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Spatial Genome Organization and Cognition

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Abstract

Non-random chromosomal conformations, including promoter--enhancer loopings that bypass kilo- or megabases of linear genome, provide a critical layer of transcriptional regulation, and move vast amounts of non-coding sequence into the physical proximity of genes that are important for neurodevelopment, cognition and behavior. Activity-regulated changes of the neuronal '3D genome' could govern transcriptional mechanisms associated with learning and plasticity, and loop-bound intergenic and intronic non-coding sequences have been implicated in psychiatric and adult-onset neurodegenerative disease. Recent studies have begun to clarify the roles of spatial genome organization in normal and abnormal cognition.

INTRODUCTION

Comprehensive exploration of the functional organization of the human genome has to go far beyond linear sequencing of 6 billion base pairs. Although high resolution mapping of the various constituents of the epigenome, including DNA methylation and histone modifications, has enabled the 'linear' genome to be understood in two dimensions, this does not account for the spatial configuration and packaging of interphase chromosomes. Despite the importance of the spatial organization of chromosomal material in the regulation of gene expression, the maintenance of genome integrity and stability, and the control of growth and differentiation, surprisingly little is known.

According to some estimates, up to 40% of the human genome is epigenomically decorated with various types of histone modifications and DNA methylation, localized transcription factor complexes or enrichment with chromatin scaffolding proteins¹. By contrast, only ~1.5% of genome sequence encodes protein. Therefore, in addition to knowledge of the genome and epigenome, mapping the 3D genome in neurons and glia is essential for a full understanding of how genes are regulated and expressed. Such an understanding could enable identification of novel distal regulatory elements and in turn, to develop an

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understand of how these elements assemble in 3D to ‘bypass’ the linear genome and regulate gene expression.

Early findings from a select set of candidate gene loci indicate that chromosomal contacts and ‘loopings’ could be heavily regulated by neuronal activity, suggesting that the 3D genome plays a part in activity-dependent regulation of gene expression in brain cells. In addition, studies on a small number of candidate genes indicate that loop-bound non-coding DNA contributes to the genetic risk architecture of cognitive disease with onset in early childhood or young adulthood, including autism² and schizophrenia³. Of note, deleterious mutations in genes encoding regulators of chromosomal scaffolding severely impact brain development and function, further underscoring that proper packaging and organization of the genomic material inside the nuclei of brain cells is of pivotal importance.

Advances in epigenomic editing techniques are now being developed that enable neuronal or glial control of transcriptional units, including genes, to be manipulated artificially by placing transcriptional activators at the side of regulatory sequences that are separated from their target genes by many thousands of base pairs. Therefore, loop-bound regulatory sequences could be harnessed to modulate expression of disease relevant genes without interfering with basal transcription. In this review, we will briefly introduce the key concepts of the spatial genome and the experimental approaches used to study it. We then discuss recent developments that have fueled the growing interest in exploring the spatial organization of chromatin fibers and chromosomes in brain cells.

The 3D genome and its Constituents

Eukaryote nuclei, separated by a nuclear membrane from the cytoplasm, contain the genome packaged into chromatin fibers as nucleosomal arrays (Figure 1). Nucleosomes are comprised of 146bp DNA wrapped around a core histone octamer, and interconnected by linker DNA and linker histones. Chromatin can exist in different ‘states’, including ‘open’ (eu-) and condensed (hetero-) chromatin. These are differentially defined by three characteristics: (1) loose or dense nucleosomal packaging euchromatin or heterochromatin, respectively), (2) specific types of post-translational histone modifications (such as acetylation), and (3) presence or absence of various chromatin regulatory proteins that either facilitate or repress transcription. For example, actively expressed genes in open chromatin show high levels of histone acetylation, with nucleosome-free intervals occupied by activator proteins (transcription factors) and the RNA polymerase II initiation complex (Figure 1). Superimposed upon these types of nucleosomal organization is the 3-dimensional conformation of chromatin fibers and entire chromosomes, often described in terms such as ‘loopings’ or ‘globules’ and *in toto* referred to as the ‘3D genome’. This includes the ‘clustering’ of euchromatic and heterochromatic sequences that tend to assemble into alternating regions of approximately ~5 megabases (Mb). These ‘compartments’, positioned along the same chromosome, are able to interact with compartments from different chromosomes⁴. Euchromatic regions are termed ‘A compartments’ and are enriched with open/decondensed chromatin and correspond to much higher overall levels of transcription, whereas ‘B compartments’ harbor inactive and heterochromatic sequences⁵ (Figure 1). In

most cell types, large clusters of heterochromatin are enriched at the nuclear periphery, in multiple pericentromeric foci in the nuclear interior and around nucleolar membranes⁶.

Scaffolding and other regulatory proteins play a crucial role in the chromosomal spatial conformations, and a prime example of this is the cohesin complex and the CCCTC-binding factor/zinc finger protein CTCF, where accessory proteins load or release the cohesion complex onto chromosomes⁷. In humans, cohesins are comprised of five core subunits SMC1, SMC3, RAD21/REC8, and STAG1-3⁷. Cohesins were initially explored in the context of sister chromatid cohesion and segregation during cell division (mitosis)⁷. However, these proteins continue to be present at high levels in the nuclear proteome of postmitotic cells including neurons⁸. Cohesins form ring-like structures, literally entrapping distant chromatin fibers into chromosomal loopings⁷ (Figure 1). Cohesins are highly enriched at actively expressed genes in a tissue- and cell-type specific manner⁷. By contrast, CTCF, although dispensable for cohesin loading onto DNA, orchestrates cohesin enrichment at select binding sites⁸. As a result, chromosomal loopings co-occupied by cohesins and CTCF at both ends often associate with broader stretches of regulatory domains at other parts of the chromosome, marking the co-regulated repression or expression of groups of genes in a cell-type specific manner⁹. The CTCF binding sites often are positioned in inward and/or convergent orientation, and to a lesser degree, tandem orientation, at the two contact sites of the loop^{5,10}. CTCF directionally recognizes binding sites via an 11 zinc finger array. Cohesin, in turn, is assembled from the CTCF's C-terminal end, resulting in loop-bound head-to-head CTCF configurations¹¹.

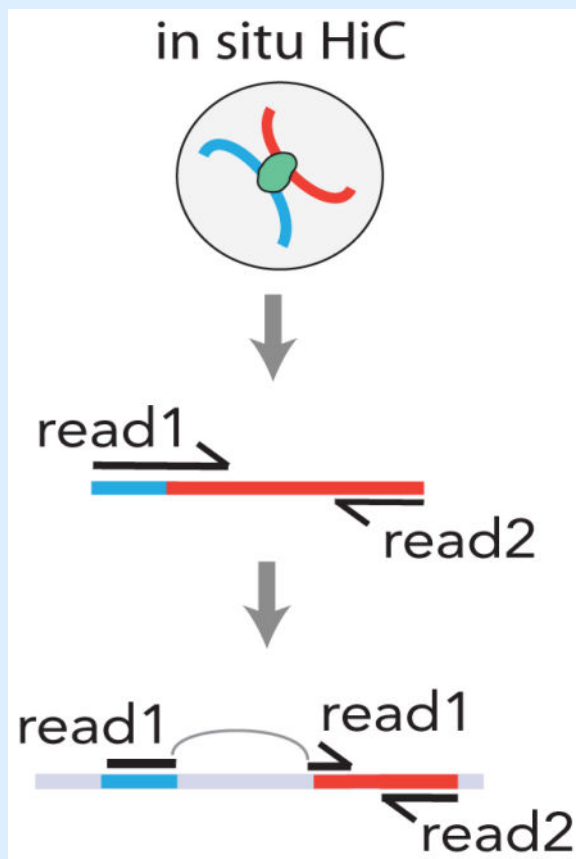
Importantly, powerful sequencing technologies in conjunction with chromosome conformation capture assays (Textbox 1, with Figure 4 in textbox) make it possible to map chromosomal contacts on a genome-wide scale, and have provided vast amounts of information on intra- and interchromosomal interactions⁵. The functional implications thereof, however, remain to be explored.

Textbox 1

Charting the 3D genome—methods and challenges

The most widely used approach to chart contacts and physical proximity of non-contiguous DNA is chromosome conformation capture (termed 3C). Intact chromatin undergoes enzyme-based restriction digest followed by enzymatic fusion of the cut DNA end with DNA ligase. This results in chimeric reads if the DNA molecule is a fusion product of (previously) two DNA molecules that each map to a different locations in the genome with different coordinates either on the same or on a different chromosome. The 3C technique is scalable, up to comprehensive mapping on a genome-wide scale ('Hi-C')⁵⁴ (Figure). The most advanced protocols can be applied to intact nuclei ('in situ Hi-C'), and allow for an incredibly high resolution (~1kb) mapping of chromosomal contacts. However, this requires cost-intensive DNA libraries sequencing at significant depth, or at least five billion reads (number of DNA molecules sequenced) per assay⁵. Not all interactions are functionally relevant and polymer features of chromatin fibers could influence to some degree the interactions between loci, in the absence of biological regulation^{115,116}. Moreover, systematic comparison of 3C derivatives with DNA

fluorescence in situ hybridization (DNA FISH), a traditional low throughput method measuring proximity of candidate genomic loci at single cell resolution, has uncovered additional confounds¹¹⁷. This is because seemingly subtle differences in chromosome conformation capture protocols and downstream bioinformatical analyses of the Hi-C data have a major effect on the quantity and quality of chromosomal contacts observed. As a result, there is still debate whether the number of ‘loopings’ (distinct contacts between non-contiguous DNA) in a typical vertebrate cell is in the order of 1×10^4 ⁵, or perhaps as many as 1×10^6 ²¹. These challenges, while not diminishing the potential of chromosome conformation capture assays to fundamentally advance knowledge in virtually all areas of genomics, clearly emphasize the importance of additional analyses and functional validation, after investigators have charted the 3D genome by chromosome conformation capture.



Promoter-enhancer loopings

Promoter-enhancer loopings, considered to be of fundamental importance for transcriptional regulation in eukaryotes, are one type of chromosomal interaction that is becoming increasingly well understood. Promoters are commonly defined as cis-regulatory sequences within 1000 base pairs from the next gene transcription start site. By contrast, enhancers are a type of cis-regulatory sequence positioned $>1\text{kb}$ from the nearest transcription start site¹². Promoters (but not enhancers) typically include a core promoter as docking site for general

transcription factors (TFIIA/B/D/E/F/H) and components of RNA polymerase II holoenzyme as part of the preinitiation complex¹². These core promoters drive low levels of basal transcription. However, gene expression is heavily stimulated by ‘activators’ or transcription factors that bind, in sequence-specific fashion, at the site of promoters and enhancers¹². The transcription factors bind to promoters and enhancer sequences in nucleosome-free intervals in open chromatin and recruit co-activator complexes, such as Mediator and CREB-binding protein (CPB)/p300¹². Promoters, in contrast to enhancers, are often CpG rich¹². Furthermore, on a genome-wide scale, promoters and enhancers show robust differences in their histone modification landscapes. These include, for example, sharp peaks of trimethyl-histone H3-lysine 4 and enrichment of mono-methyl-histone H3-lysine 4 at enhancers¹³. Each form of H3-lysine 4 methylation is thought to bind a different set of methyl-reader proteins and transcriptional regulators¹⁴. However, enhancers are separated from their target gene by many kilo- or even megabases of interspersed linear genome¹². Various mechanisms have been proposed by which enhancer chromatin could regulate the expression of target genes from distant chromosomal locations. Such types of mechanisms include sliding along the chromosome to ‘track’ promoters¹⁵, or alternatively, a physical ‘bridge’ built via protein-protein interactions¹⁶. Presently, however, most studies implicate the promoter-enhancer (chromosomal) loop model which involves physical interaction, or at least spatial proximity, between the enhancer chromatin and the promoter target¹⁷.

Recently, an important role in the shaping of promoter-enhancer loopings has been ascribed to Mediator, a multi-subunit complex acting as co-factor for many transcription factors¹⁸. Mediator loads cohesins onto chromatin fibers, potentially promoting loop formations in self-organizing fashion¹⁸. According to this model, transcription factors that recognize specific DNA motifs bind to specific genome sequences, in turn attracting the Mediator complex. This recruitment subsequently promotes the build-up of the ring-shaped cohesin complex at the site. This protein ring structure thus could promote and stabilize promoter-enhancer loopings (Figure 1).

Activity-regulated Chromosomal Contacts

Dynamic remodeling of neuronal connectivity, both in terms of structural plasticity (e.g. dendritic spines and axonal branching) and functional plasticity (e.g. changes in synaptic strength) is fundamentally important for normal brain development, learning, and memory. In the nucleus, promoter-enhancer interactions are likely to play an important role for these types of plasticity. To mention just one example, postnatal differentiation and functional maturation of cerebellar granule neurons is associated with dynamic changes in chromatin accessibility and levels of histone acetylation at thousands of putative enhancer sequences¹⁹. It is noteworthy that in differentiated tissues, including the brain, perhaps as many as 60% of all promoters and transcription start sites could be under control by multiple enhancer sequences^{20–22}. Indeed, pharmacologically induced membrane depolarization produces transcriptional changes in neurons that are associated with highly dynamic changes in chromosomal loopings at selected loci^{23–25}, which supports a role for these mechanisms in neuronal plasticity. However, in the absence of genome-wide chromosome conformation studies, it remains unknown to what extent these mechanisms operate more broadly across

the genome. Not all developmental stimuli trigger reorganization of the 3D genome because human fibroblasts exposed to Tumor Necrosis Factor alpha (TNF α), a cytokine with profound effects on growth and proliferation, do not show widespread changes in their chromosomal contact map²¹.

Promoter—enhancer interactions and plasticity

Based on recent studies, four models have been proposed for how promoter-enhancer interactions might be involved in neuronal plasticity. In the first model (Figure 2A), distal sequences carrying a cargo of transcription factors are moved into close spatial proximity to the target gene promoter. This mechanism has been proposed for enhancer loopings targeting the transcription start site of NMDA receptor subunits²³ or GABA synthesis genes²⁴. In this model, expression of a subset of immediate early gene (IEG) transcription factors is under tight control of enhancer elements, which rapidly assemble into chromosomal loopings upon synaptic stimulation²⁵. This process is combined with localized transcription from enhancer elements -- ‘enhancer RNAs’ (eRNAs) and is required for activity-induced expression of the *c-fos* IEG²⁵. According to these studies, promoter-enhancer loopings mediate the ‘re-location’ of enhancer-bound transcription factor protein (the ‘cargo’) towards activity-regulated neuronal gene promoters, thereby facilitating the transcriptional process^{24,25} (Figure 2A).

An entirely different mechanistic model ascribes a decoy function to the eRNAs, in order to liberate the target promoter from negative regulators of transcription. This model emerged from the study of loop-bound enhancer elements governing the transcription of *Activity-regulated cytoskeletal* (*Arc*), which encodes a small protein that is important for numerous aspects of synaptic plasticity²⁶. In this system, loop-bound enhancer DNA produces short-lived non-coding RNAs that bind the negative elongation factor (NELF) complex away from *Arc* promoter sequences²⁷. Because NELF functions as an inhibitor of transcription, the small RNAs produced by the *Arc* enhancer essentially act as a ‘sponge’ for NELF. As a result, NELF occupancy decreases at the *Arc* gene and paused RNA polymerase II complex, previously stalled by NELF, now becomes unlocked and is rapidly ramping up production of *Arc* RNA²⁷. Therefore, enhancer-mediated localized production of an RNA decoy for negative regulators of RNA polymerase II could temporarily disinhibit target gene expression²⁷ (Figure 2B).

Additional types of promoter-enhancer interactions are variations of the transcription factor protein cargo model put forth in Figure 2A. One example is competition between loop-bound enhancer and repressor sequences (Figure 2C). Furthermore, Figure 2D summarizes a recently described mechanism for shorter-range promoter-enhancer loopings extending a few kilobases from the transcription start site of the IEG and transcriptional regulators *Fos*, *FosB*, *Npas4*, *Egr1*, *Nr4a1* and *Nr4a3*²⁸. These IEG promoters, which show increased expression within minutes after neuronal stimulation, are positioned close to regulated DNA strand breaks induced by type II DNA topoisomerase²⁸. This effectively increases the mobility of the local cis-regulatory sequences, including shorter-range chromosomal loop formations enriched with CTCF protein. As a result, there is an increased interaction of promoters with neighboring enhancers²⁸ (Figure 2D).

With an estimated total of ~12,000 neuronal activity-regulated enhancers, and eRNA expression at ~2,000 extra- and ~1,000 intra-genic activity-regulated enhancers in cortical neurons²⁹, it is likely that neurons use a wide array of chromosomal conformations and loop structures to orchestrate gene expression programs in response to changes in synaptic activity.

The 3D Genome in neurodevelopmental disorders

Mutations in chromosomal scaffolding proteins—It seems beyond doubt that genome folding and packaging are crucial for normal brain development and function. To this end, the nuclear lamina, a protein meshwork located at the inner layer of the nuclear membrane and primarily comprised of three types of filamentous protein (lamin A/B/C) could play an important role by interacting with a wide range of repressive chromatin regulators and thereby tethering heterochromatic sequences to the nuclear periphery^{6,30}. Such types of lamina-associated heterochromatic chromosomal domains become surprisingly mobile during the course of differentiation of neural precursor cells into astrocytes³¹. As a result, many hundreds of genes undergo regulated repositioning during the course of astrocyte differentiation. By moving into or out of the nuclear lamina, these genes are thought to participate in transcriptional programs associated with astrocyte cell identity³¹. Similar phenomena have been observed during neuronal differentiation. For example, a subtype among the olfactory receptor gene family, the trace amine-associated receptors, undergo mono-allelic dissociation from lamin proteins and the nuclear periphery during the course of olfactory sensory neuron differentiation³². Early studies suggest that by relocating to inner compartments of the nucleus, olfactory receptor genes could encounter localized accumulations of transcription factors and an open chromatin environment that facilitates promoter-enhancer interactions and gene expression^{32, 33}.

In this context, it is noteworthy that deleterious mutations in genes encoding scaffolding proteins for the 3D genome have been linked to disease. These include not only neurodevelopmental disorders such as Cornelia de Lange Syndrome (CdLS)^{7, 34, 35}, but also adult-onset progressive demyelination syndromes³⁶ (Table 1). Neurodevelopmental disease phenotypes in CdLS include intellectual disability, psychosis and other psychiatric maladies. The underlying genetic defect includes microdeletions and copy number variations affecting core members of the cohesin complex including *SMC1A* and *SMC3*, and the accessory subunit *NIPBL*³⁴ (Table 1). The neurological manifestations could be due to 3D genome disorganization in brain cells and de-compaction of chromatin, however, the precise molecular mechanisms remain to be elucidated³⁷.

Genetic mutations in *CTCF*, as a key organizer for chromosomal loopings, have been linked to monogenic causes of microcephaly and cognitive disorder^{38,39}. Consistent with these findings from clinical genetics, selective ablation of *Ctcf* in postnatal mouse brain causes behavioral alterations and dysregulated transcription of hundreds of neuronal transcripts. These include a deficit in Protocadherin cell adhesion molecule expression, resulting in altered connectivity⁴⁰. It remains to be shown, however, whether the neurological manifestations of cohesin or *CTCF* gene mutations are associated with widespread 3D genome alterations in brain.

In addition to the aforementioned CTCF and cohesin complex, other examples of neurodevelopmental disease resulting from mutations in genes encoding 3D genome organizer proteins have been identified (Table 1). These include *Special AT-rich Sequence Binding Proteins 1 and 2* (*SATB1*, *SATB2*) that govern chromosomal territories extending across hundreds of kilobases⁴¹ and anchor chromatin fibers into the nuclear matrix⁴². Of note, *SATB2* is essential for craniofacial development and proper differentiation of transcallosal cortical projection neurons^{43,44}. The gene has also been linked to some cases of Glass Syndrome (*OMIM 612313*) and mental retardation^{43,45}. The related protein *SATB1* is essential for connectivity and maturation of GABAergic interneurons in the cerebral cortex^{46,47}. Furthermore, proteins encoded by the X-linked *ATRX*, and the Rett syndrome gene *MECP2* (*ATRX*, *OMIM* #301040; *MECP2*, *OMIM* #312750), regulate looping structures at a subset of imprinted (parent-of-origin-specific) loci including *H19/Igf2* and *Dlk1/Gtl2*⁴⁸. In addition, the *ATRX* and *MECP2* proteins regulate higher order chromatin at the *Dlx5/Dlx6* homeobox transcription factor locus⁴⁹ (but see also⁵⁰). Moreover, *ATRX* is recruited by *MECP2* to heterochromatic sequences⁵¹. However, because these proteins regulate a multitude of transcriptional and chromatin-associated functions inside the cell nucleus⁵², the precise role of chromosomal contacts and loopings in neurodevelopmental disorders with *ATRX* or *MECP2* mutations remains to be elucidated.

Non-coding sequences implicated in autism—In the preceding chapter, we discussed examples of neurological disease caused by mutations or duplications of genes encoding protein scaffolds for the 3D genome. Here, we discuss a second type of brain disorder that is associated with locus-specific alterations of the 3D genome. A recent whole genome sequencing (WGS) study in families on the autism spectrum identified individuals with autism who did not carry disruptive mutations and copy number variations in known neurodevelopmental risk genes⁵³. Instead, these cases carried deletions or duplications in non-coding DNA regions that in control samples were sensitive to digestion by DNase I⁵³ (note that DNase I is an indicator for nucleosome-free ('naked') DNA and a widely used method for transcription factor footprinting). This would imply that chromatin around these sequences is involved in transcriptional regulation elsewhere in the genome⁵³. These cases showed, as a group, significant enrichment for microdeletions in blocks of sequence 25–100kilobase (kb) upstream (i.e. in the regulatory non-coding region) of genes assigned with critical roles for normal human brain development. These genes included *DSCAM*, encoding Down Syndrome Cell Adhesion Molecule, and *SCN2A* encoding a sodium voltage-gated channel subunit implicated in autism and seizure disorder⁵³. Indeed, these non-coding sequences with apparently high disease penetrance are primarily located within 25–100kb from the 5' end of genes associated with autism and related disease⁵³. On a genome-wide scale, at least one half of all chromosomal loop formations targeting gene transcription start sites extend across 25–150kb (albeit some promoter-enhancer loopings can extend far beyond that range)^{20,21,54} and therefore, it is possible that some of the autism-associated non-coding sequence mutations and deletions⁵³ result in altered 3D regulation of neurodevelopmental risk genes. Similar principles may apply to other types of neurological disease. For example, aganglionic megacolon (hypertrophy and dilation of the colon) in *TashT* mutant mice, a model for Hirschsprung's disease, is caused by a 700kb transgenic insertion into a gene desert extending across 3.3 Mb. The insertion disrupts

silencer elements governing the expression of multiple target genes via long-range intrachromosomal loopings⁵⁵.

The above mentioned deletions and mutations in intergenic non-coding sequence of specific individuals diagnosed with autism⁵³ are in line with the ‘major gene’ model, which assumes either a single highly or a limited number moderately penetrant mutations in the etiology of autism⁵⁶. Other disease cases may be better defined by the polygenic risk model, implying many inherited common variants in genetic susceptibility, each with small effect⁵⁶. The majority of this type of risk-associated DNA single nucleotide polymorphisms and haplotypes do not directly affect coding sequence and protein structures⁵⁶. There is indirect evidence that some of these risk-associated non-coding polymorphisms involve chromosomal loop formations. For example, brain-specific enhancer RNAs (eRNAs), which are short non-coding RNAs transcribed in *cis* at the site of active enhancer elements, are significantly enriched for genetic variants associated with autism spectrum disorder⁵⁷. Because active enhancers are likely to positively regulate transcription of distant target genes via loop-bound chromosomal conformations, it is plausible to speculate that at least a portion of autism-associated DNA polymorphisms⁵⁷ affect regulation of neuronal or glial 3D genomes.

Adult-onset neuropsychiatric disease—In addition to the an involvement of juvenile-onset neurological disorders, there is also evidence suggesting a role for the 3D Genome in adult-onset human cognitive disorders. For example, significant over-representation of enhancer sequences has been observed within the pool of polymorphisms and haplotypes associated with schizophrenia, an adult onset cognitive disorder often accompanied by delusions and hallucinations^{3,58}. Among such type of risk-associated sequences are enhancer elements in an intragenic and intronic portion of the *CACNA1C* calcium channel gene. This gene also ranks prominently in the polygenic risk maps of other common psychiatric disease including depression⁵⁹. Risk alleles within the *CACNA1C* enhancer sequences have also been associated with decreased reporter gene activity³. Remarkably, these *CACNA1C* intragenic enhancer sequences were found to be physically bound to the gene transcription start site via a 185kb spanning chromosomal loop formation in human prefrontal cortex (Table 2)³.

Other studies have suggested that alterations in DNA methylation and other types of epigenetic dysregulation of enhancer sequences could contribute to the neuropathology underlying mood and psychosis spectrum disorders. For example, astrocyte dysfunction in depression⁶⁰ and altered neuronal gene expression in schizophrenia^{61,62} have been linked to dysregulated enhancers.

Likewise, common sequence variants conferring genetic risk for Alzheimer’s neurodegeneration show strong overlap with mammalian enhancers and other cis-regulatory elements previously implicated in immune functions⁶³. In an animal model of Alzheimer’s disease which involves hippocampal injury triggered by the Cdk5 kinase activator protein p25, m, any enhancer sequences harboring Alzheimer’s risk variants became activated⁶³. Furthermore, it was recently reported that heterochromatic compartments are disorganized in neuronal nuclei of Alzheimer’s cerebral cortex collected postmortem, accompanied by with

improper re-expression of the normally silenced genes^{64,65}. It is reasonable to speculate that the underlying molecular mechanisms involve reorganization and reactivation of enhancer-bound loop formations.

These findings, taken together, point to a strong representation of enhancer elements among the genetic and epigenetic risk architectures of some of the common neuropsychiatric disorders, including autism, schizophrenia, depression, and Alzheimer's disease. The field now eagerly awaits studies undertaking comprehensive, genome-scale (Hi-C) maps from brain tissue of diseased subjects⁶⁶. 3D genome and higher order chromatin mapping is technically feasible in postmortem brain tissue⁶⁷ and this should clarify whether chromosomal loop formations that 'recruit' enhancer sequences in the context of transcriptional regulation are dysregulated on a genome-wide scale. Chromosome conformation capture studies performed at specific candidate gene loci already have indicated that the conformation of the 3D genome might be altered in cognitive disorders (Table 2). For example, a 50kb loop formation connecting enhancer elements enriched with IEG transcription factor motifs into physical proximity of the *GADI/GAD67* GABA synthesis gene promoter, is disrupted in prefrontal cortex of subjects with schizophrenia²⁴. These alterations in the *GADI* chromatin loop occurred in conjunction with decreased gene expression and deficits in open chromatin-associated histone H3-lysine 4 methylation^{24,68}. Given that dysregulated *GADI* expression contributes to disorganized inhibitory circuitry and altered synchronization of cortical networks in psychosis^{69,70}, the reported alterations in *GADI* higher order chromatin²⁴ illustrate the importance of proper 3D genome regulation for human cognition.

Additional examples of chromosomal conformations of potential importance for human cognition exist. For example, long range (0.5–1Mb) contacts within chromosomes *2q14.1* and *16p11.2*⁷¹. Interestingly, these loop formations connect regulatory sequences defined by human-specific histone methylation 'peaks' that are absent from non-human primate brain (Table 2)⁷¹. This finding hints at the possibility that some of the cognitive abilities (and vulnerabilities) unique to human are associated with specific configurations in the 3D genome of cortical neurons⁷¹.

Dynamic NMDA receptor gene loopings affect cognition—In this section, we focus on a single gene locus, or ~1Mb of human (mouse) chromosome 12p31.1 (6, 66.36cM) encompassing the N-methyl-D-aspartate (NMDA) receptor subunit *GRIN2B*. This locus has been particularly well studied in the context of chromosomal contacts and conformations, and it provides an interesting example for the evolving view on the highly dynamic and multilayered regulation of the 3D genome as pertains to cognition and complex behavior. Furthermore, deleterious *GRIN2B* mutations rank prominently in exome sequencing studies on monogenic forms of intellectual disability, epilepsy, autism and psychosis^{72–75}, which underscores the importance of this gene for human cognition. Until recently, however, little was known about the role of non-coding DNA at the *GRIN2B* locus in health or disease. Here, we review studies on *GRIN2B* higher order chromatin and chromosomal contacts in human postmortem brain and animal models, including the complex roles of loop-bound non-coding DNA in the regulation of NMDA receptor gene expression and cognition.

Chromosome conformation studies in human and mouse prefrontal cortex identified multiple loop-bound intronic and intergenic DNA sequences, up to 450kb downstream from the *GRIN2B/Grin2b* transcription start site. These sequences were loaded with multiple transcription factors and interacted via loop formations with the *GRIN2B/Grin2b* promoter and transcription start site²³. In addition to such types of long-range promoter-enhancer loopings, the *GRIN2B/Grin2b* promoter physically interacts with intragenic repressive chromatin embedded in intron sequence²³. These repressive elements were defined by high levels of SETDB1 histone H3K9 methyltransferase and heterochromatin-associated protein 1 (HP1)²³. Therefore, it was proposed that transcriptional regulation at the *GRIN2B/Grin2b* locus involves a dynamic and competitive interplay of multiple loop formations, each of which could engage with the *GRIN2B* promoter²³. These include facilitative loopings with CTCF loop organizer protein bound at the loop contacts. The role of these loopings is to deliver IEGs and additional types of transcription factors to the *GRIN2B* promoter²³ (Figure 2C, Figure 3). However, this process is counterbalanced by promoter-bound higher order chromatin involving repressive intronic sequences 30kb downstream from the transcription start site^{23,76} (Figure 3). A subset of these chromosomal conformations were at least partially conserved in human and mouse brain and were sensitive to changes in neuronal activity²³.

Interestingly, multiple loop-bound sequences interacting with the *GRIN2B* promoter harbor single nucleotide polymorphisms (SNP) previously implicated in determining working memory efficiency²³. These included SNP rs117578877, positioned in a 450kb loop connecting the *GRIN2B* 5' end to intergenic DNA downstream from the gene's 3' end. Indeed, this polymorphism could contribute to risk for schizophrenia and personality traits associated with schizophrenia²³. Notably, the risk-associated SNP allele was associated with decreased nucleoprotein binding and motif loss for the CCAAT/Enhancer Binding Protein CEBPB (C/EBP β)²³. CEBPB is a transcription factor implicated in consolidation of cortical and hippocampal learning and memory⁷⁷⁻⁷⁹. Because many enhancer elements are defined by sequential linear alignment of multiple transcription factors within short distance^{80,81}, additional activator proteins may synergistically cooperate with loop-bound CEBPB to regulate *GRIN2B* expression²³. Taken together, these findings point to a complex and multilayered regulation of chromosomal conformations within 1Mb surrounding the *GRIN2B* gene. Therefore, loop-bound DNA targeting the *GRIN2B* promoter could either facilitate or repress expression, depending on the protein 'cargo' (Figure 3). Multiple distal loop formations compete in a highly dynamic and activity-dependent manner for access to the *GRIN2B* promoter sequences²³. It remains to be explored whether these findings from the *GRIN2B* locus also apply to other neuronal genes. It is possible, therefore, that neuronal (and glial) 3D genomes are defined by thousands of dynamically regulated chromosomal conformations that are poised to quickly re-configure in response to synaptic signaling and other stimuli.

Chromosomal Conformations – A Novel Therapeutic Target?

A number of chromatin modifying drugs have shown promising therapeutic effects in preclinical models of cognitive disease. These include histone deacetylase inhibitors and other drugs altering the balance between acetylation and deacetylation⁸²⁻⁸⁵, histone

methyltransferase inhibitors⁸⁶, topoisomerase inhibitors⁸⁷, and compounds targeting the DNA methylome^{88,89}. However, such types of drugs alter chromatin structure and function across widespread areas of the genome. Unsurprisingly then, both global and locus specific alterations in chromosomal conformations and spatial genome organization have been reported for small molecule drugs interfering with histone acetylation^{90,91} or methylation⁹². For example, exposure to histone deacetylase inhibitor drug, trichostatin A, is associated with repositioning of expressed genes towards the nuclear center and away from repressive environment such as the nuclear lamina^{90,91}. Furthermore, drug-induced inhibition of the repressive H3K9 methyltransferase, G9a, induced a spatial reconfiguration of the β -*globin* gene cluster in hematopoietic cells, thereby shifting expression pattern towards the fetal γ -globin genes⁹². Much of this work, however, was limited to cell lines. It remains to be explored whether drug-induced changes in 3D genome organization could be harnessed for therapeutic interventions in brain disorders. Moreover, the safety profile for broadly acting chromatin-modifying drugs remains unknown in the context of neuropsychiatric disease⁹³.

Recently, a novel perspective has been provided by an expanding repertoire of epigenomic editing tools that include the possibility for targeted, locus-specific interventions. For example, engineered zinc finger proteins (Zfp) were fused to the p65-NF κ B domain to recruit p300/CBP histone acetyltransferase and targeted to highly specific 18–20bp sequence motifs. This approach was used successfully to specifically activate the promoters of *glial-derived neurotrophic factor* (*Gdnf*) and *FosB/fosb* transcription factor in striatal neurons *in vivo*^{94,95}. These interventions provided robust neuroprotective effects (*Gdnf*)⁹⁴ and produced changes in reward- and depression-related behaviors (*FosB*)⁹⁵, thus confirming the feasibility of *in vivo* epigenomic editing in a preclinical model. However, *in vivo* epigenomic editing of loop-bound sequences has not yet been reported in brain. To this end, there is promising evidence from *in vitro* studies. For example, in primary neuronal culture, loading the VP64 transcriptional activator onto loop-bound distal regulatory elements bypassing 475kb of linear genome resulted in increased transcription of the *Grin2b* NMDA receptor subunit²³. Although the resulting changes in *Grin2b* expression were very modest²³, further technical improvements may deliver more robust effects. For example, a p300 histone acetyltransferase catalytic core fused to various designer DNA binding proteins, including Zfp, Transcription Activator-like Effector (TALE) or RNA-guided CRISPR/Cas could be used to regulate expression via loop-bound long range enhancer and repressor sequences⁹⁶. These epigenomic editing approaches will complement genetic studies that introduce small^{97,98} or large⁹⁹ genetic lesions of loop-bound DNA. The technique allows for correction of genetic mutations^{98,100} and testing the resulting effects on target gene transcription^{101–104}. Epigenomic editing approaches, in conjunction with *ex vivo* cell culture work, will be required to test causality and to develop therapeutic interventions based on loop-bound enhancer and other regulatory DNA elements that contribute to the genetic risk architecture of psychiatric and neurological disease. By combining the ability of human induced pluripotent stem cells (hiPSCs) to generate sufficient quantities of nearly any cell type with these novel methodologies to precisely manipulate human cells, it should be possible to test comprehensively the effect of disease-related genetic and epigenetic manipulations on the 3D genome^{105–107}. The engineering of isogenic cell lines in this way have already been used across a variety of brain disorders to precisely indicate the causal

effect of a disease associated mutation^{71,108–112}, including enhancer sequences associated with neurological disease¹¹³. We believe these approaches are poised to be used to further elucidate the functional consequences of manipulating the epigenome, including chromosomal loop formations, in human neurons and glia.

Summary and Future Directions

Regulation of 3-dimensional chromosomal conformations and spatial genome architectures (3D genome) is critical across the entire lifespan of the human brain. Mutations affecting the function of protein scaffolds and global organizers for the 3D genome frequently are associated with neurodevelopmental defects. Furthermore, many clinically relevant structural DNA variants positioned in intergenic or intragenic non-coding sequences could impact neuronal gene expression by bypassing the linear genome in order to directly interact with the target gene. The field now eagerly awaits comprehensive mapping of the 3D genome in psychiatric disorders. Multiple mechanistic types of loop-based promoter-enhancer interactions already have been identified in neurons, hinting at an unexpected functional diversity of transcriptional regulation in the context of synaptic plasticity. Furthermore, *in vivo* applications of (epi)genomic editing techniques will explore how specific manipulations of the brain's 3D genome will affect cognition and behavior. Finally, it will be exciting to apply emerging technologies for 3D genome live-imaging, such as 'Real-time Observation of Localization and Expression' (ROLEX)¹¹⁴, to the brain. Such an approach could provide deep and unprecedented insights into the dynamic reconfigurations of neuronal and glial genomes in response to internal or external stimuli.

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Glossary

Common sequence variants

DNA polymorphisms with minor allele frequencies exceeding 0.05 in the general population

Microdeletion

a loss of a fragment of a chromosome

Microduplication

a duplication of a fragment of a chromosome

Copy number variation (CNV)

genomic sequence, typically in kilo- or megabase range, that either is duplicated or deleted.

Immediate

early gene transcription factors- Activator proteins such as Fos, Jun, Egr are expressed in response to stimulus-based triggering of cAMP and other intracellular signaling cascades and are rapidly expressed within 15 min of a stimulus in sensitive neurons.

Intergenic

genome sequence between two annotated genes.

Methylome

the genome-wide distribution of DNA cytosine methylation in specific cells or tissues.

CRISPR/Cas

Clustered regularly-interspaced short palindromic repeats/CRISPR-associated protein 9, an RNA-guided DNA endonuclease enzyme in bacteria that is increasingly used a tool for targeted genomic and epigenomic editing in multicellular organisms.

Nucleolus

a specialized nuclear organelle for the production of ribosome subunits

Eukaryote

a life form with a well delineated nucleus separated from the cytoplasm by a nuclear membrane

Transcriptional unit

a stretch of DNA being transcribed into RNA molecules

Chromosomal scaffold

a three-dimensional chromatin structure whose integrity is maintained by non-histone scaffolding proteins (e.g., cohesin, CTCF).

Nucleosomal arrays

chromatin packaging in the form of repeating units of DNA-bound core histone octamers connected by linker DNA and linker histone proteins

Preinitiation complex

a large collection of proteins that are essential to begin DNA transcription, acting by recruiting RNA polymerase II, denaturing DNA, and proper positioning of DNA in the active site of polymerase.

Cis-regulatory sequence

non-coding portions of the genome that regulate transcription of nearby or distal genes (e.g., promoters, enhancers).

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Biographies

Prashanth Rajarajan trained as an undergraduate in psychology at Michigan State University in East Lansing, Michigan. Presently, he is an MD, PhD student jointly mentored by Kristen Brennand and Schahram Akbarian. He is interested in stem cell biology and the regulation of the three-dimensional genome in normal and diseased human brain.

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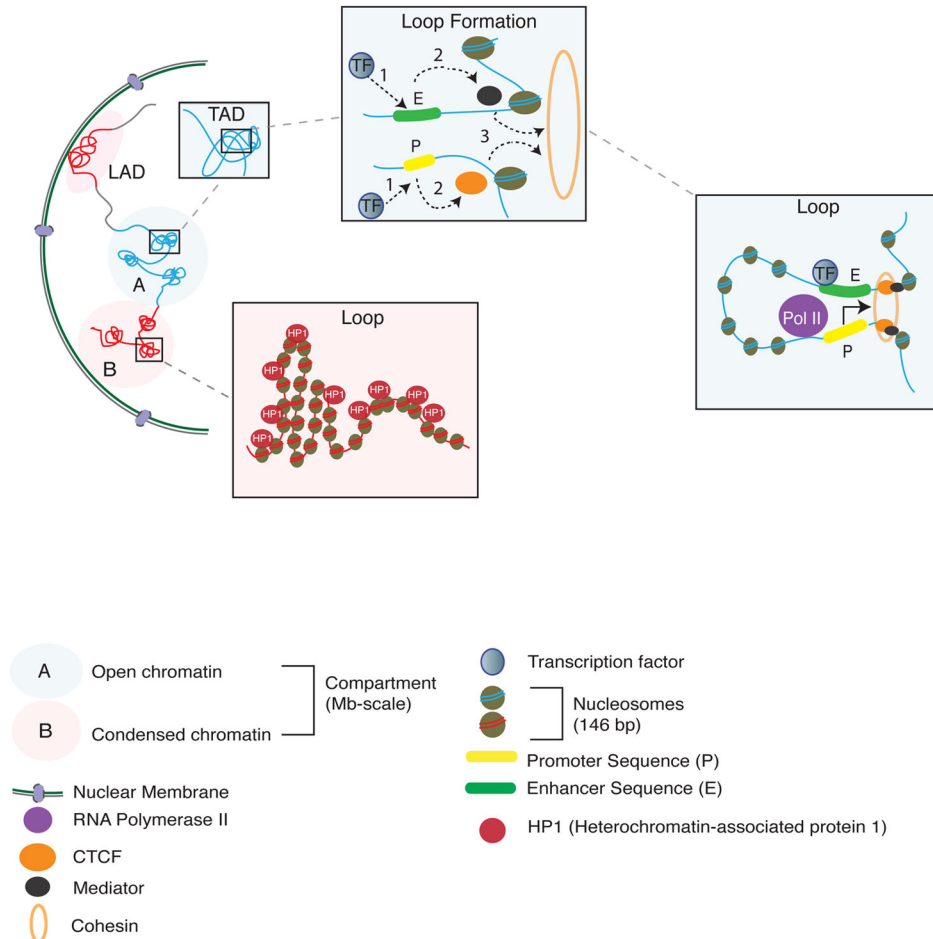


Figure 1. The 3-dimensional genome, from nucleosome to nucleus

a. Chromatin fibers that surround a DNA inside the nucleus are organized of arrays of the elementary unit, the nucleosome (146 bp of DNA wrapped in 2.5 loops around the core histone octamer). In ‘A compartments’, chromatin is in an open or active conformation and is permissive for transcription. This state is defined by high occupancy of RNA polymerase II complex and transcription factors and increased portions of nucleosome free DNA. In ‘B compartments’, chromatin is condensed and RNA polymerase and transcription factor occupancy is decreased. Each compartment can contain several megabases of 3D genome sequences. Superimposed upon this generalized model of nucleosomal organization is the 3D genome. This includes topological-associated domains (TADs) which extend on average (median size) across 185 kb and that can exist within both A compartments and in B compartments that primarily harbor repressive and condensed chromatin⁵⁴. The constituent loci within TADs come into contact with each other much more frequently than with loci from outside domains⁵. The 3D genome also includes lamina-associated domains (LAD), which are condensed heterochromatin enriched around the nuclear periphery and physically bound to lamin proteins at the inner nuclear lamina. b. In ‘B compartments’, chromatin is condensed and is enriched with a different set of proteins to A compartments, which include, among others, HP1 (heterochromatin-associated protein 1) and (not shown) repressive histone methylation markings (H3K9me3, H4K20me3). c. TAD boundaries, but also specific

chromosomal loop formations, including promoter-enhancer loopings, are often demarcated by CTCF and cohesins, and additional proteins which serve to regulate the formation of 3D genome structures¹¹⁸. Formation of promoter—enhancer loopings in A compartments, for example, involves a sequence of steps: (1) transcription factors (TF) bind to promoter and enhancer sequences of DNA; (2) the co-activator Mediator is recruited which in turn recruits the cohesin complex (3), which as ring structure creates or stabilizes the loop. CTCF operates in parallel or synergistically to Mediator¹¹⁹. This structure allows activation and modulation of the RNA polymerase II core transcription machinery

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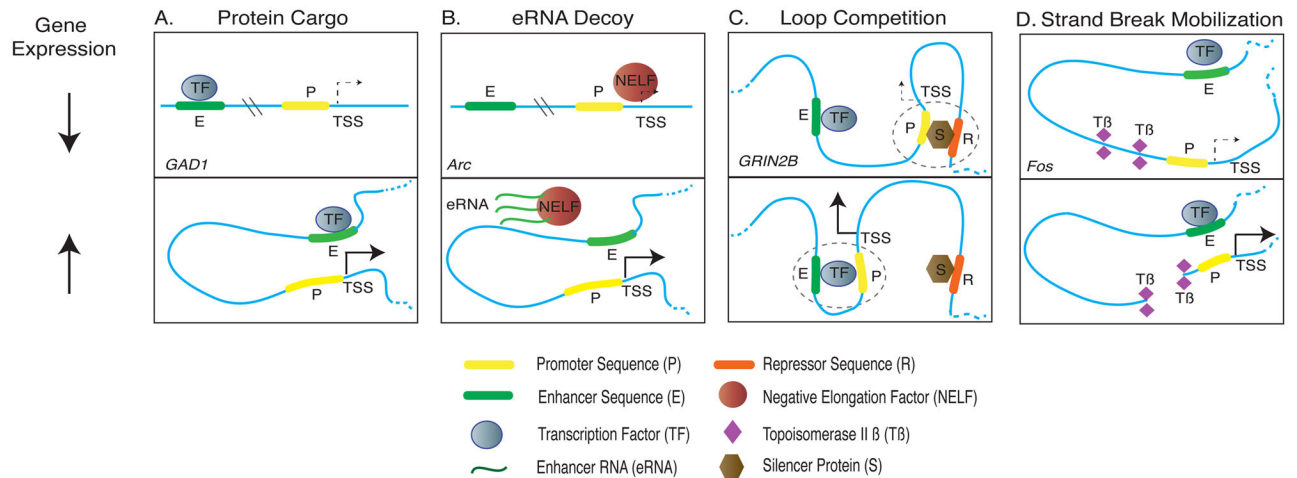


Figure 2. Enhancer-mediated regulation of neuronal gene expression

Four models for enhancer-dependent up- and down-regulation of transcription have been described in neurons (see text). The models are not mutually exclusive. **(A, top)** Protein ‘cargo’ model, which has been demonstrated for the *GAD1* gene: Distal enhancer sequences bind transcription factors. Promoter and enhancer sequences are separated and interaction between transcription factors, enhancer and promoter sequences cannot occur, and the rate of transcription is reduced (dashed arrow). Loop formations that enable physical proximity between the enhancer’s target (such as be a gene transcription start site), transcription factors and the proximal promoter region upregulate transcription (bottom). **(B, top)** enhancer RNA (eRNA) decoy model, which has been demonstrated for the neuronal gene *Arc*: When the *Arc* gene promoter is occupied by negative elongation factor (NELF), the action of RNA polymerase II complex is stalled and *Arc* transcription is low. However, the distal enhancer sequences of the gene produce enhancer RNAs (eRNAs), and when the enhancer region moves via specific loop formations into physical proximity to the *Arc* promoter, these short non-coding eRNAs bind NELF, which reduces NELF occupancy at the target gene (*Arc*), thereby liberating RNA polymerase II complex and promoting the transcriptional process (Bottom). **(C)** Loop competition model, shown for the gene *GRIN2B*: Two or more non-contiguous (separated by interspersed sequence) cis-regulatory sequences, potentially with opposing effects on transcription (i.e. a promoter sequence and a repressor sequence), are competing to access the *GRIN2B* gene promoter. Top: When the *GRIN2B* promoter interacts with a silencer protein occupying loop-bound repressor DNA elements, and transcriptional activity is reduced. Bottom: When the same promoter interacts with an active enhancer element that has bound transcription factors present, *GRIN2B* expression is increased. **(D)** Strand break mobilization at the *c-fos* immediate early gene (IEG) promoter: Promoter activity is low at baseline when the genome is in linear form (bottom). To activate transcription, topoisomerase II β enzyme induces DNA strand breaks. This results in the mobilization of promoter sequences into physical proximity with enhancer elements that were previously separated by several kb of interspersed linear genome (bottom). The physical proximity of these promoter and enhancer DNA then leads to synergistic activation of gene expression via enhancer-bound transcription factor proteins.

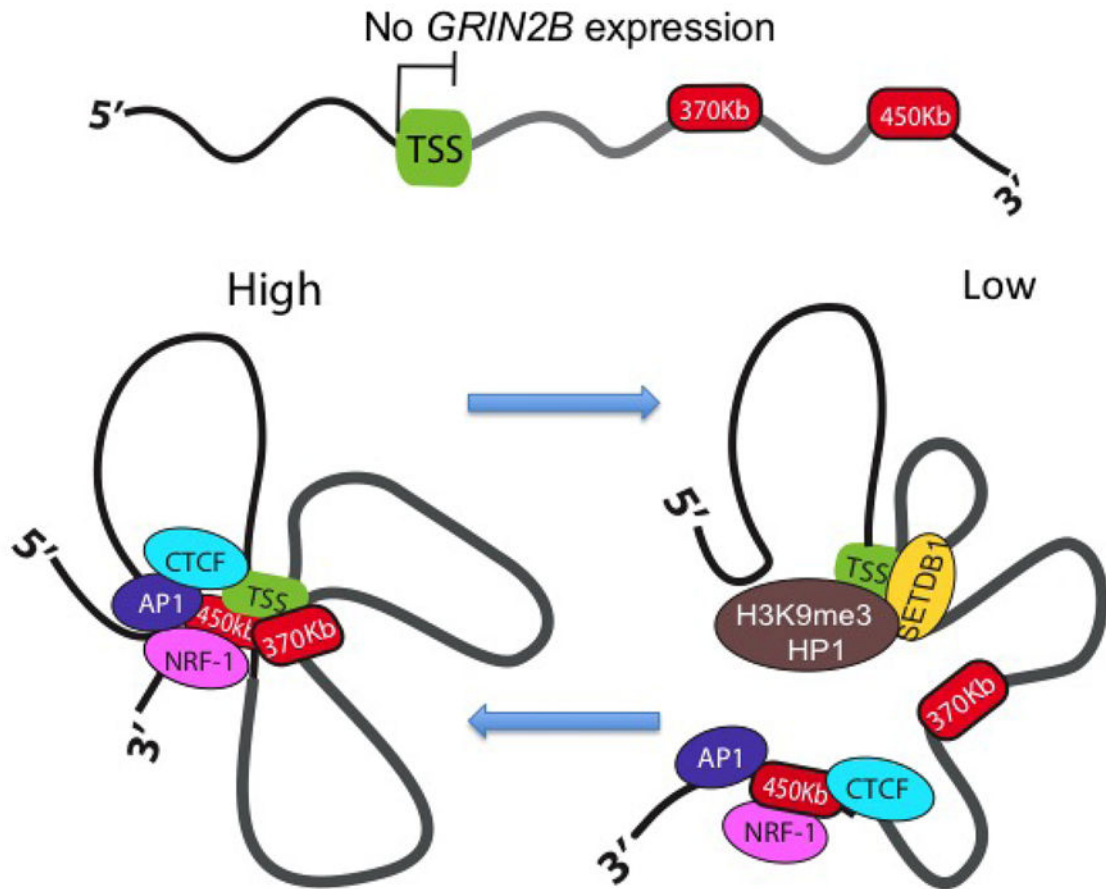


Figure 3. Dynamic model of chromosomal conformation at *GRIN2B* NMDA receptor gene locus

(top) Long-range chromosomal loop formations at the *GRIN2B* locus are not detectable in cells and tissues that do not express the *GRIN2B* protein. However, in cells that express *GRIN2B*, the expression can be finely tuned by dynamic competition between multiple chromosomal conformations competing for access to the *GRIN2B* promoter and transcription start site. Transcription is increased by a loop-bound enhancer formation of DNA, to which IEG transcription factors, NRF-1 and additional activator proteins are bound as a complex. This is counterbalanced by additional chromosomal conformations that carry repressive chromatin into physical proximity to the *GRIN2B* promoter. This repressive chromatin is characterized by localized enrichments of HP1 (heterochromatin-associated protein 1) and repressive SETDB1 histone methyltransferase, (see text and reference²³ for details).

Table 1

Monogenic neurological disease associated with altered function of 3D genome scaffolding proteins

OMIM *gene	Function	Syndrome	OMIM #disease
<i>NIPPED-B-LIKE</i> (NIPBL) *608668	Cohesin loading onto chromatin	heterozygous deletion and mutations account for ~50% of cases diagnosed with Cornelia de Lange Syndrome (CdLS) including intellectual disability (reference 4,28 in text)	#122470
<i>STRUCTURAL MAINTENANCE OF CHROMOS. 1A</i> (<i>SMC1A</i>) *300040	Cohesin core unit, component of tripartite ring to entrap chromatin fibers	<5% of CdLS, gene duplication syndrome with behavioral alterations, intellectual disability (PMID 19052029) (references 29 in text)	
<i>STRUCTURAL MAINTENANCE OF CHROMOS. 3</i> (<i>SMC3</i>) *606062	Cohesin core unit, component of tripartite ring to entrap chromatin fibers	<5% of CdLS, gene duplication syndrome with behavioral alterations, intellectual disability (PMID 19052029)(reference 29 in text)	
<i>CCCTC-binding factor</i> (<i>CTCF</i>) *604167	Chromosomal loop organizer and scaffold, insulator protein separating chromosomal domains, transcriptional regulator	heterozygous deletion and mutations microcephaly, mild mental retardation (PMID 23746550) (references 31,32 in text)	
<i>SPECIAL AT-RICH SEQUENCE BINDING PROTEIN 2</i> (<i>SATB2</i>) *608148	chromosomal domain organizer and scaffold	heterozygous mutations and deletions: Glass Syndrome (GLASS) including intellectual disability, seizure disorder, speech delay (references 36, 38 in text)	#612313
<i>LAMIN B1</i> (<i>LMNB1</i>) *150340	Nuclear lamina protein tethering heterochromatin chromosomal domains to nuclear periphery	<i>LMNB1</i> gene duplication: adult-onset autosomal dominant leukodystrophy (ADLD), progressive CNS demyelination. (reference 27 in text)	#169500

Table 2

Examples of chromosomal conformations implicated in the regulation of human cognition

Locus	Gene Function	Chromosomal conformation in Brain
<i>INSULIN GROWTH FACTOR 2 (IGF2/H19)</i>	imprinted genes, controlling metabolism, growth (including postnatal brain)	Loss of ATRX and MECP2 proteins decreases local occupancy of CTCF and disrupts long-range chromosomal contacts across several Mb at <i>Igf2/H19</i> locus, and additional loci, in mouse forebrain. PMID 24990380. References 41, 42, in text.
<i>DIPEPTIDYL-AMINOPEPTIDASE-LIKE PROTEIN 10 (DPP10)</i>	Kv4 channel auxiliary subunit regulating neuronal excitability	<i>DPP10</i> transcription start site in prefrontal cortex neurons connected via 0.5Mb long-range loop with non-coding sequences showing histone modification profile unique to human (not shared with non-human primates incl. chimpanzee). PMID: 23185133. Reference 65 in text.
<i>CALCIUM VOLTAGE-GATED CHANNEL SUBUNIT ALPHA1 C (CACNA1C)</i>	Ion channel, possible role in synaptic plasticity (PMID 20573883)	0.2Mb promoter- bound loop formation includes enhancer sequences with single nucleotide polymorphisms linked to heritable risk for schizophrenia. PMID: 25453756. Reference 51 in text.
<i>GLUTAMATE RECEPTOR, IONOTROPIC, N-METHYL-D-ASPARTATE, SUBUNIT 2B (GRIN2B)</i>	Ion channel broadly relevant for in developing and adult brain function	<i>GRIN2B</i> promoter targeted by multiple loop formations bypassing up to 0.5Mb linear genome in the context of transcriptional regulation; loop-bound DNA confers liability for working memory and cognitive disease. PMID: 25467983. Reference 17 in text.
<i>GLUTAMIC ACID DECARBOXYLASE 1 (GAD1)</i>	GABA synthesis gene	Human/mouse conserved 50 kb promoter-enhancer loop, sensitive to changes in neuronal activity and altered in prefrontal cortex of some cases with schizophrenia. PMID: 23864674. Reference 18 in text.