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Epigenetic gene regulation in the adult mammalian brain: Multiple roles in memory formation

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ABSTRACT

Brain-derived neurotrophic factor (*bdnf*) is one of numerous gene products necessary for long-term memory formation and dysregulation of *bdnf* has been implicated in the pathogenesis of cognitive and mental disorders. Recent work indicates that epigenetic-regulatory mechanisms including the markings of histone proteins and associated DNA remain labile throughout the life-span and represent an attractive molecular process contributing to gene regulation in the brain. In this review, important information will be discussed on epigenetics as a set of newly identified dynamic transcriptional mechanisms serving to regulate gene expression changes in the adult brain with particular emphasis on *bdnf* transcriptional readout in learning and memory formation. This review will also highlight evidence for the role of epigenetics in aberrant *bdnf* gene regulation in the pathogenesis of cognitive dysfunction associated with seizure disorders, Rett syndrome, Schizophrenia, and Alzheimer's disease. Such research offers novel concepts for understanding epigenetic transcriptional mechanisms subserving adult cognition and mental health, and furthermore promises novel avenues for therapeutic approach in the clinic.

1. The biology of long-term memory formation

One of the most unique features of the brain is its ability to store long-term memories (LTM). Significant advances in molecular and cellular neuroscience research have established the requirement of gene expression changes and subsequent protein synthesis in several memory-related brain regions, including in the hippocampus (Bailey, Kandel, & Si, 2004; Kandel, 2001; McGaugh, 2000). Several memory models propose that in order for LTM to be long-lasting, learning-induced molecular alterations in gene expression and protein synthesis must trigger lasting changes in cellular and synaptic properties. Thus, alterations in cellular and synaptic properties propagated by these persisting molecular changes are translated as memories by memory-recall processes which trigger activity throughout the memory circuit. However, the molecular mechanisms triggered by learning-induced signaling mechanisms to orchestrate gene transcription changes are still poorly understood and are the focus of intense study.

Numerous studies have proposed the possibility that epigenetic mechanisms might be involved in memory formation. Generally, epigenetic regulation of gene transcription has been shown to occur in response to new experiences which result in gene expression changes necessary for LTM storage and retrieval long after the original experience is introduced (Colvis et al., 2005; Gupta et al., 2010; Jiang et al., 2008; Levenson et al., 2004b, 2006; Lubin, Roth, & Sweatt, 2008; Martinowich et al., 2003; Nelson, Kavalali, & Monteggia, 2008; Wood, Hawk, & Abel, 2006b). In fact, a consensus is emerging that epigenetics is a pivotal molecular mechanism orchestrating various transcription events in response to learning and serves as a key regulator of LTM.

To facilitate a comprehensive review of epigenetic mechanisms in LTM, epigenetic regulation of one gene product that is necessary for this process, the brain-derived neurotrophic factor (*bdnf*), will be highlighted. Specifically, a review about the role of epigenetic mechanisms in the formation of long-term memories will be presented with a focus on recent studies that demonstrate *bdnf* chromatin structure regulation during memory consolidation. In particular, a discussion of two major classes of epigenetic mechanisms will be reviewed in the context of memory formation including posttranslational modifications of histone proteins and methylation of DNA that comprise the core chromatin particle. Finally, this review will conclude with the promise that epigenetic therapy holds for alleviating cognitive deficits associated with neurological disorders including epilepsy disorders, Rett syndrome, schizophrenia, and Alzheimer's disease wherein aberrant *bdnf* regulation has been implicated.

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2. Candidate epigenetic mechanisms involved in chromatin modifications in the adult brain

Epigenetics refers to regulation of chromatin structure that affect various phenotypic outcomes via a lasting control over gene expression without altering the genetic code (reviewed in Ionita-Laza, Lange, and Laird (2009), Jiang et al. (2008) and Levenson and Sweatt (2005)). Histone modification and DNA methylation are the two most widely studied chromatin-modifying mechanisms. The free N-terminal tails of histone proteins are unstructured and are amenable to addition or removal of functional groups. Addition of acetyl and phosphate groups results in exposure of the DNA and hence favors formation of the active state of chromatin or euchromatin, whereas ubiquitination and SUMOylation conceals the DNA shielding it from the transcriptional machinery. In general, the methylation of histone tails is distinct and can result in either gene activation or repression. For example, mono-, di- and tri-methylated forms of histone H3 at lysine 4 (H3K4me, H3K4me2, H3K4me3) and mono-methylation of histone H3 at lysine 9 (H3K9me) results in activation of transcription whereas di- and tri-methylation of histone H3 lysine 9 (H3K9me2, H3K9me3) results in repression of transcription (reviewed in Gupta et al. (2010)). Unlike the charged acetyl and phosphate groups, the uncharged methyl groups on histone proteins are too small to disrupt the charge between histone proteins and associated DNA (chromatin) but instead regulate transcription by functioning as docking sites to recruit activator or repressor proteins to restructure chromatin.

Understanding chromatin modifications to regulate gene transcription in the brain also involves knowledge of the methylation of DNA. CpG islands are regions of DNA near and in approximately 40% of promoters of mammalian genes (reviewed in Chen et al. (1991) and Goldberg, Allis, and Bernstein (2007)). They are regions where there are a large number of cytosine and guanine residues adjacent to each other, linked by phosphodiester bonds (CpG sites), in the backbone of the DNA. The "p" in CpG notation is used to distinguish a cytosine followed by a guanine from a cytosine base paired to a guanine. DNA methylation is catalyzed by a group of enzymes called DNA methyltrasferases (DNMTs), which transfer the methyl group from the donor S-adenosylmethionine (SAM) to 5′ position of the cytosine pyramidal ring. The methylated 'CpG' dinucleotides are found explicitly in inactive gene promoter regions. Conversely, in most instances, the CpG sites in the CpG islands are *un*methylated if genes are expressed.

The methylated CpG residues are docking sites for proteins containing the methyl-binding domain (MBD) such as the canonical methyl-CpG binding protein-2 (MeCP2) (reviewed in Deutsch, Rosse, Mastropaolo, Long, and Gaskins (2008) and Szyf (2009)). MeCP2 recruits histone modifying proteins which aid in the formation of the heterochromatin (inactive chromatin). Another influence of methylated DNA on gene transcription is due to the hindrance offered by the methylated cytosine residue which interferes with transcription factor binding and assembling of the transcriptional machinery (Takizawa et al., 2001). It is important to note that epigenetic mechanisms are not isolated events, rather interact and influence each other. Indeed, unmethylated CpG sites at gene promoter regions that are influenced by MBD activity are dependent on the chromatin microenvironment state which includes histone modifications. A striking example is of the complex interplay between DNA methylation and histone methylation observed with unmethylated histone H3K4, which becomes the docking site for DNMTs, resulting in *de novo* DNA methylation and switching off of gene expression (Szyf, 2009).

3. Epigenetics: a plausible transcriptional mechanism in long-term memory formation

Extensive research in the nervous system has supported a role for epigenetic-mediated chromatin structure regulation as being crucial for development, cellular differentiation, behavior, and memory formation. It is becoming increasingly apparent that epigenetic mechanisms are responsive to environmental influences and are linked to the cellular machinery. A prototypic example of epigenetic-facilitation in memory retention pertains to memory T-cells of the mammalian immune system (reviewed in Nakayama and Yamashita (2008)). Numerous epigenetic mechanisms such as histone modifications and DNA methylation modulate gene expression and thus play a role in T-cell survival and maintenance of T-cell function in various differentiated states. These processes underlie the formation of persistent immunological memory cells in response to transient environmental stimuli (reviewed in Nakayama and Yamashita (2008)). Thus, like immune T-cells, it is plausible that epigenetic mechanisms such as methylation of the cytosine base are changeable and occur in post-mitotic neurons to mediate neuronal function. However, unlike epigenetic mechanisms in the immune system, chromatin modifications in the CNS are greatly understudied and we still do not know how these molecular mechanisms are central to the persistence of memory.

There exists evidence for active regulation of DNMTs across the life-span and possibly across several brain regions (Feng, Chang, Li, & Fan, 2005; Feng & Fan, 2009; Feng et al., 2010). For example, there are studies that report expression of DNMT genes in neurons (Feng et al., 2005; Veldic, Guidotti, Maloku, Davis, & Costa, 2005; Veldic et al., 2004). Both DNMT1 and DNMT3a have been shown to be differentially expressed in cortical layers and specifically in interneurons from the brain of human adults (Veldic et al., 2004, 2005). Interestingly, in the brain DNMT3a is expressed during embryogenesis and decreases as DNMT3b increases into adulthood (Feng et al., 2005). The differential expression of DNMTs within the adult brain raises the question of the role of these enzymes in post-mitotic neurons and how they contribute to neuronal responses to experience-stimuli.

With this question in mind, a number of behavioral neuroscientists have begun to investigate the role of DNA methylation and DNMT activity in memory formation. Early studies have found that learning triggers DNA methylation changes in the adult hippocampus (Lubin et al., 2008; Miller & Sweatt, 2007). Additional studies demonstrate that contextual fear conditioning triggers upregulation of *de novo* DNMT gene expression in the adult hippocampus and that blockade of DNMT activity interferes with contextual fear conditioned memories (Feng et al., 2010; Lubin et al., 2008; Miller & Sweatt, 2007). Furthermore, it has been shown that global inhibitors of DNMT activity modify DNA methylation in the adult brain at specific gene promoters including *reelin*, *bdnf*, and the memory-suppressor gene *protein phosphatase 1* (*PP1*) (Levenson et al., 2006; Lubin et al., 2008; Miller & Sweatt, 2007). Together, these observations suggest that in the adult CNS dynamic regulation of DNA methylation occurs at both memory-permissive (*reelin* and *bdnf*) and memory-suppressive (*PP1*) gene promoters in response to experience and is critical for memory formation.

However, epigenetic mechanisms are not isolated events but rather they influence each other to mediate chromatin structure regulation. Thus, DNA methylation may work in concert with histone modifications to dictate the microenvironment of a given gene promoter and influence its gene transcription. Indeed, several studies have provided evidence that supports the idea that histone modifications may work in concert with DNA methylation during memory formation and storage in the adult rat hippocampus (Barrett & Wood, 2008; Graff & Mansuy, 2008; Lubin & Sweatt, 2007;

Wood et al., 2006b). A satisfying example for crosstalk between DNA methylation and histone modifications is demonstrated by work from Lubin and colleagues (Gupta et al., 2010). In this recent series of studies, they found that contextual fear conditioning triggers DNA hypermethylation at the *Zif268* gene promoter which corresponds with increases in *Zif268* mRNA expression. This was an unanticipated observation as DNA methylation is generally thought to be associated with transcriptional repression. Thus it appears that DNA methylation may also result in transcriptional activation in the adult CNS (Chahrour et al., 2008; Suzuki & Bird, 2008). Upon further investigation the authors found that contextual fear conditioning triggered increases in the active-transcription histone H3 methylation mark (H3K4me3) around the hypermethylated *Zif268* DNA promoter region. DNA methylation was associated with increased binding of the methyl-CpG binding protein, MeCP2 at the *Zif268* promoter. Interestingly, MeCP2 has been shown to bind to methylated DNA along with cyclic adenosine monophosphate (cAMP) response element binding (CREB) protein 1 to actively regulate gene transcription (Chahrour et al., 2008). Indeed, Lubin and colleagues found that a cAMP response element (CRE) binding site was present around the *Zif268* DNA methylation promoter region in hippocampal neurons. Together, these results demonstrate that histone methylation changes occur in concert with DNA methylation in the adult CNS. Additionally, these studies provide a possible explanation for recent observations that fear learning-mediated hypermethylation and hypomethylation of DNA do not often parallel with gene activation or repression, respectively, in the adult hippocampus (Gupta et al., 2010). Thus, histone methylation regulation may be more indicative of an active or repressive gene promoter than DNA methylation to facilitate gene transcription changes in post-mitotic neurons, which we are only beginning to understand.

4. The BDNF gene

Despite the known importance of BDNF function and gene expression in normal neural processes and nervous system disorders, the molecular mechanisms responsible for complex *bdnf* transcriptional readout in the brain is largely unknown. Since its discovery more than three decades ago, BDNF has been implicated in a multitude of actions pivotal for a healthy and functional CNS. BDNF not only helps to support the survival of existing neurons in the adult brain, but influences neuronal excitability, and promotes growth and differentiation of new neurons and synapses. The BDNF protein is synthesized from a gene that has a rather complex structure (Fig. 1). Recently, the *bdnf* gene has been re-characterized, and it contains nine 5′ non-coding exons (I-IX) linked to the common 3′ coding exon (IX) that produce a total of 24 transcripts all translated into the mature BDNF protein (Aid, Kazantseva, Piirsoo, Palm, & Timmusk, 2007; Liu et al., 2006).

Activity of specific promoter regions within the *bdnf* gene dictates spatial and temporal expression of specific *bdnf* transcripts. To date, the actions of BDNF in the adult CNS is the most studied and much remains to be understood on how separate signaling events converge on multiple regulatory sequences within *bdnf* gene promoters to determine transcriptional readout subserving LTM formation.

5. Histone modifications at BDNF promoters during memory formation

Initial investigations into the transcriptional mechanisms underlying differential regulation of *bdnf* transcripts in the brain have focused on the role of transcription factors, such as cAMP response element binding (CREB) protein and nuclear factor-kappa B (NF-κB), binding to *bdnf* promoter regions to initiate recruitment and activation of several coactivators of transcription. However, recent reports have highlighted another level of transcriptional regulation of the *bdnf* gene involving alterations in chromatin structure via posttranslational modifications of histones such as acetylation, phosphorylation, and methylation. It has thus been proposed that epigenetic regulation of the *bdnf* gene is a transcriptional mechanism occurring in the adult brain that functions in learning and memory processes.

In support of this hypothesis, recent studies have identified a potential role for chromatin structure regulation of the *bdnf* gene in either fear learning or spatial cognition (Bredy et al., 2007; Gupta et al., 2010; Levenson et al., 2006; Lubin et al., 2008; Wood, Attner, Oliveira, Brindle, & Abel, 2006a). For example, one behavioral paradigm often used to study learning and memory is contextual fear conditioning. In this associative form of learning, a novel context is paired with a mild footshock. The memory for this association is typically tested by measuring the freezing behavior of rodents when replaced in the chamber where they were previously shocked. Using a fear conditioning paradigm in rats, researchers found a selective increase in *bdnf* exons I and IV mRNA transcripts in the amygdala, but observed no changes in exons II and VI (Ou & Gean, 2007; Rattiner, Davis, & Ressler, 2004). In a similar study Lubin et al. (2008) found that chromatin modification of the *bdnf* gene in hippocampus occurs with fear conditioning. Specifically, fear conditioning produced an upregulation of particular *bdnf* transcripts (I and IV) that were associated with increased histone acetylation and phosphorylation at the particular promoters (Lubin et al., 2008). Furthermore, Bredy and co-workers (2007) report that histone modifications around specific *bdnf* promoters influence memory extinction. The authors demonstrate that extinction of conditioned fear in mice is associated with an increase in histone H4 acetylation around the promoter of *bdnf* exon IV, as well as increases in *bdnf* exons I and IV mRNA in prefrontal cortex. Together, these observations suggest not only that BDNF plays a role in LTM formation but selective *bdnf* transcript readout may be preferentially involved and subject to epigenetic control.

However, it remains to be determined if these patterns of exon-specific *bdnf* expression and epigenetic changes such as histone modifications are strictly related to fear conditioning or if they represent a more general phenomenon associated with learning and memory. Thus it is important to begin exploring these phenomena using other behavioral paradigms. Promisingly, in studies that are not related to memory, Nestler and colleagues have made seminal findings in chromatin modification of the *bdnf* gene in hippocampus after acute and chronic electroconvulsive seizures, in cocaine addiction, and in a mouse model of depression (Berton et al., 2006; Kumar et al., 2005; Tsankova, Kumar, & Nestler, 2004; Tsankova et al., 2006). For example, they found that chronic social defeat in adult mice produced a lasting downregulation of particular *bdnf* transcripts (IV and VI) that were associated with increased histone methylation at the particular promoters (Tsankova et al., 2006). Huang, Doherty, and Dingledine (2002) have shown increased histone H4 acetylation at specific *bdnf* gene promoters in response to status epilepticus or prolonged seizure activity by pilocarpine in rat hippocampal neurons. Together, these studies and the findings described in the preceding paragraph present a clearer understanding of the regulation of the *bdnf* gene in several behaviors, and highlight a new activity-dependent transcriptional mechanism for *bdnf* gene expression changes in the adult brain: epigenetics.

Activity-dependent transcription of the *bdnf* gene during LTM formation is thought to be mediated through N-methyl-D-aspartate (NMDA) glutamate receptor activation (Lubin et al., 2008; Tian, Marini, & Lipsky, 2010; Tian et al., 2009). However, the role of chromatin modification in NMDA-dependent *bdnf* gene expression is not completely understood. With respect to posttranslational mod-

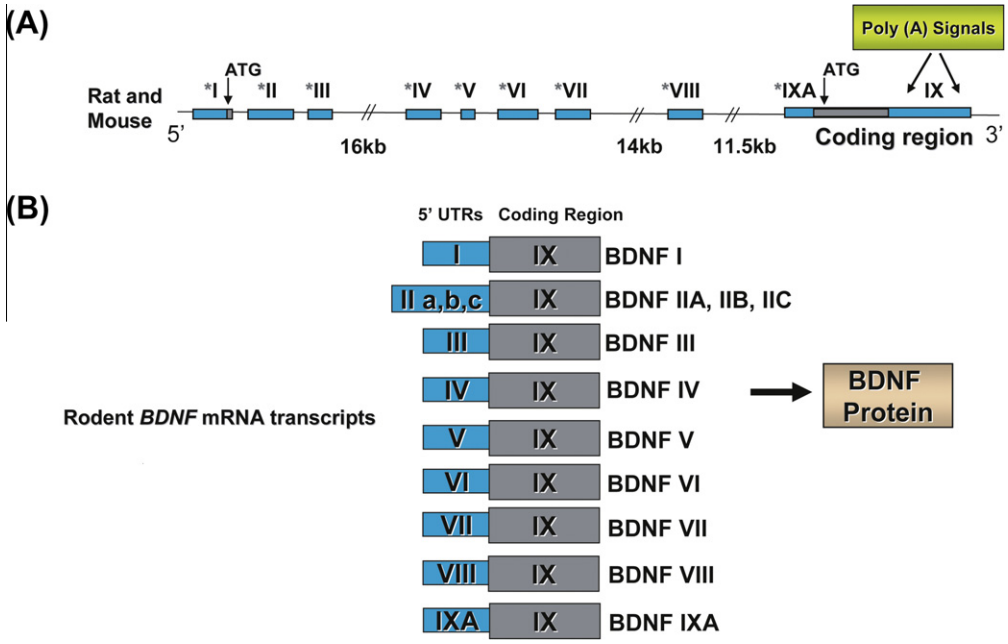

Fig. 1. The rodent brain-derived neurotrophic gene (*BDNF*). (A) The rodent *bdnf* gene contains at least nine 5′ non-coding exons, each with its own promoter, and a common coding exon. (B) Transcription of the *bdnf* gene results in BDNF transcripts containing one of the 5′ untranslated regions (5′ UTRs) spliced to the common 3′ protein coding region (exon IX). (Nomenclature based on Aid et al. (2007) and Liu et al. (2006).)

ification of histones, histone methylation shows dynamic changes in specific levels of histone H3K4me3, an active mark of gene transcription, and H3K9me2, a repressive mark of gene transcription, at *bdnf* promoter 1 in hippocampal neurons in response to NMDA receptor stimulation (Tian et al., 2009). In this regard, recent studies by Lubin and colleagues demonstrate similar changes in histone H3K4me3 at *bdnf* promoter 1 in response to fear learning (Gupta et al., 2010). Thus, these findings along with the studies of the NMDA receptor described above support the idea that distinct changes in chromatin structure occur at *bdnf* promoters following NMDA receptor stimulation and that these events mediate activity-dependent epigenetic regulation of *bdnf* transcripts in the adult brain. Furthermore, these recent studies provide a framework for future work directed at identifying the molecular targets of NMDA receptor signaling responsible for epigenetic transcriptional control of the *bdnf* gene during LTM formation. The potential molecular targets for NMDA receptor stimulation will be discussed in greater detail below in conjunction with discussions of studies investigating the role of DNA methylation in the regulation of *bdnf* transcripts triggered by learning.

6. Non-histone protein targets for chromatin-modifying enzymes in memory formation

It is important to note that although histones are well-documented protein targets for chromatin-modifying enzymes, there exist non-histone protein targets as well. The fact that chromatin-modifying enzymes act on both histone and non-histone proteins, adds a new molecular entity to epigenetically-regulated transcriptional mechanisms affecting gene expression changes in memory. In fact, referring to these enzymes as lysine-modifying rather than histone-modifying may more accurately describe their ability to add these posttranslational modifications to lysine residues on a number of proteins. A crucial implication of gene transcription regulation by epigenetic mechanisms in LTM formation is the fact that pharmacological manipulation of these lysine-modifying enzymes could possibly alter neural plasticity through their effects on non-histone targets in addition to histones.

There are many non-histone proteins targets of methylation that play important roles in regulating transcriptional activity in neuronal alterations and in memory formation. For example, the p65/RelA subunit of the NF-κB DNA-binding complex, the transcription factor p53, estrogen receptor alpha (ERα), and DNMT1 (Ng, Yue, Oppermann, & Klose, 2009; Pradhan, Chin, Esteve, & Jacobsen, 2009; Yang, Tajkhorshid, & Chen, 2010). Furthermore, an even greater variety of proteins have been shown to be non-histone targets of acetylation, including the p65/RelA NF-κB subunit, p53, tubulin, HDAC1, ERα, glucocorticoid receptor, and the HATs p300, CBP, and p300/CBP-associated protein (PCAF), which autoacetylate (Norris, Lee, & Yao, 2009; Polevoda & Sherman, 2002; Qiu et al., 2006; Sharma, 2010; Spange, Wagner, Heinzel, & Kramer, 2009; Whittle et al., 2007; Yang & Seto, 2008). Given that chromatin-modifying enzymes can act on numerous non-histone protein targets, posttranslational modification of transcription factors by chromatin-modifying enzymes such as HDACs may be involved in the regulation of *bdnf* transcriptional readout during memory formation and must be considered in future studies.

In relation to *bdnf* gene expression, the NF-κB transcription factor has been shown to be critical for regulation of this gene in neurons (reviewed in Marini et al. (2004)). Indeed, NF-κB activity has been implicated in the induction of synaptic plasticity and initial formation of long-term memory (Albensi & Mattson, 2000; Dash, Orsi, & Moore, 2005; Freudenthal et al., 2005; Kassed et al., 2003; Levenson et al., 2004a; Liou & Hsia, 2003; Mattson, Culmsee, Yu, & Camandola, 2000; Meffert, Chang, Wiltgen, Fanselow, & Baltimore, 2003; Yeh, Lin, & Gean, 2004; Yeh, Lin, Lee, & Gean, 2002). Specifically, the crucial role for NF-κB activity in memory formation is demonstrated using knockout mice of one specific member of the NF-κB transcription factor family, c-Rel (Ahn et al., 2008). In these studies c-Rel knockout mice displayed significant deficits in memory retention in the contextual fear conditioning test and in a novel object recognition test compared to wild-type littermate mice. Furthermore, hippocampal sections from c-Rel deficient mice

had normal baseline synaptic transmission but exhibited significantly less LTP than did hippocampal sections from wild-type littermates. Thus, results from these studies suggest that NF-κB/c-Rel activity is necessary for long-term synaptic potentiation *in vitro* and hippocampus-dependent memory formation *in vivo*.

These findings highlighted above suggest that specific mechanisms exist for activation of the NF-κB transcriptional signaling cascade during memory formation. Although NF-κB sites have been identified within *bdnf* promoter 4 that regulates exon IV-specific *bdnf* expression in cerebellar granule cells following NMDA stimulation, *in vitro* (Lipsky et al., 2001; Marini et al., 2004), NF-κB regulation of *bdnf* gene expression in memory formation has not previously been evaluated. Furthermore, the importance of posttranslational modification, acetylation and methylation, of the NF-κB transcription factor in the regulation of *bdnf* transcripts in neurons remains unexplored.

One finding of the effects of HDAC inhibition (HDACi) on memory formation is elegantly illustrated by work from Guan and colleagues (2009) that describe HDAC2 as a negative regulator of memory formation and synaptic plasticity. While, the authors suggest that negative regulation of histone acetylation by HDAC2 is relevant to alterations in memory formation and synaptic plasticity, they do suggest that other molecular targets, such as tubulin, may be relevant as well. Thus, such studies must consider the possibility that non-histone targets including transcription factors may be affected with HDACi. For example, the p65/RelA subunit of the transcription factor NF-κB complex interacts with HDAC2 to negatively regulate gene expression (Ashburner, Westerheide, & Baldwin, 2001). In addition, p65/RelA can be acetylated and deacetylated a process shown to be crucial for LTM formation (Yeh et al., 2004). Yeh and colleagues demonstrate that treatment with the HDAC inhibitor trichostatin A (TSA) resulted in prolonged p65 acetylation with an increase in NF-κB DNA binding activity. Treatment with TSA subsequently produced an enhancement in fear-potentiated startle. Importantly, they demonstrated that these effects were attenuated by inhibitors of NF-κB DNA binding activity. Therefore, in the studies by Guan and colleagues, a plausible effect of *Hdac2* deficiency is not only an enhancement of histone acetylation but perhaps also an enhancement of p65/RelA acetylation and enhanced NF-κB DNA binding activity, thereby leading to enhanced memory as demonstrated by Lubin and Sweatt (2007). Hence, while these studies strongly support HDAC2 activity as a negative regulator of hippocampal-dependent memory formation and is consistent with the hypothesis that enhanced histone acetylation is involved in enhanced memory formation, it cannot be ruled out that these results may also be indicative of a more general role for enhanced protein acetylation by HDACi resulting in enhanced memory formation (Swank & Sweatt, 2001; Yeh et al., 2004).

Nonetheless, there exist a significant overlap of proteins which are known targets for both acetylation and methylation, such as p65/RelA, p53, and ERα, which suggest that there may be a "code" for the precise regulation of the activity of these non-histone proteins. This is analogous to the theory of a "histone code", in which the precise combination of histone modifications is able to tightly regulate transcriptional activity within the promoter microenvironment. Thus, elucidating the regulation of posttranslational modifications of non-histone proteins is essential to our understanding of LTM formation. With this in mind, it is not completely unreasonable to imagine that alterations in acetylation and methylation of non-histone proteins may serve to regulate neural plasticity. Indeed, the process of p65/RelA acetylation and deacetylation has been shown to be crucial for LTM formation (Yeh et al., 2004). This study provides evidence that HDAC activity targets both histone as well as non-histone proteins, such NF-κB, that are involved in transcriptional regulation of genes during the formation of hippocampus-dependent memory. In addition, the regulation of a "non-histone" code may provide important information on the molecular mechanisms involved in the regulation of the "histone code", thus providing more information on the functional role of lysine-modifying enzymes in neurons. Finally, the association of transcription factors with lysine-modifying enzymes may provide important information on whether these non-histone targets may serve to recruit these enzymes to chromatin. Regardless of whether a histone code actually exists, the complex crosstalk and the timing between histone and non-histone modifications may be critical to transcriptional readout of genes involved in synaptic plasticity, activity-dependent processes or responses to experience-stimuli in the functioning adult mammalian brain.

7. Importance of BDNF DNA methylation to memory formation

There is significant crosstalk between histone modification and DNA methylation. It is well-established that the process of memory formation requires posttranslational modification of histone proteins in response to learning (Alarcon et al., 2004; Bredy et al., 2007; Chwang, O'Riordan, Levenson, & Sweatt, 2006; Fischer, Sananbenesi, Wang, Dobbin, & Tsai, 2007; Guan et al., 2002, 2009; Gupta et al., 2010; Kumar et al., 2005; Levenson & Sweatt, 2005; Levenson et al., 2004b; Lubin et al., 2008; Swank & Sweatt, 2001; Wood et al., 2006a). Now, new investigations into the role of DNA methylation as a dynamic process in the regulation of memory-related gene expression in the adult brain have recently emerged. Specifically, DNA methylation events in relation to the *bdnf* gene has been shown to occur in response to stimuli-experiences and behavior.

Research from Lubin and colleagues (2008) show that learning triggers exon-specific regulation of *bdnf* transcripts via DNA methylation using a fear conditioning rodent paradigm. These studies demonstrate that learning triggers changes in exon-specific *bdnf* transcripts. Blockade of DNA methylation via infusion of the DNMT inhibitor, zebularine, directly into area CA1 of rat hippocampus results in differential regulation of *bdnf* transcripts (Lubin et al., 2008). These findings are remarkable because they suggest that chromatin modifications is preferentially linked to selective regulation of *bdnf* variants in response to learning. In these same studies Lubin and co-workers also provide evidence that links NMDA receptor activation to *bdnf* DNA methylation changes in response to contextual fear conditioning. Importantly, these findings support the idea that DNA methylation in the adult brain can be dynamically regulated in response to environmental stimuli. In addition, these findings support the plastic nature of DNA methylation regulation in differentiated cells and the plausible presence of DNA demethylases in this phenomenon. Furthermore, a recent study from the Lubin group indicate crosstalk between histone methylation and DNA methylation at gene promoters during the process of LTM formation (Gupta et al., 2010). These recent findings indicate that histone modifications and DNA methylation occur together and not separately to influence the microenvironment of the *bdnf* gene in response to learning.

One intriguing question that remains from the NMDA receptor studies described by Lubin et al. (2008) is investigation of the signaling molecules involved in the regulation of DNA methylation in response to learning in the adult brain. Thus far, initial studies in this area have implicated the protein kinase C (PKC) and NF-κB signaling pathways in the regulation of DNA methylation in neurons (Levenson et al., 2006; Tian et al., 2009). Specifically, Levenson et al. (2006) demonstrate that PKC signaling activity coupled to DNA methylation changes and regulation of *dnmt3a* gene expression in acute hippocampal slice preparations (Levenson et al., 2006). In other signaling studies, concomitant increases in H3K4

methylated levels were found to be associated with NF-κB-mediated *bdnf* activation at κB consensus sequences containing CpG sites proximal to and distal to the *bdnf* transcription start site which correlated with increased occupancy of RNA polymerase II following NMDA receptor stimulation (Tian et al., 2009). Furthermore, NF-κB signaling has been shown to associate with histone methyltransferases to regulate H3K4 methylation changes which is strongly associated with DNA demethylation (Li et al., 2008). In another study, the scaffolding protein RACK1 has been shown to be a mediator of chromatin modifications around *bdnf* promoter IV through its association with histones H3 and H4 (He, Neasta, & Ron, 2010). Together these findings emphasize the need for a better understanding of the various signaling pathways that are involved in the regulation of epigenetic mechanisms that regulate chromatin modifications in post-mitotic neurons. This information might be essential as these molecular signaling processes occur in tandem with epigenetic mechanisms and not in isolation to influence *bdnf* gene transcription.

Traditionally, DNA methylation was thought to suppress gene transcription. However, recent work shows that like histone methylation, DNA methylation is also a two-faced modification resulting in either gene repression or activation (Chahrour et al., 2008; Gupta et al., 2010). Furthermore, the histone methylation marks can recruit DNMTs serving as a linker between histone modifications and DNA methylation to mediate memory formation (Gupta et al., 2010). Therefore, it is apparent that the microenvironment of the chromatin along with all its accessory proteins culminates in the onset or offset of gene transcription in response to the cellular signals which are reflective of the environmental stimuli affecting memory. Overall, these results suggest that DNA methylating mechanisms are associated with previously reported histone modifications (Alarcon et al., 2004; Bredy et al., 2007; Chwang et al., 2006; Fischer et al., 2007; Guan et al., 2002, 2009; Gupta et al., 2010; Kumar et al., 2005; Levenson & Sweatt, 2005; Levenson et al., 2004b; Lubin et al., 2008; Swank & Sweatt, 2001; Wood et al., 2006a) and implicate this molecular process in the regulation of the functional state of the neuron to mediate LTM formation.

Further evidence for dynamic DNA methylation changes in the adult brain include work by Nelson et al. (2008). In these studies Nelson and co-workers demonstrate that DNA methylation changes triggered in response to increased synaptic activity regulates spontaneous synaptic transmission between post-synaptic neurons. These reported effects of altered DNA methylation were validated by blockade of DNA methylation resulting in an increase in *bdnf* expression as well as decreased synaptic vesicle fusion and lower frequency of miniature excitatory post-synaptic currents (mEPSCs) in the hippocampus. The DNA methylation alterations at the gene promoters can presumably be carried out by demethylating enzymes; however, the presence of such demethylases is yet an open question. Nonetheless, these studies also delineate an activity driven role for dynamic DNA methylation in post-mitotic neurons.

8. The importance of BDNF DNA demethylation in memory formation

The DNA methylation studies described above involve not only the addition of methyl groups to the 'CpG' sites but also removal of the methyl group from the 5′-methylcytosine residue. This observation suggests the presence of DNA demethylases in post-mitotic neurons to mediate DNA demethylation. In the past few decades several candidate demethylating enzymes have been proposed for DNA methylation changes occurring in response to learning. Thus far a consensus in the field supports a DNA-repair process for active DNA demethylation in the adult nervous system which involves enzymes that can either function to remove the methyl group from 5′-methylcytosines or to remove the entire nucleotide resulting in a base-excision repair mechanism. The following paragraphs will explore these two DNA demethylating mechanisms in neuronal alterations and in the context of activity-dependent *bdnf* gene regulation in memory.

Surprisingly, one proposed DNA demethylating mechanism implicates the very same enzyme mediating DNA methylation, DNMTs. Recent studies provide evidence that DNMT3A and DNMT3B function in the conversion of 5′-methylcytosine to thymine residue in the absence of the methyltransferase donor SAM which is then followed by base-excision repair resulting in the demethylation of DNA (Kangaspeska et al., 2008; Metivier et al., 2008; Ooi & Bestor, 2008). However, whether DNMT3A and DNMT3B are efficient demethylases remain unclear and the focus of future research.

The "apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like" (Apobec) class of proteins and the MBD protein Mbd4 have also been implicated in a base-like repair mechanism to mediate DNA demethylation. Apobec proteins are deaminating enzymes that convert 5′-methylcytosine to thymine. This conversion results in a G/T mismatch pair which is removed by Mbd4, generating an abasic site that is cleaved by Apurinic/apyrimidinic (AP) endonucleases (Fig. 2). Polymerase-β removes the abasic sugar-phosphate and inserts a deoxycytidine residue. The remaining nick is sealed by a DNA ligase (Rai et al., 2008). There are several caveats to the mechanism described by Rai and colleagues (2008). For example, although Apobec enzymes deaminate cytosine residues, it is always within single stranded DNA (Activation-Induced (Cytidine) Deaminases; AID) or single stranded RNA (Apobec 1) as the amino group in question is involved in hydrogen bonding with its complementary guanine residue on the opposite strand (Conticello, Langlois, Yang, & Neuberger, 2007). In addition, Apobec proteins are known to deaminate cytosine residues more efficiently than 5′-methylcytosine (reviewed in Jiricny and Menigatti (2008)). Interestingly, during transcription the DNA is transiently

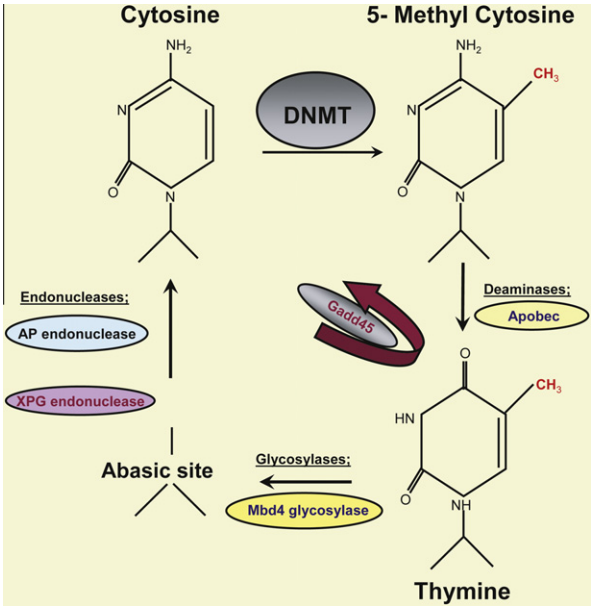

Fig. 2. A mechanistic view of the proposed enzymes involved in DNA demethylation. DNA methyltransferases (DNMT) convert Cytosines to 5′-methylcytosines (5-meC). The 5-meC is acted on by deaminases to convert it to thymine followed by the action of glycosylases which generate an abasic site. The abasic site is recognized by endonucleases and cleaved. The subsequent gap is filled with the appropriate complementary base by DNA polymerases and ligases. Circled in yellow are specific enzymes proposed by the leading theories to mediate the reactions. Gadd45a is implicated in the coupling of deamination and glycosylation reaction processes.

single stranded but the experiments by Rai and colleagues do not take this into account and their assays use a 740 bp single stranded DNA substrate. It is also important to remember that a significant number of 'CpG' sites occur in clusters, thus a symmetrically methylated CpG site would result in a GT/TG mismatch. The AP endonuclease acting on this site would result in a double-strand break and loss of the substrate DNA.

Another plausible DNA demethylating mechanism has been identified by Barreto and colleagues (2007) and implicates the Growth arrest and DNA-damage-inducible gene 45 alpha (GADD45a) as a key regulator of DNA demethylation (Fig. 2). GADD45a is an 18 kDa acidic nucleoprotein that is involved in DNA repair, cell cycle checkpoint and maintenance of genomic stability (Hollander & Fornace, 2002). In their study, Barreto and colleagues demonstrate that GADD45a promotes DNA demethylation and the subsequent activation of a methylation-silenced reporter gene (Barreto et al., 2007). Furthermore, their studies support the idea that GADD45a works in conjunction with the DNA repair endonuclease xeroderma pigmentosum complementation group G (XPG) to result in active DNA demethylation.

It is noteworthy to mention that GADD45a and XPG have previously been implicated in base-excision repair processes (Jung et al., 2007) and thus the Barreto et al. reports may hold true. However, this remains controversial as a subsequent study reported that GADD45a was not involved in active demethylation (Jin, Guo, & Pfeifer, 2008). Demethylation of methylated reporter plasmids or methylated endogenous loci was not observed when GADD45a was over-expressed, and XPG had no reported effect. Still, Barreto and colleagues demonstrate that a knockdown of GADD45a produce a 3-fold increase in global DNA methylation. Intriguingly, more than 50% of the human genome is methylated (Goll & Bestor, 2005), thus a 3-fold increase in DNA methylation would have deleterious effects on the GADD45a knockout mice. However, no severe phenotypical manifestation of the DNA hypermethylation was exhibited in these knockout mice (Barreto et al., 2007). Interestingly, these results also could not be replicated by Jin and colleagues, who in-turn proposed a role for the methyl binding protein, MBD3L2, as a potential demethylase (Jin et al., 2008).

The GADD45 family of proteins comprises of the alpha, beta, and gamma isoforms and share similar molecular functions in the process of epigenetic regulation of gene expression (Henshall, Sinclair, & Simon, 1999; Ma, Guo, Ming, & Song, 2009a; Rai et al., 2008). Therefore, not surprisingly GADD45b has also been implicated in epigenetic DNA demethylation in adult dentate gyrus neurons (Ma et al., 2009b). Interestingly, GADD45b knockout mice appeared normal; however, in a region-specific DNA methylation study GADD45b knockout mice displayed no significant demethylation at the DNA regulatory region of *bdnf* exon IX in response to electro-convulsive treatment, which resulted in a lack of *bdnf* gene expression. Together, these studies support the role of GADD45b in activity-dependent DNA demethylation in neurons. Whether GADD45b serves to mediate experience-induced DNA demethylation at *bdnf* promoters remains to be determined (Lubin et al., 2008).

Hence the emerging data supports DNA demethylation as an activity-dependent process in the nervous system but the key players and the exact mechanisms are yet to be elucidated. Although, DNA demethylation adds another level of complexity to the phenomenon of epigenetics, it provides the neurobiology field with a potential mechanism for manipulation of DNA methylation specifically as a treatment option for cognitive disorders where DNA methylation is significantly altered. Thus, the consensus of the current findings described above uncover new facets for DNA methylation changes in post-mitotic neurons and supports this molecular mechanism for regulation of gene expression, including *bdnf*, in LTM storage. Moreover, regulation of gene transcription by DNA methylation in learning and memory processes needs to be further explored as it may possibly serve to expand our understanding of memory deficits associated with neurological disorders.

9. Epigenetic regulation of the BDNF gene in cognitive disorders associated with neurological disorders

9.1. Epilepsy

Epilepsy is a neurological disorder characterized by recurrent and unprovoked seizures. Epilepsy involves episodic abnormal electrical activity in the brain that lead to increased susceptibility to cognitive impairments. Indeed, cognitive deficits are associated with epilepsy in both children and adults (reviewed in Helmstaedter, Kurthen, Lux, Reuber, and Elger (2003) and Leritz, Grande, and Bauer (2006)). However, to date, few effective treatments are available for cognitive and behavioral impairments in epilepsy. Thus, we need to begin to address how abnormal neuronal activity associated with epilepsy significantly impacts normal cognitive processes and behavior. An emerging research idea suggests that alterations in epigenetic transcriptional regulation of genes, such as *bdnf*, may underlie changes in hippocampal organization and function of mature neurons resulting in spontaneous seizures (epilepsy) and cognitive decline.

The *bdnf* gene is one of the numerous genes modulated in epilepsy models and in human temporal lobe epilepsy (Binder, 2004; Binder, Croll, Gall, & Scharfman, 2001). Seizure activity increases the expression of *bdnf* mRNA and protein (Humpel, Wetmore, & Olson, 1993; Vezzani et al., 1999). Molecular studies show that interfering with BDNF *in vivo* inhibits the development of kindling (Binder, Routbort, Ryan, Yancopoulos, & McNamara, 1999). In agreement, other studies report that intrahippocampal BDNF infusion promoted the development of epilepsy (Koyama & Ikegaya, 2005; Scharfman et al., 2005). In addition, McNamara and colleagues demonstrated that conditional deletion of TrkB, the BDNF receptor, but not BDNF prevents epileptogenesis in the kindling model (He et al., 2004). However, whether BDNF activity contributes to cognitive deficits associated with epilepsy remains unexplored. Indeed, the *bdnf* gene is activated by seizure activity and triggered by learning to mediate memory formation, yet in epileptic animal models and human patients, memory is poor. Thus, additional studies are necessary to further elucidate the role of BDNF in the development of epilepsy and related cognitive impairments.

The functional consequences of altered epigenetic mechanisms have been highlighted in cognitive disorders associated with the pathophysiology of several epileptic disorders. For example, mutations in the histone demethylase KDM5C (JARID1C/SMCX) has been associated with epilepsy and X-linked mental retardation (Abidi et al., 2008; Tzschach et al., 2006). It is hypothesized that the epigenetic interaction between KDM5C and the epigenetic regulator REST/NSRF (repressor element-1 silencing transcription factor/neuronal restrictive silencer factor) mediates these effects (Tahiliani et al., 2007). Moreover, REST orchestrates the expression of several genes that are believed to be involved in the epileptogenesis process including *bdnf* (Qureshi & Mehler, 2009). Examples of the role of REST in epileptogenesis is further illustrated in animal models of epilepsy which show that levels of the REST isoform, REST4, increase in response to seizures and is modulated by several regulatory factors, such as neuropeptide substance P and neurokinin B (Gillies, Haddley, Vasiliou, Bubb, & Quinn, 2009). Together, these studies demonstrate the important relationship between chromatin-modifying mechanisms in the development of human epilepsy and in epilepsy-related syndromes including cognitive decline.

Furthermore, given that *bdnf* gene expression is regulated in both epilepsy and memory formation, it is proposed that altered

bdnf gene transcription via regulation of chromatin structure within mature hippocampal neurons may be responsible for cognitive decline in epileptic patients. The first experimental results linking chromatin structure regulation to seizure-induced cognitive decline were recently obtained in relation an antiepileptic drug, valproic acid (VPA), which has been described as a histone deacetylase inhibitor (Jessberger et al., 2007). In this study, VPA treatment protected epileptic animals from hippocampus-dependent cognitive impairment after kainate-induced seizures. This supports the notion that altered chromatin structure may play a role in the regulation of genes, such as *bdnf*, involved in seizure-induced cognitive decline. Interestingly, Bredy and colleagues (2007) demonstrate that VPA treatment enhanced long-term memory for extinction that was associated with an increase in histone H4 acetylation at *bdnf* promoter regions and *bdnf* exon IV mRNA expression in the prefrontal cortex. Further analysis of the molecular mechanisms by which gene expression of *bdnf* is induced by seizure activity could provide novel concepts and targets for cognitive regression therapy using antiepileptic drugs such as VPA.

Although much of the epigenetics literature has focused on histone modifications across the *bdnf* gene, recent studies focused on transcriptional regulation during the induction of epileptogenesis in animal models have identified a role for DNA methylation as a potential transcriptional mechanism for differential expression of *bdnf* transcripts in the adult hippocampus in response to prolonged seizure activity (Aid et al., 2007). For example, Aid and colleagues found that seizure activity results in the demethylation of the *bdnf* gene at specific promoter regions that correspond to increased *bdnf* gene activity in the adult hippocampus. Therefore, studies aimed at understanding *bdnf* gene expression changes during prolonged seizure activity and in epilepsy should include analysis of epigenetic transcription-regulating mechanisms including altered DNA methylation at *bdnf* promoter regions.

9.2. Rett syndrome

Rett syndrome is an X-linked neurodevelopmental disorder, which primarily affects females and leads to postnatal lethality in males (Amir et al., 1999). Patients with Rett syndrome suffer from severe motor and cognitive impairments and may often display autistic features. Rett syndrome is due to a mutation in the *MeCP2* gene. MeCP2 protein binds methylated CpG sites at gene promoters and promotes gene silencing by associating with Sin3A and histone deacetylases to form corepressor complexes and through its interaction with CREB selectively regulates *active bdnf* gene transcription (Akbarian & Huang, 2009; Chahrour et al., 2008; Chen et al., 2003; Klose & Bird, 2003; Nan et al., 1998). Thus, MeCP2 mutation leads to abnormal regulation of genes necessary for proper development and cognitive function. Interestingly, recent work suggest that MeCP2 functions not only to repress gene transcription but to mediate activation of genes as well (Chahrour et al., 2008 and see Fig. 3, Gupta et al., 2010) all in the context of memory formation. These studies provide us with new insights into the gene targets that could be dysregulated due to loss of MeCP2 function in Rett syndrome.

9.3. Schizophrenia

Schizophrenia is a severe, debilitating neuropsychiatric disorder. It affects around 1.1% of the US population, age 18 and above. It is characterized by three categories of symptoms: (1) positive symptoms, these include hallucinations, delusions and thought disorder; (2) negative symptoms, include apathy, inappropriate mood, and poverty of speech; (3) cognitive dysfunction defined by impaired working memory and conceptual disorganization (reviewed in Roth, Lubin, Sodhi, and Kleinman (2009a)). Aberrant DNA methylation levels have been observed at gene promoter sites of patients suffering from schizophrenia (reviewed in Roth, Lubin, Sodhi, and Kleinman (2009b)). A study of the DNA methylation patterns in neuropsychiatric patients may help shed light on the molecular pathology of this neurological disorder. Therapeutic strategies administered to patients with schizophrenia alleviate only the symptoms. A better understanding of the cause of the disorder can potentially lead to more effective therapeutic strategies. However, to date there is limited clinical and basic research examining the effect of manipulating epigenetic mechanisms and subsequently alleviating psychotic episodes.

Promisingly, the beneficial effects of inhibiting chromatin-modifying enzymes has been shown to alleviate some of the molecular and behavioral manifestations of cognitive dysfunction (reviewed in Deutsch et al. (2008)). For example, DNA methylation is known to play a role in activity-dependent *bdnf* gene regulation and alterations in DNA methylation levels at the *bdnf* promoter or intragenic regions occurs in response to fear learning (Lubin et al., 2008; Martinowich et al., 2003). Additionally, DNMT inhibition by pharmacological means sufficiently alters basal *bdnf* transcript readout in the adult hippocampus (Lubin et al., 2008). Furthermore, analyses of post-mortem brain tissue from completed suicide subjects reveal

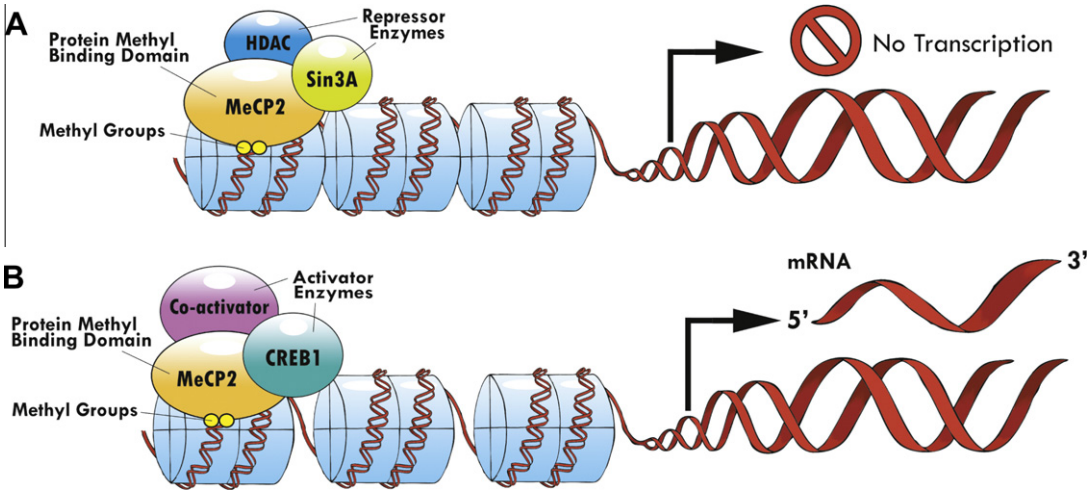

Fig. 3. Mechanisms of MeCP2-mediated gene silencing and transcriptional activation. Generally, methyl-DNA binding domain (MBD) proteins such as MeCP2 are recruited to methylated CpG sites and assemble repressor enzymes to mediate repression of gene transcripts (A). Although this is a well-characterized role of MBD proteins in transcriptional regulation, it has been reported that the MeCP2 MBD protein can also be associated with transcriptional activation via CREB1 (B).

reduced significant DNA methylation increases at *bdnf* promoter 4 which parallels with reduced *bdnf* mRNA expression in the Wernicke area (Keller et al., 2010). Overall, these findings suggest the possibility that manipulation of DNMT activity may return gene promoters to their normal methylation state and serve as therapy for schizophrenia. This therapeutic approach holds promise as it is likely that multiple genes and not a single gene contributes to symptoms associated with schizophrenia as suggested by several studies using genome-wide epigenetic methods (Connor & Akbarian, 2008; Mill et al., 2008).

9.4. Alzheimer's disease

Alzheimer's disease (AD) is one of the most common neurodegenerative disorders with an estimated 26 million people affected worldwide. Some of the symptoms associated with AD include extensive neuronal cell death initiating in brain regions implicated in cognition, learning and memory. In fact, reduced *bdnf* gene expression has been implicated in AD since the early 1990s (Phillips et al., 1991). Since then genetic polymorphisms within the *bdnf* gene have been linked to abnormal cognitive functions, personality, and memory associated with AD (Kunugi et al., 2001; Tsai et al., 2004). However, few studies have explored the molecular mechanisms involved in *bdnf* mRNA deficiency in AD. Whether epigenetic mechanisms affect *bdnf* gene regulation in AD is still to be determined, but DNA methylation has been implicated in the possible regulation of the gene coding for the amyloid precursor protein (APP), which is cleaved abnormally by either beta or gamma secretase resulting in the production of the beta-amyloid protein which is believed to underlie the neuropathology and clinical manifestations of AD. Whether the regulation of *APP* gene expression is dependent or independent of BDNF activity in AD is not yet determined. However, recent studies suggest that simultaneous activation of at least two signaling pathways, Ras/MAPK and PI3K/Akt, is necessary to mediate a full activation of the APP promoter by BDNF (Ruiz-Leon & Pascual, 2001, 2004). An interesting, yet premature, idea is that BDNF activates these signaling cascades to mediate epigenetic regulation of the APP promoter in AD.

The promoter of the APP gene is rich in CpG sites, making it a potential epigenetic target. Indeed, the promoter region of the APP gene between −226 and −101 sequence has 13 potential methylation sites, of which at least one methylcytosine was found in 26% of the patients under the age of 70 in comparison to the 8% above the age of 70 (Tohgi et al., 1999). This observation suggests hypomethylation at the APP gene promoter as a possible risk factor for AD. Research data show that external application of beta-amyloid to murine cerebral endothelial cell cultures results in DNA hypomethylation as well as extensive methylation at the neprilysin (NPE) gene promoter (Chen et al., 2009). NPE is an enzyme known to break down the beta-amyloid protein and aid in preventing formation of plaques. The hypermethylation observed at the NPE promoter was confirmed with a decreased NPE mRNA expression in the cell cultures. However, extensive research targeting differential regulation of methylated CpG sites of APP and NPE gene promoters has yet to be determined. Indeed, there are few effective treatments and no cures for AD. Thus, manipulation of DNA methylation for a given gene may serve to ameliorate the symptoms of AD and/or cognitive dysfunctions associated with AD.

10. Perspectives and summary

Epigenetic mechanisms are increasingly being recognized for their importance in exerting control over gene expression and behavior, and offer a plausible molecular mechanism responsible for stable changes in brain function and behavior. However, the findings discussed within this review highlight how little we know about the function of the key chromatin-modifying enzymes regulated in post-mitotic neurons subserving the processes of learning and memory. In relation to *bdnf* gene expression, we know little regarding regulation of its multiple gene transcripts in the CNS. What is needed now are further studies that begin to link the functional significance between differential expression of *bdnf* transcripts to changes in BDNF protein levels and how this contributes to neural plasticity or cognitive disorders. The future study of epigenetic-mediated gene transcription as a novel mechanism for *bdnf* gene changes in the CNS will add to our understanding of the chromatin biology involved in LTM formation.

Moreover, as our understanding of epigenetics in the CNS grows, it is becoming clear that the process of gene transcription in LTM formation and storage is a complex and coordinated event. The consensus in the literature is that dynamic regulation of chromatin structure does indeed occur during the consolidation phase of LTM but the findings highlighted in this review are at an early stage of discovery and leave several unanswered questions. For example, the molecular mechanism for active DNA demethylation in mammals is unknown. Furthermore, the relationship between histone posttranslational modifications at gene promoter regions in association with DNA methylation and demethylation remains unexplored. In addition, the signal transduction cascades mediating dynamic changes in DNA methylation-related chromatin modifications during memory consolidation is also unknown at present. Thus, at the outset this review is framed as a series of multiple questions that will have to be answered through future studies that incorporate all potential aspects of epigenetic mechanisms including the neuronal phenotypes involved in synaptic plasticity and molecular processes mediating LTM formation and storage.

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