Symposium

The Role of Epigenetic Mechanisms in the Regulation of Gene Expression in the Nervous System

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Neuroepigenetics is a newly emerging field in neurobiology that addresses the epigenetic mechanism of gene expression regulation in various postmitotic neurons, both over time and in response to environmental stimuli. In addition to its fundamental contribution to our understanding of basic neuronal physiology, alterations in these neuroepigenetic mechanisms have been recently linked to numerous neurodevelopmental, psychiatric, and neurodegenerative disorders. This article provides a selective review of the role of DNA and histone modifications in neuronal signal-induced gene expression regulation, plasticity, and survival and how targeting these mechanisms could advance the development of future therapies. In addition, we discuss a recent discovery on how double-strand breaks of genomic DNA mediate the rapid induction of activity-dependent gene expression in neurons.

Key words: **DNA double strand breaks; DNA methylation; MeCP2; polycomb repressive complex; topoisomerase II**

Introduction

Organs, including the brain, are determined by the pattern of gene expression that emerges in each cell lineage during development. The key drivers of these genetic programs are proteins that recognize specific combinations of nucleotides that specify a genomic address. By binding to their sites, either alone or in conjunction with others, these sequence-specific DNA binding proteins or "transcription factors" determine which genes are actively expressed and which must remain silent. Several lines of evidence suggest, however, that there are more constraints on development and differentiation than simply the availability of transcription factors. For example, although cells of the liver, skin, intestine, etc., possess the entire genome, it took many years to find ways of converting a differentiated cell into a stem cell that could once again give rise to the whole organism. Even now, reversal of differentiation is still a highly inefficient process. A likely reason is that "epigenetic marking" of the genome, laid down during the developmental history of the cell, "conditions" the genome's response to transcription factors and is therefore an essential additional factor in determining programs of gene expression. "Epigenetics" refers to the study of ways in which chromosome regions adapt structurally so as to register, signal, or perpetuate local activity states (Bird, 2007).

A key feature of epigenetic marking is that it is stable, sometimes across cell generations, but also reversible. It is mediated by proteins that add, remove, or interpret the modified structures, referred to as "writers," "erasers," and "readers" respectively. Epigenetic systems include DNA methylation (Bird, 2002), and the polycomb/trithorax system (Francis and Kingston, 2001). Other epigenetic mechanisms involve RNA (Vaistij et al., 2002; Volpe et al., 2002) or the silencing or activation of genes due to their localization within the nucleus (Brown et al., 1997; Mahy et al., 2002). These and other processes appear to be closely interwoven with histone modification, which is itself a diverse, complex system of chromosome marking (Jenuwein and Allis, 2001). Histone proteins stably associate with DNA to form a repeating structural unit that organizes the genome. The combination of DNA and periodic histone complexes is referred to as chromatin, resembling beads on a string of DNA. In addition to their structural role, histones possess exposed tails that can be marked by chemical modification. For example, acetylation of histone tails by histone acetyltransferases loosens the contact with DNA and also creates binding sites for protein readers that facilitate gene expression. Acetylation is removed by histone deacetylase-containing complexes, which therefore inhibit the activity of genes. Methylation of histone tails can be either activating or repressive, depending on the precise amino acid that is affected. For example, the function of the Polycomb Repressive Complex 2 (PRC2) is mediated by its enzymatic components Ezh1 and Ezh2, which catalyze methylation of lysine 27 on histone H3 leading to gene silencing (Müller et al., 2002). There is evidence that PRC2 is essential in developing neu-

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rons to prevent the expression of inappropriate (e.g., non-neuronal) genes (Hirabayashi et al., 2009; Pereira et al., 2010). A prevalent epigenetic mark that directly targets DNA is methylation of the 5-position of cytosine (5mC) (Jaenisch and Bird, 2003). It is deposited predominantly at CpG sites by DNA methyltransferases DNMT3a and DNMT3b and maintained in dividing cells by DNMT1. Mapping of DNA methylation in the brain has uncovered unexpected differences compared with other somatic tissues, including unprecedentedly high levels of non-CpG methylation (Lister et al., 2013; Guo et al., 2014) and also of the oxidized form of 5mC, 5-hydroxymethylcytosine.

The importance of epigenetic systems in disease has become increasingly apparent with the introduction of genome sequencing as a diagnostic tool, as mutations that affect epigenetic readers, writers, or erasers are often implicated in cases of intellectual disability (Bjornsson, 2015). These discoveries have shone a spotlight on the role of epigenetic processes in the brain, about which we still have much to learn. Here we review aspects of this rapidly expanding field. Because of space constraints, we have not attempted a comprehensive review but focus on specific areas of current activity. We discuss evidence that DNA methylation dynamics contributes to neuronal diversity and plasticity, and we also review progress in understanding how mutations affecting a DNA methylation reader, MeCP2, give rise to the neurological disorder Rett syndrome (RTT). Transcriptional repression by the polycomb complex is prevalent in the brain, and we summarize recent evidence implicating this system in silencing of genes whose expression leads to neurodegeneration in adults. Finally, we discuss a novel phenomenon that is implicated in neuronal function: the formation of double-stranded breaks (DSBs) at regulatory regions of a subset of genes that are activated when neurons fire. Through these exemplar discoveries, we hope to convey the excitement and rapid progress now associated with the burgeoning field of neuroepigenetics.

Dynamic DNA modifications in neurons and their functions

It was believed for decades that cytosine methylation in the genomic DNA of terminally differentiated cells is largely irreversible (Ooi and Bestor, 2008). Only recently have several advances in the neuroscience field collectively helped to overturn the dogma and started to reveal functional roles of dynamic DNA demethylation in neurons (Martinowich et al., 2003; Miller and Sweatt, 2007; Nelson et al., 2008; Ma et al., 2009; Feng et al., 2010, 2015; Guo et al., 2011b, c; Kaas et al., 2013; Lister et al., 2013; Rudenko et al., 2013; Meadows et al., 2015; Yu et al., 2015; Yao et al., 2016).

First, studies have convincingly demonstrated dynamic changes of DNA methylation levels in postmitotic neurons. Because of the complexity of the mammalian brain consisting of many cell types and each cell type exhibiting a distinct methylome, it has been challenging to demonstrate robust methylation changes in a particular cell type using gold standard approaches used in the epigenetic field, such as bisulfite sequencing (Shin et al., 2014). Culture studies showed that depolarization of neurons leads to significant decrease of methylation levels at the promoter IV region of the *brain-derived neurotrophic factor* (*Bdnf*) (Martinowich et al., 2003). Using a relative pure population of dentate granule neurons in the adult mouse hippocampus that can be easily switched from an inactive state to an active state by electroconvulsive stimulation, it was shown that neuronal activation leads to demethylation at promoter IX region of *Bdnf* gene (Ma et al., 2009). Subsequent genome-wide analysis further revealed large scale modification of the neuronal methylation landscape by neuronal activation (Guo et al., 2011c). Approximately 1% of CpG sites examined exhibit methylation changes within 4 h, including both *de novo* methylation and demethylation. Notably, physiological stimulation, such as running, also leads to similar dynamic methylation changes. Dynamic DNA methylation in neurons mostly occurs in low CpG density regions and intergenic regions. More recently, genetic approaches have been developed to facilitate nuclear isolation from different neuronal subtypes of adult mouse brain and set the stage for future studies of changes in cell-type specific DNA methylation dynamics in response to behavioral stimulations for epigenetic analysis (Mo et al., 2015).

Second, the molecular machinery mediating active DNA demethylation has been recently identified (Guo et al., 2011a). Accompanying the rediscovery of another DNA modification, 5-hydroxylmethylation (5hmC), in adult mouse neurons (Kriaucionis and Heintz, 2009), Tet proteins were identified to oxidize 5mC to 5hmC (Tahiliani et al., 2009; Ito et al., 2010). These findings immediately raised the possibility that Tet proteins initiate the active DNA demethylation process via the 5hmC intermediate step. Indeed, evidence from dentate granule neurons *in vivo* provided the first support of this model and further showed that Tet-initiated active DNA demethylation is mediated by 5hmC deamination followed by a base-excision repair mechanism (Guo et al., 2011b). Later studies revealed that Tet proteins can also further oxidize 5hmC sequentially into 5fC and then 5CaC, both of which can be converted to unmethylated cytosine via a TDG-mediated base-excision repair mechanism (Wu and Zhang, 2014).

Third, identification of the molecular machinery mediating active DNA demethylation provides essential tools, for the first time, to directly address the physiological role of active DNA demethylation in neuronal functions at cellular, circuitry, and behavioral levels. Not surprisingly, dysregulation of TET functions leads to deficits in neural stem cells and their differentiation (Zhang et al., 2013). In addition, genetic manipulation of Tet functions provides evidence for a causal role of DNA demethylation in memory formation and extinction as well as drug addiction (Kaas et al., 2013; Rudenko et al., 2013; Feng et al., 2015). At the cellular level, Tet3 regulates glutamatergic synaptic transmission by controlling the surface expression of glutamate receptors (Yu et al., 2015). Notably, Tet3 expression in neurons is bidirectionally regulated by systemic circuitry activity, as reducing network firing leads to decreased Tet3 expression, whereas elevating network activity results in increased Tet3 expression. Global changes in neuronal network firing induce synaptic scaling, a form of homeostatic synaptic plasticity that affects all synapses within a neurons (Turrigiano, 2008). Interestingly, dysregulation of Tet3-mediated DNA demethylation signaling prevents both synaptic scaling-up and scaling-down (Yu et al., 2015). These studies identify Tet3 as a novel global synaptic activity sensor and suggest that even the most fundamental properties of neurons, such as synaptic transmission and surface GluR1 levels, are dynamically regulated by DNA demethylation via DNA oxidation and subsequent base-excision repair. Together with the recent finding that neuronal activity-triggered formation of DNA DSBs on promoters of early-response genes controls their expression in neurons (Madabhushi et al., 2015), these results suggest a previously underappreciated role for DNA repair in normal neuronal physiology and plasticity. Interestingly, blocking of *de novo* DNA demethylation by pharmacological inhibition of DNMTs also affects synaptic scaling (Meadows et al., 2015), suggesting a critical role of dynamic DNA methylation levels in homeostatic plasticity.

The recent several years have witnessed tremendous progress in our understanding of DNA methylation in neurons. First,

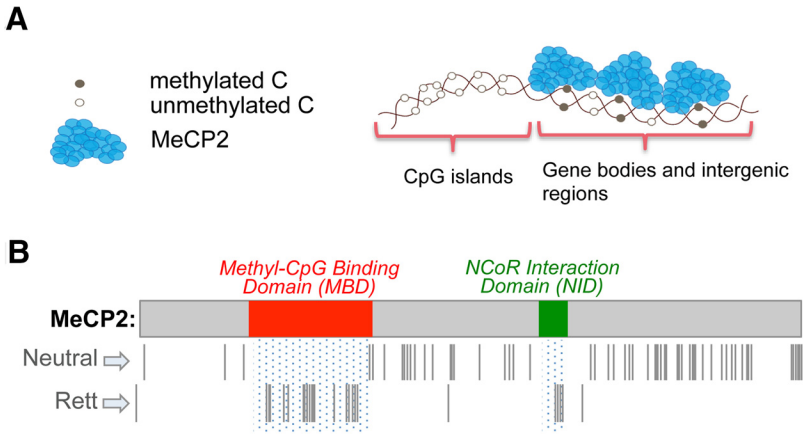

Figure 1. Understanding MeCP2 function. ***A***, MeCP2 binds to chromosomes at sites of DNA methylation, which are absent at CpG island promoters, but present in gene bodies and intergenic regions of the genome. ***B***, Missense mutations that cause RTT are concentrated in the MBD and NID of MeCP2. Missense mutations in the population at large avoid these domains.

many more forms of DNA modifications have been identified in neurons. In addition to oxidation production of 5mC, including 5hmC, 5fC, and 5CaC, single-based genome-wide studies have revealed enrichment of DNA methylation in the non-CpG context in neurons and suggested their role in suppressing gene expression (Lister et al., 2013; Guo et al., 2014). Intriguingly, non-CpH methylation appears to correlate even better with gene expression than CpG methylation (Lister et al., 2013; Guo et al., 2014; Mo et al., 2015). More recently, N(6)-methyladenine (N6mA), another form of DNA modification, was identified in the mammalian genome (Wu et al., 2016). Second, large-scale profiling of different forms of DNA modifications has revealed dynamic nature of different types of neuronal methylation landscape during development and in response to different stimuli. Third, emerging evidence converges and suggests that DNA methylation in neurons largely suppresses gene expression, whereas dynamic changes in DNA methylation play a critical role in meta-plasticity, a phenomenon in which the history of a neuron's activity determines its current state and its ability to undergo synaptic plasticity (Abraham and Bear, 1996). Given the new tools and rapid development of the field, new principles are likely to emerge soon.

MeCP2: how loss of a DNA methylation reader affects the brain

A long-known DNA methylation reader is Methyl-CpG binding Protein 2 (MeCP2), which binds specifically to 5-methylcytosine in a mCG or mCA contexts. The protein binds to chromosomes in a DNA methylation-dependent manner (Fig. 1*A*) and is most highly expressed in neurons, where it approaches the abundance of histones. MeCP2 behaves as a transcriptional repressor that binds methylated DNA and recruits the multiprotein corepressor NCoR, which removes acetyl groups from histones. Importantly, mutations in the *MECP2* gene are the almost exclusive cause of a neurological disorder called RTT, which manifests as loss of acquired language and motor skills, seizures, mental retardation, and autistic behaviors. RTT is of particular interest as a monogenic disorder caused by effective loss of an epigenetic reader that is essential for brain function. Moreover, convincing animal models closely mimic the human disorder. As the *MECP2* gene is on the X chromosome (Amir et al., 1999), RTT occurs almost exclusively in girls, who typically develop normally for 6–18 months and then regress. Males who are hemizygous for these mutations rarely survive infancy. Having the correct amount of MeCP2 in neurons is clearly critical, as abnormally high levels due to duplication of the *MECP2* gene also result in severe intellectual disability (Collins et al., 2004; Luikenhuis et al., 2004). It seems that a high, but balanced, concentration of MeCP2 is crucial for neurons to operate properly. Both RTT and *MECP2* overexpression syndrome phenotypes are reversible in mice (Guy et al., 2007; Sztainberg et al., 2015), indicating that neurons are not permanently damaged by development with too little or too much of the protein and raising the possibility that these conditions will be curable in humans, too.

Significant progress has been made in understanding the molecular basis of MeCP2-related disorders (Lyst and Bird, 2015). Numerous different mutations have been reported in the *MECP2* gene of patients with RTT, providing an important resource that pinpoints crucial protein domains (Fig. 1*B*). Analysis of missense mutations, which alter only one amino acid, reveals a strikingly nonrandom distribution (Lyst et al., 2013). Mutations cluster into two discrete regions that coincide with domains mediating protein-protein or protein-DNA interactions. Closest to the N terminus is the Methyl-CpG Binding Domain (MBD) and mutations interfere with DNA binding and often destabilize the protein (Nan et al., 1993). The second domain is the NCoR Interaction Domain (NID), which has been shown to bind the histone deacetylase complex NCoR (Lyst et al., 2013). The importance of these two domains is underlined by the finding that missense mutations occurring in the human population at large (neutral variants) occur throughout the length of the protein, yet are largely excluded from these two sensitive regions (Fig. 1*B*). These observations suggest that MeCP2 functions as a "bridge" between methylated DNA and the corepressor complex, with breakage of either end of the bridge resulting in RTT. As other roles for MeCP2 have also been proposed, including transcriptional activation, alternative splicing, maintenance of global structure of chromatin, regulation of protein synthesis, or combinations of these functions (Lyst and Bird, 2015), it is now vital to critically test the bridge hypothesis.

The role of MeCP2 in transcriptional regulation is hotly debated. Some evidence suggests that the protein activates transcription (Chahrour et al., 2008; Li et al., 2013), whereas other experimental results support a repressive role. In favor of a repressor model, the primary target sequence, methyl-CpG, is a repressive signal in a variety of systems and MeCP2 colocalizes in mouse cell nuclei with heavily methylated foci that are "heterochromatic" and therefore transcriptionally silent. Moreover, the association between MeCP2 and HDAC-containing corepressor complexes, including NCoR and Sin3a, is closely correlated with its ability to repress transcription in cell-based assays (Nan et al., 1997, 1998; Lyst et al., 2013). Mutations that preclude binding to NCoR, or treatment with inhibitors of the histone deacetylases upon which these corepressors depend, abolish MeCP2-dependent gene silencing. It is also reported that depletion of MeCP2 increases chromatin acetylation levels *in vivo* and elevates expression of normally silenced DNA repeat elements and retrotransposons (Skene et al., 2010). High levels of the methylated sequence mCA, recently discovered in brain (Guo et al., 2014), do

not alter this picture, as this modified sequence also appears to behave as a repressive signal. Methylated CA accumulates in the mouse brain postnatally, coincident with the increase in MeCP2 abundance, and MeCP2 binds to mCA as well as mCG (Gabel et al., 2015). Specifically, MeCP2 has been found to regulate transcription of long genes in the brain and its loss causes their upregulation, particularly those that are mCA-enriched (Gabel et al., 2015). Unlike the repressor model, for which candidate partners have been identified, the activating effect of MeCP2 is not well understood. Deficiency of MeCP2 evidently leads to smaller neurons with less RNA (Yazdani et al., 2012; Li et al., 2013), but whether this effect is direct or indirect is unknown, as are the molecular mechanisms that might be involved. A proposal that MeCP2 may activate through binding to hydroxymethylcytosine (Mellén et al., 2012) has been questioned, as the great majority of hmC is in the dinucleotide sequence hmCG, which does not bind MeCP2 (Valinluck et al., 2004; Gabel et al., 2015). It is reported, however, that non-CG methylation is associated with both upregulation and downregulation of gene expression by MeCP2 (Chen et al., 2015). Despite some remaining uncertainty regarding the function of MeCP2, there are grounds for optimism that recent experimental and technological advances have begun to illuminate its fundamental role in the brain. In time, these advances promise to facilitate the search for therapies for *MECP2*-related disorders.

PRC2 protects neurons against neurodegeneration

Normal brain function critically depends on the interaction between the highly specialized neurons. A failure to maintain neuronal specification in the adult brain is commonly associated with aging and age-related neurodegenerative disorders, including Huntington's and Alzheimer's disease. Alterations in neuron type-specific gene expression in medium spiny neurons (MSNs) in the striatum, for example, represents one of the earliest signs of Huntington's disease (HD) pathology, preceding neuronal dysfunction, cell death, and behavioral alterations (Zabel et al., 2009). These findings suggest a possible link between the fidelity of neuronal transcriptional specification and neuronal survival in the adult brain.

Neuronal specification is governed by transcriptional programs that are established during the early stages of neuronal development (Molyneaux et al., 2007; Hobert, 2011) and remain in place in the adult brain (Deneris and Hobert, 2014). Programs of gene activation by transcription factors are combined with systems that suppress genes whose expression might enforce the differentiation of neurons of other types (Cobos et al., 2006; Molyneaux et al., 2007; Hobert, 2011, 2016; Greig et al., 2013; Deneris and Hobert, 2014). Gene regulatory processes that play a pivotal role in neuronal specification include the epigenetic silencing of non-neuronal/other neuron fate-determining genes. Much of the negative gene regulation in developing neurons is achieved by the PRC2 (Hirabayashi et al., 2009; Pereira et al., 2010; Di Meglio et al., 2013; Corley and Kroll, 2015; Zemke et al., 2015). While tremendous progress has been made in elucidating the role of PRC2 in neuronal specification during development (Corley and Kroll, 2015), little is known about the role of PRC2 in the adult brain. Recent data highlight the role of PRC2 in the prevention of systemic neurodegeneration, as PRC2 contributes to the selective suppression of a transcription program that is detrimental for adult neuron function and survival (von Schimmelmann, 2016). More than 2000 PRC2 target genes that are associated with high levels of the methylated histone H3K27me3 at their TSS have been identified. Surprisingly, many of these transcriptionally silent genes at which H3K27me3 and PRC2 colocalize, are also associated with the transcriptionally activating H3K4me3 chromatin modification (von Schimmelmann, 2016). In ES cells as well as in cells of other types, the combination of H3K27me3 and H3K4me3 at promoters is referred to as chromatin "bivalency" and supports the plasticity of cell differentiation (Barski et al., 2007; Mohn et al., 2008; Dobenecker et al., 2015). It remains unclear, however, if or how fully differentiated neurons could benefit from the bivalent state. The results suggest that, whereas the majority of H3K27me3 target genes in MSNs are surprisingly insensitive to PRC2 deficiency, PRC2 selectively controls the transcriptional silencing of these bivalent genes in adult neurons (von Schimmelmann, 2016). Indeed, PRC2 deficiency in adult MSNs leads to the derepression of a specific group of bivalent PRC2 target genes that are dominated by self-regulating transcription factors, as well as several death-promoting genes normally suppressed in these neurons. The majority of the PRC2-suppressed genes encode transcription factors that are associated with the transcriptional specification of other cell and neuron types (von Schimmelmann, 2016). The data suggest that these transcription factors may become expressed at relatively high levels due to their ability to form autoregulatory and coregulatory transcriptional network(s) that reinforce their own expression. Therefore, the formation of mutually reinforced transcription loops among PRC2 target genes represents a possible mechanism for the selective and progressive upregulation of PRC2 target genes. The upregulation of non–MSN-specific transcriptional regulators in PRC2-deficient MSNs, however, does not lead to the expected neuronal dedifferentiation or induction of other neuron type-specific gene programs (i.e., of dopaminergic neurons of Purkinje cells) but is associated with the subsequent downregulation of highly expressed MSN-specific genes (von Schimmelmann, 2016). These age-dependent transcriptional changes in PRC2-deficient neurons are associated with the development of progressive and fatal neurodegeneration in mice. Moreover, the molecular, cellular, and behavioral changes associated with postnatal neuron-specific PRC2 deficiency overlap with the neurodegenerative phenotypes observed in mice with HD, giving credence to the potential contribution of epigenetic mechanisms, particularly to PRC2-mediated gene expression regulation, in HD pathology and possibly in other neurodegenerative processes.

In summary, current data reveal that PRC2 links the maintenance of adult neuron specification to neuronal survival (von Schimmelmann, 2016). Altered neuron specification may interfere with coordinated function of neuronal networks and may be harmful for overall brain function. It is plausible that neurons possess an intrinsic mechanism that triggers the elimination of neurons with defective specification during development. It is moreover tempting to speculate that the activation of the PRC2-controlled transcriptional network, including the death-promoting genes, may represent a type of checkpoint mechanism that enables the elimination of neurons with impaired PRC2 function in the adult brain. Changes in PRC2 function could be triggered either transiently, by nonpersisting events such as seizures (Reynolds et al., 2015) or chronic stress, or permanently, by the expression of pathogenic proteins such as mutant huntingtin protein, which can directly interfere with PRC2 recruitment and function (Seong et al., 2010; Dong et al., 2015). While short-term activation of such a mechanism could be beneficial for ensuring brain integrity, it seems that persistent changes in the activity or recruitment of PRC2, as well as other H3K27me3-controlling enzymes, may lead to systemic neurodegeneration. It is proposed that PRC2 is essential for the protection of neurons from neurodegeneration by the selective suppression of a self-enforcing transcriptional network that, once initiated, can set neurons on an irreversible path of neurodegeneration.

The role of activity-induced DNA breaks in neuronal physiology and disease

Our behavior is remarkably shaped by our experiences. The ability of experience to influence the development of long-lasting adaptive responses requires an intricate "dialogue between genes and synapses" in which an incoming stimulus activates signaling cascades in neurons that culminates in new protein synthesis and the activation of new gene transcription programs (Kandel, 2001). The earliest genes to be induced in this program are the early response genes, and these are enriched for transcription factors, such as *Fos*, *Npas4*, *Nr4a1*, and *Egr1*. These transcription factors, in turn, prime the expression of the so-called late response genes, such as *Bdnf* and *Cpg15*, and together these immediate early gene products ultimately regulate experience-driven changes to synapses, learning, and memory (West and Greenberg, 2011). A great body of research conducted over the past 30 years has provided crucial insights into the mechanisms that govern the rapid induction of these immediate early genes (West and Greenberg, 2011). Despite these details, the precise mechanisms that constrain early response genes in the absence of a stimulus, and those that trigger their rapid induction, remain poorly understood.

An important recent finding in this regard has been the observation that numerous paradigms of neuronal stimulation, including exposure of mice to physiological learning behaviors *in vivo*, result in the formation of DNA double-strand breaks (DSBs) (Suberbielle et al., 2013; Madabhushi et al., 2015). These neuronal activity-induced DSBs are restricted to only handful of loci in the genome, and these loci are also enriched for the early response genes, including *Fos*, *Npas4*, *Egr1*, and *Nr4a1* (Madabhushi et al., 2015). Attempts to understand the mechanisms underlying this phenomenon have revealed that neural activity-induced DNA breaks are generated by a Type II topoisomerase, Topo IIβ, and these Topo IIβ-mediated DSBs are essential for the rapid induction of early response genes. While *Top2b* knockdown attenuates both activity-induced DSB formation and early response gene induction, engineering targeted DSBs in the promoters of early response genes allows for the induction of these early response genes even when *Top2b* is knocked down (Madabhushi et al., 2015). Together, these results reveal DSB formation as a novel triggering mechanism for the induction of early response genes following neuronal activity.

While these mechanisms were tested in neurons, a recent report demonstrated that stimulation of proliferating cells with serum also results in Topo IIβ-mediated DSB formation and γH2AX accumulation at immediate early genes, including *Fos* and *Egr1* (Bunch et al., 2015). Inhibition of Topo IIβ activity increases RNAPII pausing at these genes (Bunch et al., 2015). In addition to this, Topo IIβ-mediated DSBs have been shown to induce gene expression in other cell types in response to stimulation by androgens, estrogen, and insulin (Ju et al., 2006; Wong et al., 2009). Together, these studies suggest that activity-induced DSB formation by Topo IIβ might be a conserved mechanism to rapidly induce gene expression.

The precise mechanisms by which activity-induced DSBs facilitate gene expression also remain poorly understood. Interestingly, an analysis of motifs that were enriched at Topo IIβ binding sites in neurons revealed that the binding motif of the architectural protein, CTCF, was the most significantly enriched at these sites (Madabhushi et al., 2015). The enrichment of CTCF at Topo IIβ binding sites and the sites of activity-induced DSBs suggest an intriguing model in which CTCF-mediated chromatin looping imposes a topological constraint that precludes early response gene expression by preventing enhancer-promoter interactions, and this constraint is overcome through the formation of DSBs, which stimulates enhancer-promoter interactions at early response genes (Fig. 2). Recent observations indicate that a combination of enhancer elements interacts with the promoters of early response genes in a stimulus-dependent manner to drive their expression (Joo et al., 2016). Furthermore, neuronal stimulation causes widespread transcription at enhancer elements in neurons, resulting the production of a class of noncoding RNAs called enhancer RNAs (Kim et al., 2010). Upon neuronal stimulation, these enhancer RNAs bind and inhibit NELF, a factor that maintains RNAPII in a paused state at the promoters of early response genes (Schaukowitch et al., 2014). Together, these studies could explain how topological architecture of the genome allows for the fine-tuning of gene expression in response to neuronal activity.

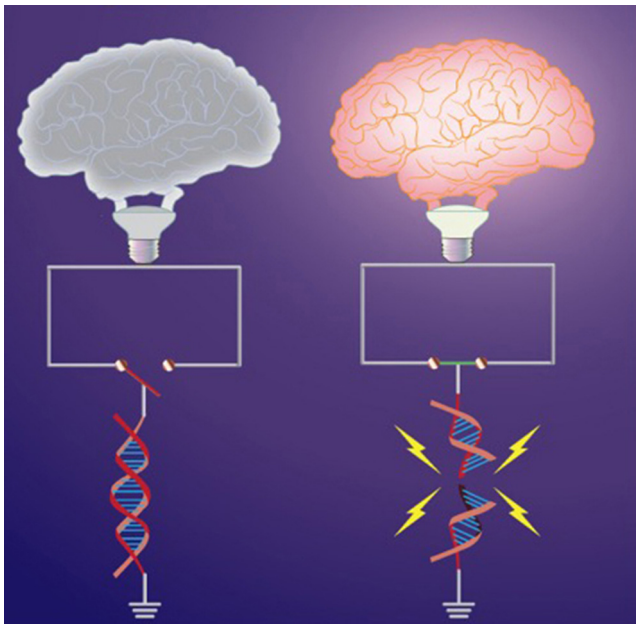

Figure 2. Activity-induced DNA breaks "switch-on" gene expression. Left, IEGs are important for synaptic plasticity in the brain and are "switched off" under basal conditions due to the presence of topological constraints. Right, Neuronal activity triggers the formation of Topo IIβ-mediated DNA breaks in the promoters of a subset of IEGs, which overrides these topological constraints, and "switches on" gene expression. Here, the topological constraint to IEG expression under basal conditions is represented as an open switch that is tethered by intact DNA. Formation of the break severs the constraint and promotes the circuit to be closed. The "brain bulb" represents the manifestation of neuronal activity.

While DSB formation and their timely repair by the DSB repair machinery govern the dynamics of early response genes, a recent report demonstrated how late response genes, such as *Bdnf,* are regulated through active demethylation that requires the use of the base-excision repair pathway (Yu et al., 2015). The utilization of such strategies to regulate the expression of crucial activity-dependent genes in neurons has important pathophysiological implications. Defects in DNA repair have been linked to various congenital and age-related neurological disorders (Madabhushi et al., 2014). The unexpected link between DNA lesions and activity-induced gene expression suggests that changes in the ability to either form or repair activity-induced DNA lesions could have a huge impact on cognitive performance. Interestingly, in a recent report, homozygous mutations in the gene *TDP2* were discovered in patients with a neurological disease characterized by seizures, cognitive deficits, and ataxia (Gómez-Herreros et al., 2014).

TDP2 encodes for the enzyme, tyrosyl-DNA phosphodiesterase 2 (TDP2), which specializes in the error-free repair of Topo II-mediated DSBs. Understanding whether neurological abnormalities caused by mutations in DNA repair factors, including TDP2, arise from the defective repair of activity-induced DSBs will therefore be of enormous significance.

In conclusion, here we review current efforts in understanding how readers, writers, and erasers of DNA methylation, the polycomb repressive complex, and DNA DSBs, coordinate the epigenetic changes that occur in response to neuronal activity. Future discoveries in these areas should provide a more refined understanding of how epigenetic mechanisms regulate crucial neuronal functions, such as synaptic plasticity and learning behaviors, and how dysregulation of these processes underlies developmental and neurodegenerative disorders.

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