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Dynamic DNA methylation: a prime candidate for genomic metaplasticity and behavioral adaptation

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DNA methylation was once considered to be a static epigenetic modification whose primary function was restricted to directing the development of cellular phenotype. However, it is now evident that the methylome is dynamically regulated across the lifespan: during development as a putative mechanism by which early experience leaves a lasting signature on the genome and during adulthood as a function of behavioral adaptation. Here, we propose that experience-dependent variations in DNA methylation, particularly within the context of learning and memory, represent a form of genomic metaplasticity that serves to prime the transcriptional response to later learning-related stimuli and neuronal reactivation.

Introduction

A range of epigenetic modifications, including the covalent modification of DNA by cytosine methylation, confers the transcriptional activity of a given gene. DNA methylation was once considered to be a relatively static epigenetic modification, with its primary function restricted to the regulation of transcriptional programming during early cellular development. However, a surge of recent studies point to a continued role for DNA methylation across the lifespan, particularly with respect to alterations in neuronal gene expression that directly impact behavior [1–8]. Drawing from a conservative developmental perspective, investigations into the function of DNA methylation in the adult brain have predominantly explored instances where learning- or activity-induced changes in methylation within gene promoters correlate with changes in gene expression. Instances where basal levels of gene expression remain unaltered following a change in DNA methylation within the corresponding gene [9,10] have been largely overlooked, which has led to a limited appreciation of the functional variations in DNA methylation, both within gene promoters and elsewhere in the genome. However, recent advances in next-generation sequencing indicate that the relation between DNA methylation and transcriptional activity is more complex than previously realized. In the adult brain, neuronal activity-induced changes in DNA methylation frequently occur outside gene promoters [2], and 5-hydroxymethylcytosine, a newly discovered base derived from 5-methylcytosine that represents a functional intermediary in the active demethylation process [11], accounts for almost half of DNA methylation detected in the brain [12]. Furthermore, DNA methylation can interact with other epigenetic marks to jointly regulate transcription [13,14]. However, the relevance of this expanded repertoire of epigenomic modifications, particularly within the context of behavioral adaptation across the lifespan, remains to be determined.

One of the most remarkable features of the adult brain is its plasticity in response to experience. To have a lasting impact on behavior, learning-induced neuronal activity must be accompanied by a functional reprogramming of gene expression with corresponding modifications of protein synthesis and synaptic connectivity [15]. However, sustained changes in gene expression could severely constrain plasticity and jeopardize the ability of a neuron to respond to later stimuli. Instead, similar to the dormancy of memory until recall, learning-related reprogramming of gene expression may be encoded in the genome and reflected in changes in gene expression only when required, such as during neuronal reactivation. This form of latent responsivity, termed 'metaplasticity', or the plasticity of synaptic plasticity, is a fundamental mechanism of behavioral adaptation [16–18]. Experience-dependent metaplasticity allows prior learning to register a signature that directs later plasticity without disrupting cell homeostasis. For example, reward-seeking behavior is governed by the induction of 'silent' synapses, which do not influence the basal efficacy of synaptic transmission but are prominent mediators of plasticity in response to later stimulation, the result of which is enhanced behavioral sensitivity to subsequent exposure to cues related to prior learning [18].

Although the existence of metaplasticity has been recognized for some time, the molecular mechanisms underpinning this adaptation are largely unknown. We propose that activity-induced variations in DNA methylation, particularly within the context of learning and memory, represent a form of genomic metaplasticity that serves to prime the transcriptional response to later neuronal activation. In collaboration with other epigenetic marks, experience-dependent changes in DNA methylation would direct later transcription and plasticity in several ways, including the regulation of alternative splicing [19] and transposable elements [20], the development of bivalent chromatin marks that render genes poised for transcriptional activity [21],

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or by directing nucleosome repositioning to bookmark recently activated genes [22]. DNA methylation is intimately related to the functional capacity of the genome and may therefore contribute substantially to behavioral adaptation across the lifespan through its direct effects on neural plasticity and cognition.

Mechanisms of dynamic DNA methylation

The activity of three DNA methyltransferases, DNMT1, DNMT3a, and DNMT3b, regulate cytosine methylation in mammals. DNMT1 is considered to be a maintenance methyltransferase, whereas DNMT3a and DNMT3b mediate *de novo* methylation. Although each of these enzymes plays a crucial role in establishing genomic methylation patterns during early neurodevelopment, only DNMT1 and DNMT3a are expressed in mature neurons, where they appear to play a complementary role in regulating synaptic plasticity [23] (Figure 1).

Active DNA demethylation permits the dynamic regulation of the methylome in response to neuronal activity [2,24,25] and learning [26]. Active demethylation involves enzymatic removal of 5-methylcytosine and occurs via several mechanisms, including: (i) deamination of 5-methylcytosine to thymine by activation-induced cytidine deaminase (AID) [27] and subsequent removal of a T–G mismatch by the base excision repair pathway (Figure 2a); (ii) conversion of 5-methylcytosine to 5-hydroxymethylcytosine by the ten-eleven translocation 1-3 (Tet1-3) family of DNA hydroxylases followed by base excision repair [24] (Figure 2b); or (iii) nucleotide excision repair, which involves Gadd45a [28] (Figure 2c). Moreover, it is likely that these mechanisms act in conjunction with each other to dynamically regulate DNA demethylation and the transcriptional activity at a specific genomic locus.

DNA methylation and cellular differentiation

A tightly timed interplay between DNA methylation, hydroxymethylation, and active demethylation regulates gene expression and cellular differentiation in the developing nervous system [29–31]. Pluripotency is strongly associated with high levels of 5-hydroxymethylcytosine in embryonic stem cells [32,33] and the loss of 5-hydroxymethylcytosine and subsequent accumulation of 5-methylcytosine corresponds with lineage commitment [32]. Critical developmental stage-specific patterns of DNMT expression further reflect the importance of DNA methylation in directing early neurodevelopment [34]. For example, active demethylation within the promoters of several astrocytic markers [35,36] directs astrocyte lineage commitment from pluripotent neural precursor cells. Furthermore, epigenetic reprogramming via DNA methylation is required for the development of neural precursor cells [37] and is associated with neuronal differentiation [38]. Based on these observations, it is widely believed that the induction, or loss, of DNA methylation during early development drives unidirectional and sustained changes in gene expression, which ultimately give rise to cellular identity [30]. However, differentiating neurons retain a comparatively high level of 5-hydroxymethylcytosine, indicating that perhaps the neuronal methylome retains a greater degree of plasticity throughout development [33]. In humans, neurons show significantly greater interindividual variation compared with non-neuronal cells of the brain, supporting the idea that the neuronal methylome may have an enhanced propensity for plasticity in response to environmental cues [39].

The relation between DNA methylation and gene expression in development is also more complex than previously appreciated. For example, although promoter methylation appears to be a key regulator of cell type-specific programming [29,40], recent evidence suggests that non-promoter DNA methylation also coordinates the expression of neurogenic genes [41]. Moreover, the association between promoter methylation and gene expression appears to be contingent on CpG density [42], and there are instances where altered DNA methylation fails to

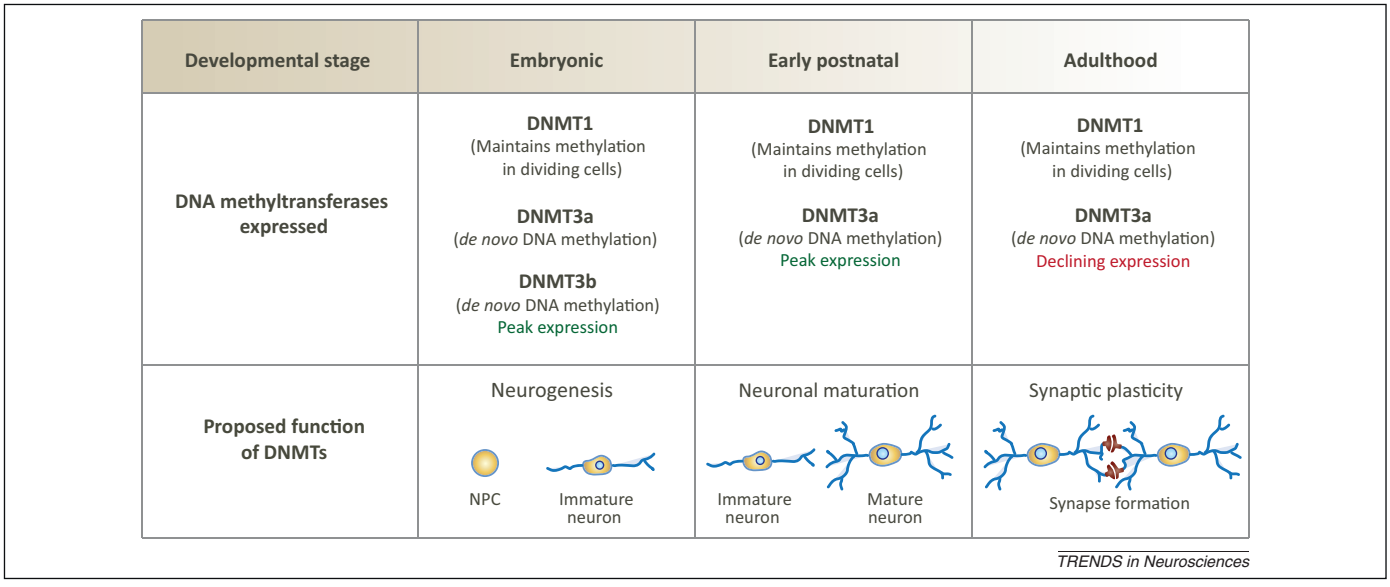

Figure 1. DNA methylation across the lifespan. DNA methylation is mediated by two *de novo* DNA methyltransferases, DNMT3a and DNMT3b, and one maintenance methyltransferase, DNMT1. The expression of these DNMTs varies across the lifespan: the expression of DNMT3b is restricted to embryonic development and corresponds to a period of early neurogenesis, whereas an increase in DNMT3a expression coincides with early postnatal neuronal maturation [34]. DNMT3a and DNMT1 are expressed in the CNS throughout the lifespan and may be important for synaptic plasticity [5,34]. Abbreviation: NPC, neural progenitor cell.