Neurobiology of Learning and Memory 96 (2011) 68–78

Contents lists available at ScienceDirect

Neurobiology of Learning and Memory

journal homepage: www.elsevier.com/locate/ynlme

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

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ARTICLE INFO

Article history:
Available online 16 March 2011

Keywords:
bdnf
NF-kappaB
DNA methylation
Histone methylation
Hippocampus
Amygdala
DNMT
Gadd45

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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doi:10.1016/j.nlm.2011.03.001

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;