Epigenetic regulation and chromatin remodeling in learning and memory

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Understanding the underlying mechanisms of memory formation and maintenance has been a major goal in the field of neuroscience. Memory formation and maintenance are tightly controlled complex processes. Among the various processes occurring at different levels, gene expression regulation is especially crucial for proper memory processing, as some genes need to be activated while some genes must be suppressed. Epigenetic regulation of the genome involves processes such as DNA methylation and histone post-translational modifications. These processes edit genomic properties or the interactions between the genome and histone cores. They then induce structural changes in the chromatin and lead to transcriptional changes of different genes. Recent studies have focused on the concept of chromatin remodeling, which consists of 3D structural changes in chromatin in relation to gene regulation, and is an important process in learning and memory. In this review, we will introduce three major epigenetic processes involved in memory regulation: DNA methylation, histone methylation and histone acetylation. We will also discuss general mechanisms of long-term memory storage and relate the epigenetic control of learning and memory to chromatin remodeling. Finally, we will discuss how epigenetic mechanisms can contribute to the pathologies of neurological disorders and cause memory-related symptoms.

INTRODUCTION

Many studies have shown evidence of active epigenetic marker changes during learning and memory processes.1,2 The term ‘neuroepigenetics’ describes memory processes as consequences of dynamic experience-dependent changes in the genome.3 Epigenetic mechanisms cause DNA compaction and relaxation, which lead to transcriptional repression and activation, respectively. Chromatin is made of histone units, with each unit composed of an 8-subunit histone core and the DNA coiling around it.4 As 146 bps of DNA coil around one histone, DNA is compacted and is able to fit into the nucleus. Chromatin may adopt one of two major states in an interchangeable manner. These states are heterochromatin and euchromatin. Heterochromatin is a compact form that is resistant to the binding of various proteins, such as transcriptional machinery. In contrast, euchromatin is a relaxed form of chromatin that is open to modifications and transcriptional processes (Figure 1)5.

The term epigenetics was coined by Waddington in 1942, and was used to describe the 'interactions of genes with their environment that brings the phenotype into being'.6 Waddington originally used the term epigenetics to explain the phenomena in which changes not encoded in the DNA occur in the cell during development in response to environmental stimuli. Since then, an extensive number of studies has shown that long-lasting epigenetic changes occur in the genomes of cells. These changes include changes to post-mitotic neurons, which are used to incorporate experience-dependent changes.7 An early study showing the important relationship between epigenetics and synaptic plasticity is that of Kandel and colleagues. This study investigated long-term effect of excitatory and inhibitory signaling in Apbyaisa sensory neurons. The authors discovered that the facilitatory transmitter 5-HT activates cyclic AMP-responsive element-binding protein 1, which causes histone acetylation. On the other hand, the inhibitory transmitter FMRFa causes CREB2 activation and histone deacetylation.8 These results indicate that gene expression and epigenetic changes are required for long-term memory-related synaptic plasticity in Apbyaisa. Thus, epigenetic modifications are made independently of actual genome sequence changes and lead to the creation of the ‘epigenome.’ The epigenome undergoes
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Figure 1 Schematic drawing of histone methylation and acetylation in relation to chromatin remodeling. Addition of methyl groups to the tails of histone core proteins leads to histone methylation, which in turn leads to the adoption of a condensed state of chromatin called 'heterochromatin.' Heterochromatin blocks transcription machinery from binding to DNA and results in transcriptional repression. The addition of acetyl groups to lysine residues in the N-terminal tails of histones causes histone acetylation, which leads to the adoption of a relaxed state of chromatin called 'euchromatin.' In this state, transcription factors and other proteins can bind to their DNA binding sites and proceed with active transcription.

biochemical changes in response to environmental stimuli and leads to the remodeling of chromatin structure. The term chromatin remodeling mainly refers to the ATP-dependent process of genomic transformation by enzymes that shift nucleosomes, such as the SWI/SNF complex.9 This mechanistic change in genomic conformation sets out a platform for various cellular processes, such as the exposure of the promoter of a specific gene to its transcriptional machinery.9 Chromatin remodeling, DNA methylation and histone posttranslational modifications (PTMs) are all known to be important long-term memory processes. There are many types of histone modifications, such as acetylation, methylation, phosphorylation, ubiquitination and ADP-ribosylation.10 In this review, we will discuss DNA methylation, histone modifications and chromatin remodeling, which are the most studied epigenetic mechanisms in relation to memory processes.

MEMORY FORMATION AND STORAGE
Memory allows us to acquire new information and to store it in our brains. Memory enables animals to behave adaptively to changing environments. While memory formation and storage require many processes at the cellular level, the 'memory engram' can be considered to be a biological trace of memory. This term was first coined by Richard Semon and denoted the 'hypothetical material basis of learned information'.11 Memory engram-bearing cells are specific cells that retain biochemical changes induced by learning and sustain the information until it is later retrieved based on appropriate cues.11 How experiences are transformed into the memory engram and stored for a long time is now an extensively studied field in neuroscience.

After a memory is formed, a chain of biological reactions occurs to store it for the long term. Roberson and Sweatt described these reactions as 'mnemogenic,' which is a term based on the Greek word for memory formation.12 Mnemogenic reactions, such as de novo protein synthesis and DNA-histone modifications, chemically alter the biological system so that the acquired information is stably protected from protein turnover.13 Another important aspect of memory is the change in synaptic connection strength. This phenomenon is called long-term potentiation (LTP), during which synaptic connections are strengthened and synaptic efficacy is increased.14 Bliss and Lomo described LTP for the first time in 1973 through an experiment that showed that a train of high-frequency stimulation causes an increase in synaptic transmission efficiency in the rabbit brain. This synaptic strengthening was effective for several hours and required a number of biological changes.15 On the postsynaptic side, glutamate signaling through α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid and N-methyl-D-aspartate receptors trigger Ca2+/calmodulin-dependent protein (CaMKII) activation. CaMKII is a well-known protein that triggers secondary signaling processes that play central roles in promoting synaptic plasticity.16 The rhythmic bursts of activity that induce LTP mimic the naturally occurring theta rhythm that is present in the hippocampus during learning.17 Several studies have shown that injecting LTP blockers, such as CaMKII inhibitors and extracellular signal-regulated kinase inhibitor into the hippocampus cause impairments in hippocampus-dependent tasks, such as the Morris water maze.16,18 There are two major types of memories: short-term memories, which last for a few hours, and long-term memories, which persist for several days or longer. Long-term memory formation requires a cascade of processes, which are needed to produce new messenger RNAs and proteins related to synaptic plasticity.19 When dendritic spines were imaged over different time courses in different regions of the mouse brain, the fraction of spines undergoing turnover persistently decreased. This shows that synaptic connections are continually stabilized during adulthood. In addition, spine turnover rates were different in the different brain regions. This indicates that different brain regions differ in their capacities for experience-dependent plasticity.20 The fact that the plasticity of a neuron can be altered for a long time can be explained by the synthesis of new proteins in the soma. Active transcription and local translation aid the maintenance of LTP and memory consolidation (Figure 2).

To be stably stored, a memory must overcome its vulnerability to external disruptions. After initial acquisition, memory transforms from a transient state into a stable state during 'memory consolidation'.21 Cellular and circuit level structural changes support long-term memory storage and involve different brain regions and networks. After fear conditioning training, the mouse hippocampus and anterior cingulate cortex exhibit dendritic spine growth in a time-dependent manner.22 How memories persist for a lifetime may be explained by
DNA METHYLATION AND MEMORY REGULATION

DNA methylation is known to occur on nucleotide base cytosines that are next to guanine (CpG) and regulates chromatin state transitions. During DNA methylation, DNMTs aid the covalent binding of a methyl group from S-adenosyl-methionine to the 5’ position of cytosine. DNA methylation typically represses transcription by inhibiting the binding of the transcriptional machinery to binding sites. DNA methylation is often referred to as a lifelong inhibitory change that induces gene silencing. DNA methylation in neuronal cells can persist because mature neurons do not divide. Furthermore, if a methylated cytosine is damaged, the new cytosine is methylated to reconstruct the epigenetic change. Thus, we can say that DNA methylation, like other epigenetic processes, self-perpetuates through DNMT activity.

There is extensive evidence that DNA methylation is regulated in an experience-dependent manner by neural activity. An early discovery indicating the involvement of active DNA methylation in hippocampus-dependent memory showed that neuronal activation induces sufficient changes in the DNA methylation of memory-related genes. Treating with DNMT blockers inhibits memory formation and synaptic plasticity induction. There are three types of DNMTs: DNMT1, DNMT3a and DNMT3b. Before the discovery of the demethylation activity of Tet enzymes, it was once thought that DNA methylation was irreversible and that demethylation is unlikely to occur in mature neurons. However, this idea was proven wrong by work showing that Tet enzymes have active demethylation activity and affect memory formation and stabilization. Tet1 knockout (KO) transgenic mice have impaired gene expression and memory extinction. In addition, when DNA methylation was blocked in the anterior cingulate cortex 30 days after memory formation by inhibiting DNMTs using drugs, remote memory was impaired. These data imply the presence of important roles for DNA methylation and demethylation in memory maintenance and extinction.

How does DNA methylation regulate gene transcription? A pair of methyl groups at the major groove of DNA blocks transcription factors from recognizing their binding sites. DNA methylation also increases chromatin compaction by attracting histone-remodeling enzymes. Histone-remodeling proteins are attracted to methylated DNA sites by methyl CpG-binding domain (MBD) proteins. MBD proteins recruit transcriptional corepressors, such as histone deacetylases, which increase the positive charge of the nucleosome and alter local chromatin structure into the transcriptionally incompetent heterochromatin state. Mutations in MBD can cause developmental disabilities, such as Rett syndrome, which is caused by an MBD mutation in the MECP2 protein.

Cognitive ability tends to decline with age. Oliveira et al. found that cognitive decline associated with aging is concomitant with a reduction in DNA methyltransferase Dnmt3a2 expression in the hippocampus. When the authors restored Dnmt3a2 levels in aged mice, cognitive ability was restored to normal levels in tasks such as trace fear conditioning.
In another study, fear learning caused DNA methylation changes in different brain regions, as DNMT3a and 3b expression in the hippocampus was increased after contextual fear conditioning. Furthermore, while histone H3 acetylation and DNMT3a expression were increased in the lateral amygdala after auditory fear conditioning, blocking DNMT pharmacologically or using the intra-CA1 infusion of inhibitors such as 5-azacytidine or zebularine resulted in impairment of contextual fear conditioning. These data appear to be rather controversial because DNA methylation has been known to negatively regulate memory. However, recent evidence suggests that DNA methylation is involved in silencing the expression of memory suppressor genes, such as PPI, and that DNA methylation may regulate memory by interacting with histone acetylation and changing its levels. Although further investigations are needed to clarify the roles of DNA methylation in memory consolidation, a body of evidence clearly indicates that memory formation requires hypermethylation of memory suppressor genes and hypomethylation of memory promoting genes. Thus, DNA methylation is dynamically regulated in memory processes.

**PTMS OF HISTONES**

In addition to DNA methylation, post-translational histone modifications are also important for chromatin geometry and gene expression. The positive charges of unmodified histone proteins promote a tight interaction with negatively charged DNA and cause chromatin to be in a transcriptionally unfavorable state. However, histones undergo modifications, including acetylation, phosphorylation and methylation, that alter their charges and binding properties. Histone acetylation involves the histone acetyltransferase (HAT) enzyme. It is often associated with transcriptional activation, as acetylated histones act as binding sites for transcriptional machinery. Histone phosphorylation is also associated with transcriptional activation, while histone methylation can promote both transcriptional activation and repression. Newly formed hippocampus-dependent memories need to be stabilized into a persistent memory trace. Mice with decreased PPI levels had enhanced remote memory performance with increasing histone PTMs. This suggests that histone PTMs are important for memory consolidation and retention. The levels of PTMs of histones associated with the promoter region of Zif268, which is an immediate early gene involved in memory, were shown to increase and their expression was shifted from the hippocampus to the prefrontal cortex as memories matured. The above data show the important roles of histone PTMs in different brain regions and how they facilitate memory consolidation.

Increasing histone acetylation levels by blocking the HDAC can cause an increase in memory storage. Therefore, altering histone modifications or the activity of histone-modifying enzymes, including CREB binding-protein (CBP), affect memory storage. Histone modifications may gate the transcription of plasticity genes that change the response of individual neurons and regulate behavior. These histone modification patterns may alter the structure of chromatin and its interaction interface for transcriptional proteins.

**HISTONE ACETYLATION IN MEMORY FORMATION**

Among the various types of histone modifications, histone acetylation is one of the most studied mechanisms. It involves the addition of an acetyl group to a lysine present at the N-terminal tail of the nucleosome, which is the basic unit of DNA packaging in eukaryotic cells. It has been known for a long time that long-term memory formation requires de novo protein synthesis. It has also been shown that there exist critical time periods of protein synthesis after learning, indicating that there is a time limit for gene expression events for memory consolidation. Growing evidence indicates that histone acetylation is just as important for the persistence of long-term memory. HDAC inhibitors, such as like tyrosine A and sodium butyrate (NaBu), enhance LTP in acute hippocampal slices and memory consolidation during contextual fear conditioning. Histone acetylation occurs at various lysine positions within the core histone. Therefore, it is possible that different forms of learning induce different patterns of acetylation at specific gene promoters. There are three main classes of HDAC based on sequence similarity to yeast proteins. HDAC2 has been found to negatively regulate memory. When Guan et al. overexpressed HDAC2 in neurons, dendritic synaptic plasticity and memory formation were impaired, while Hdac2 deletion caused memory facilitation. CBP is a well-known transcriptional coactivator that has HAT activity and has been shown to be important for long-term memory formation. Mutations in CBP can contribute to the pathology of Rubinstein-Taybi syndrome, a neurodevelopment disorder characterized by cognitive impairment and characteristic facial features, such as microcephaly. The Bdnf promoter is known to be responsive to histone acetylation changes after learning. Bredy et al. found that fear conditioning leads to a distinct pattern of acetylation at histones H3 and H4 around bdnf promoters. It has also been shown that CBP is recruited to the c-fos gene promoter in an activity-dependent manner, and that CBP’s acetyltransferase activity is required for the expression of the c-fos gene, which is involved in memory formation and consolidation.

**HISTONE METHYLATION**

Chromatin structure is also regulated through histone methylation. Although methylation is generally thought of as
Chromatin Remodeling

It is clear that transcriptional regulation involves active interactions between transcription factors and chromatin. Chromatin structure is dynamic and precisely controls cellular processes, including gene expression. Taking a step back from investigating gene expression at the protein level, we find that global changes resulting from chromatin remodeling have received considerable attention in association with gene expression and memory processes. Chwag et al. revealed that the extracellular signal-regulated kinase-1/mitogen-activated protein kinase signaling pathway regulates histone phosphorylation and acetylation in the hippocampus after contextual fear conditioning and long-term memory formation. Their results support the idea that chromatin acts as a dynamic signal platform through active histone modifications. The amino-terminal tails of histone proteins subjected to post-translational modifications create a stage for dynamic interplay between histone and DNA modifications and the combinatorial possibilities for gene regulation. Chromatin structure is modified through two mechanisms. First, interactions between nucleosomes are broken. Second, various protein factors are recruited to the unraveled nucleosomes. There are various types of histone-modifying enzymes, such as acetyltransferases, methyltransferases, serine/threonine kinases, ubiquitin ligases and proline isomerases. Enzymes that carry out chromatin modifications and those that remove the modifications have been identified. For example, while G9a and CLL8 methylate lysine, enzymes such as LSD1/BHC110 and JDM1a have demethylase activity. Methyltransferases and kinases are the most characterized histone modifying enzymes. Learning and memory processes are associated with chromatin remodeling. Tsai and colleagues used CK-p25 transgenic (Tg) mice to show the critical effects of environmental enrichment (EE) on learning and memory. The CK-p25 Tg mouse is a neurodegenerative mouse model, in which the expression of the Alzheimer’s disease-associated protein p25 is controlled by doxycycline. The authors showed that learning and long-term...
memory deficits in CK-p25 Tg mice are rescued following EE. This memory-restoring effect was achieved through EE-induced hippocampal and cortical chromatin remodeling, which leads to enhancements in synaptic efficacy.4

CONCLUSION
A few years ago, a group of scientists investigated the inheritance of parental experience using olfactory molecular specificity. They exposed F0 mice to acetophenone for odor fear conditioning and found that F1 and F2 generation mice similarly display an increased sensitivity to the F0-conditioned odor. Moreover, they found that sperm cells from F0 mice exhibit hypomethylation of the odorant receptor gene Olfr151 and that the F1 mice display neuroanatomical alterations of the olfactory system. These results indicate that experience-induced behavioral and structural adaptations can be biologically passed on to offspring through neuroanatomical and epigenetic changes.67 Mansuy and colleagues observed altered microRNA expression levels and trans-generational phenotype inheritance in mice that experience early life traumatic stress. They obtained similar results by injecting the traumatized males’ sperm RNA into a fertilized wild type egg. Specifically, the resulting offspring exhibited similar behavioral and epigenetic characteristics to their traumatized parent.68

Epigenetic modifications can serve as enduring changes in the genome and help drive stable gene expression, which manifests as a long-lasting change in the animals’ behavior. This may be accomplished by direct alteration of DNA or by post-translational modifications of histones. These modifications may include methylation, acetylation and phosphorylation. These covalent modifications affect the physical remodeling of chromatin structure or regulate recruitment of signaling complexes that drive or repress transcription.69 It is becoming increasingly clear that histone modifications and chromatin remodeling are critical for gene expression during memory processes. About 1–4% of mammalian genomic DNA consists of CpG dinucleotides and about 75% of these dinucleotides are known to be methylated.70

Failure of epigenetic processes can lead to pathogenesis characteristic of various neurological disorders (Table 1). One such example is Huntington’s disease (HD), which is a fatal, inheritable disorder with a mid-life onset. HD patients show symptoms such as chorea, emotional distress and cognitive decline. HD is caused by a CAG repeat expansion in exon 1 of the huntingtin (Htt) gene. Epigenetic modifications are found to be critical in HD pathogenesis. In patients with HD and HD transgenic mice, a number of genes, such as Sox2 and Pax6, were found to be hypermethylated, and to have accordingly reduced gene expression levels.71 Since these genes are important for neurogenesis, HD patients suffer from hippocampal neurogenesis deficits and cognitive decline. HDAC inhibitor treatment has been found to restore histone modification dysfunction and rescue some symptoms of HD.72 This study indicates that dysregulation of epigenetic processes can have prominent effects on the pathogenesis of neurological disorders. It is clear that epigenetic modifications and chromatin remodeling are necessary for proper memory formation and consolidation. As a number of chromatin-modifying drugs are being tested in preclinical studies,73 it will be important to extensively investigate the exact mechanisms of epigenetic regulation and to provide a basis for translational research and potential therapies for neurological diseases.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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