

## DNA damage and repair regulate neuronal gene expression

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**Neuronal activity-induced gene expression is crucial for the development and plasticity of the brain and for learning and memory. In a recent study published in *Cell*, Madabhushi *et al.* report that neuronal activity triggers the formation of DNA double-strand breaks on promoters of early response genes, which in turn controls their expression in neurons.**

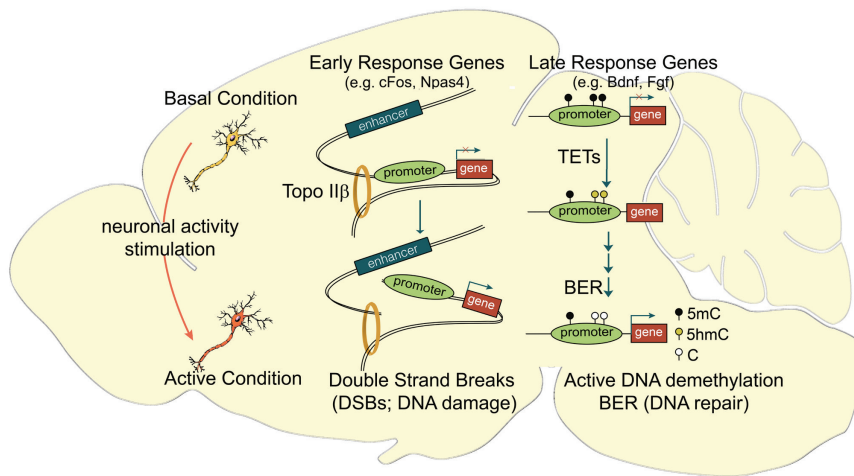
DNA damage, including altered bases, abasic sites, and single- and double-strand breaks, is known to be neurotoxic factors in aging and in the pathogenesis of multiple neurological disorders, such as Parkinson's disease, amyotrophic lateral sclerosis and Alzheimer's disease [1, 2]. Mammalian cells have evolved at least six DNA repair pathways to deal with various types of DNA damage [3]. Some pathways are only compatible with DNA replication mechanisms and therefore could not proceed in terminally differentiated post-mitotic cells. For example, there are two main pathways that are involved in DNA double-strand break (DSB) repair in mammalian cells: homologous recombination, which requires a sister chromatin to act as a template for accurate repair and is confined to late S and G2 phases of cell cycle, and non-homologous end joining (NHEJ), which is active throughout the entire cell cycle but most prevalent in G1 and early S phases. DNA DSBs cannot be accurately repaired through these repair pathways in mature neurons, presumably because they do not divide. Consequently, it is generally believed that neurons gradually accumulate unrepaired DNA damage over time, which could potentially lead to pathogenesis of neurodegenerative disorders. A recent

study further showed that the expression of an Alzheimer's disease-linked hallmark protein, amyloid  $\beta$ , exacerbates the accumulation of the unrepaired DNA damage [4]. However, emerging evidence suggests that the impact of DNA damage and repair is not restricted to cellular stress and brain disorders as previously thought, but is also involved in fundamental physiological functions of neurons. In a recent study published in *Cell*, Madabhushi and colleagues showed that neuronal activity triggers DNA DSB formation on promoters of multiple neuronal early response genes, which is required for their expression in neurons, and they further uncovered the underlying molecular mechanism [5].

Early response genes, also called immediate-early genes, are a set of genes that are activated rapidly, and usually transiently, in response to a wide variety of cellular stimuli. Naturally, many early response genes encode transcription factors (e.g., *c-Fos*, *c-myc*, and *c-jun*), which in turn govern the expression of late response genes (e.g., *bdnf* and *homer1* in neurons) to regulate various cellular responses. In neurons, early response genes play important roles in cellular, circuitry and cognitive functions, including neurite outgrowth, synapse development and maturation, balance between excitatory and inhibitory synapses, and learning and memory [6]. Although the phenomenon of early response gene expression was discovered three decades ago, the precise molecular mechanism is still unclear. In this study, Madabhushi *et al.* [5] examined the transcriptome of cultured neurons treated with etoposide, an inhibitor of topoisomerase II (Topo

II) that induces DNA DSBs. Strikingly, they found that upregulated genes are enriched for neuronal activity-regulated genes, particularly early response genes. They further performed a series of elegant experiments to support their conclusion that DNA DSB formation is both required and sufficient to induce neuronal early response gene expression. First, neuronal activation causes DSBs in neurons both *in vivo* and *in vitro*. Remarkably, genome-wide profiling via  $\gamma$ H2AX Chip-seq showed that neuronal activity induces DSB formation mostly in early response genes. Second, Chip analysis showed that Topo II $\beta$  cleavage patterns coincide with sites of activity-induced DNA DSBs, which occurs within topological domains defined by CCCTC-binding factors (CTCF). Finally, they utilized the CRISPR-Cas9 system to show that generation of targeted DSBs within the promoters of early response genes is sufficient to induce gene expression without neuronal stimulation. Together, these results suggest the model that topological constraints cause RNA Pol II pausing at promoters of early response genes under basal conditions, and activity-induced DSB formation rapidly breaks topological barriers to initiate gene transcription (Figure 1).

Activity-induced neural plasticity is a fundamental mechanism through which the brain adapts to the external environment. The transient nature of neuronal early response gene expression requires activity-induced DSBs to be temporary and can be quickly repaired to reset. Indeed, Madabhushi *et al.* [5] showed that pharmacological inhibition of DSB repair via NHEJ extends the duration of



**Figure 1** Neuronal activity-induced DNA damage and repair regulates expression of both early response and later response genes in neurons. Topo II $\beta$ : Topoisomerase II $\beta$ ; DSBs: Double strand breaks; TET: Ten-eleven translocation methylcytosine dioxygenase; BER: base-excision repair; 5mC: 5-methylcytosine; 5hmC: 5-hydroxymethylcytosine; C: cytosine.

early response gene expression. Future studies of the dynamics of activity-induced DSB formation and repair under both physiological and pathological conditions will offer an exciting entry point to determine its scope and function in gene regulation by neuronal activity and cellular processes that contribute to higher order brain functions and also how its dysregulation may contribute to the pathogenesis of neurological and psychiatric diseases. Additionally, more quantitative and genome-wide methods to identify DSBs, using markers other than  $\gamma$ H2AX or 53BP1, will be extremely helpful to gain further insight.

The interesting results from Madabhushi and colleagues support an emerging view that DNA damage and repair

responses are not limited to pathological conditions as previously thought, but instead they are important for neuronal activity-induced gene expression under physiological conditions. In addition to DSBs, the base-excision repair (BER) pathway functions downstream of TETs to mediate active DNA demethylation in neurons [7] and previous studies have identified critical roles of the BER pathway in neuronal activity-induced expression of late response genes (e.g., *bdnf*, and *fgf1*) and in synaptic transmission and plasticity (Figure 1) [8]. Neurons appear to evolve different mechanisms to mediate DNA damage and repair for precise temporal control of gene expression based on unique functional requirements. Interestingly,

a recent study provided evidence to support a connection between DSBs, defined by H2AX S139 phosphorylation, and TGF $\beta$ -induced, high mobility group protein (HMGA2)-mediated transcriptional initiation during epithelia-mesenchymal transitions [9]. Therefore, the involvement of DNA damage and repair in regulating gene expression could be a general mechanism critical for other cell types and organisms and thus much more remains to be discovered.

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