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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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Abstract

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.

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

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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- 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) [39–41] (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 [39–41]. 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 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 (H3S10ph), 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 relocation 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 relocation 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 II β in break formation. Moreover, through genome-wide profiling of the DSB-associated phosphorylated histone variant γ H2AX, they identified just twenty-one genomic loci that accrue DSBs in response to NMDA-mediated neuronal activity. Remarkably, a majority of the sites exhibiting γ H2AX 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.

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References

1. Kandel ER. The molecular biology of memory storage: a dialog between genes and synapses. *Biosci Rep.* 2001; 21:565–611. [PubMed: 12168768]
2. Greer PL, Greenberg ME. From synapse to nucleus: calcium-dependent gene transcription in the control of synapse development and function. *Neuron.* 2008; 59:846–860. [PubMed: 18817726]
3. Leslie JH, Nedivi E. Activity-regulated genes as mediators of neural circuit plasticity. *Prog Neurobiol.* 2011; 94:223–237. [PubMed: 21601615]
4. Saneyoshi T, Fortin DA, Soderling TR. Regulation of spine and synapse formation by activity-dependent intracellular signaling pathways. *Curr Opin Neurobiol.* 2010; 20:108–115. [PubMed: 19896363]
5. Day JJ, Sweatt JD. Epigenetic mechanisms in cognition. *Neuron.* 2011; 70:813–829. [PubMed: 21658577]
6. Rudenko A, Tsai LH. Epigenetic regulation in memory and cognitive disorders. *Neuroscience.* 2014; 264:51–63. [PubMed: 23291453]
7. Kandel ER, Dudai Y, Mayford MR. The molecular and systems biology of memory. *Cell.* 2014; 157:163–186. [PubMed: 24679534]
8. Ebert DH, Greenberg ME. Activity-dependent neuronal signalling and autism spectrum disorder. *Nature.* 2013; 493:327–337. [PubMed: 23325215]
9. McGaugh JL. Memory—a century of consolidation. *Science.* 2000; 287:248–251. [PubMed: 10634773]
10. Flexner JB, Flexner LB, Stellar E, De La Haba G, Roberts RB. Inhibition of protein synthesis in brain and learning and memory following puromycin. *J Neurochem.* 1962; 9:595–605. [PubMed: 13945540]
11. Flexner JB, Flexner LB, Stellar E. Memory in mice as affected by intracerebral puromycin. *Science.* 1963; 141:57–59. [PubMed: 13945541]
12. Igaz LM, Vianna MR, Medina JH, Izquierdo I. Two time periods of hippocampal mRNA synthesis are required for memory consolidation of fear-motivated learning. *J Neurosci.* 2002; 22:6781–6789. [PubMed: 12151558]
13. Davis HP, Squire LR. Protein synthesis and memory: a review. *Psychol Bull.* 1984; 96:518–559. [PubMed: 6096908]
14. Neale JH, Klinger PD, Agranoff BW. Camptothecin blocks memory of conditioned avoidance in the goldfish. *Science.* 1973; 179:1243–1246. [PubMed: 4689018]
15. Stork O, Welzl H. Memory formation and the regulation of gene expression. *Cell Mol Life Sci.* 1999; 55:575–592. [PubMed: 10357228]
16. Alberini CM. Transcription factors in long-term memory and synaptic plasticity. *Physiol Rev.* 2009; 89:121–145. [PubMed: 19126756]
17. Tischmeyer W, Grimm R. Activation of immediate early genes and memory formation. *Cell Mol Life Sci.* 1999; 55:564–574. [PubMed: 10357227]
18. Bailey CH, Bartsch D, Kandel ER. Toward a molecular definition of long-term memory storage. *Proc Natl Acad Sci U S A.* 1996; 93:13445–13452. [PubMed: 8942955]
19. Guzowski JF, McNaughton BL, Barnes CA, Worley PF. Environment-specific expression of the immediate-early gene *Arc* in hippocampal neuronal ensembles. *Nat Neurosci.* 1999; 2:1120–1124. [PubMed: 10570490]
20. Vann SD, Brown MW, Aggleton JP. Fos expression in the rostral thalamic nuclei and associated cortical regions in response to different spatial memory tests. *Neuroscience.* 2000; 101:983–991. [PubMed: 11113347]

21. Hall J, Thomas KL, Everitt BJ. Cellular imaging of zif268 expression in the hippocampus and amygdala during contextual and cued fear memory retrieval: selective activation of hippocampal CA1 neurons during the recall of contextual memories. *J Neurosci.* 2001; 21:2186–2193. [PubMed: 11245703]
22. Lin Y, Bloodgood BL, Hauser JL, Lapan AD, Koon AC, Kim TK, Hu LS, Malik AN, Greenberg ME. Activity-dependent regulation of inhibitory synapse development by Npas4. *Nature.* 2008; 455:1198–1204. [PubMed: 18815592]
23. Li B, Carey M, Workman JL. The role of chromatin during transcription. *Cell.* 2007; 128:707–719. [PubMed: 17320508]
24. Saha RN, Wissink EM, Bailey ER, Zhao M, Fargo DC, Hwang JY, Daigle KR, Fenn JD, Adelman K, Dudek SM. Rapid activity-induced transcription of Arc and other IEGs relies on poised RNA polymerase II. *Nat Neurosci.* 2011; 14:848–856. [PubMed: 21623364]
25. Kim TH, Barrera LO, Zheng M, Qu C, Singer MA, Richmond TA, Wu Y, Green RD, Ren B. A high-resolution map of active promoters in the human genome. *Nature.* 2005; 436:876–880. [PubMed: 15988478]
26. Barski A, Jothi R, Cuddapah S, Cui K, Roh TY, Schones DE, Zhao K. Chromatin poises miRNA- and protein-coding genes for expression. *Genome Res.* 2009; 19:1742–1751. [PubMed: 19713549]
27. Adelman K, Lis JT. Promoter-proximal pausing of RNA polymerase II: emerging roles in metazoans. *Nat Rev Genet.* 2012; 13:720–731. [PubMed: 22986266]
- **28. Madabhushi R, Gao F, Pfenning AR, Pan L, Yamakawa S, Seo J, Rueda R, Phan TX, Yamakawa H, Pao PC, et al. Activity-Induced DNA Breaks Govern the Expression of Neuronal Early-Response Genes. *Cell.* 2015; 161:1592–1605. This study demonstrates that physiological neuronal activity causes DNA DSBs at the promoter of IEGs, and that those breaks are necessary and sufficient to induce transcription. The findings have strong implications for chromatin topology in enabling activity-dependent transcription. Moreover, this study suggests that abnormalities in break formation and/or repair at IEGs could have long-term consequences on important transcriptional programs relevant to cognition. [PubMed: 26052046]
29. Lieberman-Aiden E, van Berkum NL, Williams L, Imakaev M, Ragoczy T, Telling A, Amit I, Lajoie BR, Sabo PJ, Dorschner MO, et al. Comprehensive mapping of long-range interactions reveals folding principles of the human genome. *Science.* 2009; 326:289–293. [PubMed: 19815776]
30. Tolhuis B, Palstra RJ, Splinter E, Grosveld F, de Laat W. Looping and interaction between hypersensitive sites in the active beta-globin locus. *Mol Cell.* 2002; 10:1453–1465. [PubMed: 12504019]
31. Vernimmen D, De Gobbi M, Sloane-Stanley JA, Wood WG, Higgs DR. Long-range chromosomal interactions regulate the timing of the transition between poised and active gene expression. *EMBO J.* 2007; 26:2041–2051. [PubMed: 17380126]
32. Bickmore WA, van Steensel B. Genome architecture: domain organization of interphase chromosomes. *Cell.* 2013; 152:1270–1284. [PubMed: 23498936]
33. Cremer T, Cremer C. Chromosome territories, nuclear architecture and gene regulation in mammalian cells. *Nat Rev Genet.* 2001; 2:292–301. [PubMed: 11283701]
34. Zhang Y, McCord RP, Ho YJ, Lajoie BR, Hildebrand DG, Simon AC, Becker MS, Alt FW, Dekker J. Spatial organization of the mouse genome and its role in recurrent chromosomal translocations. *Cell.* 2012; 148:908–921. [PubMed: 22341456]
35. Geyer PK, Vitalini MW, Wallrath LL. Nuclear organization: taking a position on gene expression. *Curr Opin Cell Biol.* 2011; 23:354–359. [PubMed: 21450447]
36. Burke B, Stewart CL. The nuclear lamins: flexibility in function. *Nat Rev Mol Cell Biol.* 2013; 14:13–24. [PubMed: 23212477]
37. Zuleger N, Robson MI, Schirmer EC. The nuclear envelope as a chromatin organizer. *Nucleus.* 2011; 2:339–349. [PubMed: 21970986]
38. Taddei A, Van Houwe G, Hediger F, Kalck V, Cubizolles F, Schober H, Gasser SM. Nuclear pore association confers optimal expression levels for an inducible yeast gene. *Nature.* 2006; 441:774–778. [PubMed: 16760983]

39. Sexton T, Yaffe E, Kenigsberg E, Bantignies F, Leblanc B, Hoichman M, Parrinello H, Tanay A, Cavalli G. Three-dimensional folding and functional organization principles of the Drosophila genome. *Cell*. 2012; 148:458–472. [PubMed: 22265598]
- *40. Rao SS, Huntley MH, Durand NC, Stamenova EK, Bochkov ID, Robinson JT, Sanborn AL, Machol I, Omer AD, Lander ES, et al. A 3D Map of the Human Genome at Kilobase Resolution Reveals Principles of Chromatin Looping. *Cell*. 2014; 159:1665–1680. Using *in situ* Hi-C, this study provides a comprehensive and high-resolution map of chromatin contacts that builds upon previous observations that the genome is folded in a hierarchical manner to form functional domains. This mapping also provided insights into the factors involved in domain formation, as domain boundaries often bound CTCF sites that predominantly occur in a convergent motif orientation. [PubMed: 25497547]
41. Dixon JR, Selvaraj S, Yue F, Kim A, Li Y, Shen Y, Hu M, Liu JS, Ren B. Topological domains in mammalian genomes identified by analysis of chromatin interactions. *Nature*. 2012; 485:376–380. [PubMed: 22495300]
42. Nora EP, Lajoie BR, Schulz EG, Giorgetti L, Okamoto I, Servant N, Piolot T, van Berkum NL, Meisig J, Sedat J, et al. Spatial partitioning of the regulatory landscape of the X-inactivation centre. *Nature*. 2012; 485:381–385. [PubMed: 22495304]
43. Wijchers PJ, Geeven G, Eyres M, Bergsma AJ, Janssen M, Verstegen M, Zhu Y, Schell Y, Vermeulen C, de Wit E, et al. Characterization and dynamics of pericentromere-associated domains in mice. *Genome Res*. 2015; 25:958–969. [PubMed: 25883320]
44. Raab JR, Chiu J, Zhu J, Katzman S, Kurukuti S, Wade PA, Haussler D, Kamakaka RT. Human tRNA genes function as chromatin insulators. *EMBO J*. 2012; 31:330–350. [PubMed: 22085927]
45. Zullo JM, Demarco IA, Pique-Regi R, Gaffney DJ, Epstein CB, Spooner CJ, Luperchio TR, Bernstein BE, Pritchard JK, Reddy KL, et al. DNA sequence-dependent compartmentalization and silencing of chromatin at the nuclear lamina. *Cell*. 2012; 149:1474–1487. [PubMed: 22726435]
46. Splinter E, Heath H, Kooren J, Palstra RJ, Klous P, Grosveld F, Galjart N, de Laat W. CTCF mediates long-range chromatin looping and local histone modification in the beta-globin locus. *Genes Dev*. 2006; 20:2349–2354. [PubMed: 16951251]
47. Hou C, Dale R, Dean A. Cell type specificity of chromatin organization mediated by CTCF and cohesin. *Proc Natl Acad Sci U S A*. 2010; 107:3651–3656. [PubMed: 20133600]
- *48. Guo Y, Xu Q, Canzio D, Shou J, Li J, Gorkin DU, Jung I, Wu H, Zhai Y, Tang Y, et al. CRISPR Inversion of CTCF Sites Alters Genome Topology and Enhancer/Promoter Function. *Cell*. 2015; 162:900–910. This study indicates the importance of CTCF binding sites in the formation of topologically associating domains (TADs) and enhancer-promoter interactions. Using both the β -globin and protocadherin model gene loci, the authors invert CTCF binding motifs by CRISPR/Cas9 editing to demonstrate that the relative orientation of CTCF binding sites determines specificity of long-range interactions. [PubMed: 26276636]
49. Dundr M, Misteli T. Biogenesis of nuclear bodies. *Cold Spring Harb Perspect Biol*. 2010; 2:a000711. [PubMed: 21068152]
50. Iborra FJ, Pombo A, Jackson DA, Cook PR. Active RNA polymerases are localized within discrete transcription “factories” in human nuclei. *J Cell Sci*. 1996; 109(Pt 6):1427–1436. [PubMed: 8799830]
51. Pederson T. The nucleolus. *Cold Spring Harb Perspect Biol*. 2011;3.
52. Manuelidis L. Different central nervous system cell types display distinct and nonrandom arrangements of satellite DNA sequences. *Proc Natl Acad Sci U S A*. 1984; 81:3123–3127. [PubMed: 6587343]
53. Fraser J, Ferrai C, Chiariello AM, Schueler M, Rito T, Laudanno G, Barbieri M, Moore BL, Kraemer DC, Aitken S, et al. Hierarchical folding and reorganization of chromosomes are linked to transcriptional changes in cellular differentiation. *Mol Syst Biol*. 2015; 11:852. [PubMed: 26700852]
- **54. Won H, de la Torre-Ubieta L, Stein JL, Parikshak NN, Huang J, Opland CK, Gandal MJ, Sutton GJ, Hormozdiari F, Lu D, et al. Chromosome conformation elucidates regulatory relationships in developing human brain. *Nature*. 2016; 538:523–527. By analyzing global chromatin interactions via Hi-C in neural progenitor cells and cortical plate neurons from developing human brain tissue, this study highlights the importance of higher-order chromatin organization in

transcriptional regulation during neural development. Moreover, they show that specific non-coding variants associated with schizophrenia lie in putative regulatory regions that engage in long-range interactions with critical neurodevelopmental genes. [PubMed: 27760116]

55. Takizawa T, Gudla PR, Guo L, Lockett S, Misteli T. Allele-specific nuclear positioning of the monoallelically expressed astrocyte marker GFAP. *Genes Dev.* 2008; 22:489–498. [PubMed: 18281462]
56. Williams RR, Azuara V, Perry P, Sauer S, Dvorkina M, Jorgensen H, Roix J, McQueen P, Misteli T, Merkenschlager M, et al. Neural induction promotes large-scale chromatin reorganization of the *Mash1* locus. *J Cell Sci.* 2006; 119:132–140. [PubMed: 16371653]
57. Peric-Hupkes D, Meuleman W, Pagie L, Bruggeman SW, Solovei I, Brugman W, Graf S, Flicek P, Kerkhoven RM, van Lohuizen M, et al. Molecular maps of the reorganization of genome-nuclear lamina interactions during differentiation. *Mol Cell.* 2010; 38:603–613. [PubMed: 20513434]
- *58. Therizols P, Illingworth RS, Courilleau C, Boyle S, Wood AJ, Bickmore WA. Chromatin decondensation is sufficient to alter nuclear organization in embryonic stem cells. *Science.* 2014; 346:1238–1242. This study elegantly demonstrated that spatial nuclear reorganization is driven by remodeling of chromatin rather than transcription *per se*. [PubMed: 25477464]
59. De Boni U, Mintz AH. Curvilinear, three-dimensional motion of chromatin domains and nucleoli in neuronal interphase nuclei. *Science.* 1986; 234:863–866. [PubMed: 3775367]
60. Fung LC, De Boni U. Modulation of nuclear rotation in neuronal interphase nuclei by nerve growth factor, by gamma-aminobutyric acid, and by changes in intracellular calcium. *Cell Motil Cytoskeleton.* 1988; 10:363–373. [PubMed: 3141072]
61. Billia F, Baskys A, Carlen PL, De Boni U. Rearrangement of centromeric satellite DNA in hippocampal neurons exhibiting long-term potentiation. *Brain Res Mol Brain Res.* 1992; 14:101–108. [PubMed: 1323006]
62. Borden J, Manuelidis L. Movement of the X chromosome in epilepsy. *Science.* 1988; 242:1687–1691. [PubMed: 3201257]
63. Jordan BA, Fernholz BD, Khatri L, Ziff EB. Activity-dependent AIDA-1 nuclear signaling regulates nucleolar numbers and protein synthesis in neurons. *Nat Neurosci.* 2007; 10:427–435. [PubMed: 17334360]
64. Wittmann M, Queisser G, Eder A, Wiegert JS, Bengtson CP, Hellwig A, Wittum G, Bading H. Synaptic activity induces dramatic changes in the geometry of the cell nucleus: interplay between nuclear structure, histone H3 phosphorylation, and nuclear calcium signaling. *J Neurosci.* 2009; 29:14687–14700. [PubMed: 19940164]
65. Brami-Cherrier K, Valjent E, Herve D, Darragh J, Corvol JC, Pages C, Arthur SJ, Girault JA, Caboche J. Parsing molecular and behavioral effects of cocaine in mitogen- and stress-activated protein kinase-1-deficient mice. *J Neurosci.* 2005; 25:11444–11454. [PubMed: 16339038]
66. Ghavi-Helm Y, Klein FA, Pakozdi T, Ciglar L, Noordermeer D, Huber W, Furlong EE. Enhancer loops appear stable during development and are associated with paused polymerase. *Nature.* 2014; 512:96–100. [PubMed: 25043061]
- **67. Joo JY, Schaukowitz K, Farbiak L, Kilaru G, Kim TK. Stimulus-specific combinatorial functionality of neuronal *c-fos* enhancers. *Nat Neurosci.* 2016; 19:75–83. This study builds on previous observations of the correlation between enhancer RNA transcription and inducible, neuronal activity-dependent transcription. In an elegant series of experiments, the authors demonstrate that various stimuli promote differential eRNA transcription, which is correlated with enhancer-promoter interaction and transcriptional induction of the immediate early gene (IEG) *c-fos*. Together, this suggests that neuronal activity-dependent transcription results from stimulus-dependent alterations in enhancer-promoter interactions. [PubMed: 26595656]
68. Kim TK, Hemberg M, Gray JM, Costa AM, Bear DM, Wu J, Harmin DA, Laptevich M, Barbara-Haley K, Kuersten S, et al. Widespread transcription at neuronal activity-regulated enhancers. *Nature.* 2010; 465:182–187. [PubMed: 20393465]
69. Sanyal A, Lajoie BR, Jain G, Dekker J. The long-range interaction landscape of gene promoters. *Nature.* 2012; 489:109–113. [PubMed: 22955621]
70. Schaukowitz K, Joo JY, Liu X, Watts JK, Martinez C, Kim TK. Enhancer RNA facilitates NELF release from immediate early genes. *Mol Cell.* 2014; 56:29–42. [PubMed: 25263592]

71. Li W, Notani D, Ma Q, Tanasa B, Nunez E, Chen AY, Merkurjev D, Zhang J, Ohgi K, Song X, et al. Functional roles of enhancer RNAs for oestrogen-dependent transcriptional activation. *Nature*. 2013; 498:516–520. [PubMed: 23728302]
72. Lai F, Orom UA, Cesaroni M, Beringer M, Taatjes DJ, Blobel GA, Shiekhattar R. Activating RNAs associate with Mediator to enhance chromatin architecture and transcription. *Nature*. 2013; 494:497–501. [PubMed: 23417068]
73. Hsieh CL, Fei T, Chen Y, Li T, Gao Y, Wang X, Sun T, Sweeney CJ, Lee GS, Chen S, et al. Enhancer RNAs participate in androgen receptor-driven looping that selectively enhances gene activation. *Proc Natl Acad Sci U S A*. 2014; 111:7319–7324. [PubMed: 24778216]
74. Spilianakis CG, Flavell RA. Long-range intrachromosomal interactions in the T helper type 2 cytokine locus. *Nat Immunol*. 2004; 5:1017–1027. [PubMed: 15378057]
75. Osborne CS, Chakalova L, Brown KE, Carter D, Horton A, Debrand E, Goyenechea B, Mitchell JA, Lopes S, Reik W, et al. Active genes dynamically colocalize to shared sites of ongoing transcription. *Nat Genet*. 2004; 36:1065–1071. [PubMed: 15361872]
76. Oti M, Falck J, Huynen MA, Zhou H. CTCF-mediated chromatin loops enclose inducible gene regulatory domains. *BMC Genomics*. 2016; 17:252. [PubMed: 27004515]
77. Carter D, Chakalova L, Osborne CS, Dai YF, Fraser P. Long-range chromatin regulatory interactions in vivo. *Nat Genet*. 2002; 32:623–626. [PubMed: 12426570]
78. Walczak A, Szczepankiewicz AA, Ruszczycki B, Magalska A, Zamlynska K, Dzwonek J, Wilczek E, Zybura-Broda K, Rylski M, Malinowska M, et al. Novel higher-order epigenetic regulation of the *Bdnf* gene upon seizures. *J Neurosci*. 2013; 33:2507–2511. [PubMed: 23392678]
79. Dhar SS, Ongwijitwat S, Wong-Riley MT. Chromosome conformation capture of all 13 genomic Loci in the transcriptional regulation of the multisubunit bigenomic cytochrome C oxidase in neurons. *J Biol Chem*. 2009; 284:18644–18650. [PubMed: 19439416]
80. Dhar SS, Wong-Riley MT. Chromosome conformation capture of transcriptional interactions between cytochrome c oxidase genes and genes of glutamatergic synaptic transmission in neurons. *J Neurochem*. 2010; 115:676–683. [PubMed: 21064266]
- **81. Crepaldi L, Policarpi C, Coatti A, Sherlock WT, Jongbloets BC, Down TA, Riccio A. Binding of TFIIC to sine elements controls the relocation of activity-dependent neuronal genes to transcription factories. *PLoS Genet*. 2013; 9:e1003699. This study provides some of the first evidence that neuronal activity-dependent transcription is correlated with spatial relocalization of the IEGs *c-fos* and *Gadd45b* to transcription factories, providing a link between activity-dependent chromatin rearrangements and transcription. [PubMed: 23966877]
- **82. Suberbielle E, Sanchez PE, Kravitz AV, Wang X, Ho K, Eilertson K, Devidze N, Kreitzer AC, Mucke L. Physiologic brain activity causes DNA double-strand breaks in neurons, with exacerbation by amyloid-beta. *Nat Neurosci*. 2013; 16:613–621. This study provides the first evidence that physiological neuronal activity induces DNA double-strand breaks in neurons of relevant brain regions. [PubMed: 23525040]
83. Bunch H, Lawney BP, Lin YF, Asaithamby A, Murshid A, Wang YE, Chen BP, Calderwood SK. Transcriptional elongation requires DNA break-induced signalling. *Nat Commun*. 2015; 6:10191. [PubMed: 26671524]
84. Ju BG, Lunyak VV, Perissi V, Garcia-Bassets I, Rose DW, Glass CK, Rosenfeld MG. A topoisomerase IIbeta-mediated dsDNA break required for regulated transcription. *Science*. 2006; 312:1798–1802. [PubMed: 16794079]
85. Gorbunova V, Seluanov A. DNA double strand break repair, aging and the chromatin connection. *Mutat Res*. 2016; 788:2–6. [PubMed: 26923716]
- *86. Wei PC, Chang AN, Kao J, Du Z, Meyers RM, Alt FW, Schwer B. Long Neural Genes Harbor Recurrent DNA Break Clusters in Neural Stem/Progenitor Cells. *Cell*. 2016; 164:644–655. Using high-throughput genome-wide translocation sequencing (HTGTS), this study demonstrates that neural stem/progenitor cells exhibit recurrent DNA DSBs in specific gene loci that are important for neural circuit formation and are often mutated in neurodevelopmental disorders and cancer. This supports previous findings that cells of the neural lineage harbor localized DSBs that may be relevant to neurodevelopment, learning and memory. [PubMed: 26871630]

87. Schwer B, Wei PC, Chang AN, Kao J, Du Z, Meyers RM, Alt FW. Transcription-associated processes cause DNA double-strand breaks and translocations in neural stem/progenitor cells. *Proc Natl Acad Sci U S A*. 2016; 113:2258–2263. [PubMed: 26873106]
88. Gregor A, Oti M, Kouwenhoven EN, Hoyer J, Sticht H, Ekici AB, Kjaergaard S, Rauch A, Stunnenberg HG, Uebe S, et al. De novo mutations in the genome organizer CTCF cause intellectual disability. *Am J Hum Genet*. 2013; 93:124–131. [PubMed: 23746550]
89. McKinnon PJ. Maintaining genome stability in the nervous system. *Nat Neurosci*. 2013; 16:1523–1529. [PubMed: 24165679]

Highlights

- Epigenetic regulation represents a key mechanism of learning, memory and cognition
- Chromatin topology is emerging as a major regulator of neuronal gene expression
- Dynamic chromatin topology changes correlate with activity-dependent transcription
- DNA double-strand breaks facilitate induction of immediate early gene transcription

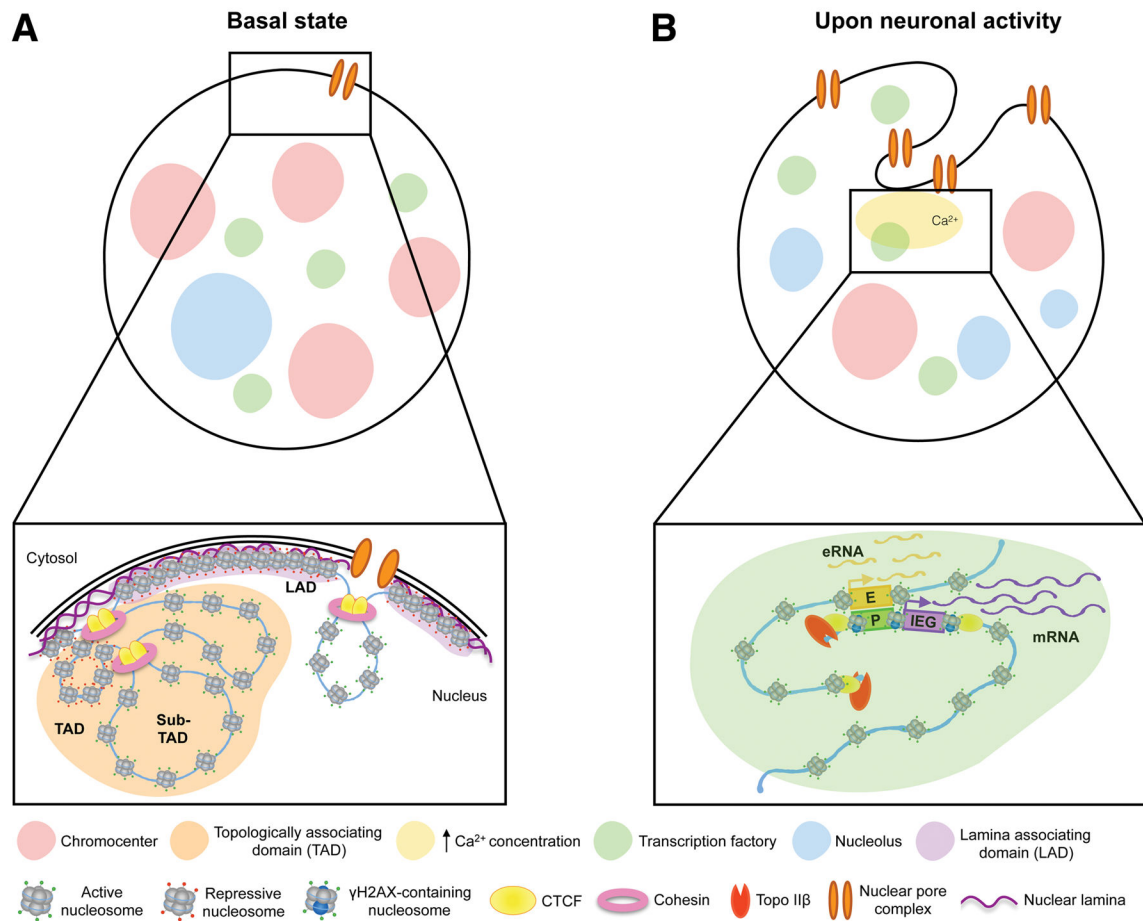


Figure 1. Neuronal activity is associated with alterations in nuclear geometry, subnuclear domains, and chromatin topology

(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 relocation of IEG loci to transcription factories and transcription of eRNAs (inset). Moreover, activity causes Topo II β -mediated DNA double strand break (DSB) formation at the promoter of specific immediate early genes (IEGs), resulting in γ H2AX enrichment across the gene body and target mRNA transcription (inset).