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SPATIAL GENOME EXPLORATION IN THE CONTEXT OF COGNITIVE AND NEUROLOGICAL DISEASE

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Abstract

The ‘non-linear’ genome, or the spatial proximity of non-contiguous sequences, emerges as an important regulatory layer for genome organization and function, including transcriptional regulation. Here, we review recent genome-scale chromosome conformation mappings (‘Hi-C’) in developing and adult human and mouse brain. Neural differentiation is associated with widespread remodeling of the chromosomal contact map, reflecting dynamic changes in cell-type-specific gene expression programs, with a massive (estimated 20–50%) net loss of chromosomal contacts that is specific for the neuronal lineage. Hi-C datasets provided an unexpected link between locus-specific abnormal expansion of repeat sequences positioned at the boundaries of self-associating topological chromatin domains and associated with monogenic neurodevelopmental and neurodegenerative disease. Furthermore, integrative analyses of cell-type-specific Hi-C and transcriptomes uncovered an expanded genomic risk space interacting with sequences conferring

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liability for schizophrenia and other cognitive disease. We predict that spatial genome exploration will deliver radically new insights into the brain's nucleomes in health and disease.

General Introduction to the 'Spatial Genome'

The 6Gb human diploid genome is spatially organized in the cell nucleus via myriads of chromosomal conformations, many of which bypass the 'linear genome' and thereby position stretches of non-contiguous stretches of intra- or, to a lesser degree, inter-chromosomal sequence into close physical proximity with each other. Orderly configuration of chromosomal conformations is thought to be an important prerequisite for proper genome organization and function. Recent articles have discussed the experimental and computational methods commonly used to explore the basic building blocks of mammalian '3-dimensional genomes' (3DG) [1,2]. Here, we discuss some of the recent findings from 3DG studies that employ genome-wide ('all sequences surveyed against all sequences') Hi-C DNA-DNA proximity assays, involving chromatin restriction digestion followed by re-ligation, ideally within intact nuclei [2]. It should be noted that the actual proximity of non-contiguous DNA fragments 'stitched together' by DNA-ligase treatment—as the critical step in the Hi-C protocol following the chromatin digest—is thought to vary across 2–3 orders of magnitude on the nanometer scale [3] in the interphase nucleus. Importantly, Hi-C studies across a wide range of species and tissues revealed, for each chromosome, various degrees of 'modular' organization, or chromatin domains frequently referred to as *topologically-associated domains (TADs)*, with smaller domains ('subTADs') nested into them. Each TAD and subTAD is comprised of long stretches of sequence, typically extending across hundreds of kilobases showing much higher chromosomal contact frequencies within as compared outside their domain boundaries, including the neighboring TADs [4]. Furthermore, TADs often assemble inside the nucleus with others TADs, preferentially those with of a similar chromatin architecture, ultimately giving rise to A/B (open/repressive) megabase-scale compartments, or chromosomal megadomains[5]. The function of TADs includes insulation by preventing spurious interactions between elements such as promoters and enhancers, which in turn could lead to aberrant levels of gene transcription, for instance of an oncogene [4,6]. Early reports indicated that TAD boundaries were enriched for a heterogenous set of genomic entities, such as housekeeping genes, transfer RNAs and short interspersed element (SINE) retrotransposons [7]. Furthermore, and somewhat surprisingly, the majority of TADs are considered to be conserved among cell types, and even species [7–10]. However, their potential for insulation and connections to other regions, both measured quantitatively, can be subject to cell-type-specific changes, primed perhaps by transcription factor binding at least during lineage specification [11]. Additionally, TAD insulation is highly correlated with transcription, such that novel TAD or subTAD borders could assemble around promoters of developmentally regulated genes [12].

Furthermore, the spatial genome also includes including tens of thousands of *chromosomal loop* formations, commonly defined as distinct pairwise contacts that, in Hi-C maps, sharply stand out from the surrounding 'linear' genome sequence, often interconnecting specific regulatory elements such as, for example, a gene promoter with a distal enhancer[2]. In contrast to TADs, a significant portion of chromosomal loopings is subject to cell type-

specific regulation[13]. Note that the computational algorithms defining 'loops' in Hi-C datasets may differ between studies, with some authors reporting as many as 1×10^6 loop-like conformations in their Hi-C datasets [14]. Furthermore, higher order forms of promoter-enhancer loopings may exist, as exemplified by euchromatic enhancer islands 'bundled' together with odorant receptor genes via inter-chromosomal interactions[15]. Such type of olfactory receptor gene-specific chromosomal contacts apparently are positioned within, or at least in close proximity to constitutive and facultative heterochromatin that in olfactory sensory neurons—in contrast to many other cell types—includes a significant portion of central territories of the nuclear interior[16, 17]. Such types of higher order chromatin architectures are considered important for proper transcriptional regulation of the (single) active odorant receptor allele in an individual olfactory sensory neuron[18,19]. While compartments, TADs, and chromatin loops are important for genome organization including epigenetic and transcriptional regulation, the mechanisms governing the formation of such types of higher order chromatin are only partially understood. For instance, CTCF binding sites often are positioned in inward/convergent orientation, and to a lesser degree, tandem orientation, at the two contact sites of a particular loop or certain TADs [2,7,10,20]. Experimental inversion of CTCF binding sites at promoter-enhancer loops could in some instances alter normal patterns of gene expression [21]. Estimates of the proportion of loopings with convergent CTCF sites at loop anchor sequences range from 65% to 92% [2,20]. Of note, CTCF directionally recognizes binding sites via an 11 zinc finger array. Cohesin, as a multi-subunit protein complex, in turn, is assembled from the CTCF's C-terminal end, resulting in loop-bound head-to-head CTCF configurations [21]. The orientation is purported to matter due to the biophysical "loop extrusion" model, whereby structural proteins such as cohesin continuously extrude chromatin until blocked by CTCF that is in an appropriately oriented site [22,23]. Cohesin removal, by deletion of cohesion-loading factor Nipbl or by auxin-induced degradation of RAD21 (a cohesin complex component), revealed a loss of CTCF loops and TADs but preservation of A/B compartment segregation, suggesting two independent mechanisms for 3DG and chromatin domain organization [24,25]. Similar to the decoupling of topological features impacted by cohesin depletion, depletion of CTCF also eradicated, in dose-dependent fashion, CTCF-bound chromosomal loops and TADs while compartments remained largely unaffected [26].

Neuronal differentiation is associated with genome-scale remodeling of the chromosomal contact map

Recent studies in developing mouse brain described developmentally regulated changes in 3DG folding in the context of multiple mechanisms including CTCF-dependent loop alterations, repressive chromatin remodeling and dynamic changes in cell- and lineage-specific transcription factor networks[12], and mobilization of genes into or out of heterochromatic environments such as the (nuclear) lamina-associated domains[27]. Similarly, during the course of isogenic differentiation of human neural precursors cells (NPCs), the genes bound to loopings that underwent pruning during the course of NPC-to-neuron-transition were significantly enriched for regulators of cell proliferation, morphogenesis and neurogenesis [13]. This is likely a reflection of the cells' developmental dynamics including departure from precursor stage towards neuronal lineage commitment

and neuronal differentiation [28]. Likewise, loops lost during NPC-to-glia-transition were significantly enriched for various neuron-specific functions, consistent with non-neuronal lineage commitment[28]. In addition, loss of many shorter range contacts and loopings during NPC-to-neuron transition was associated with concomitant increases in longer range (>100–200kb) contacts in both human and mouse *in vitro* model systems [12,13]. Of note, smaller subsets of short-range loops and contacts, including CTCF- and neuron-specific transcription factor occupied loop anchors, may indeed increase rather than decrease during the course of neuronal differentiation, reflecting functional dynamics in chromatin folding intimately linked to cell identity [13].

Such types of dynamic 3DG remodeling during the course of neural differentiation are unsurprising and in line with the overall association between chromosomal loopings and active gene expression. However, there is a somewhat unexpected, if not perplexing finding that emerged from the first wave of Hi-C studies in developing human and mouse brain: massive (estimated 25–50%) genome-wide net loss of chromosomal contacts in postmitotic neurons and adult cerebral cortex, as compared to neural progenitors and fetal cortex (Figure 2A). This large-scale net pruning of chromosomal contacts appears to be specific for neuronal differentiation[13]. The same phenomenon was independently reported in a study comparing Hi-C libraries in postmortem (human) fetal versus adult cortex[29]. Importantly, the two studies applied (i) different variations of the Hi-C protocol including different choices of enzymes for chromatin digest, and (ii) different biocomputing algorithms to count more broadly defined chromosomal contacts[29] versus chromosomal ‘loops’ as more conservatively defined distinct pair-wise contacts[13]. Furthermore, both studies [13,29] corroborated the findings from their Hi-C data by analyses of (previously published) additional Hi-C datasets from developing mouse and human brain [12,30]. In addition, genome-scale pruning of chromosomal loopings was specific for NPC-to-(glutamatergic) neuron generation and not observed in the isogenic parallel differentiation NPC-to-glia, and not associated with genome-wide shifts in the proportion of open chromatin [13]. Thus, based on the available evidence generated to date, one could summarize the above discussion that in mammalian brain, the process of neuronal differentiation involves a net loss of chromosomal contacts, apparently disproportionally affecting many ‘shorter range’ chromosomal contacts (<100–200Kb range) and accordingly, many of the smaller TADs, including nested subTADs[13,29] (Figure 2A). The biological significance of such developmental pruning of the chromosomal contact map in neurons is presently unclear and the field eagerly awaits additional studies that are to be expected to provide deeper insight into the massive 3DG remodeling in developing neurons.

Exploring mechanisms of neurological disease via spatial genome studies

References [31–34] provide an overview of early 3DG studies in the nervous system, which mostly explored candidate gene loci using polymerase chain reaction-read out of chromosome conformation capture (3C) assays and, by that approach, described phenomena such as activity-dependent regulation of chromosomal contacts in the context of gene expression changes during learning and memory[35] and in chronic neuropsychiatric disease[36–38)], or drug addiction[39]. In addition, findings from clinical genetics leave little doubt that proper 3DG regulation is critical for brain development and function, given

that mutations and DNA structural variants impacting genes encoding chromosomal scaffolding proteins (including classical ‘loop organizers’ assembling as Cohesin-CTCF complex) are associated with neurological maladies including neurodevelopmental syndromes and—as exemplified by mutations of the nuclear lamina-associated protein *LMNB1*—also adult-onset demyelinating disease (reviewed in [31]).

Localized disruption of TAD architectures in neurological disease

To date, very few studies have explored regulatory mechanisms governing structure and function of chromatin domains, or TADs, in the nervous system. A recent study in conditional mutant mice with neuron-specific ablation of the repressive histone H3-lysine 9 methyltransferase and neurodevelopmental risk gene, *KMT1E (SETDB1)*[40,41], reported that a small subset of megabase-spanning neuronal TADs (‘superTADs’) are dependent on SETDB1-mediated H3K9 methylation at the site of intra-TAD sequences flanking the TAD boundaries[42] (Figure 1A). Ablation of *Setdb1* resulted in excessive CTCF occupancies, which together with the concomitant loss of H3K9 methylation is thought to disrupt repressive chromosomal conformation, including long-range contacts interconnecting the distal portions of the TADs [42] (Figure 1B). Such a disruption led to a partial disintegration of the superTADs, best exemplified by a large domain on mouse chromosome 18 that encompasses >70 genes, including the clustered *Protocadherin* genes[42]. This family of cell adhesion molecules is critically important for orderly development of neuronal connectivity[43], with the stochastic restraint and expression levels of individual Protocadherin genes in neurons regulated by a delicate balance of promoter-bound DNA methylation and sense transcription, CTCF promoter occupancy and specific promoter-enhancer loopings[42,44]. Importantly, localized disruptions of TAD architectures were associated with an abnormal expression of the affected Protocadherin genes in the adult mutant brains[42] or neural cell cultures [44], strongly suggesting that orderly formation or maintenance of TAD-bound chromosomal conformations associated with specific TADs provide a critical layer of transcription regulation in the affected neurons.

The potential importance of proper chromatin structures specifically at the site of TAD boundaries became dramatically clear when a recent study uncovered that a surprisingly large share, or 22 out of 27 neurological and medical conditions associated with abnormal expansion of short tandem repeat (STR) sequences had their disease-associated STR sequence located to the site of a TAD boundary[45]. These disease-associated STRs at TAD boundaries were defined by a very high CpG island density and included the specific STR sequences subject to an abnormal expansion, each associated with a monogenic neurodevelopmental or neurogenerative disease, including but not limited to FMR1 (fragile X syndrome), FXN (Friedreich’s ataxia), HTT (Huntington’s disease) and C9ORF72 (motor neuron disease) and ATXN1 (spinocerebellar ataxia 1)[45]. Unsurprisingly, given that TAD landscapes are largely invariant to cell type, the disease-relevant STR location at TAD boundaries was present in embryonic stem cell, blood, neural progenitor and postmortem brain tissue[45]. Interestingly, cell lines from FMR1 patients showed subtle changes in chromosomal conformations at the affected TAD boundaries including abnormal CTCF peak profiles within 100kb of the abnormally expanded STR[45]. However, it remains to be clarified whether the localized disruption of TAD architectures plays a role in the epigenetic

dysregulation, including abnormal DNA methylation, and transcriptional shutdown of the FMR1 gene in fragile X cases through the positioning of disease-associated unstable DNA repeats at the site of TAD boundaries. The questions could be expected to be resolved soon.

3DG mappings uncover an expanded genomic risk space associated with schizophrenia

Schizophrenia is a common disorder, affecting 0.8% of the population world-wide[46], defined by core symptoms such as cognitive impairment and thought dysfunction, delusions and hallucinations, social withdrawal and a host of additional psychiatric symptoms[47]. The overwhelming majority of cases escape a mono- or oligogenic disease etiology thus far[48], while on the other hand common variants contributing to heritability risk are overwhelmingly positioned in non-coding DNA, with to date 145 loci identified by genome-wide association in the largest study involving 105,318 subjects [49]. Each of the 145 genomic loci linked to schizophrenia heritability harbors common variants, extending across 1bp to up to >1Mb of sequence in linkage disequilibrium (LD), making it exceedingly difficult to identify the causal variants[49]. Because the majority of functional elements in human non-coding DNA, including enhancers and repressors, are *not* bound to the nearest TSS but instead tethered via chromosomal contacts to genes located elsewhere on the chromosome[50], it is unsurprising therefore that, as discussed in[26], non-3DG based approaches such as, for example, gene expression quantitative trait loci (eQTL) and SNP prioritization algorithms had only limited success in assigning specific target genes to risk loci. In a pioneering Hi-C study, Won and colleagues integrated chromosomal conformations from fetal brain with schizophrenia GWAS noncoding variants and were able to highlight many candidate genes interacting with them, including those integrated in disease-relevant pathways such as cholinergic signaling and neurogenesis [30]. Another Hi-C study charting 3DG maps from fetal and adult human cortex reported that 1,197 (8.1%) of all brain-expressed protein coding genes are linked to a schizophrenia GWAS locus, with a majority of such genes being separated from the risk locus by hundreds of kilobases (median 305kb), and interact with a specific risk locus via chromosomal conformations bypassing the linear genome[29]. Furthermore, an integrative study by the PsychENCODE consortium[51], analyzed transcriptome and open chromatin landscape and transcriptional histone marks from altogether 2000 postmortem brains including hundreds of cases diagnosed with schizophrenia and integrating these profiles from the ‘linear genome’ with Hi-C data from fetal and adult reference brains[52]. The study mapped ~79,000 brain-active enhancers with their associated chromosomal contacts and TAD landscapes[52] and identified a vast number of eQTLs and gene regulatory networks and perhaps most importantly, applied deep machine learning algorithms that, for the first time, were able to predict presence or absence of disease (schizophrenia) based on a subject’s brain transcriptome and chromatin profiles[52]. The study approached disease prediction at probability level of 75%, reflecting a significant advancement over more conventional genomic approaches predicting disease only marginally above chance (50%) [52]. Likewise, Hi-C chromosomal contact mapping in iPSC-derived NPCs and their differentiated neurons and glia, increased the number of actively transcribed genes associated with a schizophrenia GWAS locus by approximately 2–3 fold (total *N* expressed genes interconnected with or located within a schizophrenia GWAS

locus ranged from 201–386, depending on cell type (NPC, neuron, glia) [13] (Figure 2B). Because neurons, together with NPC, had the largest number of cell-type-specific chromosomal contacts anchored in a risk locus (as compared to glia and other non-neuronal cells), one could conclude that (as discussed above) while the overall spatial genome space contracts when NPC differentiate to neurons, the 3DG space associated with schizophrenia risk disproportionately increases as neurons differentiate[13]. Remarkably, the disease-related chromosomal connectome specific to NPC or neurons was associated with “clusters” of coordinated gene expression and protein interactions, with at least one cluster strongly enriched for regulators of neuronal connectivity and synaptic plasticity, and another cluster for chromatin-associated proteins, including transcriptional regulators[13]. Likewise, an expanded genome space involving higher order chromatin and chromosomal contacts anchored in schizophrenia risk loci has also been described for cultured primary sensory neurons from the olfactory neuroepithelium[53] (Figure 2B,C).

Outlook and future studies

Here, in this *Current Opinions* article, we report recent 3DG discoveries highly relevant for two very different categories of brain disorders. The first category includes rare monogenic neurological disorders associated with locus-specific abnormal expansion of short DNA repeats. The second disease category includes schizophrenia, a common psychiatric disease defined by an exceedingly complex genetic risk architecture. The fact that two such disparate disease categories, each afflicting the human brain in very different ways, were both newly informed via Hi-C-based approaches clearly speaks to the promise of spatial genome exploration in the fields of genomic medicine and neurobiology. We predict that 3DG studies, in cell-type-specific fashion, will provide new and critical insights into the genetic risk architectures of a broad range of neurological and psychiatric disorders and thereby providing a critical link between genome, epigenome and the ‘nucleome’ in normal and diseased brain. To mention just one example for work expected to be pursued in the near future, the next generation of schizophrenia-focused 3DG work could focus on the chromosomal risk connectome that showed a surprisingly strong correlation at the level of the transcriptome and, at least for a subset of expressed genes, also at the level of the proteome[13]. It is an open question currently whether or not the (schizophrenia) GWAS-bound genomic sequences converge on intra- and inter-chromosomal hubs enriched for specific transcription or splicing factors, in analogy to similar principles governing coordinated regulation of gene expression in sensory and peripheral systems [15,54,55]. To this end, it is encouraging that the three major functional categories associated with the genetic risk architecture of schizophrenia—neuronal connectivity, synaptic signaling and chromatin remodeling[56,57]—are also heavily represented within the developmentally regulated cell-type-specific chromosomal connectomes of cultured neurons and their precursors[13] and fetal brain tissue *in vivo* [30,58]. We have argued that cell-type-specific intersection of 3DG and genetic risk maps may further deepen understanding of the genomic underpinnings of normal and diseased cognition, and may lead to improved disease risk prediction as compared to cumulative schizophrenia risk allele burden estimates such as “polygenic risk score” (PRS) or “biologically-informed multilocus profile scores” (BIMPS), which currently do not take into account the spatial genome, a critical limitation that may

explain why PRS and BIMPS are presently only minimally informative about disease risk [59].

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Highlights

- Review of Hi-C genome-scale chromosome conformation (‘3D Genome’) mappings in brain
- Discussion of developmental 3DG reorganization in differentiating neurons
- Discussion of chromosomal contacts associated with schizophrenia risk sequences
- Monogenic neurological disorders and DNA repeats at chromatin domain boundaries.

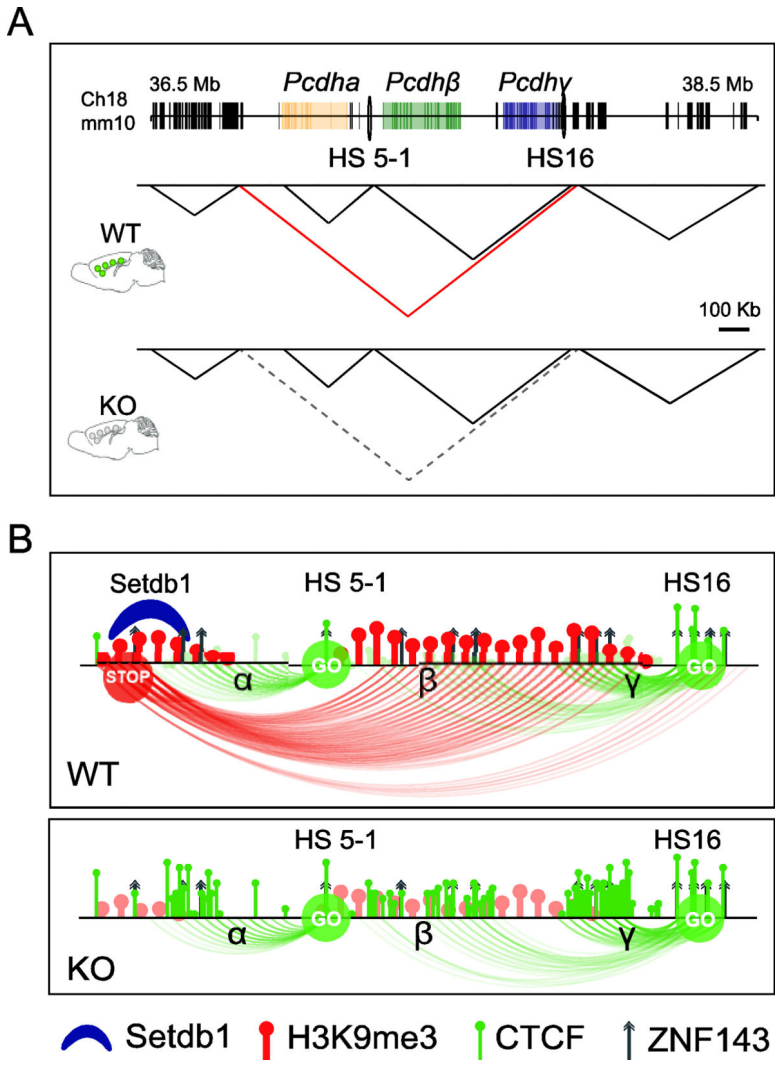

Figure 1: Selective loss of a large megabase TAD at the clustered Protocadherin gene locus after neuronal ablation of *Kmt1e/Setdb1* methyltransferase.

(A) Schematic overview of TAD landscape of adult cortical neurons in 2Mb portion of chromosome 18, shown for wildtype (WT) and conditional *Setdb1* knock-out (KO), as indicated. Dotted triangle in KO (corresponding to red triangle in WT) demarcates TAD that is massively weakened in KO neurons. Notice that subTADs and surrounding TAD landscape is minimally affected. (B) Chromatin structure and function at the clustered Protocadherin locus. Schematic presentation of 1 megabase TAD at site of Protocadherin genes (see A), illustrating repressive chromatin ('STOP') in TAD periphery connecting to intraTAD enhancer sequences ('GO'). Top, WT, Bottom, KO. Loss of SETDB1 in neurons results in histone hypomethylation, massive excess of CTCF across the TAD, loss of repressive loopings at enhancers and zinc finger protein (incl. ZNF143) binding sites, triggering excessive transcription. See ref. [42] for details.

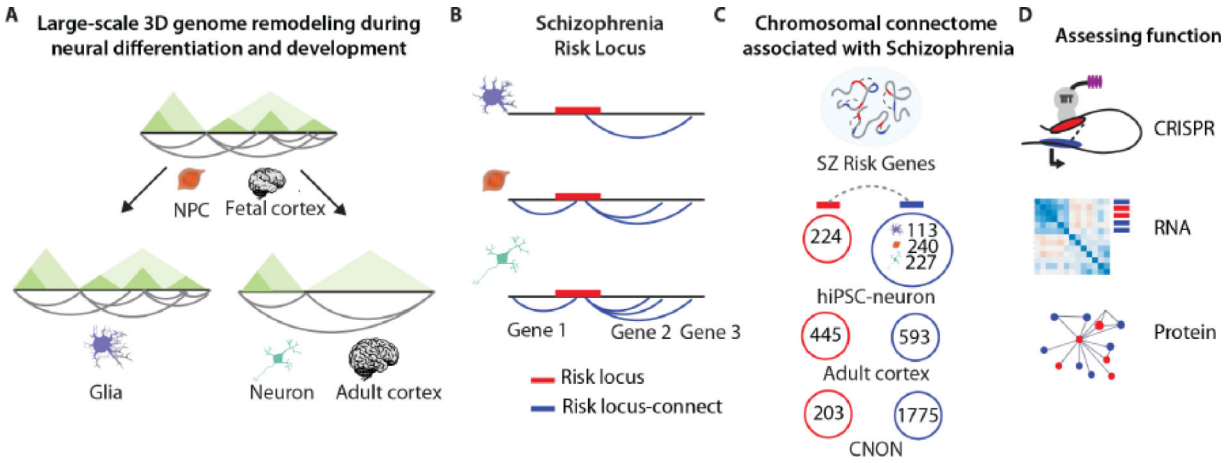

Figure 2: Developmental remodeling of the spatial genome during the course of neural differentiation, with implications for the expanded genomic risk architecture of schizophrenia.

A) Independent Hi-C studies exploring (i) hiPSC-derived neural progenitor cells (NPCs) and neurons and glia derived from them by isogenic differentiation, and (ii) 3DG mappings in adult and fetal postmortem cortex, reveal large-scale changes in chromosomal conformation including pruning of loops and contacts, and widening of TADs across neuronal differentiation and development [13,29]. B) Cell-type-specific chromatin contacts anchored at schizophrenia risk loci identify genes from outside of the loci ("risk locus-connect") interacting with common variants. Contacts defined in hiPSC NPCs/neurons/glia (n = 2 per cell type or 6 total, [13]) by binomial statistics, and in adult postmortem cortex (n = 3) [3+26], and neuronal cells derived from olfactory neuroepithelium (CNON) (n = 2) [53] by Fit Hi-C. Identification of risk locus-connect genes in multiple model systems expanding the known set of schizophrenia risk-associated genes. Rhie et al data [53]were re-assessed to consider vantage point from the latest 145 risk loci [49] used in Rajarajan et al [13] and Giusti-Rodriguez et al [29]as opposed to the older 132 used in the original study. Rajarajan et al [13] only considered cell-type-specific interactions. Giusti-Rodriguez et al [29] considered only interactions labeled as "promoter-promoter" or "promoter-enhancer" determined by open chromatin and histone modification signatures. Rhie et al [53] only considered interactions originating in bins containing high linkage disequilibrium regulatory (i.e., enhancer) variants as determined by histone modification and CTCF ChIP-seq. All three studies demonstrate the power of Hi-C to substantially increase the number of putative risk genes beyond location alone. Red circle = risk locus genes participating in Hi-C interactions; Blue circle = risk locus-connect genes participating in Hi-C interactions. D) Functionality of contacts of interest can be assessed through CRISPR (epi)genomic editing, transcriptome, and proteome studies.