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In the loop: how chromatin topology links genome structure to function in mechanisms underlying learning and memory

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Different aspects of learning, memory, and cognition are regulated by epigenetic mechanisms such as covalent DNA modifications and histone post-translational modifications. More recently, the modulation of chromatin architecture and nuclear organization is emerging as a key factor in dynamic transcriptional regulation of the post-mitotic neuron. For instance, neuronal activity induces relocalization of gene loci to 'transcription factories', and specific enhancer–promoter looping contacts allow for precise transcriptional regulation. Moreover, neuronal activity-dependent DNA double-strand break formation in the promoter of immediate early genes appears to overcome topological constraints on transcription. Together, these findings point to a critical role for genome topology in integrating dynamic environmental signals to define precise spatiotemporal gene expression programs supporting cognitive processes.

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Introduction

Sensory, cognitive, and emotional experiences induce long-lasting changes in neuronal circuits by stimulating intracellular signaling cascades to induce synaptic remodeling and nuclear changes that promote important transcriptional programs. Neuronal activity-dependent signaling responses are critical for adaptation to novel environments, learning behaviors and memory formation [1,2], and are correlated with cellular morphological changes such as increased dendritic growth and branching, synaptogenesis, and hippocampal neurogenesis [3,4]. DNA, RNA, histones and their post-translational modifications act together to define chromatin states that dictate genomic functions. Emerging evidence suggests that epigenetic modification of chromatin constitutes a powerful mechanism of memory regulation [5,6]. Here, we review recent studies that indicate an important role for nuclear architecture in regulating critical aspects of neuronal functions pertinent to learning and memory encoding. First, we will review physiological mechanisms of learning and memory, with a focus on activity-dependent gene expression as an upstream regulator of the transcriptional programs associated with cognition. We will then describe our current understanding of chromatin folding and compartmentalization in cells of the central nervous system. Finally, we will discuss some very recent findings that suggest an important role for chromatin topology and DNA break formation in the regulation of activity-dependent transcription.

Sensory experience induces transcriptional programs important for synaptic plasticity

Experience modulates neurotransmitter release at specific synapses, which can induce long-lasting forms of synaptic plasticity such as long-term potentiation (LTP). Glutamate, the most common excitatory neurotransmitter, binds to both AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) and NMDA (*N*-methyl-D-aspartate) receptors to induce membrane depolarization. Importantly, activated NMDA receptors flux calcium, a critical neuronal second messenger that influences stabilization of LTP through activation of intracellular signaling cascades to locally alter synapses and stimulate transcription in the nucleus [7,8].

The formation and maintenance of past experiences requires the transition between labile short-term memory traces to stable long-term memories, a process known as consolidation [9]. *De novo* protein synthesis is a distinctive hallmark of memory consolidation across many species [10–13], and decades of research utilizing methods to modulate transcription and translation implicate transcription as a key component of long-term memory [14]. At least two waves of transcription are required for the process of memory consolidation [15,16]. First, a group of stimulus-responsive genes encoding transcription factors (immediate early genes; IEGs) are activated immediately after a learning event [17]. Second, the protein products of IEGs control the expression of a

broader set of neuroplasticity genes, ultimately resulting in stable changes in synaptic connections that modulate neurotransmission [18].

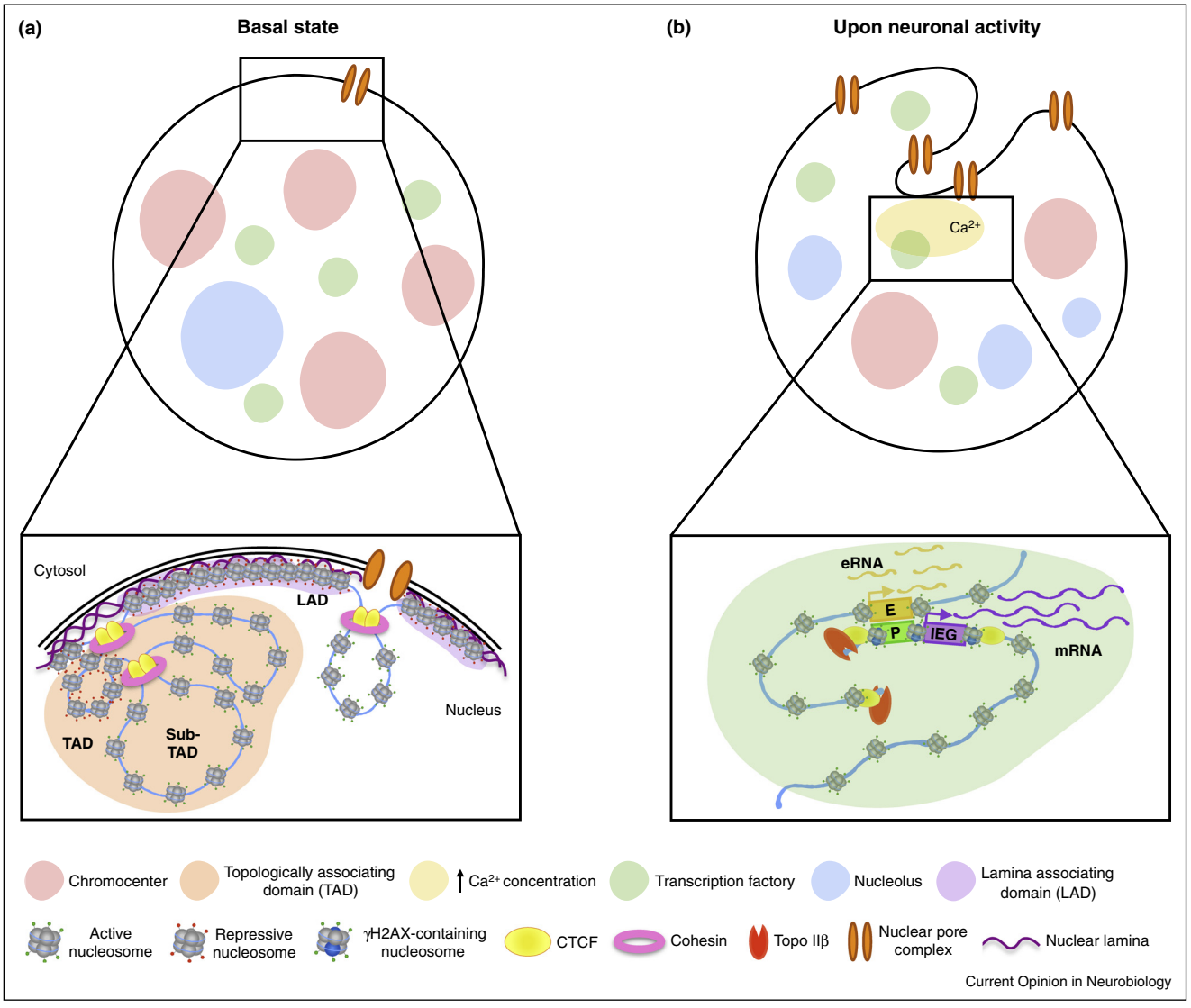
IEGs, such as *c-fos*, *egr-1*, *Arc*, and *Npas4*, are rapidly and transiently transcribed in response to synaptic activation [19–22]. Since IEGs are an apical feature of the transcriptional changes associated with learning and memory processes, their activation has been extensively investigated. Several interconnected mechanisms of transcriptional control regulate the activation of IEGs. The first layer of control involves the specific chromatin state of a given gene, which functions to define the local structural conformation of DNA and provide docking sites for transcriptional activators and repressors [23]. Stimulus-responsive genes like IEGs appear to be 'poised' for activation [24]. These classes of genes are characterized by stalled RNAPII [25] and enrichment of active histone modifications at their promoter and enhancer elements, but are only fully transcribed in response to specific stimuli [26]. The 'poising' of genes is proposed to enable synchronous processivity and rapid responses to external transcriptional cues [27]. Another key feature in the regulation of stimulus-responsive genes is the requirement for DNA break formation [28••], which will be discussed in more detail in the section titled 'Physiological neuronal activity induces DNA double-strand breaks'. The final level of transcriptional regulation involves the three-dimensional (3D) spatial context of a given gene, which enables functional compartmentalization of the nucleus into active and repressive chromatin domains [29], as well as local enhancer–promoter looping interactions for precise transcriptional control [30,31]. In the next sections, we will discuss the relationship between nuclear compartmentalization, chromatin looping, and transcription in neurons and how these genomic features may be altered in response to environmental stimuli relevant to learning and memory processes.

Chromatin folding and compartmentalization in the nucleus enables efficient genome packaging and dynamic regulation of DNA metabolism

Nuclear architecture, which refers to chromatin topology, nuclear compartments, and spatial genome organization [32], is dynamically regulated by internal and external cues to dictate genome function. The fundamental unit of chromatin is the nucleosome, which is comprised of ~147 base pairs of DNA wrapped around a $(H3–H4)_2$–$(H2A–H2B)_2$ histone octamer. The nucleosome is organized into the chromatin fiber, which is further condensed to generate chromosomes. Within the nucleus, chromosomes occupy distinct territories, and chromatin folds in *cis* to mediate interactions between regulatory elements as well as bring genomic regions from long distances or in *trans* to bring different chromosomes into close spatial proximity for co-regulation [33]. This type of genome organization is confirmed by chromosome conformation capture (3C)-based experiments, demonstrating that nuclear compartments differ with regards to chromatin and genic features: DNAse I hypersensitive, active, and gene-rich loci cluster together and are separate from gene-poor, transcriptionally silent chromatin regions [29,34]. Furthermore, different chromosomes occupy specific territories within the nucleus [33]. The arrangement of chromosome territories, and their interaction with one another and with the nuclear lamina, has a profound effect on gene expression [35]. The nuclear lamina, comprised of a meshwork of A-type and B-type lamins attached to the inner surface of the nuclear envelope [36], exhibits a strong inhibitory effect on gene expression and is hypothesized to provide mechanical stability and a structural framework for chromatin organization in the nucleus [37] (Figure 1a). Spatial proximity to the inner nuclear membrane does not always correspond to gene silencing, however, as nuclear pore complexes embedded within the nuclear membrane are important for rapid export of transcribed messenger RNA (mRNA) species into the cytosol for translation [38] (Figure 1a).

Chromosome compartments are further organized into domains of 0.1–1 Mb that are topologically separated from one another (forming topologically associating domains; TADs) [39,40•,41] (Figure 1a). TADs are largely conserved across cell types, while intra-TAD chromatin interactions exhibit some cell type-specificity [41,42]. TADs are defined by an increased frequency of chromatin interactions within a domain compared to the rest of the genome, and interactions within TADs represent the majority of enhancer–promoter interactions [39,40•,41]. Several genomic features correlate with TADs, such as chromatin marks [39,41], lamina associating domains [41], and chromocenters [43]. For instance, comparison between TADs and chromatin modifications has revealed several different types of domains that correspond to TADs. Those domains exhibit broad enrichment for histone marks and/or protein binding such as those enriched for H3K27me3 and binding of polycomb proteins, heterochromatin domains enriched for repressive modifications and HP1 binding, as well as active domains that are gene rich and marked by H3K4me3, H3K36me3, and histone acetylation [39]. Deletion of TAD boundary regions causes partial fusion of the flanking TADs [42], suggesting that TAD boundaries are genetically defined. Indeed, TAD boundaries are enriched for housekeeping genes, tRNAs, short interspersed element (SINE) retrotransposons, and binding sites for the architectural proteins CCCTC-binding factor (CTCF) and cohesin [39,41,44,45]. However, CTCF/cohesin binding is not specific to TAD boundaries and the proteins also function together to define intra-TAD loop formation [46,47]. Interestingly, the majority (>90%) of loop contacts contain CTCF motifs in convergent orientation [40•] and inversion of CTCF binding site orientation can alter

Figure 1



Neuronal activity is associated with alterations in nuclear geometry, subnuclear domains, and chromatin topology. **(a)** Under basal conditions, the nucleus is organized into specialized domains such as chromocenters (red), nucleolar regions (blue), and transcription factories (green). Moreover, the genome is highly organized into chromatin domains that interact with one another and with the nuclear architecture (inset). Topologically associating domains (TADs; orange) that segregate the genome into regulatory neighborhoods enriched for chromatin–chromatin interactions that facilitate sub-TAD formation. CTCF/cohesin are enriched at the boundaries of loop domains and TADs. Repressive chromatin is enriched at the nuclear lamina to form lamina associating domains (LADs; purple). Chromatin found in close proximity to nuclear pores is often enriched for active chromatin marks and highly expressed genes. **(b)** Neuronal activity is associated with infolding of the nuclear membrane, increased abundance nuclear pore complexes, and elevated calcium concentration (yellow), as well as changes in chromocenter and nucleolar organization. Neuronal activity also induces relocalization of IEG loci to transcription factories and transcription of eRNAs (inset). Moreover, activity causes Topo IIβ-mediated DNA double strand break (DSB) formation at the promoter of specific immediate early genes (IEGs), resulting in γH2AX enrichment across the gene body and target mRNA transcription (inset).

enhancer–promoter interactions and reshape TAD domains [48•], suggesting CTCF/cohesin binding is critically important for multiple types of higher chromatin organization.

The nucleus also contains dense assemblies of functionally related factors known as nuclear bodies, which help partition the genome into specialized domains [49] (Figure 1a). Examples of nuclear bodies include transcription factories, the nucleolus, and chromocenters. Transcription factories are subnuclear domains enriched for RNAPII that appear to congregate co-regulated genes and thus act as transcriptional hubs [50]. The nucleolus is the central region for rRNA transcription and ribosome biogenesis [51], and thus constitutes a major transcription factory with links to global protein synthesis. On the other

hand, chromocenters are repressive domains of constitutive pericentromeric heterochromatin that are easily visualized by DNA stains since they tend to cluster together within the nucleus [52].

Decades of research has thus indicated that the nucleus is precisely organized into specialized compartments, and that chromatin folding occurs in a hierarchical nature to partition the genome into functional domains.

Neurodevelopment and neuronal activity are associated with nuclear architecture reorganization

To date, characterization of large-scale chromatin topology has primarily been accomplished in non-neuronal cells, however emerging evidence implicates the importance of 3D chromatin organization in neural development and in regulating key neuronal transcriptional programs. High-resolution chromosome conformation capture (Hi-C) mapping of global chromatin contacts in the transition between embryonic stem (ES), neural progenitor cells (NPCs), and neurons described large-scale reorganization of TADs during differentiation [53,54••]. Additionally, neural differentiation is associated with dramatic remodeling of the genomic sites that contact the nuclear lamina [55–57]. Many of the genes that move away from the lamina become transcriptionally activated, while others appear to become 'primed' for activation in the next differentiation step [57]. These studies imply a relationship between transcriptional activity and chromatin topology, but it remains somewhat ambiguous whether chromatin interactions are a cause or consequence of transcriptional activity. An elegant study of nuclear organization in ES cells indicated that chromatin remodeling, rather than transcription, drives repositioning of gene loci [58•], suggesting that the former may be more likely.

The first study to describe non-random chromatin organization in neurons identified distinct chromocenter localization patterns in Purkinje and granule neurons of the cerebellum that were conserved across species [52]. Time-lapse imaging studies of slice cultures from the brain enabled visualization of nucleoli motion relative to DNA that occurred independently of cytoplasmic structures [59] and correlated with changes in intracellular calcium concentration [60]. Likewise, induction of LTP in rat hippocampal slices caused spatial reorganization of centromeres [61], and differences in X chromosome positioning were observed in neurons from cortical epileptic foci compared to healthy neurons bordering such lesions [62]. The number of nucleoli in neurons is also altered in response to neuronal activity, potentially to meet the increased protein demands of stimulated neurons [63]. These initial observations indicate substantial fluctuations in nuclear domain organization in response to external signals that accompany neuronal activity.

Evidence also suggests that, in addition to the global reorganization of subnuclear structures, the nuclear lamina of hippocampal neurons experiences dramatic remodeling in response to action potential bursts that could be visualized as infoldings of the nuclear membrane [64] (Figure 1b). The infoldings were stimulated by synaptic NMDA receptor-dependent calcium entry, and correlated with increased abundance of nuclear pore complexes and phosphorylation of serine 10 on histone H3 (H3S10 ph), a chromatin mark that is induced in response to neuronal activity [64,65]. Since actively transcribed genes are often located proximal to nuclear pore complexes [38], the authors proposed that membrane infolding might function to generate microdomains of enhanced calcium signaling by increasing surface area of the nuclear envelope, enabling efficient signal-induced transcriptional responses (Figure 1b). Given that repeated stimulation increased the stability of nuclear membrane alterations [64], infolding may represent a long-lasting form of structural plasticity.

External stimuli induce transcriptional responses via topological chromatin reorganization

While nuclear changes associated with neuronal activity have been documented for several decades, only very recently have studies connected these architectural changes to alterations in transcription and spatial relocalization to transcription factories.

Enhancer–promoter looping interactions are an important event in transcriptional initiation, but looping is not always sufficient to drive expression *per se*. This is exemplified by the finding that many looping contacts are established prior to gene activation [66]. A small percentage of loci, however, exhibit transcription-dependent looping specificity and IEGs appear to belong to this category [67••]. Looping interactions between IEG enhancer elements and their target gene promoters are associated with RNAPII-dependent bidirectional transcription of enhancer domains, generating enhancer RNAs (eRNAs; Figure 1b) [54••,68,69]. Transcription from eRNAs is correlated with target gene induction; at least five enhancers have been described for the IEG *c-fos*, and differential eRNA transcription elicited by external stimuli is correlated with specific and combinatorial enhancer–promoter interactions [67••]. The function of eRNAs remains unclear, but emerging evidence points to a functional role for eRNAs in sequestering the negative elongation factor (NELF) complex to promote productive elongation by RNAPII [70]. Additionally, other studies demonstrate that eRNAs facilitate enhancer–promoter interactions by recruiting architectural proteins such as cohesin and the mediator complex [71–73].

Chromatin loops promoted by distal regulatory elements are also capable of triggering the relocation of stimulus-responsive gene loci to active chromatin hubs

[30,74–77]. For instance, activity-dependent induction of brain-derived neurotrophic factor (*Bdnf*) correlates with spatial relocalization of the *Bdnf* gene from the nuclear lamina to interior [78]. 3C-based analyses have also identified that neuronal activity can modulate the colocalization of cytochrome oxidase family gene loci with some glutamatergic neurotransmitter receptor genes within transcription factories [79,80], potentially providing an efficient mechanism for the coordination of energy metabolism and neurotransmission [80]. Furthermore, in response to neuronal activity, the relocation of IEGs *c-fos* and *Gadd45b* to transcription factories is a necessary event that mediates activity-dependent transcription [81••].

Collectively, large-scale changes in neuronal nuclear architecture occur in an activity-dependent manner and appear to provide an additional layer of regulation for precise temporal transcriptional control. In the future, it will be important to explore the factors that mediate these topological changes, and how they function to define specific chromatin interactions and coordinate neuronal transcriptional responses.

Physiological neuronal activity induces DNA double-strand breaks

Recently, several groups have described a perplexing phenomenon that occurs in response to neuronal activity: the formation of DNA double-strand breaks (DSBs; Figure 1b). Physiologically relevant neuronal activity, such as that elicited by exploration of a novel environment, induced DSB formation in neurons of memory-relevant brain regions such as the hippocampus [82••]. Madabhushi *et al.* [28••] recently provided a mechanism for these activity-induced DSBs by demonstrating a role for the type II topoisomerase Topo IIβ in break formation. Moreover, through genome-wide profiling of the DSB-associated phosphorylated histone variant γH2AX, they identified just twenty-one genomic loci that accrue DSBs in response to NMDA-mediated neuronal activity. Remarkably, a majority of the sites exhibiting γH2AX enrichment encompassed the bodies of IEGs, were flanked by CTCF binding sites, and break induction was shown to be necessary and sufficient for transcriptional activation of *c-fos* and *Npas4*. Furthermore, inhibition of the non-homologous end joining (NHEJ) DNA repair pathway resulted in prolonged IEG expression, indicating that effective and timely repair of activity-dependent DSBs is important for dynamic regulation of IEGs. Together, these findings suggest that DSBs are critical for IEG expression dynamics and that CTCF may define a specific chromatin topology that is modified upon neuronal activity to enable appropriate transcriptional activation of IEGs.

While it is unknown exactly why neurons would choose break formation and repair as a strategy to regulate stimulus-dependent transcription, DSBs at gene promoters may enable topological alterations and subsequent changes in chromatin organization that facilitate the transition between a 'poised' genomic environment to one that promotes transcription and productive elongation [83,84]. Importantly, these observations raise the intriguing possibility that the deterioration of DNA damage response mechanisms during normal and pathological aging [85] may influence the repair of neuronal activity-induced DSBs and dysregulate important transcriptional programs. Recent genome-wide profiling of DSBs in neural progenitor cells indicate that recurrent breaks form in genes involved in cell adhesion and synapse formation, as well as in genes rearranged in some cancers [86•,87]. Moreover, mutations in epigenetic regulators, such as *CTCF* [88], as well as factors involved in DNA repair and damage response signaling [89], often cause intellectual disability and neurological defects, indicating the importance of chromatin topology and genomic stability in cognitive function.

Together, neuronal activity-dependent DNA breaks represent a novel mechanism for transcriptional induction of IEGs, revealing an unexpected link between DSB formation and crucial neuronal functions. Moreover, in addition to IEG promoters in neurons, recurrent DSBs are localized to a set of long genes that are necessary for proper cognitive function in neural progenitor cells [86•,87]. While the exact mechanism bridging these two observations is unknown, evidence to date suggests that there is a subset of genes whose transcriptional activation/elongation is regulated by DNA breaks, and that regulatory specificity may relate to the DNA topological environment in which those genes reside. Neural cells thus exhibit high rates of localized DSBs, and the location of these DSBs suggest that inefficient repair would negatively impact cognitive function. These exciting new findings raise numerous questions about how extracellular stimuli are perceived by neurons to induce long-lasting forms of plasticity. Moving forward, it will be important to characterize the specific changes in nuclear morphology, compartmentalization, chromatin looping, and DSB formation that occur in response to different environmental stimuli, and how defects in these processes during aging and disease may influence cognition.

Conclusions

Decades of research indicate that epigenetic regulation is a critical component of learning and memory processes. More recently, nuclear architecture is emerging as a dynamic physiological template that acts to integrate environmental inputs into cellular adaptation. Studies examining the relevance of chromatin topology to neuronal transcriptional programs are still in their infancy, though early findings suggest important roles for these processes in cognitive function. Emerging technologies to study large-scale chromatin interactions such as Hi-C and

chromatin interaction analysis of paired-end tags (ChIA-PET) will indisputably uncover the functional significance of dynamic genome organization and reorganization in neurons and its relevance to cognitive function.

Conflict of interest statement

Nothing declared.

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