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Three-dimensional chromosome architecture and drug addiction

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Abstract

Aberrant gene expression underlies drug addiction. Therefore, studying the regulation of gene expression in drug addiction may provide mechanistic insights into this disease, for which there are still only limited treatments. Recently, the three-dimensional (3D) compaction and organization of linear DNA in the nucleus has been recognized as having a major influence on gene transcription. Here, we review its roles in both basic brain function and neuropsychiatric disorders, while also highlighting its emerging implications in drug addiction. Unraveling the 3D organization of chromosomes in drug addiction is adding to our understanding of this disease and has the potential to trigger novel approaches for better diagnosis and therapy.

Introduction

Substance use disorders, or drug addiction, are characterized by maladaptive neural plasticity in response to drugs of abuse that persists throughout the lifetime of an individual. Susceptibility to addiction is believed to be the convergence of genetic predisposition and exposure to environmental risk factors, which results in dysregulation of specific genes [1,2]. Recent research into the molecular basis of drug addiction has focused on epigenetic mechanisms, such as posttranslational histone modifications and covalent DNA modifications, and demonstrated that drugs of abuse lead to widespread alterations across the epigenome, which are often associated with aberrant gene transcriptions in key brain regions of the reward system [3,4]. However, these have been predominantly studied on the level of one-dimensional linear genome.

In each eukaryotic cell, billions of DNA nucleotides span about two meters when stretched linearly, requiring extensive three-dimensional (3D) folding to fit into a nuclear space of only several micrometers in diameter. Through the mobilization of distal (i.e. up to several million bases away) regulatory elements into spatial proximity of their target genes, 3D genomic organization regulates cell type-specific transcriptional programs throughout

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Declaration of Conflicts

None to declare.

development and culminates into discrete organizational profiles in mature cells [5–7]. The discovery of such 3D chromosome interactions points to a functional interplay between genome topology and gene expression, revolutionizing the current view of transcriptional regulation. However, its implications in brain function, particularly in relation to substance use disorders, remain largely unknown [8–12].

Three-dimensional chromosome organization and brain function

The recent development of chromosome conformation capture (3C)-based techniques [13–15] has complemented traditional microscopy-based studies in characterizing 3D genomic architecture with much higher throughput and genomic resolution than previously possible. The basic strategy of these techniques is nuclear proximity ligation, which allows for the detection of linearly distant genomic segments that reside within spatial proximity of each other *in vivo* (Figure 1). Over the years, these methods have evolved to focus on interactions at different scales, which include between a single pair of genomic loci (3C), between one locus and all other genomic loci (4C), between all restriction fragments within a given region (5C), or between all possible pairs of fragments across the genome (Hi-C).

It is now understood that the genome is highly organized at several hierarchical 3D levels superimposed above nucleosomal organization (Figure 2). First, each chromosome occupies a defined nuclear volume known as a “chromosome territory” [16]. Additionally, the genome as a whole can be partitioned into two spatial compartments: an active “A” compartment and an inactive “B” compartment [15]. Located in the core of the nucleus, the “A” compartment is characterized by a high density of transcription factories which promotes the expression of chromosomal regions that are typically open, gene-rich, and high in GC content. In contrast, the “B” compartment is characterized by compact, gene-poor genomic regions that are minimally expressed. The “B” compartment exists in the nuclear periphery and interacts with nuclear lamina, a network of lamina proteins on the inner membrane of the nucleus. Such interactions with the lamina lead to formation of lamina-associated domains (LADs), which typically contain heterochromatic segments and function to anchor inactive chromosomal regions to the periphery of the nucleus [17]. Furthermore, the borders of LADs are defined by enrichment of active promoters, CpG islands, and the insulator protein CCCTC-binding factor (CTCF), which may prevent the mobilization of silenced elements into the nuclear core [17]. Additionally, each chromosome is comprised of a number of topologically associating domains (TADs), which contain chromosomal regions that preferentially interact with each other *in cis* and can be hundreds of kilobases to several megabases in length. Furthermore, TADs are maintained throughout cell divisions, and consistent between different cell types [18–20]; due to their high degree of conservation across species, these chromatin domains are considered to be the fundamental units of chromosome folding. Finally, the lowest level of 3D genomic organization occurs via DNA looping which, through the activity of architectural proteins (*e.g.* CTCF), enables distal genomic segments (*e.g.* enhancers) to be positioned into close spatial proximity of target genes and influence their expression [5–7].

Though only a handful of studies have investigated higher-order genome organization in the brain, they have all highlighted its essential roles in brain function [8–12]. One of the first

pieces of evidence was revealed nearly 70 years ago with the demonstration of nuclear reorganization in motor neurons following electrical stimulation of the feline hypoglossal nucleus [21,22]. It was also found that during neural cell differentiation, respective sets of genes associated with cell identity undergo repositioning from peripheral heterochromatin domains to the inner compartment of the nucleus [23]. Using Hi-C to profile the chromosomal connectome, it was recently demonstrated that neuron- and astrocyte-like cells undergo cell type-specific 3D architecture remodeling during differentiation from human induced pluripotent stem cells [24]. Similarly, the role of 3D nuclear organization has been examined in the context of olfactory and retinal sensory neurons [25–27]. Though heterochromatin normally resides at the nuclear periphery, whereas euchromatin situates toward the nuclear interior, it was found their organization in rod photoreceptor neurons of nocturnal retinas is inverted [27,28]. The dense heterochromatin localized in the nuclear center may therefore serve as collecting lenses to facilitate light transduction efficiency, which provides an example on how nuclear architecture implicates in neuron function. In the mouse nose, odorants are detected by more than 1,000 different olfactory receptors (ORs) with each olfactory sensory neuron expressing a single receptor allele. ORs are used in combination to detect odorants, which explains how a myriad of odorants can be discriminated. From a series of studies from Lomvardas' group, it was found that selective chromatin-mediated silencing and spatial positioning of OR genes ensure aggregation of inactive OR genes into heterochromatic foci which allows such monogenic and monoallelic expression of a single OR gene in olfactory sensory neurons [29–31]. Similarly, the expression of trace amine-associated receptors (TAARs), whose expression resembles that of ORs, is accompanied by an escape of the encoding genes from peripheral heterochromatin to the permissive interior chromatin environment [26]. Furthermore, it was demonstrated that the transcription factor and adapter protein that bind to intergenic olfactory receptor enhancers (or "Greek islands") also mediate trans-chromosomal interactions so to facilitate multi-chromosomal super-enhancer that is associated with the active single olfactory receptor gene [32]. Recently, a single neuron 3D structure was characterized by using diploid chromatin conformation capture (Dip-C) [28], which further illustrated multiple aggregates of OR genes and enhancers from different chromosomes in the olfactory sensory neuron. Combined together, these studies provide convincing evidence for the link between chromatin organization and neuronal function.

Upon neuronal activation, the expression of many genes, particularly immediate early genes, require changes in chromatin accessibility [33] and formation of DNA loops involving regulatory regions such as enhancers [34]. Furthermore, neuronal activity induces the transcription of regulatory sequences to produce noncoding enhancer RNAs (eRNAs) [35], which may also participate in DNA looping to regulate transcription factor binding at target genes [36]. Such enhancer activities are often co-regulated to drive the coordinated expression of subsets of gene [37] or perform combinatorial functions to ensure proper expression in response to a variety of stimuli [38]. Chromosomal organization, therefore, is a crucial modulator of neuronal gene expression in the brain. Unsurprisingly, the malfunction of key molecules involved in chromosomal looping is associated with several brain disorders. For example, mutations of cohesin-related genes cause the neurodevelopmental disorder Cornelia de Lange syndrome (CdLS) [39,40], while mutations to the gene encoding

for CTCF have been implicated in the etiology of microcephaly and intellectual disabilities [41].

Three-dimensional chromosome organization in neuropsychiatric disorders

The implications of changes to 3D genome organization in neuropsychiatric disorders have also recently been examined [8,9]. For example, it was recently discovered that all known disease-associated short tandem repeats, such as those responsible for Fragile X syndrome, are located at the boundaries of 3D chromatin domains and that topological disruption of these boundaries may contribute the etiology of disease [42]. Using a FISH assay, it was also found that accumulation of H2BGFP (green fluorescent protein-tagged histone H2B) in hippocampal CA1 neurons of transgenic mice disrupts chromatin compaction and nuclear lamina interaction [43], which is also associated with detrimental effects on cognitive functions. Though this does not affect neuronal viability, it is accompanied with specific transcriptional changes in serotonin and dopamine signaling pathways, suggesting that some neuropsychiatric disorder-associated genomic loci may be vulnerable to changes in higher-order genome organization.

Akbarian and colleagues have done extensive investigations into 3D genomic organization in schizophrenia. From their work, it was found that the expression of NMDA receptor subunit GRIN2B/Grin2b is regulated by H3K9 methyltransferase SETDB1 through a conserved *cis*-regulatory element which forms activity-dependent DNA loopings encompassing over 500 kb of linear sequences; dysregulation of this locus affects mood-related behaviors and contributes to cognitive defects [44,45]. The group also reported destabilization of a megabase-scale neuron-specific topological chromatin domain following conditional knockout of *Setdb1* in mice, which was accompanied by increased CTCF binding, DNA hypomethylation, histone hyperacetylation, and up-regulated gene expression [46] (Figure 3). Similarly, they discovered that conserved chromosomal looping governs the expression of *GAD1/Gad1* in the prefrontal cortex (PFC), and that these structures are significantly weakened in humans with schizophrenia [47]. Furthermore, increased DNA looping of a putative *MEF2C* (encoding for a neurodevelopmental factor associated with schizophrenia) super-enhancer to risk loci over 500 kb away occurs in induced stem cell-derived cultured neurons of subjects with schizophrenia, and binding sites for MEF2C are enriched in *cis*-regulatory enhancer and promoter regions marked by aberrant H3K4 hypermethylation in postmortem PFC samples [48].

Given the aforementioned dynamic 3D structure changes during neural development [23,24,26] and a wide spectrum of neuropsychiatric disorders (*e.g.* schizophrenia) with developmental etiologies, it is reasoned that studying chromosome conformations in the developing brain would provide novel genetic insights of these neuropsychiatric disorders. Indeed, through Hi-C mapping generated during human brain corticogenesis, Won *et al.* not only recognized many novel long range enhancer-promoter interactions, but also displayed several candidate schizophrenia risk genes/pathways by integrating this 3D interactome with non-coding variants identified in schizophrenia genome wide association studies (GWAS) [49]. A recent report also found that risk variants for schizophrenia are overrepresented in genomic regions decorated with nucleosomal marks associated with active transcription in

the dorsolateral PFC and anterior cingulate cortex of subjects with schizophrenia [50]. Additionally, these 3D genomic changes associated with schizophrenia risk sequences are also highly enriched in neurons instead of glial cells [24]. Together, these studies highlight the epigenomic mechanisms by which the genome confers liability to the development of schizophrenia and how disruption to genomic architecture may contribute to neuropsychiatric disorders in general.

Three-dimensional chromosome organization in drug addiction

Addiction is regarded as a malfunctioning of neural plasticity. Several genes implicated in this process have been shown to undergo 3D genome organization changes in various contexts. For example, *Bdnf*, a well-studied gene in addiction, detaches from the peripheral nuclear lamina in rat hippocampal neurons after kainate-induced seizures [51]. The resulting translocation of *Bdnf* to the open chromatin environment of the nucleus center is believed to allow for its exposure to transcription factors in the inner “A” compartment, which facilitate promoter-enhancer interactions and gene activation [51]. Similarly, in mice, a single dose of cocaine induces translocation of Sig-R1 from the endoplasmic reticulum to the nuclear envelope, resulting in increased Sig-R1 interactions with emerin (a nuclear envelope transmembrane protein) and recruitment of chromatin compacting factors [52]. Interestingly, these alterations were found to specifically decrease expression of monoamine oxidase B, providing a possible mechanism of enhanced dopaminergic responses following drug exposure that involves 3D chromatin reorganization in the nuclear periphery. Furthermore, Arc (an immediate early gene product that is associated with addiction) was found to accumulate to the nucleus of striatal medium spiny neurons following cocaine-induced rapid upregulation. Arc downregulates phospho-Ser10-histone H3 (an important component of nucleosomal response) and is suspected to dull the decompaction of heterochromatic regions [53]. These results are consistent with findings from other studies that have demonstrated the impact of nucleosome remodeling on addiction-related behaviors and learning and memory processes [54,55].

Currently, there is still limited evidence that directly demonstrates the higher-order 3D genomic structures in addiction. In one study, however, Engmann *et al.* used chromosome conformation capture-based techniques (3C, 4C) to demonstrate cocaine-induced destabilization of a DNA loop that spans 1524 kb in the mouse nucleus accumbens, a central brain region involved in reward behaviors [56] (Figure 4). It was found that chromosomal looping which brings *Auts2* and *Caln1* within close proximity of each other is disrupted following repeated cocaine exposure. This was accompanied by a range of epigenetic changes, such as altered levels of H3K4me3 and DNA methylation, as well as disruption to CTCF binding at these loci. Importantly, these alterations occur in a neuronal subtype-specific manner, preferentially affecting D2-like medium spiny neurons (MSNs) of the nucleus accumbens, but not D1-like MSNs. Additionally, these effects were only observed in male mice, but not females. These results highlight the need for further investigation into the molecular divergences between the sexes that may contribute to differential susceptibility to drug addiction, as well as the specific roles of neuronal subtypes in brain regions containing heterogeneous cell types.

Conclusion

The 3D folding of chromosomes has been shown to have a major influence on gene transcription by facilitating interactions between gene promoters and distal non-coding regulatory elements. Emerging evidence indicates its roles in both basic brain function as well as in neuropsychiatric disorders. However, how 3D genomic architecture is organized and functions in drug addiction is still unclear.

Though there are still few studies that directly investigate 3D looping in relation to substances of abuse, previous observations suggest that 3D nuclear reorganization in drug addiction could be widespread. For example, numerous studies have demonstrated cocaine-induced epigenetic changes across intergenic non-coding regions (*e.g.* [57]). It is predicted that these epigenetic changes are associated with hundreds of thousands of regulatory elements located great distances away from their target genes. This indeed has been demonstrated by a study on the DNA loop connecting *Auts2* and *Caln1* genes, which bypasses 1524 kb linear DNA sequence; the epigenetic alterations on the non-coding DNA sequences within this loop are associated with cocaine-induced dissociation of both genes and their expression changes [56].

With coding sequences representing about 3% of the genome, the function of the vast majority of genome that is comprised of non-coding sequences remains elusive. We expect that the study of 3D chromatin organization will provide insights into regulation of gene transcription in drug addiction. For example, it is currently still obscure as to what the gene targets are, if any, for the large number of intergenic regions that display drug-induced epigenetic alterations. To address this question, future studies should take advantage of evolving tools for investigating 3D nuclear organization [58], such as recent advances in Hi-C methods that specifically probe 3D interactions at promoters and allow for characterization of the promoter interactome with unprecedented resolution [59]. Furthermore, GWAS have identified numerous SNP variants in addiction-relevant gene loci, many of which occur in noncoding regions (*e.g.* [60,61]). It has been shown that these SNP sites are highly enriched at intergenic regulatory regions, such as enhancers, which suggests a role for 3D chromatin organization in the pathogenesis (*e.g.* [46,49,62]). Therefore, elucidating higher-order genome organization in drug addiction should help us to prioritize candidate gene targets and decode disease-relevant pathways.

Although the study of 3D genome in the brain holds great promise, there are several questions that will need to be addressed moving forward. One technical challenge is the heterogeneous cell population of even brain micronuclei. As 3D chromosomal organization is highly cell-type specific, it is essential to probe the higher-order genomic architecture within the contexts of defined cell types. Though neuronal NeuN labeling and neuronal subtype fluorescent protein-expressing transgenic mice have shown success in this approach [46,56], isolation processes usually result in the loss of most genetic material. It is still a challenge to study the 3D genome in small brain structures (*e.g.* nucleus accumbens) at a global level (*e.g.* Hi-C), which usually requires far more cells than a single brain can provide. Furthermore, given the enormous number of chromatin interactions across the genome, thorough characterization of 3D genomic organization requires significant

sequencing depth of coverage. This also demands advanced bioinformatic analytical tools that may be developed through creative collaborations between biologists and bioinformaticians. Fortunately, technological advances are now allowing us to identify the spatial organization within a single cell [63] and across both space and time (i.e. the 4th dimension) [58]. Through the application of these and other tools, further investigation of 3D chromosome organization in drug addiction should reveal novel molecular insights into this disease that may have the potential to trigger novel approaches for better diagnosis and therapy.

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•• of outstanding interest

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Highlights

- Three-dimensional (3D) chromosome architecture is highly organized.
- 3D chromosome organization plays important roles in basic brain function
- Altered 3D interactions are associated with neuropsychiatric disorders
- Cocaine disrupts a particular DNA loop formation in the brain.

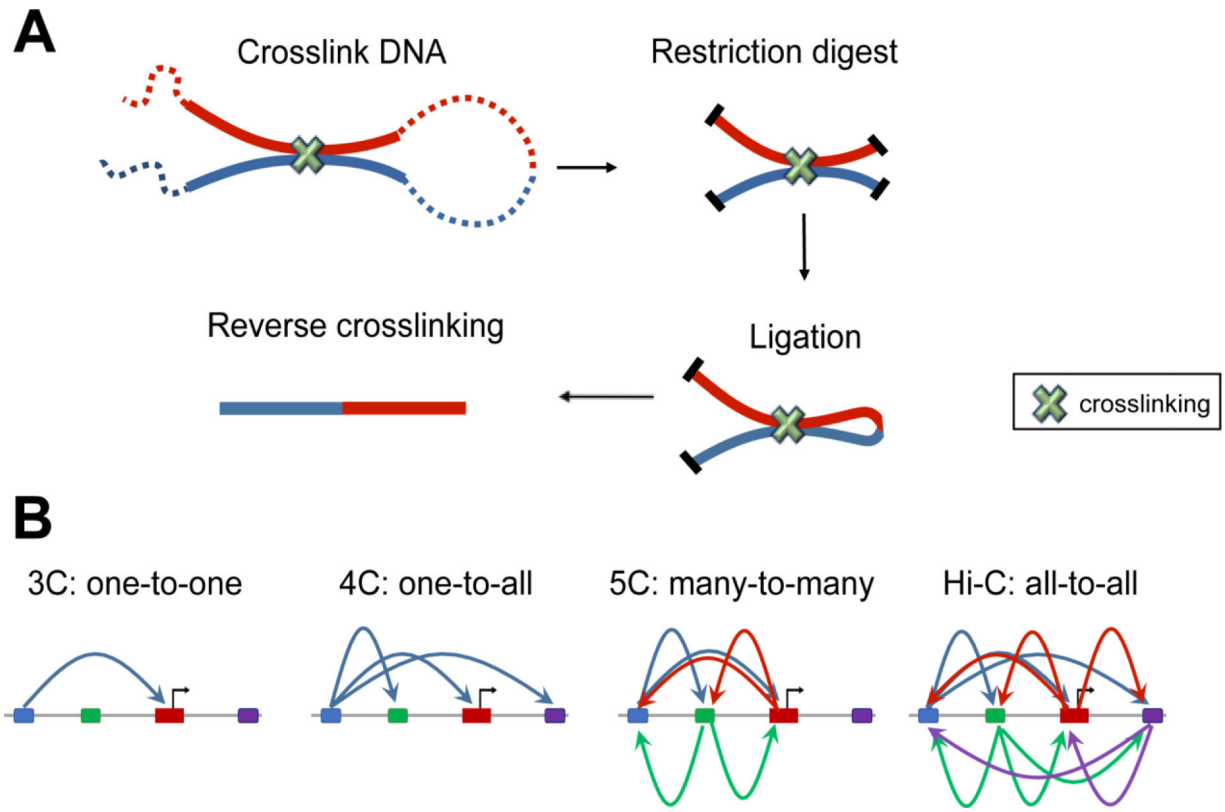

Figure 1. Chromosome conformation capture based techniques

(A) In Chromosome conformation capture (3C), three dimentional proximal DNA fragments are crosslinked and digested while in the nucleus. Open ends of DNA fragments are then repaired and chimeric fragments are ligated, which allows the detection of linearly-distant genomic segments that reside in spatial proximity. **(B)** The 3C-based techniques have evolved to focus on interactions between a single pair of genomic loci (3C), between one locus and all other genomic loci (4C), between all restriction fragments within a given region (5C), or between all possible pairs of fragments across the genome (Hi-C).

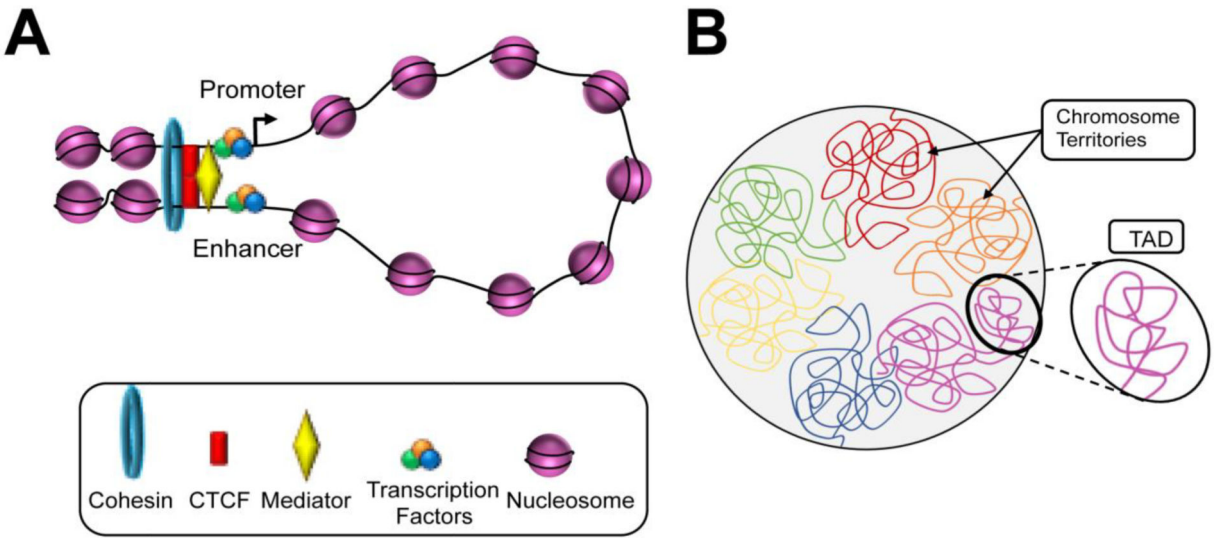

Figure 2. Three-dimensional chromosome organization

(A) Locally, at the submegabase scale, chromatin loop brings the distant cis-regulartory elements (e.g. enhancers) into close spatial proximity with its target promoter to regulate transcription. Architectural proteins and complexes including CTCF, mediator, and cohesin facilitate the formation of DNA loops. **(B)** On a larger scale, multiple loci undergoing regulatory interactions are grouped into topologically associated domains (TADs). The sequences within the same TADs have a high degree of interaction, which correlates with coordinated gene expression. Furthermore, all TADs are assembled into two spatial compartments (not shown). Lastly, each chromosome tends to locate in a discrete area within the nucleus, namely chromosome territories

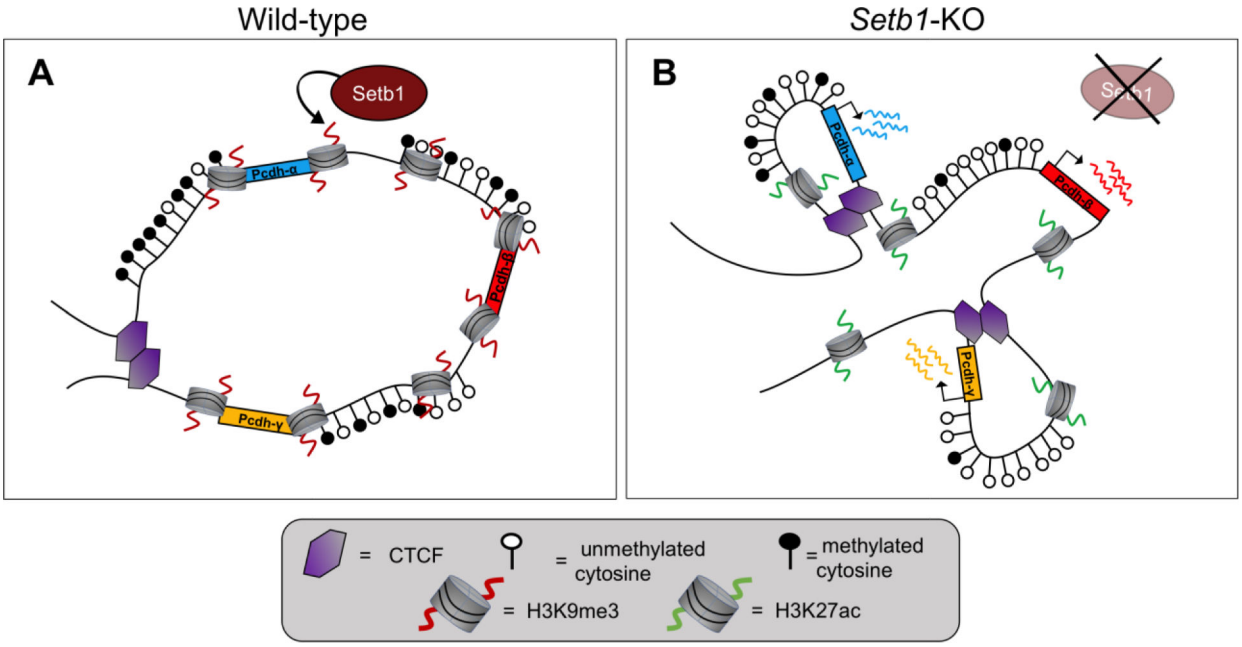

Figure 3. Setb1 repressor complex regulates a large topologically associated domain (megaTAD) formation and shields from excessive CTCF binding in the neuronal genome

(A) Setb1 maintains H3K9me3, which acts in tandem with high levels of DNA cytosine methylation to create a heterochromatic gene environment in mature neurons. **(B)** Ablation of Setb1 activity disrupts formation of this megaTAD. *Setb1*-KO neurons display decreased H3K9me3, increased H3K27ac. This results in increased CTCF binding at cryptic binding sites, decreased cytosine methylation, and upregulated expression of subsets of clustered *Pcdh* genes (genes *Pcdhα, Pcdhβ*, and *Pcdhγ* are shown in blue, red, and amber respectively).

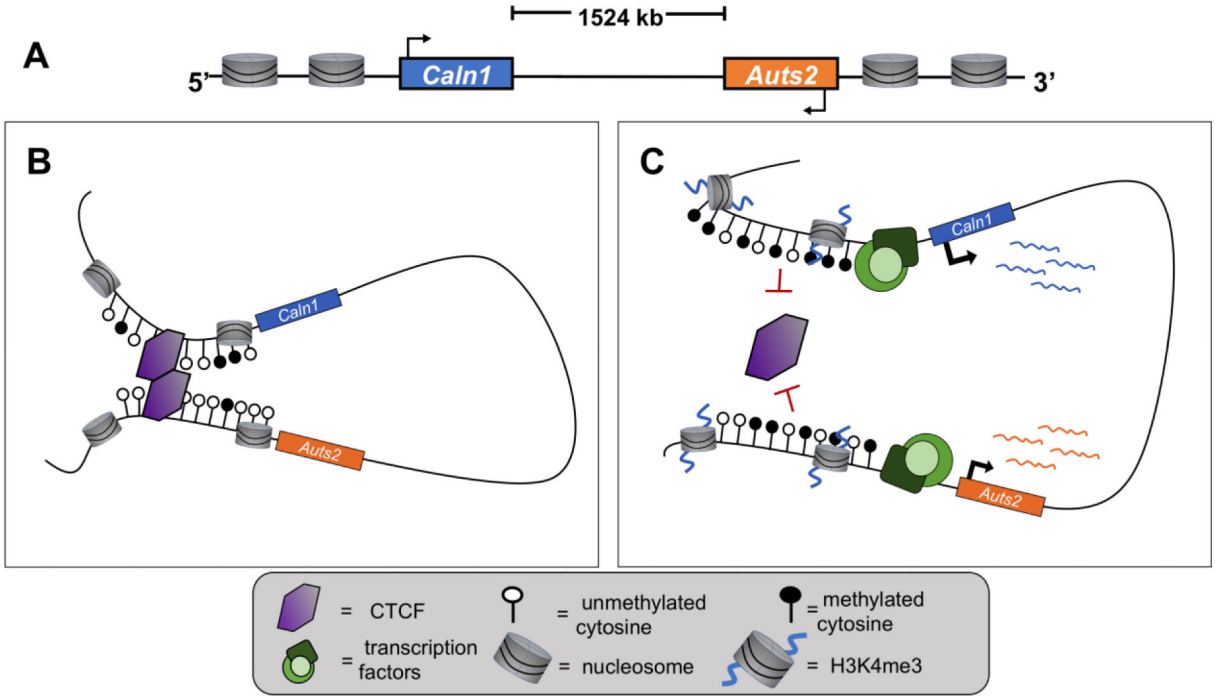

Figure 4. Cocaine-induced disruption of DNA loop encompassing *Auts2* and *Caln1*

(A) Linear representation of *Auts2* (orange) and *Caln1* (blue) genes separated by ~1524 kilobases in rodents and humans. **(B)** DNA looping encompassing *Auts2* and *Caln1* under baseline conditions in the brain. Low levels DNA methylation upstream of *Auts2* and *Caln1* allow for CTCF binding and homodimerization, resulting in DNA loop formation. *Auts2* and *Caln1* are within close 3D space of each other and both genes remain minimally expressed. **(C)** Repeated cocaine exposure disrupts *Auts2-Caln1* interactions. Increased levels of DNA methylation upstream of *Auts2* and *Caln1* prevents CTCF binding in regulatory regions and prevents DNA loop formation. The associated increased levels of H3K4me3 facilitate the recruitment of transcription factors and therefore upregulation of both genes' expression.