Opinion

EMBO reports

Activity-DEPendent Transposition

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The fundamental unit of the brain, the neuron, has a remarkable ability to respond to environmental stimuli. Neuronal stimulation results in rapid transcriptional changes in immediate-early (IE) genes. Their regulation has been the focus of intense research, which revealed that enhancer/promoter activity of IE genes is facilitated by topoisomerase IIβ-mediated induction of double-strand breaks (DSBs) [1]. Building on these data, we suggest a novel mechanism, termed Activity-DEPendent Transposition (ADEPT). We propose that DSBs resulting from neuronal activity are exploited by transposable elements (TEs) to generate adaptive somatic mosaicism in adult neurons, which is distinct from the well-documented developmental somatic mosaicism seen during neurogenesis. The genomic rearrangements caused by ADEPT can lead to either *de novo* integration of transposable elements or homology-directed recombination of repetitive sequences contained within the transposable elements. In our opinion, the evidence in favor of ADEPT is mounting and carries considerable importance for neuronal plasticity, neural network adaptation, and, importantly, genome stability in the aging brain.

Transposable elements are mobile genetic elements that comprise 40–50% of the mouse and human genomes and are known to harbor eukaryotic transcription factor binding sites (TFBS). After germline integration, newly available TFBS can be exaptated as species- or tissue-specific transcriptional enhancers [2]. Several studies have confirmed copy number and TE integration loci to vary significantly between individual neurons, a developmental somatic mosaicism that appears to occur during neurogenesis [3]. This has led to the notion that somatic mosaicism is generated in large part by *de novo* insertions of LINE retrotransposons (long interspersed nuclear elements) in neurons while undergoing the last neural progenitor divisions. As a population, neurons with high or low TE insertions may express different subsets of ion channels or neuronal adhesion molecules and thus have different firing properties. The generation of such a diverse neuronal population initiates the stochastic input required for network formation. This theory is a particularly attractive biological correlate for those who model the development and adjustment of neural networks computationally, as network properties can vary significantly depending on the amplitude of the input noise.

However, recent evidence suggests that developmental mosaicism is unlikely to be caused by LINE element transposition directly. A study by Wei and colleagues [4] described genomic areas that break and translocate at high frequency in neuronal progenitors. Such breaks were not random and clustered in the vicinity of neuron-specific genes. Notably, TEs are known to be able to exploit available DNA breaks for insertion [5], which indicates that developmental mosaicism is more likely generated by large chromosomal rearrangements that occur at these high-frequency break clusters, which are in turn exploited by LINEs. The timing of TE activity during neurogenesis correlates clearly with the generation of developmental mosaicism. Intriguingly, recent studies have shown that TE expression persists in the adult brain, which begs the question: Why does TE transcription persist in adult neurons, and could expressed TEs capitalize on activity-induced DSBs?

A handful of studies have explored transposable element expression in stimulated adult neurons. By way of background, it is well-established that autonomous (functional) LINEs are able to facilitate the transposition of other non-autonomous transposable elements *in trans*. This includes facilitating transposition of defunct LINEs, or smaller SINEs (short interspersed nuclear elements) and, in primates, the compound SVA (SINE/VNTR/Alu) transposons. Transposition *in trans* means that a single functional LINE transcript may enable transposition of non-autonomous transposons of which there are millions of copies in the genome. In humans, SVA transposons display a high rate of transposition in the brain, likely due to autonomous LINE activity. Notably, the B2 subfamily of SINE elements increases in expression following stimulation in mature neurons [6]. In a pivotal study, *de novo* LINE insertions in hippocampal neurons appear to occur preferentially at hippocampus-specific neuronal enhancers [7], which we suggest is due to ADEPT. Transposable element insertion into the enhancer/promoter region of neuronal genes may bestow a neuron with one of two *ADEPTATIONS:* either *ADEPT-mediated potentiation* or *ADEPT-mediated inactivation*. These phenomena confer adaptive somatic mosaicism in functional neurons, which is distinct from the aforementioned developmental somatic mosaicism occurring in neural progenitors.

ADEPT-mediated potentiation occurs when the transcription factor binding sites carried by the newly transposed element enhance future activity-dependent transcription of the neuronal gene. *ADEPT-mediated inactivation* occurs when the insertion disrupts the enhancer/promoter architecture or endogenous regulatory sequences, resulting in a decrease or abolition of future activity-dependent transcription.

ADEPT-mediated inactivation seems to be more likely. Some evidence comes from the study of evolutionarily older LINE1 and

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DOI 10.15252/embr.201643797 | Published online 20 February 2017

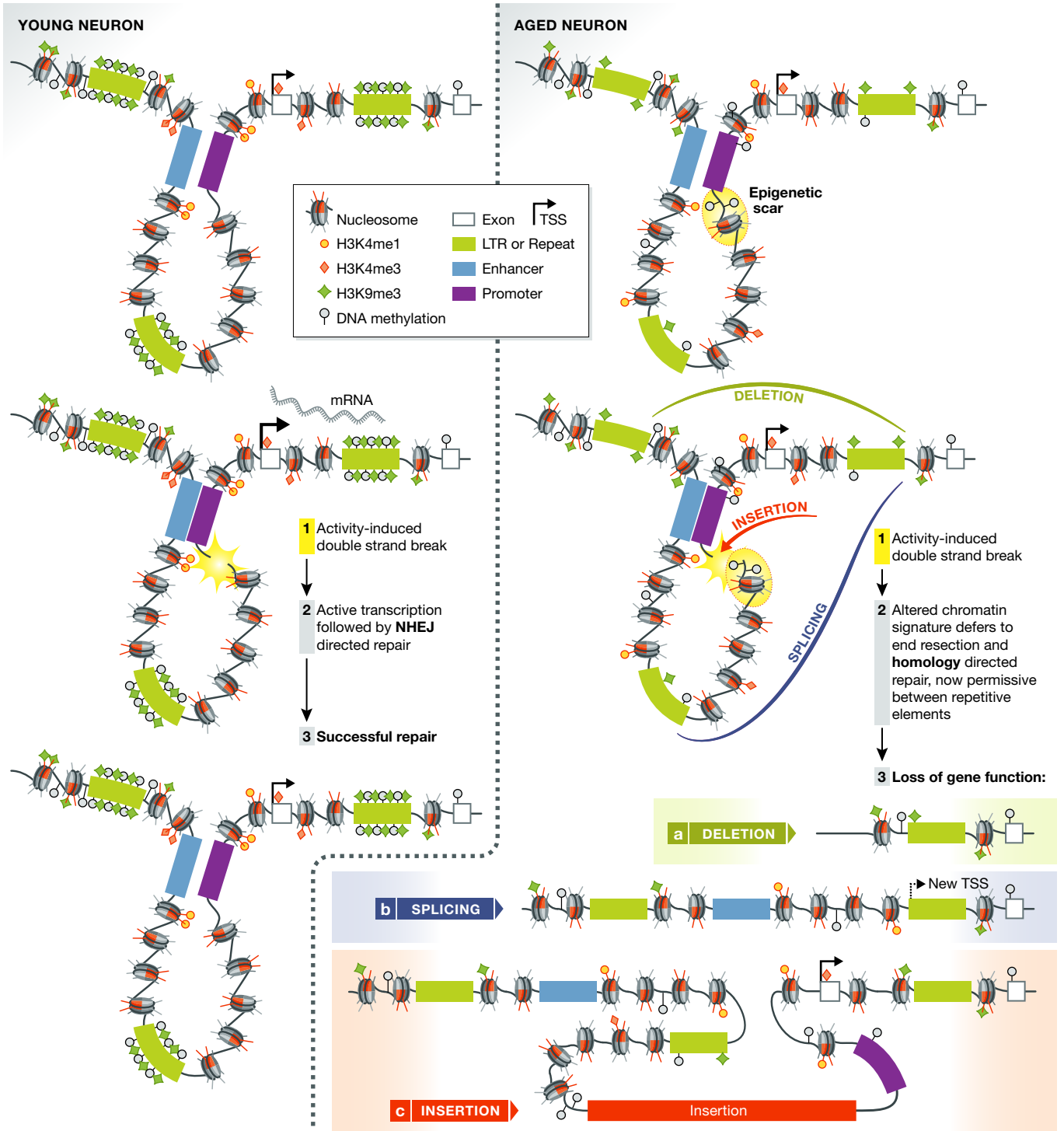

Figure 1. Age-related augmentation of Activity-DEPendent Transposition.
Upon stimulation, neurons induce DNA double-strand breaks (DSBs) in the enhancer/promoter region of immediate-early genes (middle panel), a known requirement for their expression. Left column: In young neurons, these activity-induced DSBs are repaired by the non-homologous end-joining (NHEJ) pathway (bottom panel) [1]. Right column: In aged neurons, epigenetic drift results in poor definition of chromatin compartments as defined by histone modifications and DNA methylation. Active marks such as H3K4me1 and H3K4me3 no longer sharply define promoters or the transcription start site (TSS). In the vicinity of the enhancer/promoter DSB region, an "epigenetic scar" is likely to form, due to improper re-establishment of epigenetic marks following a lifetime of DNA repair. Repetitive sequences are no longer repressed by H3K9me3 and DNA methylation, resulting in their transcription as lncRNA. It is a likely scenario that this epigenetic drift diverts DNA repair toward transcription-coupled homologous recombination (TCHR), which uses the available repeat-rich lncRNA as a template. Homologous recombination is permissive between homologous repetitive elements flanking genic exons, most often resulting in (a) large deletions or (b) alternative TSS generation or alternative splicing due to loss of one exon. Insertions (c) or duplications into the enhancer/promoter region may also occur at a higher frequency with diminished DSB repair efficacy and higher abundance of transposable elements and repeat sequences.

LTR (long terminal repeat) elements found in the germline. Older TEs are found to be under-represented five kilobases from gene promoters, which is likely due to a negative effect of TE insertions on proximal gene expression [8].

At the network level, ADEPT-mediated inactivation may function as a "genomic hand brake" in assemblies prone to unstable (Hebbian) hyper-excitation by permanently disabling IE gene activation in one or more neurons of the network. Accordingly, neurons that are hyper-excitable would generate more activity-induced DSBs and hence carry a higher probability of TE insertion. Since it is more likely that an insertion would disrupt IE gene enhancer/promoter loci, long-term potentiation in neurons with *de novo* integrations would be permanently disrupted, halting positive feedback and permanently returning homeostasis. To the end of obviating excitotoxicity, this mechanism might endow neural networks with a biological limit on coupled excitation and provide a stabilizing force in Hebbian plasticity. This would be in line with the findings of Upton *et al* [7] who observed deleterious effects after *de novo* LINE integrations that occurred at enhancers of neurons of the hippocampus—the *de facto* seat of new memory formation and infamously sensitive to seizures.

The magnitude of ADEPT is likely augmented in an aging epigenome, as both TE silencing and DNA repair are regulated by overlapping epigenetic pathways. A consensus of age-related changes to the epigenetic landscape is gradually emerging, which has shown that the defined boundaries between densely packed heterochromatin and transcriptionally active euchromatin are gradually lost. Deposition of H3K4me1 and H3K4me3 in euchromatin becomes more diffuse in aged cells and no longer clearly demarcates promoters and transcription start sites, respectively. Similarly, heterochromatin shows declining levels of histone methyltransferases and loss of repressive epigenetic modifications H3K9me3, H3K27me3, and DNA methylation, as well as loss in chromatin-bound HP1. Heterochromatin normally packages and silences repetitive elements and retrotransposons, and age-related loss of heterochromatin consistently precedes increases in TE transcription [9]. As transcription of TE elements is often a prerequisite for transposition, this epigenetic change is likely to result in increased transposition.

Epigenetic drift is likely to affect ADEPT not only by increasing TE transcription but also by causing a bias in DSB repair pathway (Fig 1). Madabhushi *et al* [1] reported that activity-induced DSBs are repaired by the non-homologous end-joining (NHEJ) DNA repair pathway with preservation of sequence fidelity through retention of topoisomerase IIβ. Indeed, up until recently, the accepted view was that NHEJ is the only active pathway in post-mitotic neurons. However, recent studies have shown that an RNA-templated version of homologous recombination repair occurs in humans and that this transcription-coupled homologous recombination repair (TCHR) is the preferred mode at actively transcribed loci [10]. Given the plethora of studies that have shown that the epigenetic landscape surrounding the DSB site can determine repair pathway choice, we suggest that epigenetic drift causes a bias in DSB repair pathway choice in aged neurons—away from NHEJ and toward TCHR repair. As a consequence, homologous recombination can occur between adjacent TE-derived repetitive sequences, resulting in deletions, insertions, or the generation of alternative splice sites (Fig 1). In aged neurons, this process may be repeated leading to collapse of IE transcriptional networks, which we suggest could be the underlying primary etiology of most, if not all, neurodegenerative diseases.

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