E. (1999). Cloning, expression and chromosome locations of the human DNMT3 gene family. *Gene* **236,** 87–95.

Xu, G. L., Bestor, T. H., Bourc'his, D., Hsieh, C. L., Tommerup, N., Bugge, M., Hulten, M., Qu, X., Russo, J. J., and Viegas-Pequignot, E. (1999). Chromosome instability and immunodeficiency syndrome caused by mutations in a DNA methyltransferase gene. *Nature* **402,** 187–191.

Zhao, X., Ueba, T., Christie, B. R., Barkho, B., McConnell, M. J., Nakashima, K., Lein, E. S., Eadie, B. D., Willhoite, A. R., Muotri, A. R., Summers, R. G., Chun, J., *et al.* (2003). Mice lacking methyl-CpG binding protein 1 have deficits in adult neurogenesis and hippocampal function. *Proc. Natl. Acad. Sci. USA* **100,** 6777–6782.

Zhu, J. K. (2008). Epigenome sequencing comes of age. *Cell* **133,** 395–397.