NEUROSCIENCE

Reversing DNA Methylation: New Insights from Neuronal Activity–Induced Gadd45b in Adult Neurogenesis

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Neurogenesis in the adult mammalian brain involves activity-dependent expression of genes critical for the proliferation of progenitors and for neuronal maturation. A recent study suggests that the stress response gene *Gadd45b* (growth arrest and DNA-damage–inducible protein 45 beta) can be transiently induced by neuronal activity and may promote adult neurogenesis through dynamic DNA demethylation of specific gene promoters in adult hippocampus. These results provide evidence supporting the provocative ideas that active DNA demethylation may occur in postmitotic neurons and that DNA methylation-mediated dynamic epigenetic regulation is involved in regulating long-lasting changes in neural plasticity in mammalian brains.

A fascinating characteristic of nervous systems is the continuous, activity-dependent remodeling of existing neural circuits, which is the basis for the development of adaptive responses or behaviors that permit the organism to thrive in the ever-changing environment. The essence of this process of neural plasticity is that the brain turns transient, and perhaps repetitive, experiences (i.e., learning) into retrievable long-term memory. For instance, a single exposure of certain drugs is sufficient to cause addictive behaviors (*1*). Another striking example is the early postnatal interactions between mother and babies, which can shape life-long anxiety-related behaviors (*2*). To date, it is largely unclear how such long-term memories are formed. Generation and functional integration of new neurons, although limited to specific regions of the adult brain, such as the hippocampus, represents one type of neural plasticity that potentially participates in certain forms of memory. Although structural and biochemical alterations at the synapses are likely to be involved in regulating neural plasticity, gene expression during learning is also required for long-term memory. Thus, epigenetic regulations, which include DNA methylation, histone modifications, and noncoding RNAs, elicit stable and heritable changes in gene expression without alterations in DNA sequences and are now being recognized as critical mechanisms underlying various cognitive functions, including learning and memory.

Methylation of cytosine (5-methylcytosine) at CpG dinucleotides is a predominant form of epigenetic modification of the mammalian genome. In mouse, the zygotic DNA methylation pattern is first established by de novo DNA methyltransferases (Dnmt3a and Dnmt3b) (*3*) and maintained or inherited mitotically through actions of the maintenance methyltransferase Dnmt1 (*4*). DNA methylation is a stable epigenetic mark involved in diverse developmental and pathological processes, including genomic imprinting, transposon silencing, repression of germline-specific genes in somatic cells, and aberrant inactivation of tumor suppressor genes in cancer cells (*5*, *6*). In general, methylation of proximal promoters containing a high density of CpG dinucleotides (CpG-rich regions or CpG islands) results in long-term gene silencing, through blocking the binding of transcriptional activators or recruiting methyl-CpG binding proteins and transcriptional corepressor complexes (*7*).

Recent studies have begun to reveal new functions of DNA methylation in dynamic gene regulation (*2*, *8*–*12*). DNA methylation can be reversed either passively, as when maintenance DNA methyltransferase activity is inhibited in proliferating cells, or by an active, replication-independent process in which DNA methylation is enzymatically removed. In flowering plants, biochemical and genetic evidence suggests that DNA glycosylases involved in base excision repair pathways are responsible for directly removing 5-methylcytosine from DNA (*13*, *14*); however, there are no known mammalian homologs for those enzymes. Although genome-wide active DNA demethylation occurs in the male pronucleus of the zygote before the first cell division (*15*), mechanisms of active DNA demethylation in mammals are poorly understood. Furthermore, it is largely unknown to what extent DNA demethylation takes place in the genome of adult organisms and how it is regulated in postmitotic cells, such as terminally differentiated neurons.

Ma *et al*. have recently identified one isoform of a family of stress response genes, *Gadd45b* (growth arrest and DNA-damage-inducible protein 45 beta), as an immediate early gene in mature hippocampal neurons, the expression of which is induced by neuronal activity, such as electroconvulsive treatment (ECT) or increased exercise (*16*). This activation process of *Gadd45b* expression in neurons was dependent on the well-characterized signaling pathway involving *N*-methyl-D-aspartate receptors (NMDARs), Ca^{2+} influx, and calmodulin kinase (CaMK) (*17*). Building on findings from the Niehr laboratory, which implicated members of *Gadd45* gene family in DNA demethylation in *Xenopus laevis* oocytes (*18*), the authors showed that Gadd45b promoted rapid DNA demethylation at specific regulatory regions of brain-derived neurotrophic factor (*Bdnf*) and fibroblast growth factor 1 (*Fgf1*), two genes crucial for adult neurogenesis. Moreover, DNA demethylation at these promoters was accompanied by transcriptional activation of *Bdnf* and *Fgf1* in hippocampal neurons. Given that the activation of *Gadd45b* is largely restricted to postmitotic neurons, the observed DNA demethylation was likely mediated by a replication-independent enzymatic pathway. Moreover, consistent with another study carried out in human embryonic kidney 293 cells (*19*), Ma *et al*. showed that Gadd45b does not reduce global DNA methylation amounts in mouse hippocampal neurons, suggesting that Gadd45b-dependent DNA demethylation is highly locus-specific. The Gadd45b-dependent DNA demethylation seemed to be transient and reversible, because rapid de novo DNA methylation takes place after

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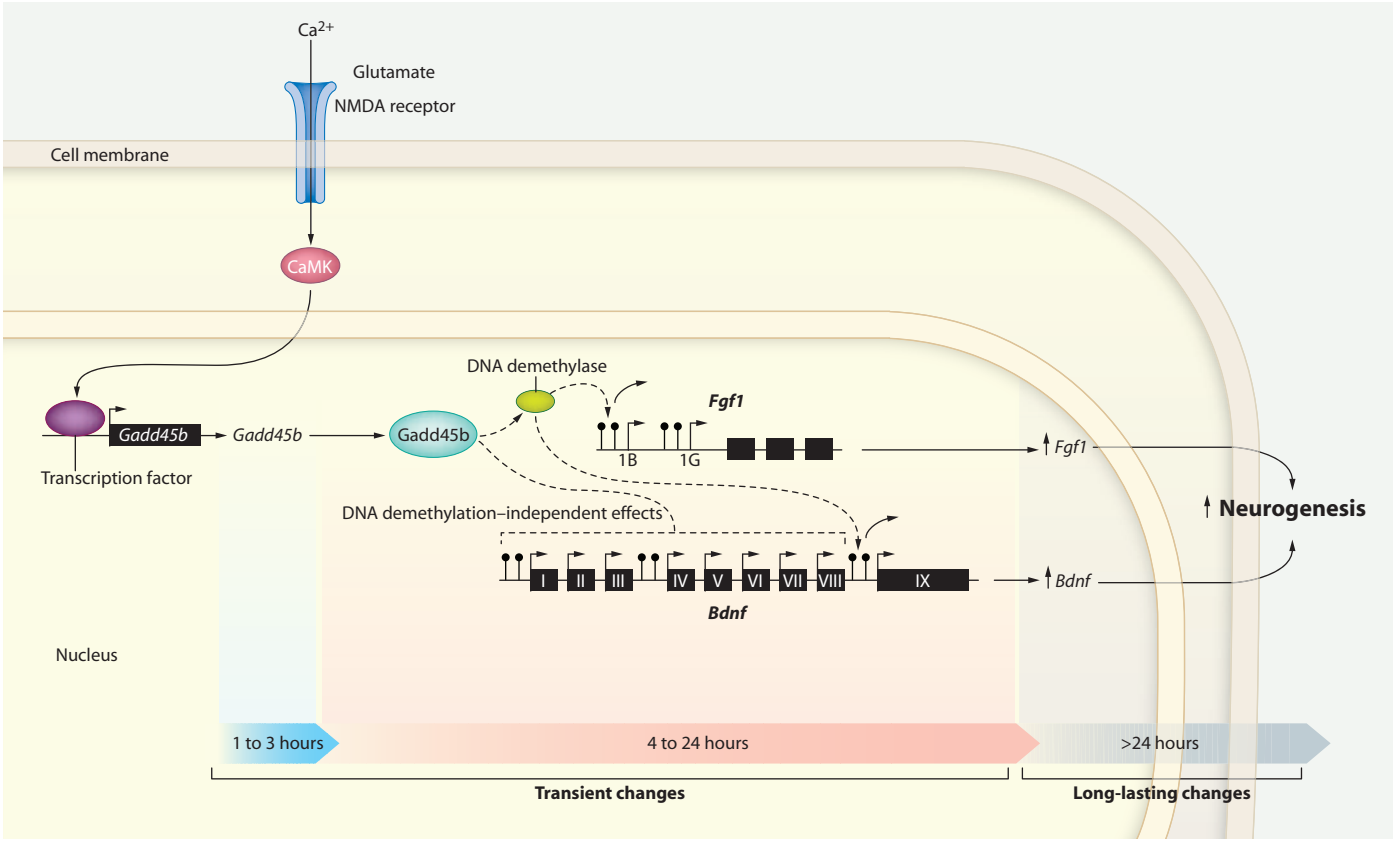

Fig. 1. Proposed model of Gadd45b-dependent promoter-specific DNA demethylation in neuronal activity–induced adult neurogenesis. Transient activation of mature dentate granule cells causes Ca^{2+} influx through NMDARs, which activate CaMK and subsequently a transcription factor, leading to the transient induction of *Gadd45b* expression. Nonenzymatic Gadd45b promotes rapid and transient DNA demethylation by stimulating the activity or enhancing the chromatin binding affinity of a putative DNA demethylase on specific promoters of *Fgf1* (two promoters are shown: the kidney- or liver-specific 1G and the brain-specific 1B) and *Bdnf* (which has nine alternative promoters). DNA demethylation of the *Fgf1* 1B promoter and of the *Bdnf* exon IX promoter causes long-lasting increases in *Fgf1* and *Bdnf* expression, which in turn result in a long-lasting enhancement of neurogenesis. Gadd45b may also act in a DNA demethylation–independent manner on the promoters for exons I to VIII of *Bdnf*. Because the promoters for exons I and IV *Bdnf* are also DNA-methylated and because transcription at these promoters is also induced by neuronal activity, it will be necessary to determine how Gadd45b is specifically targeted to the exon IX promoter.

expression of *Gadd45b* decreases, within 24 hours after ECT.

These new results raise a number of issues. First, Gadd45b-dependent DNA demethylation seems to be highly specific to certain genomic loci. Although expression of *Bdnf* can be initiated by nine alternative promoters (for exons I to IX) in mouse (*20*) (Fig. 1), the authors showed that only the promoter for exon IX, but not that for exon IV, undergoes rapid DNA demethylation upon neuronal activity induction. How is Gadd45b specifically recruited to the exon IX promoter in neurons? Is activity-dependent induction of other promoters of *Bdnf*, such as that for exon IV, also regulated indirectly by Gadd45b-dependent DNA demethylation or by Gadd45b-dependent but DNA demethylation–independent mechanisms? Quantitative polymerase chain reaction analyses of exon-specific *Bdnf* mRNAs in resting and active *Gadd45b*-mutant neurons will reveal new insights as to whether Gadd45b has DNA demethylation–independent functions in regulating activity-dependent *Bdnf* expression. Moreover, future studies using high-resolution genome-wide DNA methylation analysis of purified neuronal nuclei are needed to fully elucidate the scale and specificity of activity-dependent DNA demethylation within the genome of postmitotic neurons.

One obstacle in the in vivo study of epigenetic mechanisms, including DNA methylation, in complex organs such as the brain is the heterogeneity of tissue samples. Unlike some studies that assume DNA methylation amounts detected from brain genomic DNA faithfully reflects DNA methylation in neurons, Ma *et al.* carefully measured the composition of neuronal cells in the dentate tissue they examined before they reached the conclusion that ECT- or exercise-induced DNA demethylation was largely restricted to postmitotic neurons. Alternatively, one could isolate neuronal nuclei on the basis of neuronal-specific nuclear proteins, such as neuronal nuclei (NeuN), for analysis of DNA methylation or histone modifications to ensure that only neuronal chromatin is studied (*21*).

An additional observation from Ma *et al.* is that the detected DNA demethylation events at promoters of *Bdnf* (exon IX) and *Fgf1* were transient and largely

returned to baseline within 24 hours after ECT but that Gadd45b-dependent increases in adult neurogenesis were long-lasting and persisted for at least 10 days after ECT. It is therefore important to elucidate whether the *Bdnf* exon IX–specific transcription, as well as the activities at the other eight exons, remain elevated after exon IX promoter demethylation has been nearly reversed (for example, 24 hours after ECT). Future studies should address whether other epigenetic mechanisms also participate in potentially long-lasting changes in *Bdnf* expression at the mRNA or protein levels (*22*, *23*) and whether it is DNA demethylation per se or some other function of Gadd45b independent of its DNA demethylation activity that causes long-lasting changes in *Bdnf* expression (Fig. 1).

Other questions concern the molecular mechanism of DNA demethylation in general (*13*, *14*, *24*). Despite intense investigation, whether and how members of the Gadd45 gene family promote active DNA demethylation is still disputed (*18*, *19*, *25*–*27*). In a proposed model of active DNA demethylation in zebrafish embryos (*26*), the G•T mismatch-specific thymine glycosylases MBD4 (methyl-CpG-binding domain protein 4) and TDG (thymine DNA glycosylase) can remove 5-methylcytosine (5-meC) after it is first converted to thymine by the 5-meC deaminases AID (activation-induced deaminase) and Apobec1 (apolipoprotein B mRNA editing enzyme, catalytic polypeptide 1). Because members of Gadd45 gene family lack enzymatic activity, Gadd45 proteins may facilitate DNA demethylation by promoting functional or physical interactions between the enzymes proposed to be involved in DNA demethylation (*18*, *26*). It will be of interest to examine whether inhibition of these enzymes in the hippocampus results in deficits in adult neurogenesis similar to those observed in *Gadd45b*-deficient mice. One additional implication of the Ma *et al*. study is that active DNA demethylation may not be restricted to one-cell embryos or embryonic germ cell primordium but instead may be a widespread phenomenon that persists into adulthood. Because the active DNA demethylation processes observed in one-cell embryos or zygotes compared with those in neurons occur on different scales (genome-wide versus locus-specific), there may be more than one enzymatic pathway that carries out active DNA demethylation in mammals. Technological advances in high-throughput DNA methylation analysis and RNA interference–based genetic screening methods may lead to better characterization of the enzymatic pathways involved in active DNA demethylation.

Taken together, the findings of Ma *et al*. reveal unexpected, highly dynamic DNA methylation changes in postmitotic neurons in response to neuronal activity, indicating that active DNA demethylation enzymes and de novo DNA methyltransferases may function coordinately to achieve such dynamic regulation. The missing link, however, remains the mechanism by which transient changes in DNA methylation lead to long-lasting changes in adult neurogenesis.

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