REVIEW

The Genome in Three Dimensions: A New Frontier in Human Brain Research

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Less than 1.5% of the human genome encodes protein. However, vast portions of the human genome are subject to transcriptional and epigenetic regulation, and many noncoding regulatory DNA elements are thought to regulate the spatial organization of interphase chromosomes. For example, chromosomal "loopings" are pivotal for the orderly process of gene expression, by enabling distal regulatory enhancer or silencer elements to directly interact with proximal promoter and transcription start sites, potentially bypassing hundreds of kilobases of interspersed sequence on the linear genome. To date, however, epigenetic studies in the human brain are mostly limited to the exploration of DNA methylation and posttranslational modifications of the nucleosome core histones. In contrast, very little is known about the regulation of supranucleosomal structures. Here, we show that chromosome conformation capture, a widely used approach to study higher-order chromatin, is applicable to tissue collected postmortem, thereby informing about genome organization in the human brain. We introduce chromosome conformation capture protocols for brain and compare higher-order chromatin structures at the chromosome 6p22.2-22.1 schizophrenia and bipolar disorder susceptibility locus, and additional neurodevelopmental risk genes, (*DPP10, MCPH1*) in adult prefrontal cortex and various cell culture systems, including neurons derived from reprogrammed skin cells. We predict that the exploration of three-dimensional genome architectures and function will open up new frontiers in human brain research and psychiatric genetics and provide novel insights into the epigenetic risk architectures of regulatory noncoding DNA.

Key Words: Chromatin fiber, chromosomal looping, chromosome conformation capture, genome in 3D, higher-order chromatin, human brain

A comprehensive exploration of the human genome will have to extend far beyond the fine mapping of its linear sequence, which comprises 6 billion base pairs (bp) in diploid cells. Importantly, there is a rapidly increasing number of studies that explore, in the human brain, the regulation of various epigenetic markings including DNA cytosine methylation and hydroxymethylation, and many of the (estimated more than 100) residue-specific posttranslational modifications such as acetylation, phosphorylation, and methylation of the nucleosome core histones (a nucleosome is the elementary unit of chromatin, with 146 bp of DNA wrapped around a histone H2A/H2B/H3/H4 octamer) (1). These epigenetic markings, in various combinations, show a complex sequence-specific distribution in brain and many other tissues, resulting in specific epigenetic signatures of proximal promoters, enhancers, actively expressed genes, silenced genes, and facultative or constitutive heterochromatin (such as the pericentric or telomeric repeat sequences at the extreme end of chromosomes) (2–11). However, there is also evidence for coordinated regulation of the epigenome, which includes a nonrandomness in the spatial organization of interphase chromosomes, often referred to as "loopings," "tetherings," and "globules." These structures enable noncontiguous DNA sequences, which on the linear genome are separated by various amounts of interspersed sequence, to move into close physical contact with each other (12). Chromosomal "loopings," in particular, are pivotal for the orderly process of gene expression, by enabling distal regulatory enhancer or silencer elements positioned on the same chromosome to interact directly with that specific proximal promoter and the transcription start site of the gene (13,14). Furthermore, actively expressed genes are more likely to be clustered together and positioned toward a central position within the nucleus, whereas heterochromatin and silenced loci tend to locate toward the nuclear periphery (15,16).

Unsurprisingly, therefore, in recent years an increasing number of studies explored three-dimensional (3D) genome architectures both in vertebrate (17) and invertebrate (18) model systems, and there is little doubt that the regulation of chromosomal conformations is of critical importance for human health, including orderly brain development and function. One well known example relates to Cornelia de Lange Syndrome (CdLS), with an estimated incidence of 1:10,000–30,000 live births among the more frequent genetic disorders (source http://ghr.nlm.nih.gov). Of note, CdLS often is associated with severe developmental delay and a range of neuropsychiatric symptoms, including autism spectrum disorder and psychosis (19). The CdLS syndrome (including *Online Mendelian Inheritance of Man* 122470 and 300590) is linked to mutations in the cohesin complex, a multisubunit protein that includes, among others, nipped B-like protein, structural maintenance of chromosomes proteins SMC1A and SMC3, and histone deacetylase HDAC8 (20,21). Cohesin is thought to form ring-like structures bringing together DNA segments from different locations, and by interaction with transcriptional co-activators such as Mediator, the complex is thought to promote promoter-enhancer loopings that define cell-type specific gene expression programs (22).

However, despite of the growing realization of the importance of 3D chromatin architectures for transcriptional regulation, very little is known about their role in the nervous system. Until recently, to the best of our knowledge, only three studies in the literature reported on loop formations in brain tissue

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(23–25). Other studies used brain as negative control for studies that were primarily focused on the sensory epithelium of the nose (26) or the hematopoetic system (27). Interestingly, there is evidence that at least some of the higher-order chromatin structures, including physical interactions of noncontiguous DNA elements and chromosomal loopings, are preserved in human brain collected after death (28). Furthermore, DNA-histone nucleosomal structures are largely maintained even after 35 hours of autolysis (29). Therefore, it seems feasible to study not only the posttranslational decoration of nucleosome core histones but also more complex layers of epigenetic regulation. Recently, for example, chromosome conformation capture (3C) assays and genome-wide histone methylation mappings were employed to the prefrontal cortex of the human and nonhuman primate brain, revealing multiple physical interactions of DNA elements separated by hundreds of kilobases sites within several neurodevelopmental risk loci (including 2q14.1/*DPP10* and 16p11.2) (28).

On the basis of the aforementioned findings then, the "genome in 3D" should be open for investigation in the human brain, including its surrogates, such as cultured neurons and glia. Here, we provide an overview on chromosome conformation capture (commonly referred to as 3C) technique, which to date is considered the optimal approach to map and quantify 3D genome structures (30). We provide illustrative examples to highlight the advantages and limitations of 3C. Specifically, we present chromosome conformation data for the major histocompatibility complex II (MHC II) locus on chromosome 6, long implicated in schizophrenia and other psychiatric disease and recently confirmed by large genome-wide association studies (GWAS) (31–33).

The 3C technique involves mild crosslinking of nuclei followed by chromatin extraction and restriction enzyme digest, followed by religation of cut DNA fragments with DNA ligase, followed by polymerase chain reaction (PCR)-based quantification of ligation product. The central tenet of 3C is that, during the critical religation step, the fusion of the digested DNA ends at the site of chromosomal loopings will result in an artificial DNA sequence that normally is not encountered in the linear genome (Figure 1) (34–39). The 3C technique does not require special reagents such as antibodies and is easily quantifiable and scalable from locus/candidate sequence-specific assays to genome-scale chromosomal interaction maps (39,40).

Methods and Materials

3C

See Supplement 1 for detailed, step-by-step description of protocol.

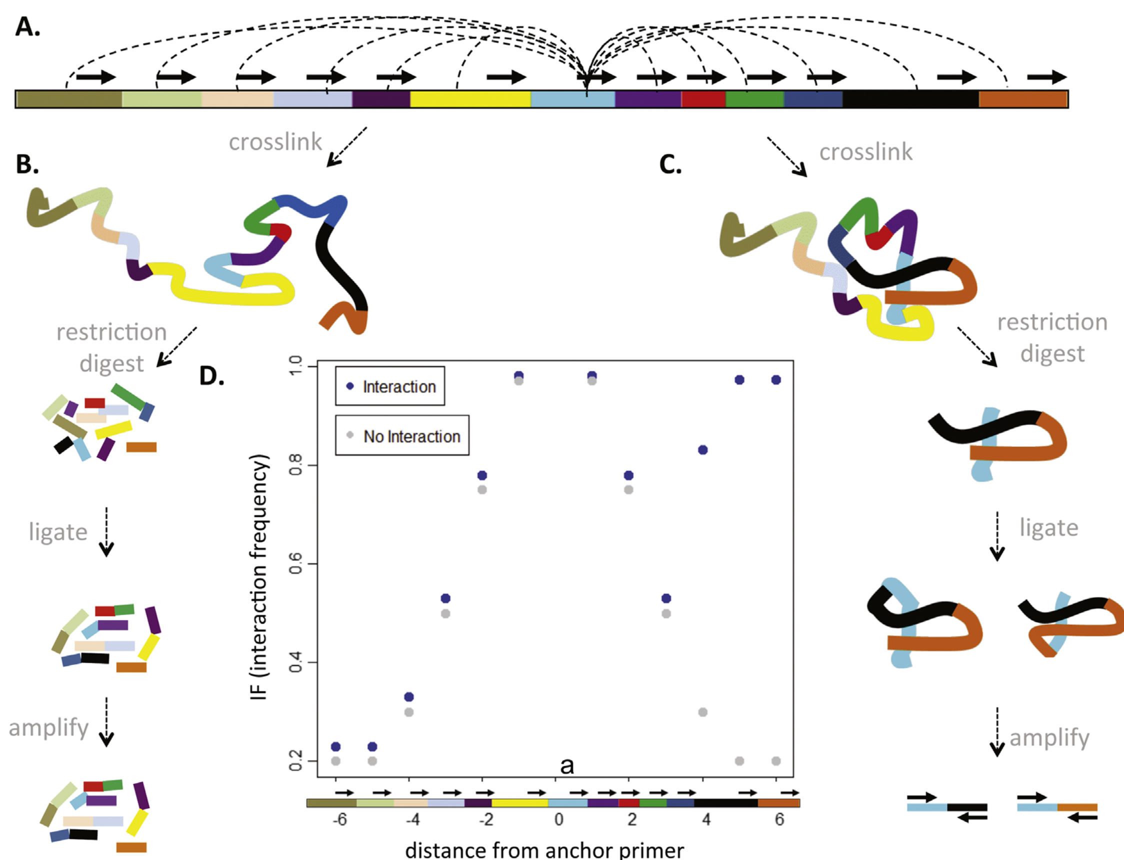

Figure 1. Chromosome conformation capture (3C). **(A)** For 3C assays, primers (represented as arrows) are designed within 30 base pairs (bp) of the restriction fragments (shown as colored rectangles). For 3C interactions in *cis* (same chromosome), primers typically are placed in 5′ to 3′ orientation along the same DNA strand to detect looping interactions. Typically the first primer is in a fixed position and the 3C assays are anchored to that specific restriction fragment (light blue in **A**), and interactions are tested with a set of second primers, each from a different fragment positioned at increasing distances from the anchor (A). Hypothetical chromosome without **(B)** or with **(C)** a loop formation. Theoretically, only **(C)** will result in PCR product from noncontiguous DNA elements with the anchor. **(D)** Hypothetical outcome from a typical 3C experiment, revealing in the absence of a loop formation ("no interaction") exponentially declining 3C product intensity with increasing distance from the anchor (a) (fragment 0). In contrast, there is a robust interaction between the anchor (a) and the more distal fragment (black/brown fragments 5, 6) in chromosome with loop formation.

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