Neuronal activation and insight into the plasticity of DNA methylation

"*Together, these studies implicate modification of the neuronal DNA methylome as a previously under-appreciated mechanism for activity-dependent epigenetic regulation in the adult CNS.*"

KEYWORDS: demethylation ■ epigenetic ■ Gadd45 ■ learning ■ neurogenesis

Ryan J Felling

Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, MD, USA

Junjie U Guo

Institute for Cell Engineering, Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, MD, USA

Hongjun Song

Author for correspondence:
Institute for Cell Engineering, Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, MD, USA
Tel.: +1 443 287 7499
Fax: +1 410 614 9568
shongju1@jhmi.edu

In a carefully choreographed sequence of events during vertebrate development, a single cell divides and differentiates into hundreds of distinct cell types. What is most remarkable about this process is that each of these cell types retains essentially the same genomic DNA, yet differentially expresses its own subset of genes. Epigenetic regulation is the means by which chromatin structure is altered without changing the underlying nucleotide sequence, thereby selectively exposing or denying specific regions of the genome accessibility to the cell's transcriptional machinery. Ultimately, it is the epigenetic marks that determine which genes a cell can express. Three predominant mechanisms of epigenetic regulation and their interactions have been well characterized: DNA methylation, histone modifications and ncRNAs.

DNA methylation is the most highly characterized means of the known epigenetic modifications. In the mammalian genome, methylation occurs primarily at the cytosine residue of CpG dinucleotides. 5-methylcytosine was first identified as a component of nucleic acids in 1925 by Johnson and Coghill [1], and subsequently confirmed by others [2,3]. It was not until 1975, however, that a potential role for DNA methylation in propagating genomic information through cell generations was formally proposed [4]. Since then the molecular machinery that coordinates this process has been extensively characterized. In animals, *de novo* methylation is mediated by specific methyltransferase enzymes (Dnmt3a and Dnmt3b). Methylation patterns are then maintained through the cell cycle by Dnmt1, another methyltransferase that preferentially recognizes hemimethylated DNA. Historically, DNA methylation has been viewed as a highly stable epigenetic mark, but there is now significant evidence indicating that DNA methylation is actually a very flexible epigenetic mark that fluctuates over a life cycle, both in a highly structured and choreographed manner at a large scale during development, and in a loci-specific fashion in response to individual environments and experiences.

How is it that a chromatin modification that was once viewed as the most highly stable and heritable epigenetic mark can be changed to reflect unique cellular memories? This requires a process of active DNA demethylation and implies inherent plasticity in DNA methylation signatures. DNA demethylation is known to occur at specific time points during mammalian development [5] and is necessary during dedifferentiation that occurs in instances such as cancer or induced pluripotency. While we know DNA 'demethylation' can occur passively during cell division, the concept of active DNA demethylation in postmitotic cells has been controversial [6]. The dynamic nature of DNA methylation is supported by the fact that individual experiences and environmental exposures can exert significant effects on chromatin structure [7]. The contribution of epigenetic mechanisms to changes in gene expression during normal development has long been recognized, but we now know that chromatin modifications, including DNA modifications, occur throughout life in what were once thought to be stable, mature populations of cells including neurons [8]. Twin studies have shown that as newborns, monozygotic twins exhibit high concordance in DNA methylation patterns. This concordance diminishes throughout the lifespan, suggesting that individual factors can modify genetic potential [9]. The increasing appreciation of the importance of DNA demethylation has spurred numerous studies investigating the dynamics between methylation and demethylation.

In the CNS, the initial evidence supporting the dynamic nature of DNA methylation

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came from studies of learning and memory [10]. Studies of fear conditioning showed that *de novo* DNA methylation in hippocampal neurons is required for memory formation, but that this methylation is transient. In fact, these same studies also suggested demethylation of a specific plasticity-related gene, lending credence to the presence of a 'demethylase' activity in the mature brain [11]. In contrast to the dynamic changes observed in the hippocampus, fear conditioning induces long-lasting changes in DNA methylation in cortical neurons [12]. This finding supports the original hypothesis that stable chromatin modifications may be important for memory storage, which occurs in the cortex. In the hippocampus, consolidation of learning and memory relies on a more dynamic process of DNA methylation changes. The concept of experience-driven changes in chromatin structure provides an enticing link between neuronal activity and DNA methylation status in the mature brain.

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One model of neuronal activation has provided important insight into one potential mechanism of active DNA demethylation in the mammalian CNS. Electroconvulsive stimulation (ECS) in animals models human electroconvulsive therapy, a widely used treatment in psychiatry known to influence neurogenesis and synaptic plasticity in the adult hippocampus [13]. Examining the effects of ECS in the dentate gyrus of the hippocampus has a number of advantages. The dentate gyrus harbors a large, highly enriched population of a single neuronal subtype dentate granule cells, allowing the investigation of a relatively homogeneous cell population compared with other regions of the brain. ECS provides a synchronized activation of this population of cells so that the time course of subsequent changes can reliably be defined. ECS induces expression of Gadd45b in the dentate gyrus as well as CpG demethylation of specific BDNF and FGF1 promoters, growth factors related to neurogenesis and synaptic plasticity. Mice deficient in Gadd45b show reduced ECS-induced neurogenesis and abolished demethylation of these promoters [14]. Gadd45b is a member of a family of proteins proposed to mediate DNA demethylation through a DNA base-excision and repair mechanism [15]. Studies of ECS have further refined this model, demonstrating that neuronal activity-induced DNA demethylation also requires oxidation of 5-methylcytosine by the hydroxylase TET1 followed by deamination by APOBEC 1 before entry into the base-excision-repair pathway [16]. Defining the mechanisms by which active DNA demethylation occurs is essential to further determining the biological significance of this process in normal neuronal physiology as well as disease states.

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The finding that neuronal activity induced by ECS triggers targeted demethylation of specific genes involved in neuronal plasticity is important, but more recent work has shown that these changes are much more widespread than anticipated. Next-generation sequencing methods have provided powerful tools, such as methyl-sensitive cut counting, to profile methylation status with a single-base resolution and on a genome-wide scale. Using these techniques we have demonstrated broad changes in the methylation landscape following synchronous neuronal activation, involving both demethylation and *de novo* methylation [17]. Importantly, similar dynamic DNA methylation changes also occur after other experiences, such as physical exercise. These investigations showed that most observed methylation modifications occurring early after synchronous neuronal activation localized to regions of low-density CpG sites. This is in contrast to most previous studies, which have focused largely on islands of high-density CpG sites, revealing a previously underappreciated significance for low-density CpGs in activity-induced changes in the DNA methylome. The acute modifications of DNA methylation were also associated with changes in the expression of genes associated with alternative splicing, synaptic function, neuronal differentiation, protein phosphorylation and calcium signaling. The changes in gene expression were only associated with CpG methylation changes within the 5′ regions right upstream of the transcription start sites, and the biological relevance of methylation changes at other intragenic or intergenic regions needs to be further defined. Together, these studies implicate modification of the neuronal DNA methylome

as a previously under-appreciated mechanism for activity-dependent epigenetic regulation in the adult CNS.

The dynamic regulation of DNA methylation status in the CNS is a fascinating area of investigation that benefits from the wave of excitement generated by the field of epigenetics. Often described as a new frontier in the popular media, people are intrigued by the idea that fate is not written in our genes themselves but how these genes are modified over a lifetime. Significant questions persist regarding epigenetic regulation in general, and DNA demethylation in particular. The molecular mechanisms through which this occurs must be further defined. The means by which active DNA demethylation is targeted to specific genomic loci, cells or brain regions must also be identified. It is also unknown why some CpGs are more dynamically regulated than others, namely, what controls the 'meta-plasticity' of the DNA methylome. The various functions of DNA modifications in the mature CNS remain to be explored. From a translational research perspective, investigating the roles that DNA methylation plays in neurologic and psychiatric disease may provide better pathophysiologic understanding and even novel therapeutic targets. DNA methylation pathways are known to be involved in neurologic disease, as in the case of Rett's Syndrome, a neurodevelopmental disorder caused by mutations in *MeCP2*. Gadd45b is aberrantly expressed in brain regions of patients with autism and schizophrenia, and can also be influenced pharmacologically [18,19]. DNA methylation has also been implicated in epilepsy [20], and potential roles for DNA methylation changes in acute neurologic injury such as stroke are also promising avenues of investigation. As the field of epigenetics continues to evolve, and high-throughput methods of epigenetic evaluation improve, the area of DNA methylation plasticity in the CNS promises to be very fruitful territory for future investigation.

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