Manipulating the brain with epigenetics

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A study finds that the DNA methylation enzymes Dnmt1 and Dnmt3a are needed to maintain the epigenetic landscape in nondividing, postmitotic neurons and that this process is required for normal learning and memory.

One of the mysteries of life is that cells with identical genetic material maintain unique identities and specialized functions. This phenomenon is, in part, attributed to epigenetically controlled cellular memory, a fundamental basis for the existence of multicellular forms of life. Epigenetic codes include patterns of DNA methylation and histone acetylation that regulate the expression of various gene products[1] (**Fig. 1**). Histones are DNA-interacting proteins in the quaternary structure of chromatin, the functional form of DNA[2]. More recently, it has become apparent that transient alterations of histone acetylation–mediated epigenetic states of chromatin in forebrain excitatory neurons in adult mammalian brain are critical for the changes that underlie memory[3]. If covalent histone modifications are indeed involved in new memory formation, as has been broadly postulated[3–6], then what is the role of more stable and silencing covalent modifications? In other words, can DNA methylation control psychological memory? In this issue, Feng *et al.*[7] provide genetic evidence for the importance of DNA methylation in nondividing, postmitotic neurons in synaptic plasticity and in behavioral learning and memory in mice.

Methylated DNA prevents transcription by directing gene-silencing mechanisms to specific promoters[8]. Methyl-binding proteins such as MePC2 bind directly to the methylated cytosine and, subsequently, recruit repressor complexes with histone deacetylases, which are required to maintain chromatin remodeling events and retain a transcriptionally nonpermissive environment[9]. DNA hypermethylation involves a rather stable set of DNA-covalent tags that are frequently targeted to cytosine- and guanine-rich regions, referred to as GpC islands, which are associated with 76% of human genes[2]. DNA hypermethylation has a silencing effect on gene promoters. Conversely, DNA hypomethylation, together with histone acetylation, promotes gene expression. It has been postulated that cellular memory is critical for establishing and maintaining terminal cellular differentiation[1]. In this model, such differentiation is viewed as a progression through several epigenetically controlled reprogramming phases that restrict developmental options (progressing from omnipotency to monopotency to terminal differentiation). Such epigenetic mechanisms are considered important not only for the establishment of embryonic glia and neuronal populations derived from common neural stem cells, but also for adult neurogenesis[10].

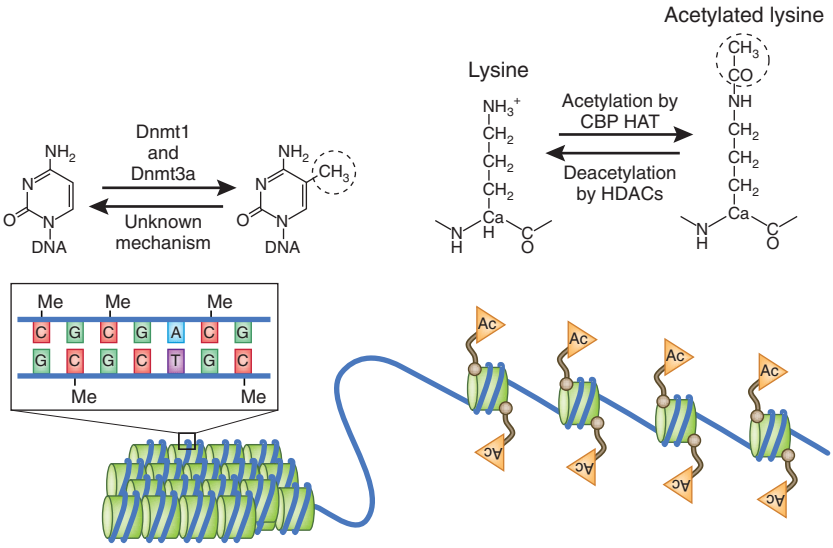

Figure 1 DNA methylation and histone acetylation are two critical epigenetic mechanisms controlling chromatin structure and function in postmitotic mammalian neurons. Hypermethylated DNA recruits silencing transcription chromatin remodeling complexes with histone deacetylases (HDACs) and promotes chromatin condensation. Hypomethylated DNA unfolds into a 'beads-on-a-string' structure in which histones are accessible for chromatin remodeling factors such as CREB-binding protein histone acetyltransferase (CBP HAT), the transcriptional coactivator implicated in epigenetic mechanisms controlling memory consolidation[3]. Ac, acetyl group; Me, methyl group.

Disruption of cellular memory in mature differentiated cells as a result of aberrant reprogramming has been directly linked to cancer. However, transient reprogramming in terminally differentiated cells might also be critical. Current models of the epigenetic control of memory consolidation[3] postulate that neuronal activity can induce transient reprogramming of epigenetic codes required for memory consolidation. Transient DNA methylation has recently been observed during memory formation[11]. Two abundant DNA methyltransferases (Dnmt1 and Dnmt3a) have been implicated in transient DNA methylation in mammalian postmitotic neurons[12,13]. A conventional knockout of either of these two major DNA methyltransferases in mouse, however, causes global DNA hypomethylation and early lethality[9], which makes it impossible to use these mutants to study the role of DNA methylation in nondividing, postmitotic neurons. Single conditional knockouts that eliminated the activity of Dnmt1 or Dnmt3a in postmitotic excitatory forebrain neurons showed no phenotype[7].

Feng *et al.*[7] circumvented this problem by generating Dnmt1 and Dnmt3a double conditional knockout (DKO) mice. In DKO mice, the two null mutations were delivered into the forebrain excitatory neurons of single mice during late development shortly after birth[7]. They found that Dnmt1 and Dnmt3a are somewhat redundant, but together are critical for maintaining patterns of DNA methylation that are required for long-lasting neural stability, morphology and function. Feng *et al.*[7] found that a number of abnormally demethylated genes found in mutant mice were also aberrantly expressed. Although no neuronal

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loss was observed, neurons appeared smaller. Basal synaptic transmission was normal, but there was an impairment of hippocampal long-term potentiation and a decreased threshold for hippocampal long-term depression.

Notably, the DKO mice also showed behavioral deficits for two tasks that are frequently used to assess hippocampal-dependent memory: the Morris water maze and contextual fear conditioning. In the contextual version of Pavlovian fear conditioning, mice learn the association between foot shock and the training cage environment. When normal mice are placed back into the training cage after 24 h, they freeze, which is an indicator of fearful memory. Successful training in the Morris water maze depends on the ability of mice to navigate to a submerged platform in murky water.

The involvement of DNA methylation in memory has been proposed previously[11]. A contextual fear conditioning task induced a transient increase in DNA methylation levels of protein phosphatase 1 (*Ppp1*), a gene that is considered to repress memory, 1 h after learning. The increased levels of DNA methylation were accompanied by a transient elevation of Dnmt expression (after 24 h, these levels returned to normal). This pattern of DNA methylation suggests an active and gene-specific demethylation mechanism, whose identity remains elusive. Feng *et al.*[7] could not confirm or disprove that a transiently high pattern of DNA methylation and demethylation was a requirement for learning and memory. However, they found strong genetic evidence that specific patterns of DNA methylation are critical for the long-lasting preservation of neuronal functions and morphology, including those supporting memory.

Defining the specific demethylation mechanisms in postmitotic neurons has also been controversial[14], with inconsistent and isolated reports implicating different putative DNA methylases in mammalian cells. Passive mechanisms for DNA demethylation have also been proposed. A study[15] of oxidative stress in neurons proposed that DNA damage induces a DNA base-excision and repair cascade, producing a repaired, but unmethylated, base. In this model, DNA methyltransferases (including Dnmt1 and Dnmt3a) cooperate with the DNA repair machinery to restore neuron type–specific DNA methylation patterns. Feng *et al.*'s results[7] are certainly consistent with this model.

Feng *et al.*[7] had two particularly interesting results. First, DNA methylation patterns in specific, postmitotic neuronal populations were altered in DKO mice. Consequently, this genetic manipulation caused the alteration of both DNA methylation patterns and gene expression, suggesting that Dnmts indeed are required for the maintenance of DNA methylation in postmitotic neurons. Second, they were able to link a distinctive behavioral deficit to these altered DNA methylation patterns in defined neuronal populations in otherwise normally functioning mutant mice. It is fair to conclude that Feng *et al.*[7] were able to alter brain function by direct alteration of gene expression in specific neurons via an epigenetic mechanism. These data indicate that fine-tuning of epigenetic program in postmitotic neurons is possible without disruption of basal synaptic transmission and without grossly altering brain function. The phenotype of the DKO mice is subtle and the mice retain both short-term memory and the ability to learn.

The implication of DNA methylation in psychological memory is intriguing. Future work should answer the pressing question of which genes show methylation patterns that are critical for the mechanism(s) underlying synaptic plasticity and memory. Genome-wide screening should help to unveil candidates, including those proposed by Feng *et al.*[7]; however, it appears that a 'one-gene-at-the-time' approach will be required to assess such mechanistic aspects. Another pressing question is how the specificity of DNA methylation patterns can be maintained. The repression mechanism driven by methylated DNA is also largely unknown. One of the proteins that directly binds to hypermethylated CpG is MeCP2 (ref. 9). However, despite initial biochemical indications that MeCP2 may repress methylated promoters, genetic data does not confirm that prediction[9].

In conclusion, Feng *et al.*[7] confirm the importance of DNA methylation for neuronal function and morphology. Their research opens new avenues for epigenetic studies of relationships between epigenetically encoded cellular memory in postmitotic neuronal populations and cognitive functions in the context of both normal brain processing, as well as in mental disorders.

COMPETING FINANCIAL INTERESTS
The author declares no competing financial interests.

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Mouse brains wired for empathy?

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A study in this issue reports that mice can be fear conditioned through observation of other mice receiving aversive stimuli and identifies some of the brain regions involved in this observational fear learning.

Do mice have empathy? This question may elicit a wide range of answers, including "yes, of course", "impossible" and "we'll never know". One of the reasons behind such a diversity of opinions is simply a matter of definition. Empathy implies at least some emotional sensitivity in an individual to the affective state of another. But emotional sensitivity to another can refer to many specific phenomena. Some are automatic, such as emotional contagion (for example, babies starting to cry when they hear another baby crying), whereas others have a strong cognitive component, such as sympathy and compassion. Some apply the term empathy to a wide range of these phenomena (for example, see refs. 1,2). Others prefer to restrict it to a more specific case with criteria such as a similarity between the emotional states of the observer and the observed, and

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