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CHROMOSOMAL CONFORMATIONS AND EPIGENOMIC REGULATION IN SCHIZOPHRENIA

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Abstract

Chromosomal conformations, including promoter-enhancer loops, provide a critical regulatory layer for the transcriptional machinery. Therefore, schizophrenia, a common psychiatric disorder associated with broad changes in neuronal gene expression in prefrontal cortex and other brain regions implicated in psychosis, could be associated with alterations in higher order chromatin. Here, we review early studies on spatial genome organization in the schizophrenia postmortem brain and discuss how integrative approaches using cell culture and animal model systems could gain deeper insight into the potential roles of higher order chromatin for the neurobiology of and novel treatment avenues for common psychiatric disease.

Keywords

schizophrenia; epigenetics; chromatin; 3D genome; gene regulation; promoter-enhancer loops

INTRODUCTION TO THE EPIGENOMICS OF SCHIZOPHRENIA

Schizophrenia is a major psychiatric disorder often with onset in adolescence or young adulthood, with a wide range of symptoms ranging from delusions and hallucinations to severe social withdrawal and apathy, along with reduced lifespan primarily due to cardiovascular disease and suicide [1–3]. Antipsychotic medications are widely prescribed and mostly target dopaminergic and serotonergic receptor systems[4, 5] but often fail to deliver a satisfactory therapeutic response [6, 7]. Cognitive symptoms in particular are severe, chronically disabling, and often persistent during the course of illness[8]. Regrettably, these symptoms are ineffectively treated with antipsychotic medication[9]. The genetic risk architecture of schizophrenia is exceedingly complex. For example, in a study involving 150,000 subjects, the Psychiatric Genomics Consortium identified altogether 108 haplotypes that by individual small effect contribute to the heritable risk for schizophrenia[10]. In addition, there are rare mutations and variants discovered by comprehensive sequencing of all protein coding genes (the ‘exome’, which comprises approximately 1% of total genome

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sequence)[11], some of which may be causal to the disease etiology in some of the cases with schizophrenia[12].

Importantly, there can be little doubt that dysregulation of neuronal gene expression in prefrontal cortex (PFC) and various other brain regions implicated in the neural circuitry of psychosis contributes to the pathophysiology of schizophrenia, broadly affecting excitatory and inhibitory neurotransmission, metabolism, myelination and immune signaling[13–19]. With the transcriptional process intimately connected to chromatin structure and function in human cells and model organisms alike[20, 21], one would therefore expect that epigenomic markers associated with open ('active', 'loose') chromatin permissive for gene expression, versus repressed and silenced chromatin, will show significant alterations in brain tissue from subjects diagnosed with schizophrenia. Indeed, work conducted over the course of the last 15 years has provided first insights into epi-(*Greek for 'over', 'above'*)-genomic aberrations encountered in brain tissue from subjects diagnosed with schizophrenia. Such types of epigenomic explorations in the postmortem brain initially focused on DNA methylation, one of the key epigenetic mechanisms involved in the regulation of gene expression[22]. Methylation at the cytosine C5 position, primarily in the context of cytosine-guanine (CpG) dinucleotides, when located in gene promoters often is implicated in gene repression by directly impeding the binding of transcription factors and by inducing repressive chromatin structure non-permissive to transcription[23]. Early studies, examining the DNA methylation status of candidate genes affected by dysregulated expression in brains of schizophrenia reported differential DNA methylation profiles in diseased cerebral cortex for key regulators of neuronal connectivity such as *REELIN (RELN*) [24, 25] and key transcription factors such as sex-determining region Y-box containing gene 10 (SOX10) [26], to mention just two examples. Recent genome-wide mapping of the DNA methylome [27, 28] reported DNA methylation changes for various genes implicated in excitatory or inhibitory neurotransmission, with one study exploring the DNA methylome in the prefrontal cortex of 191 subjects with schizophrenia in comparison to 335 controls collected across the lifespan identifying >2000 CpG sites with altered methylation levels in diseased tissue[29]. However, the functional implications of the overall extremely subtle methylation differences (on average, 1.3% difference between schizophrenia and control brains for significantly affected CpG sites) in the aforementioned study[29] remain unclear.

Future studies, exploring the DNA methylome of specific brain cell populations in diseased vs. control brains[30, 31] as opposed to the earlier studies which utilized tissue homogenate or correlational analyses between the brain's DNA methylomes and structural or functional defects in neurons[32, 33], may provide deeper insight into the role of epigenetic dysregulation affecting the brains of subjects with schizophrenia. Of note, some of the epigenomic determinants of chromatin structure and function in fetal and adult human brain, including histone methylation and acetylation markings associated with cis-regulatory sequences such as gene promoters, enhancers and repressors, show an impressive, up to 26-fold enrichment for single nucleotide polymorphisms associated with heritable risk for schizophrenia[34]. These effects were highly tissue-specific, because brain histone methylation landscapes showed no enrichment for polymorphisms associated with rheumatoid arthritis and other common disorders usually not affecting the central nervous system[34]. Therefore, given that 1) DNA methylation alterations have been reported in postmortem brain of subjects with

schizophrenia, and 2) the significant association of histone modification landscapes from brain cells with the genetic risk architecture of the disease, it is very likely that ‘epigenomic dysregulation’—including alterations in the expression of specific genes or disruptions in the coordinated regulation of multiple transcriptional units—could be key drivers underlying cortical dysfunction in schizophrenia.

REGULATION OF CHROMOSOMAL CONFORMATIONS IN NEURODEVELOPMENT

As discussed in the Introduction, evidence arising both from candidate gene studies and genome-scale mappings of DNA methylation and histone modifications provided strong support for the hypothesis that alterations in chromatin structure and function could contribute to transcriptional dysregulation in brains of subject with schizophrenia. However, as discussed in a recent review[35], comprehensive exploration of the epigenome in schizophrenia and other cognitive disease has to go far beyond DNA methylation, histone modifications and other regulators of gene expression that are typically ‘charted’ as epigenetic signals directly onto and above the ‘linear’ genome in two dimensions (Figure 2.1). Instead, it is increasingly recognized that the spatial configuration and packaging of interphase chromosomes, the equivalent of 250 cm of DNA from a single nucleus when unwound, involves myriads of non-randomly occurring chromosomal loop structures and DNA-DNA proximity conformations that bypass the linear genome across a wide range of genomic distance, from hundreds of base pairs (such as a short loop formation connecting a transcription start site and proximal gene promoter with a nearby enhancer) to many megabases of interspersed sequence (as in the case of some long-range contacts on the inactive X chromosome in female somatic cells). Proper regulation of these types of 3D genome structures are considered critical for the regulation of gene expression, maintenance of genome integrity and stability, control of growth and differentiation, among many other functions. Surprisingly, however, there is extremely little knowledge about the regulation of the three-dimensional (3D) genome in developing or mature brain, including potential alterations in neuropsychiatric disease, such as schizophrenia.

Building blocks of the 3D genome

We will start our discussion of the 3D genome and its implications for schizophrenia research with a brief overview of its basic principles and organization. Nuclei, separated by a nuclear membrane from the cytoplasm, contain the genome packaged into chromatin fibers as nucleosomal arrays. 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, (2) specific types of post-translational histone modifications, 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 2.2). Superimposed upon this nucleosomal organization is the 3D conformation of chromatin fibers and entire

chromosomes, often described in terms such as ‘loops’ or ‘globules’ and *in toto* referred to as the ‘3D genome’. These chromosomal conformations, to a certain extent, reflect the two alternative chromatin states mentioned above. For example, euchromatic and heterochromatic sequences tend to assemble into alternating regions of approximately ~5 megabases (Mb). These ‘compartments’, positioned along the same chromosome, could interact with compartments from different chromosomes[36]. Furthermore, A or B compartment designations are correlated with features such as DNA accessibility and transcriptional activity. Thus, A compartments are enriched for euchromatic regions with much higher overall levels of transcription, while B compartments primarily harbor inactive and heterochromatic sequences[37] (Figure 2.2). Some of the largest clusters of heterochromatin are microscopically visible and enriched at the nuclear periphery and around nucleolar membranes[38].

Studies exploring scaffolding and regulatory proteins with a critical role for the spatial conformations of the chromosomal materials have primarily focused on the cohesin complex and the CCCTC-binding factor/zinc finger protein CTCF. Cohesins are comprised of five core subunits SMC1, SMC3, RAD21/REC8, and STAG1–3 in humans[39]. In addition, accessory proteins load or release the complex onto chromosomes[39]. Cohesins were initially explored in the context of sister chromatid cohesion and segregation during cell division (mitosis) [39]. However, these proteins maintain a heavy presence in the nuclear proteome of postmitotic cells including neurons[40]. Cohesins form ring-like structures, literally entrapping distant chromatin fibers into chromosomal loops[39] (Figure 2.2). Cohesins are highly enriched at actively expressed genes in a tissue- and cell-type specific manner[39]. In contrast, CTCF, while dispensable for cohesin loading onto DNA, orchestrates cohesin enrichment at select binding sites[40]. As a result, chromosomal loops co-occupied by cohesins and CTCF at both ends often associate with broader stretches of regulatory domains, marking the co-regulated repression or expression of groups of genes in a cell-type specific manner[41]. The CTCF binding sites are thought to often (but not exclusively) be positioned in inward/convergent and, to a somewhat lesser degree, tandem orientation at the two contact sites of the loop[37, 42]. CTCF directionally recognizes binding sites via an 11 zinc finger array, while cohesin undergoes assembly from the CTCF’s C-terminal end, often resulting in higher order chromatin with loop-bound head-to-head CTCF configurations[43].

Chromosomal scaffolding protein mutations and neuropsychiatric disease

Importantly, a rapidly increasing list of deleterious mutations in genes encoding scaffolding proteins for the 3D genome is linked to neuropsychiatric disease. These include neurodevelopmental disorders such as Cornelia de Lange Syndrome (CdLS) [39, 44, 45] and adult-onset progressive demyelination syndromes[46]. 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* [44]. The neurological manifestations could be due to 3D genome disorganization in brain cells and de-compaction of chromatin, albeit the precise molecular mechanisms remain to be elucidated[47]. In addition, genetic mutations in *CTCF*, as a key organizer for chromosomal loops, have been linked to monogenic causes of microcephaly

and cognitive disorder[48, 49]. 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[50]. It remains to be shown, however, whether the neurological manifestations of *Cohesin* gene mutations or *CTCF* 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. These include *Special AT-rich Sequence Binding Proteins 1* and *2* (*SATB1, SATB2)* that govern chromosomal territories extending across hundreds of kilobases[51] and anchor chromatin fibers into the nuclear matrix[52]. Of note, *SATB2* is essential for craniofacial development and proper differentiation of transcallosal cortical projection neurons[53, 54]. The gene has also been linked to some cases of Glass Syndrome (*OMIM 612313*) and mental retardation[53, 55]. The related protein, *SATB1,* is essential for connectivity and maturation of the GABAergic interneuron population in the cerebral cortex[56, 57]. These findings are of particular interest to the field, given that epigenomic dysregulation of GABAergic gene expression has been reported both for schizophrenia postmortem brain and in the animal and cell culture models[33, 58–61].

LOOP DISRUPTIONS POTENTIALLY INVOLVED IN SCHIZOPHRENIA–EARLY FINDINGS

Promoter-enhancer loops and transcriptional regulation

Because, as discussed above, the molecular pathology of schizophrenia includes transcriptional dysregulation in cerebral cortex and other brain regions, chromosomal conformations associated with the regulation of gene expression are of particular interest to the field. To this end, promoter-enhancer loops represent one type of chromosomal interaction that is becoming increasingly understood. Promoters are often defined as cis-regulatory sequences within 1000 base pairs from the next gene transcription start site. In contrast, enhancers are a type of cis-regulatory sequence positioned >1kb from the nearest transcription start site[62]. 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 complex[62]. 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 enhancers[62]. The transcription factors bind to nucleosome-free intervals in open chromatin of promoters and enhancer sequences and recruit co-activator complexes, such as Mediator and CREB-binding protein (CPB)/p300[62], to mention a few examples. Promoters in contrast to enhancers are often CpG rich[62], which is of interest given that alterations in promoter CpG DNA methylation have been reported in the brains of schizophrenia patients[24–27, 33]. Importantly, enhancers, as distal regulatory elements, while critically important for transcriptional regulation are separated from their target gene often by many hundreds of base pairs, and in some cases many kilo- or even mega-bases of interspersed linear genome[62]. Various mechanisms have been proposed by which enhancer chromatin could regulate the expression of target genes from distant chromosomal locations. Such mechanisms include sliding along the chromosome to ‘track’ promoters[63], or

alternatively, a physical 'bridge' built via protein-protein interactions[64]. 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 target[65].

Evidence for Loop Disruption in Brains of Subjects with Schizophrenia

Importantly, chromosome conformation capture (3C), a widely used DNA-DNA proximity assay on chromatin preparations treated by restriction digest followed by re-ligation, is applicable to postmortem tissue, with many chromosomal loop formations showing some level of preservation in brain tissue collected several hours after death[66]. One interesting and early example of 3C applied on human brain involved *GAD1*[67], encoding the 67Kda glutamic acid decarboyxlase GABA synthesis enzyme, a gene frequently showing dysregulated expression in multiple areas of the forebrain of subjects with schizophrenia [18, 68–86]. One of the loop formations that were altered in a small pilot cohort of diseased prefrontal cortex[67] also existed in neuronal cultures derived from induced pluripotent stem cells. Furthermore, this type of chromatin loop, which bypassed approximately 50kb of linear genome, was conserved in mouse and human brain[67], which could indicate an important regulatory function for GAD1 expression across multiple mammalian lineages. In any case, according to the aforementioned study[67], promoter-enhancer loops are potentially disrupted in diseased brain, thereby contributing to dysregulated gene expression. In the case of this 50kb GAD1 promoter-enhancer conformation, it has been suggested that the distal sequence (the enhancer) carries, via the loop, a cargo of transcription factors into close spatial proximity with the target gene promoter[67](Figure 2.3A*)*.

Another interesting example of higher order chromatin relevant for schizophrenia was recently described for the *GRIN2B* gene locus, encoding an NMDA glutamate receptor subunit[87]. Importantly, deleterious *GRIN2B* mutations rank prominently in exome sequencing studies capturing rare monogenic forms of neuropsychiatric disease, including intellectual disability, epilepsy, autism and psychosis spectrum disorders including schizophrenia[88–92]. Multiple loop-bound intronic and intergenic DNA sequences, up to 450kb downstream from the *GRIN2B/Grin2b* transcription start site (TSS), compete for access to the TSS[87]. These sequences are loaded with multiple transcription factors and, via chromosomal loop formations, are in physical proximity to the *GRIN2B/Grin2b* transcription start site[87]. However, in addition to such long-range promoter-enhancer loops, the *GRIN2B/Grin2b* promoter also interacts with intragenic repressive chromatin embedded in intron sequences[87]. 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* promoter[87](Figure 2.3B). However, this process is counterbalanced by promoter-bound higher order chromatin involving repressive intronic sequences 30 kb downstream from the transcription start site[87, 93] (Figure 2.3B). Interestingly, multiple loop-bound sequences interacting with the *GRIN2B* promoter harbor single nucleotide polymorphisms (SNP) implicated with liability for working memory[87], and schizophrenia and personality traits associated with schizophrenia[87]. Notably, one these risk-associated SNP alleles was associated with a promoter-enhancer loop formation and thought to convey decreased nucleoprotein binding

and motif loss for the CCAAT/Enhancer Binding Protein CEBPB (C/EBPβ)[87]. CEBPB is a transcription factor implicated in consolidation of cortical and hippocampal learning and memory[94–96]. Because many enhancer elements are defined by sequential linear alignment of multiple transcription factors within short distances[97, 98], additional activator proteins may synergistically cooperate with loop-bound CEBPB to regulate *GRIN2B* expression[87]. Taken together, these findings point to a complex and multilayered regulation of chromosomal conformations across at least one megabase of sequence surrounding the *GRIN2B* locus. Therefore, loop-bound DNA targeting the *GRIN2B* promoter and carrying disease-associated sequence polymorphisms, could either facilitate or repress expression, depending on the protein 'cargo', with multiple distal loop formations competing in a highly dynamic and activity-dependent manner for access to the *GRIN2B* promoter sequences[87]. Importantly, the DNA sequence variants affecting *GRIN2B* enhancer function, and working memory and liability for schizophrenia are located nearly half a megabase downstream from the *GRIN2B* transcription start site[87]. Therefore, these *GRIN2B* higher order chromatin studies provide the first example how chromosome conformation capture approaches could be harnessed to assign neurological function to risk-associated non-coding sequences that on the linear genome appear to be too far removed from gene promoters to impact expression.

Similarly, risk-associated sequences positioned in enhancer elements within the *CACNA1C* calcium channel gene body (a gene locus which ranks prominently in the polygenic risk maps of common psychiatric disease including schizophrenia and depression[99]) were recently identified as potent modulators of reporter gene activity[34]. Remarkably however, these *CACNA1C* intragenic enhancer sequences were, in human prefrontal cortex, physically bound to the gene transcription start site via a 185kb spanning chromosomal loop formation[34], providing yet another example how the study of chromosomal conformations in human brain tissue could help to illuminate the role of non-coding DNA that, on the linear genome, is positioned far away from transcription start sites (Figure 2.3C*)*.

However, there is evidence pointing to the importance of chromosomal conformations and the '3D genome' for human cognition and schizophrenia far beyond the aforementioned candidate gene examples. For example, significant over-representation of enhancer sequences has been observed within the pool of polymorphisms and haplotypes associated with genetic risk for schizophrenia[34, 100]. Furthermore, postmortem studies using more conventional epigenomic assays, including genome-scale DNA methylation survey, have suggested that epigenetic dysregulation of enhancer sequences could contribute to the neurobiology of mood and psychosis spectrum disorders, including astrocyte dysfunction in depression[101], and broad changes in neuronal gene expression in schizophrenia[29, 102]. Finally, recent genome-wide chromosomal conformation mapping in neural progenitor cells and fetal brain tissues reveals many additional examples of promoter-enhancer loops at the sites of schizophrenia relevant genes[103].

(EPI)GENOMIC EDITING OF LOOP-BOUND REGULATORY SEQUENCES IN THE PRECLINICAL MODEL

In the paragraphs above, we provided specific examples of regulatory non-coding DNA that is associated with the genetic risk architecture of schizophrenia and via chromosomal loops physically associates with promoters and transcription start sites of important neuronal genes such as *GAD1*, *GRIN2B* and *CACNA1C*. One could argue that such types of promoter-enhancer loops provide unique opportunities for highly locus- and sequence-selective interventions, targeted against individual risk polymorphisms associated with schizophrenia, and aimed at conveying a therapeutic effect by affecting target gene expression. Thus, the field will soon be challenged with the task to 'convert' this molecular information into testable hypotheses aimed at gaining deeper insights into the neurobiology of schizophrenia. Perhaps more importantly, such newly gained information could be harnessed to develop novel therapies aimed at improving cognitive dysfunction. While there are already significant efforts to translate these evolving findings from schizophrenia genetics, genomics and epigenomics into drug discovery pipelines and clinical testing[104], we predict that behavioral studies in mice and other small laboratory animals will serve as a critical preclinical intermediates towards this goal, in conjunction with molecular and cellular exploration of human cells in culture dishes[105].

Importantly, the molecular toolboxes to test this approach, at least for the preclinical model, already exist. Specifically, genome editing strategies via RNA-guided nucleases, including the Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR)-CRISPR-associated protein systems (CRISPR-Cas), introduced to the field only a few years ago[106], have now been widely adopted in all areas of genomic medicine, including the neurosciences[107]. Mutations in and CRISPR-mediated disruption of enhancer sequences have been observed and accomplished for several neuropsychiatric risk genes, including the NMDA receptor gene *GRIN2B* [87] and the *FOXG1* transcription factor[103]. Strikingly, mutations and manipulations in the enhancers have been linked with changes in the target gene expression even though the enhancer sequences lie distal to the target gene promoters on the linear genome. While the precise molecular mechanisms underlying these phenomena, such as allele-specific binding of specific transcription factors, often remain incompletely understood, it is noteworthy that such experiments have been conducted to date primarily in cell culture systems. Typically, transcriptional changes are explored after targeted mutations in the regulatory element, or by allele-specific comparisons of reporter gene expression activity. Therefore, the next phase of experiments should include *in vivo* genomic editing of risk-associated promoter and enhancer sequences, and then test for changes in cognition and behavior in the animal. In doing this, we must keep in mind that such genomic editing may carry drawbacks given that mutagenic interventions are likely to be irreversible. However, CRISPR-Cas and other RNA-guided nuclease systems can easily be converted into epigenomic editing tools (by using mutant protein with inactivated nuclease function, fused to a transcriptional activator such as VP64 or P300[108], or a repressor such as KRAB[109], even with multi-locus manipulation[110]. With the underlying DNA sequence left intact, it will be interesting to explore whether simultaneous epigenomic targeting of enhancer and promoter sequences within multiple risk haplotypes could offer a

promising approach to effectively alter cognition and behavior. Just as in the aforementioned examples of genomic editing, proof-of-principle studies for epigenomic editing of schizophrenia risk loci by CRISPR-Cas-mediated loading of loop-bound sequences with artificial transcriptional activators and repressors already have been published. These experiments, performed on the clustered *PROTOCADHERIN* locus (regulating neuronal connectivity), have one caveat: they were conducted in cell culture and not in the animal[111].

Obviously, preclinical assessment of cognitive and behavioral changes after genomic and epigenomic editing of psychiatric risk haplotypes is only feasible for genomic sites that show at least some degree of conservation between human and mouse (or other laboratory animals') genomes. Such an 'epigenomic conservation' could include similarities in sequential arrangements of genes and transcriptional units at the locus of interest, conservation of histone modification landscapes, and similarities in chromosomal conformations including promoter-enhancer loops important for transcriptional regulation. While a more detailed investigation on the epigenomic conservation across species for genomic sites harboring schizophrenia risk haplotypes awaits further investigation, genome-scale[112, 113] studies suggest that chromatin structure and function is conserved in human and mouse for a large number of regulatory non-coding sequences, even if these are not necessarily accompanied by DNA sequence conservation. This general observation also holds for brain chromatin, and there are striking similarities in locus-specific higher order chromatin landscapes in human and mouse for several of the aforementioned neuronal genes and loci, including *GAD1, GRIN2B* and the clustered Protocadherins[67, 87, 111]. With region-specific multiplex gene editing in adult mouse brain *in vivo* [114] now possible, we predict that the genomic and epigenomic editing of loop-bound regulatory DNA sequences will soon become an important avenue for preclinical schizophrenia research.

FUTURE DIRECTIONS

The application of 3D genome mapping technologies in the field of neuropsychiatry is still a relatively new endeavor. A few studies, as enumerated above, have delved into locus-specific dynamics in chromatin conformations and their potential impact in schizophrenia etiology. However, using agnostic, genome-wide approaches, we need to map the baseline 3D interaction landscape across various brain-relevant cell types, from neurons to microglia, to truly understand the nuanced ways in which disease risk variants impact loops in one or some but not in all cell types. Such an approach would not only elucidate any fundamental differences in overall genome topologies across cell types but would also allow us as a field to parse out cell-type-specific impacts of noncoding variants on promoter-enhancer loops and, as a result, variation in gene expression programs. Especially considering that various neuropsychiatric disorders are suspected to have neurodevelopmental origins, mapping chromatin states across differentiation from neural progenitor stage to other cell fates could prove fruitful.

With the advent of newer iterations in genome-wide as well as locus-targeted high throughput chromatin conformation mapping, paired with the trend in decreasing sequencing costs, the field is poised to uncover elements in the relatively underexplored epigenetic layer of three dimensional regulation. Moreover, innovations in CRISPR-Cas technologies,

allowing for fine-tuned gene activation/inactivation, locus deletion, and multiplexing to target various disease-relevant loci at once will further enhance our abilities to tease out the functionality of newly discovered looping interactions, with the potential of uncovering novel therapeutic targets for complex neuropsychiatric illness.

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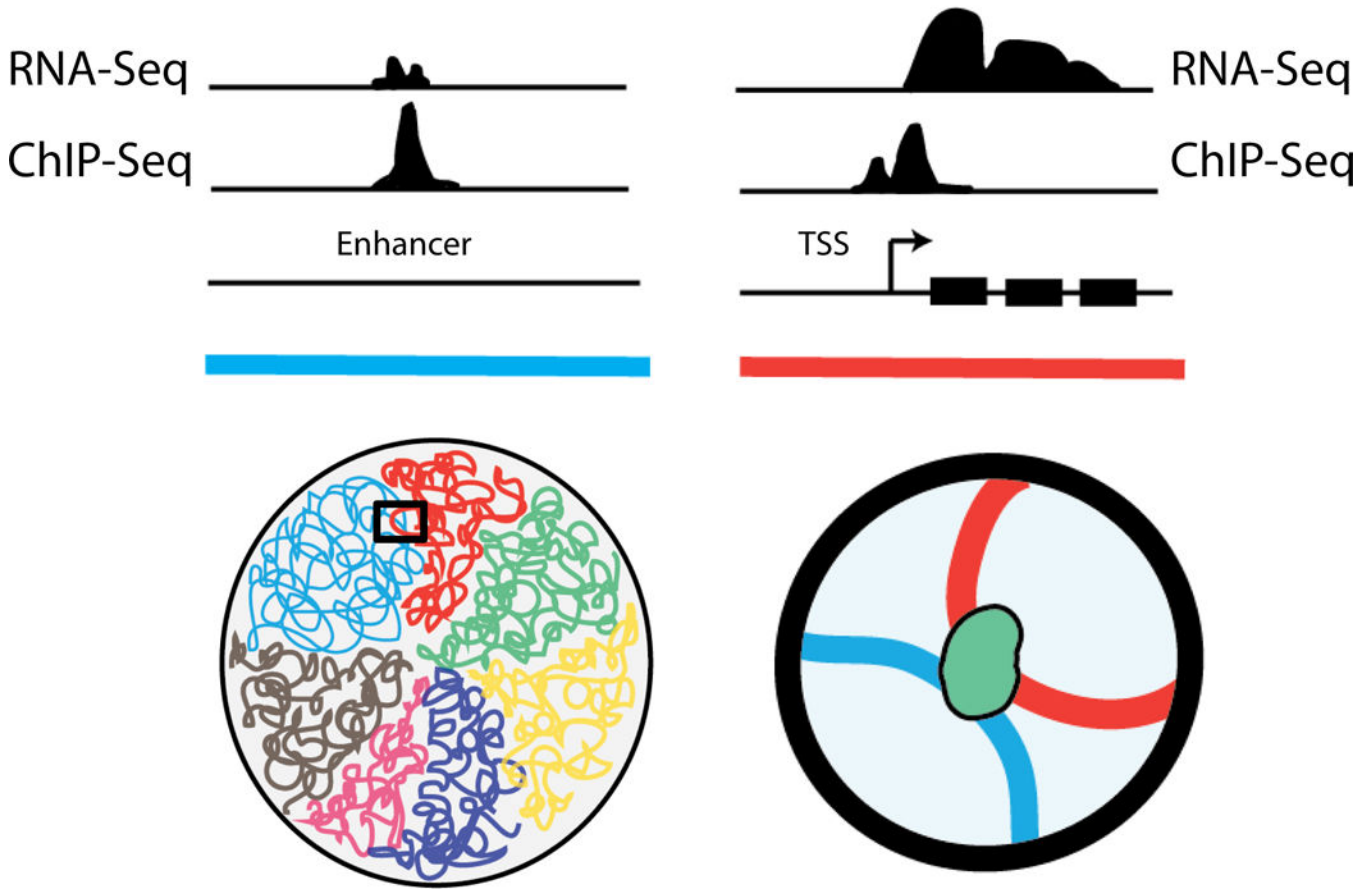

Figure 2.1:
(Top) Conventional ChIP-Seq and RNA-seq tracks on the linear genome demonstrate enhancer and promoter features. (Bottom) However, these traditional approaches miss the fact that the two elements, although quite distal in the linear genome, are actually in close physical proximity through looping interactions.

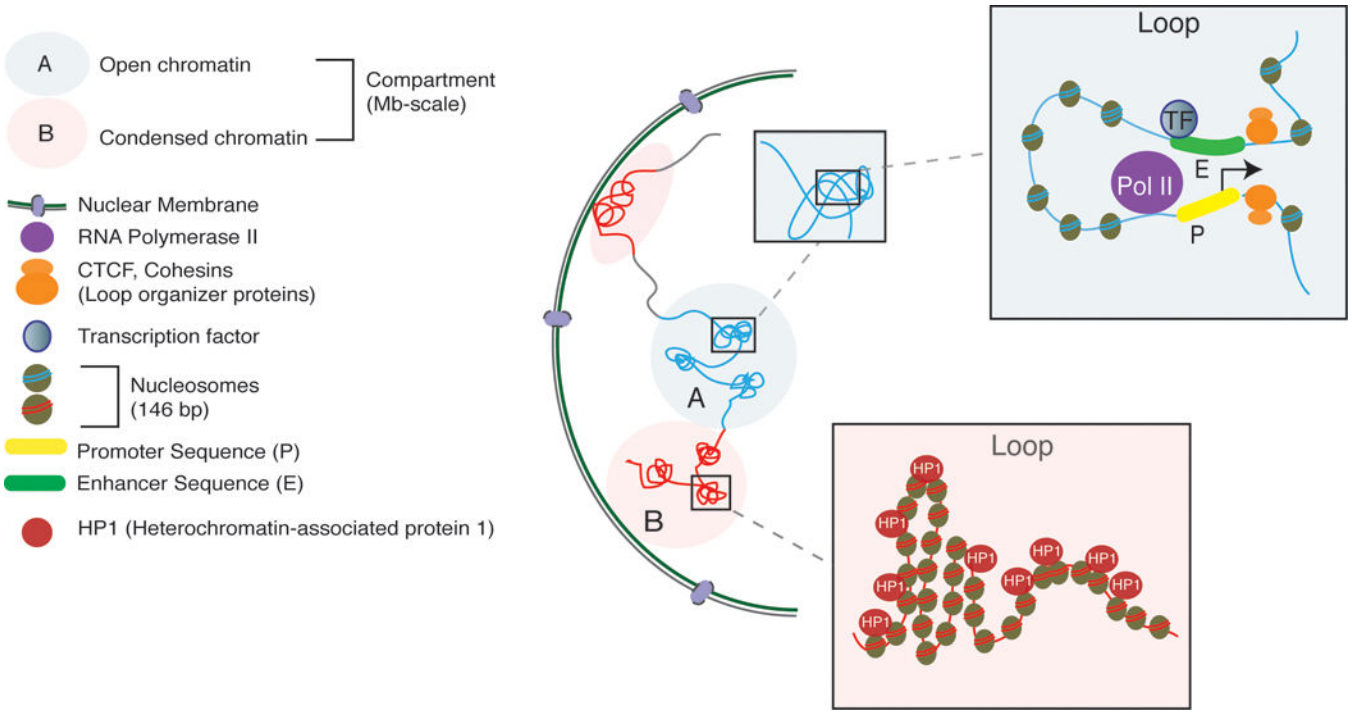

Figure 2.2:

Within the nucleus, chromatin is organized into megabase-scale active or inactive compartments (blue and red circles, respectively), which contain many loops that are facilitative and allow promoter-enhancer contacts (top box) or are repressive and make heterochromatic the contained regions (bottom box). Loops are often scaffolded by key proteins, CTCF and cohesin (orange circles).

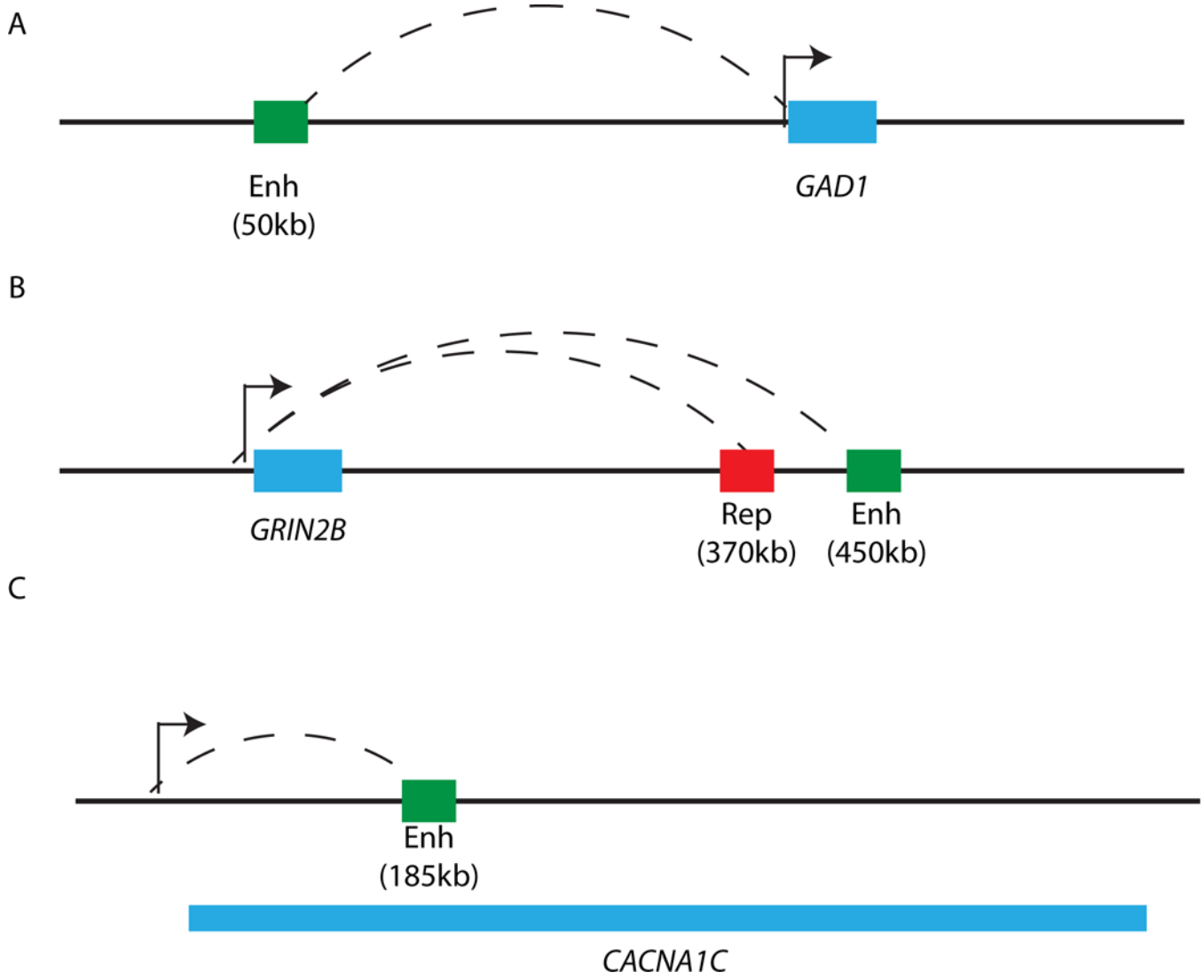

Figure 2.3:

Depictions of distal promoter-enhancer loop interactions on the linear genome.

A) *GAD1* (blue box) and its enhancer (green box) that lies 50kb upstream.

B) *GRIN2B* (blue box) and its enhancer (green box) that lies 450kb downstream and its repressor that lies 370kb downstream.

C) *CACNA1* (introns and exons span the region demarcated by blue box) and its enhancer, which lies in an intronic sequence 185kb downstream from the promoter.