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Mini-Review

# The chromatin tapestry as a framework for neurodevelopment

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**The neuronal nucleus houses a meticulously organized genome. Within this structure, genetic material is not simply compacted but arranged into a precise and functional 3D chromatin landscape essential for cellular regulation. This mini-review highlights the importance of this chromatin landscape in healthy neurodevelopment, as well as the diseases that occur with aberrant chromatin architecture. We discuss insights into the fundamental mechanistic relationship between histone modifications, DNA methylation, and genome organization. We then discuss findings that reveal how these epigenetic features change throughout normal neurodevelopment. Finally, we highlight single-gene neurodevelopmental disorders that illustrate the interdependence of epigenetic features, showing how disruptions in DNA methylation or genome architecture can ripple across the entire epigenome. As such, we emphasize the importance of measuring multiple chromatin architectural aspects, as the disruption of one mechanism can likely impact others in the intricate epigenetic network. This mini-review underscores the vast gaps in our understanding of chromatin structure in neurodevelopmental diseases and the substantial research needed to understand the interplay between chromatin features and neurodevelopment.**

Neurodevelopment is orchestrated by an intricate ballet of epigenetic mechanisms that regulate gene expression through dynamic changes in chromatin architecture; for review, see Salinas et al. (2020) and Cummings and Rowley (2022). Within the nucleus, chromatin organizes into a functional 3D landscape critical for development and differentiation (Rowley and Corces 2018; Zheng and Xie 2019; Harris and Rowley 2024). Whole-genome methodologies such as chromatin immunoprecipitation sequencing (ChIP-seq), assay for transposase-accessible chromatin using sequencing (ATAC-seq), and in situ Hi-C have been valuable in revolutionizing our understanding of how chromatin architecture contributes to both normal and diseased states (Box 1; Mardis 2007; Buenrostro et al. 2013; Rao et al. 2014; Hsieh et al. 2015; Skene and Henikoff 2017; Zhong et al. 2023). These genome-wide assays, along with others, have revealed how features of chromatin architecture function in concert as part of an interwoven tapestry.

Here, we examine the role of chromatin architecture within this tapestry and its contribution to neurodevelopment. We begin by defining several features of chromatin architecture, including the interplay between histone modifications, DNA methylation, and 3D genome organization. In the context of neurodevelopment, the dramatic alterations throughout normal neurodevelopment highlight the responsiveness and connectedness between components of chromatin architecture. Illustrating these interconnections, we highlight representative single-gene human neurodevelopmental disorders in which genetic disruptions of DNA methylation or genome architecture, for example, have cascading effects across the multiple epigenomic layers.

This review of the chromatin landscape in neurodevelopment highlights the many complex changes across multiple layers of epigenetic regulation that characterize both normal neurological development and neurodevelopmental disorders. Current and future advances in methodology to assess chromatin architecture will further reveal the essential role of the 3D genome within the larger tapestry of epigenetic regulation in health and disease.

## 3D chromatin organization within the epigenetic network

Several recent technological and methodological achievements, combined with deep sequencing, have revolutionized our understanding of 3D genome organization; for review, see Kempfer and Pombo (2020). These methods, which measure long-range chromatin interactions, reveal intricate features of chromatin organization at fine-scale resolutions, commonly visualized by 2D contact maps (Lieberman-Aiden et al. 2009; Hsieh et al. 2015; Deshpande et al. 2022; Kalluchi et al. 2023).

### Compartments

From even the very first Hi-C study, the segregation of loci into active (A) and inactive (B) chromatin compartments has been apparent, highlighting it as a fundamental feature of chromatin organization (Lieberman-Aiden et al. 2009). Further advancements and deeper sequencing depth refined our understanding, and recent work demonstrated that A and B compartmentalization occurs at ultra-fine-scale, such that individual 1–2 kb regions can segregate into compartmental domains (Goel et al. 2023; Harris et al. 2023). Indeed, small distal active enhancers and the promoters of genes can segregate from neighboring inactive loci to interact within distinct compartment patterns (Goel et al. 2023; Harris et al. 2023). These studies found that coarse binning missed these small compartment intervals; therefore, many maps may underestimate or miss important organizational features due to resolution limits.

The A compartment correlates with transcription, active marks such as histone 3 lysine 27 acetylation (H3K27ac), histone

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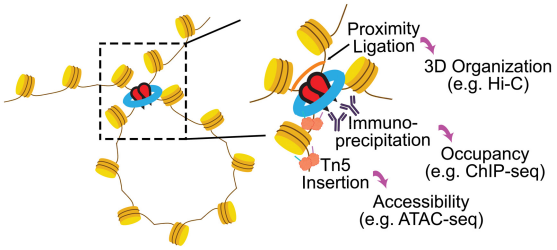
**Box 1. Identification of chromatin architecture**




A CCCTC-binding factor (red)/cohesin (blue) chromatin loop diagram illustrates three prominently used methods to identify different aspects of chromatin architecture. *3D organization*—Digestion and ligation of 3D proximal loci are often used to identify long-range chromatin interactions genome-wide. Typical implementations include Hi-C (restriction enzyme digestion) and Micro-C (micrococcal nuclease [MNase] digestion). *Protein occupancy*—Immunoprecipitation is used to identify loci occupied by a protein of interest. A typical implementation is ChIP-seq, as well as recent methods which use a modified protein A (pA). These include Cleavage Under Targets and Release Using Nuclease (CUT&RUN), which uses pA-MNase, and Cleavage Under Targets and Tagmentation (CUT&Tag), which uses pA-Tn5. *Accessibility*—Transposase-based insertion is often used to identify "accessible" sites. ATAC-seq uses Tn5 to insert sequencing adapters at accessible loci.

3 lysine 4 trimethylation (H3K4me3), and chromatin accessibility. In contrast, the B compartment correlates with quiescent chromatin or repressive marks such as histone 3 lysine 9 trimethylation (H3K9me3) and GC-rich methylated regions (Du et al. 2021; Monteagudo-Sánchez et al. 2024). However, the specific mechanisms that drive the formation of these compartments remain unclear. The resolution of compartment identification has been a limiting factor, and advances in methodologies, algorithms, and sequencing depth will undoubtedly continue to reveal important insights into what we call A and B compartments (Harris and Rowley 2024). However, it is also likely that categorizing loci as one of two states, namely, either A (active) or B (inactive), is an oversimplification that has potentially influenced our interpretations of these features. Indeed, the field has already shown the existence of either subcompartments or, perhaps, even a third compartment that is wholly distinct from either A or B (Rao et al. 2014, 2017; Bonev et al. 2017; Nichols and Corces 2021). Given the recent support for a complex and fine-scale compartmental organization and a lack of evidence for one single mechanistic driver, it is becoming increasingly evident that a two-state model may oversimplify this critical principle. Indeed, similar to the original histone-code hypothesis, we have proposed that compartments come in a variety of "flavors" with nuanced preferences for each other depending on shared compatibility (Jenuwein and Allis 2001; Harris and Rowley 2024). However, the degree of interconnectedness between compartments, histone modifications, and other aspects of chromatin remains mysterious.

## 3D chromatin domains

3D chromatin research often examines topologically associated domains (TADs), which manifest as triangles of high signal intensity in chromatin contact maps, indicative of interactions among loci in close linear proximity (Dixon et al. 2012; Hou et al. 2012; Nora et al. 2012; Sexton et al. 2012). Recent advancements in TAD identification have allowed for the detection of nested TAD structures within overarching TADs (Zufferey et al. 2018; Mourad 2022; Sefer 2022). However, despite the frequent examination of TADs, it is crucial to recognize that the interpretation of what constitutes a "TAD" varies significantly due to the ambiguity of the term. This variability arises because triangular interaction patterns can embody distinct structural features, ranging from compartment domains, CCCTC-binding factor (CTCF) loop domains, "ordinary" domains, and intragenic interaction domains (Rao et al. 2014; Dong et al. 2017; Rowley et al. 2017; Rowley and Corces 2018; Beagan and Phillips-Cremins 2020). These diverse features all can form "triangular" interaction patterns, some of which can overlay each other, underscoring the complexity of chromatin organization and the nuanced differences in how genomic elements interact within the nucleus (Hyle et al. 2019; Szczepińska et al. 2021; Chakraborty et al. 2023; Spracklin et al. 2023).

## CTCF/cohesin loops

In addition to compartments, advancements in genome-wide chromatin contact mapping have revealed the presence of CTCF loops, represented by intense localized interaction signals (Rao et al. 2014). These loops are created by the cohesin complex, which is thought to extrude chromatin until stopped by CTCF proteins bound in a convergent orientation (de Wit et al. 2015; Guo et al. 2015; Nichols and Corces 2015; Fudenberg et al. 2016; Nora et al. 2017; Rao et al. 2017; Nuebler et al. 2018). The critical roles of CTCF and cohesin in establishing these loops have been emphasized by numerous studies that demonstrate the impact of their depletion on loop integrity and gene expression (Nora et al. 2017; Rao et al. 2017; Lyu et al. 2023); for review, see Cummings and Rowley (2022). Furthermore, many CTCF loops are cell type– or developmental stage–specific (Grubert et al. 2020; Bond et al. 2023; Lyu et al. 2023), indicating that signals other than the simple presence versus absence of the motif can control these features. Several studies indicate that the binding of CTCF to chromatin can sometimes be affected by CpG methylation; for review, see Monteagudo-Sánchez et al. (2024). Methylation alone cannot be the sole epigenetic determinant of cell type–specific CTCF binding, as many of these sites do not even contain CpG dinucleotides, with an estimate of as little as 10% of bound CTCF motifs having a CpG in mouse embryonic stem cells (mESCs) (Monteagudo-Sánchez et al. 2024). To further highlight the interconnectedness of chromatin architecture, cohesin itself can impact other aspects of the chromatin landscape in addition to loops. For example, the

cohesin loader can activate chromatin remodelers to reposition and evict nucleosomes indicating that the loading of cohesin is not only important for creating chromatin loops, but can also change the local nucleosome landscape (Lopez-Serra et al. 2014; Muñoz et al. 2019). Altogether, CTCF loops are one part of a complex chromatin ecosystem and cell type specificities likely correspond to multiple impacted chromatin features within a complex mechanism (Segueni and Noordermeer 2022).

### Intergenic and intragenic interactions

Hi-C maps display bright "foci"; in mammals, most of these superintense interaction signals correspond to CTCF loops (Rao et al. 2014; Rowley et al. 2020; Harris et al. 2023). However, other types of interactions exist and likely help control gene expression. For example, enhancer–promoter (E–P) interactions are particularly well-acknowledged and facilitate targeted gene expression by bringing distant enhancers in proximity to their respective promoters. CTCF loops may help bridge some of this distance or even block enhancers from connecting with their promoters (Guo et al. 2015; Tarjan et al. 2019). However, recent work has demonstrated some E–P interactions can also bypass CTCF boundaries, and several researchers have found that gene expression and even E–P interactions are fairly maintained upon acute loss of CTCF loops, making the relationship somewhat unclear (Nora et al. 2017; Rao et al. 2017; Hsieh et al. 2022; Chakraborty et al. 2023). RNA polymerase II elongation correlates with E–P and intragenic interactions, forming strong cell type–specific interactions during human neural differentiation (Titus et al. 2024).

### An interconnected chromatin tapestry

When considering chromatin, we often examine components separately as if they are distinct, independent features. This may be due to the popularity of "single-profile" methods which provide rich data for only one aspect of chromatin at a time (Box 1). However, features such as chromatin accessibility (Fig. 1A), DNA methylation (Fig. 1B), compartments (Fig. 1C), CTCF loops, E–P, and other interactions (Fig. 1D) are all part of an interwoven network "tapestry" that provides context for the regulation of gene expression, cell differentiation, and cell fate (Fig. 1). Within this framework, and as discussed below, disruption of one epigenetic thread may or may not impart rippling impacts across the other features, depending on how tightly they are woven together.

## Chromatin restructuring during neurodevelopment

Neural progenitor cells (NPCs), which emerge during neural tube formation during the third week of embryogenesis in humans (Sadler 2019), are multipotent entities capable of differentiating into neurons, astrocytes, or oligodendrocytes (Ke et al. 2023). Characterized by extensive gene expression changes, this cellular differentiation process involves a series of carefully coordinated changes at all levels of the epigenetic landscape.

Neural differentiation-associated changes to chromatin architecture are numerous and involve changes to CTCF loops, chromatin accessibility, chromatin remodelers, and histone modifications. Chromatin compartments undergo genome-wide reorganization alongside neuronal cell type–specific gene expression throughout neural differentiation, resulting in chromatin compartment profiles uniquely reflective of that cell type (Dixon et al. 2015; Rahman et al. 2023). Ahanger et al. (2021) also describe a considerable difference in lamina-associated chromatin domains (LADs) and corresponding gene expression between excitatory neurons and inhibitory neurons due to a difference in function and region of origin, coming from the dorsal and ventral brain, respectively. This finding elucidates the necessity for cell type–specific compartmental signatures and the use of the lamina for heterochromatic "storage" of unrequired genes. Additionally, LADs in the cortex and those conserved across mouse, macaque, and human samples cortex were enriched in transcriptionally active genes, compared to other LADs, indicating that LADs are not simply synonymous with repression and that there may be subdomains within LADs of active chromatin that are important for proper neural development (Ahanger et al. 2021). A and B compartments shift throughout neural cell differentiation in mice, where interactions between B compartments strengthen, coinciding with an increase in the inactive histone mark, H3K9me3. At the same time, A compartments decrease in strength coinciding with a weakening of active marks (H3K27ac) (Fig. 2; Meshorer et al. 2006; Bonev et al. 2017; Liu et al. 2023). These effects are thought to result in overall increased compaction of chromatin transcription, as embryonic stem cells (ESCs) exhibit globally elevated levels of mRNA, which decreases alongside chromatin accessibility toward differentiation to NPC and neurons (Efroni et al. 2008; Meléndez-Ramírez et al. 2021).

The role of chromatin changes in neurodevelopment has been reinforced in mouse studies of single-cell populations, where chromatin accessibility, DNA methylation, and 3D chromatin interactions change throughout differentiation from NPC to neurons (Noack et al. 2022). In particular, the transcription factor

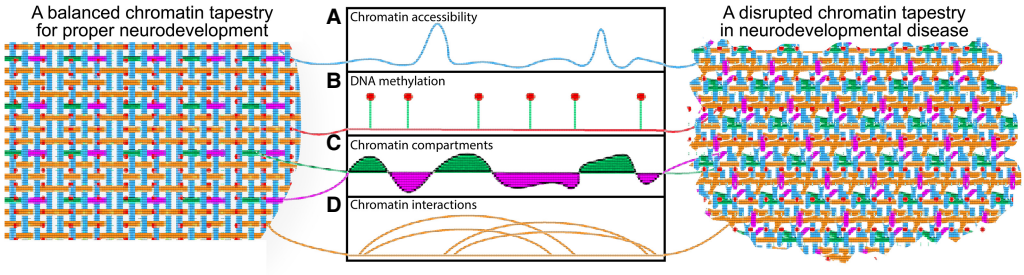



**Figure 1.** An interconnected chromatin tapestry. Illustration depicting the interwoven nature of chromatin features that create a balance necessary for proper neural differentiation and development. Some examples of the individual threads of this chromatin tapestry include (*A*) chromatin accessibility, (*B*) DNA methylation, (*C*) 3D chromatin compartments, and (*D*) other long-range chromatin interactions. Because of their relationship, disruption of one feature likely ripples throughout the others, which may explain multiple correlated epigenetic aspects of aberrant neural development.

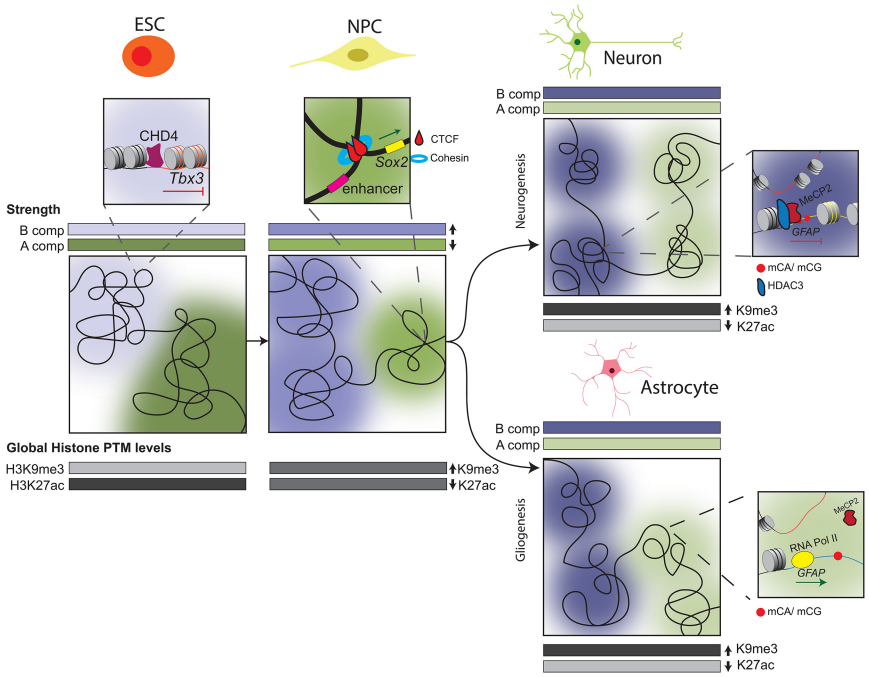



**Figure 2.** Chromatin restructuring during neurodevelopment. Visual representation of the chromatin restructuring that occurs during neurodevelopment, from ESCs to NPCs and finally to either neurogenesis or gliogenesis. The strength of chromatin compartments fluctuates during this process. ESCs exhibit strong A compartments (dark green), gradually decreasing in strength (light green) during differentiation from NPCs to neurons/glial cells. Conversely, the B compartment in ESCs is relatively weak (light blue) and becomes stronger through differentiation (blue and dark blue). Global histone levels follow a similar pattern as H3K9me3 (histone mark of inactive chromatin) increases through differentiation (light gray to dark gray), in converse to H3K27ac (histone mark of active chromatin), which decreases relative to ESC levels (dark gray to light gray). Zoomed-in examples show the regulation of specific genes. In ESCs, CHD4 (purple) mediates transcriptional silencing of *Tbx3*, a differentiation factor required for cells leaving the ESC state. In NPCs, there is contact between an NPC-specific enhancer and the neural transcription factor gene *Sox2* (yellow box), mediated by a CTCF (red teardrop)/cohesin (blue ring) loop. In neurons, MeCP2 (red) and HDAC3 (blue) govern the transcriptional repression of *GFAP*, while in astrocytes MeCP2 absence from the gene allows RNA polymerase II (yellow) to transcribe *GFAP*.

NEUROG2 has been implicated in epigenome remodeling to safeguard correct lineage differentiation (Noack et al. 2022). Additionally, in vivo mouse astrocyte-to-neuron reprogramming was associated with an epigenomic response at multiple levels, with an increase in accessibility and expression of neuronal genes, like *Dcx*, and a compartmentalization profile matching that of cortical neurons, indicative of the necessity of the epigenome in multiple aspects of cell programming (Pereira et al. 2024). Despite a clear correlation between compartments and transcription, the independence and influence between chromatin organization and transcription are complex and unclear, primarily due to a lack of resolution sufficient to identify interactions at subkilobase levels; for review, see Harris and Rowley (2024). With the advent of fine-scale analysis revealing microcompartments and the dynamics of loop extrusion at the subgenic resolution, it will be exciting to examine finer-scale changes during neurodevelopment (Gabriele et al. 2022; Harris et al. 2023).

CTCF loops also change during neurodevelopment and likely play a vital role in differentiation (Arzate-Mejía et al. 2018). Indeed, targeted depletion of CTCF in mESCs disrupts neural differentiation (Kubo et al. 2021). As an illustrative example, the mouse *Sox2* locus forms a long-range contact with an NPC-specific enhancer during differentiation to NPCs (Bonev et al. 2017; Chakraborty et al. 2023). This change in 3D chromatin interaction is also associated with a change in the local CTCF and H3K27ac landscape, indicative of coordinated changes to enhancer activity, CTCF looping, and E–P contacts (Fig. 2—NPC; Bonev et al. 2017; Chakraborty et al. 2023). As another example, while few, some CTCF-occupied sites do get altered by aberrant DNA methylation, which has been linked with neurological disorders affecting cognitive function such as Alzheimer's disease, Fragile X syndrome, and Huntington's disease, despite a primary single-gene genetic origin in the case of the latter two conditions (Barbé and Finkbeiner 2022; Patel et al. 2023). In terms of neurodevelopment, there has been some evidence that DNA methylation can influence the dynamic nature of CTCF-binding sites across cell types (Chen et al. 2012; Wang et al. 2012), which correlates with an overall strengthening of CTCF binding and an increase in loop formation during neural differentiation and exit from pluripotency in mice (Bonev et al. 2017; Pękowska et al. 2018). Furthermore, CTCF does not work alone in the coordinated chromatin organization and architecture-driven nature of neural development. Histone methyltransferase SETDB1 is required for the repressive chromatin mark H3K9me3 and subsequent regulation of CTCF occupancy in neurons, highlighting the multilayer connection across chromatin features (Jiang et al. 2017; Tam et al. 2024).

In addition to changes to CTCF loops and compartments, chromatin accessibility shifts dramatically during neurodevelopment (de la Torre-Ubieta et al. 2018). Mannens et al. (2024) describe paired chromatin accessibility and transcription changes across the entire human brain throughout the first trimester, revealing the interconnectedness between chromatin accessibility and transcriptional activity for facilitating proper neural development (Mannens et al. 2024). A study in mice showed that splicing isoforms of DPF2, (-S and -L), a subunit of the BRG1/BAM (BAF) chromatin remodeling complex, are controlled by splicing regulator PTBP1 to switch isoforms during the NPC stage, an integral part of neuronal differentiation and stem cell maintenance (Valletta et al. 2020; Nazim et al. 2024). More specifically, in mESCs, DPF2-S preferentially binds near and regulates key pluripotency genes (*Sox2, Pou5f [also known as Oct4]*). The replacement of DPF2-S with DPF2-L directs the BAF complex toward neurogenic targets and CTCF-binding sites, particularly in NPCs and neurons (Valletta et al. 2020; Nazim et al. 2024).

Another critical observation during neurodevelopment is the reduction of long-range interactions between active chromatin regions and an increase between inactive chromatin regions (Fig. 2). These changes occur as differentiation progresses from ESCs to NPCs and then to cortical neurons and correlate with increased B compartment interval locations and strength as well as increased H3K27me3 and H3K9me3, marking a pivotal aspect of neuron differentiation (Fig. 2; Bonev et al. 2017; Kishi and Gotoh 2018).

Alongside the above, the changes to chromatin accessibility are likely driven by chromatin remodelers (Reyes et al. 2021), such as the chromodomain-helicase-DNA-binding (CHD) family of proteins, which is responsible for the regulation of a large cohort of genes in directed neurogenesis (Chai et al. 2018; Goodman and Bonni 2019; Goodman et al. 2020; Sood et al. 2020). The nucleosome remodeling and deacetylation complex (NuRD) include CHD3/4/5 in a mutually exclusive fashion, where each protein guides the complex to a unique role in neural differentiation, including gene repression and activation of layer specification and neural lineage-defining genes (Chai et al. 2018; Goodman and Bonni 2019; Goodman et al. 2020; Sood et al. 2020). Specifically, NuRD with CHD4 ensures transcriptional activation of *Pax6, Sox2,* and *Tbr2* in NPCs, later repressed in postmitotic neurons by NuRD with CHD3 (Nitarska et al. 2016). CHD4 was also shown to maintain mouse ESC proliferation as CHD4-depletion results in increased expression of markers for endoderm, ectoderm, and mesoderm through *Tbx3* expression of lineage proteins, demonstrating the early on importance of CHD4 in preventing premature differentiation (Fig. 2—ESC; Zhao et al. 2017). Later on in differentiation, CHD4 controls premature astrocyte differentiation through contact with EZH2, the catalytic subunit of Polycomb Repressive Complex 2 regulating H3K27me3, to suppress the expression of gliogenesis factor gene *GFAP* (Sparmann et al. 2013). Recent in vivo work in mice revealed that conditional knockout of *Chd4* not only alters chromatin accessibility, but also alters cohesin recruitment, chromatin loops, interaction domains, and compartments (Goodman et al. 2020). These findings highlight the importance of the NuRD complex in neuro-differentiation, emphasize the interdependence of a variety of epigenetic modulators of chromatin function, such as the combined influence of CHD3/4 NuRD complexes and CTCF-directed enhancer contacts for transcriptional regulation of *Sox2*, and, combined with several other studies, explain why mutations in NuRD and other CHD genes are frequently associated with neurodevelopmental disorders; for review, see Boulasiki et al. (2023). In particular, another CHD family member, CHD7, responsible for the neurodevelopment disorder CHARGE syndrome, was found to disrupt correct neural and glial differentiation in mouse cell culture, with *Chd7* knockout reducing H3K27ac and RNA polymerase II binding at enhancers (Yao et al. 2020). However, compartmental domains or E–P interactions were not significantly altered after *Chd7* knockout, although this may be a result of changes occurring at fine-scale that warrant high-depth Hi-C maps to interrogate.

In addition to the aforementioned interplay around chromatin accessibility, coordinated regulation between histone modifications and DNA methylation of CpG sites is prominently featured in neural development. These modifications are thought to guide ESCs to NPCs and finally to neurons and glial cells, where "switches" take place in NPCs to guide development toward neurogenesis, followed by gliogenesis; for review, see Gavin et al. (2017). These changes in differentiation path are directed by an interplay between DNA methylation and an increase in repressive histone modifications H3K9me3 and H3K27 methylation and are governed, at least in part, by MeCP2, a CpG-binding protein that recruits histone deacetylase 3 (HDAC3) at transcription start sites (TSSs) of highly methylated genes, resulting in a reduction in transcription initiation at those genes, including the astrocyte marker genes *GFAP* and *S100*β in neurons (Fig. 2—Neuron; Setoguchi et al. 2006; Boxer et al. 2020). Additionally, MeCP2 is particularly abundant in neurons, where MeCP2 levels are sevenfold higher than in nonneuronal cell types, indicating a stark reliance of neuron populations on this keen chromatin organizational and transcriptional regulator (Skene et al. 2010). Environmental impacts to these epigenetic factors, such as those caused by prenatal alcohol exposure, correspond to aberrant neural development (Chater-Diehl et al. 2017; Gavin et al. 2017). Therefore, it would be interesting to determine if and how early stage exposure to other environmental factors, such as pollution and other chemicals, might alter chromatin architecture and impact neurodevelopment.

Changes at the chromatin level during cell differentiation are a feature of all cell lineages (Schmitt et al. 2016). Pluripotent stem cells exhibit a global open chromatin state, with the ectoderm lineage exhibiting a similar chromatin accessibility and methylation configuration, in contrast to mesoderm and endoderm lineages (Hawkins et al. 2010; Argelaguet et al. 2019). Ectoderm neural lineages are intrinsically suited to an open chromatin state, and a lack of external growth factors in ectoderm cells often results in differentiation toward a neural lineage, which has sparked a model where a neuro-like state is the default (Muñoz-Sanjuán and Brivanlou 2002). This "neuro-default" model might explain why single-gene mutants of chromatin modifiers or remodelers so often result in an aberrant neurodevelopmental phenotype (Zahir and Brown 2011).

Overall, the dynamic and intricate interplay among multilayered epigenetic factors underlies the regulation of chromatin states, pivotal for steering neurodevelopment. Such mechanisms can display species-specificity, as was recently shown with the existence of a "lower" epigenetic barrier in mice (Ciceri et al. 2024). For example, the decrease in EZH2/H3K27me3 at NPC neuronal maturation loci is much more substantial in humans than in mice (Ciceri et al. 2024). Regardless, it is becoming clear that the epigenetic system is feedback-sensitive and responds when one aspect of the tapestry is perturbed.

## Human epigenetic neurodevelopmental disorders

Given their essential roles in normal neural development, it is not surprising that alterations to the highlighted epigenetic processes above have been identified in the mechanism of a variety of human neurodevelopmental disorders. This includes environmental factors known to disrupt epigenetic processes, as well as a wide number of single-gene disorders involving variants in the epigenetic machinery themselves. Notably, a variant in a single gene can alter multiple layers of epigenetic regulation, even when that gene's canonical role is to control only one aspect of chromatin. These phenomena further highlight the interdependence and coregulation of these features. A clinically useful application of this interplay has been the development of DNA methylation signatures to diagnose a wide variety of epigenetic conditions (Kerkhof et al. 2024). Indeed, dozens of condition-specific DNA methylation signatures have been validated to aid in the diagnosis of a wide variety of single-gene disorders affecting various genes involved in epigenetic regulation broadly, far beyond genes directly involved in methylating DNA. In addition to this diagnostic potential, investigating the effects of a single-gene disorder on epigenetic regulation broadly, including genome organization, is essential in understanding the mechanism of action. Below, we highlight two single-gene disorders, Rett syndrome and Cornelia de Lange syndrome (CdLS), to emphasize that the interplay of chromatin organization with other epigenetic features extends beyond normal neurodevelopment into neurodevelopmental disorders.

## Rett syndrome

Due to variants in the X-linked gene MeCP2, Rett syndrome is a neurodevelopmental condition characterized by a brief period of typical neurodevelopment, followed by regression and loss of skills in early childhood (Amir et al. 1999). In addition to its prominent role as a DNA methylation sensor as part of a repressor complex with a histone deacetylase, the MeCP2 function also intersects with chromatin organization. In a mouse model of Rett syndrome, differentially expressed genes within the cerebral cortex were associated with mCA methylated regions, which interact in chromatin compartments found via Hi-C (Clemens et al. 2020). Studies investigating earlier stages of cerebral development found that the development of interaction domains preceded the propagation of mCA methylation (Clemens et al. 2020). This suggests that chromatin organization may set the stage for DNA methylation patterns that later recruit MeCP2 in its canonical role of gene repression, which is impaired in Rett syndrome (Fig. 3A). In a second study illustrating the necessity of proper chromatin organization in facilitating MeCP2 function, investigators demonstrated in a MeCP2-mutant Rett syndrome mouse model that the ability of MeCP2 to engage in transcription repression relies on its ability to contact the TSS, even when MeCP2 is bound within the gene body itself (Figs. 2, 3B—Neuron; Boxer et al. 2020). Genes upregulated in the MeCP2 knockout are ones where there are interactions between the gene body and the TSS, suggesting that these interactions may facilitate MeCP2-mediated gene repression (Boxer et al. 2020). The two-way nature of this relationship between genomic architecture and MeCP2 function was further highlighted in a model of Rett syndrome that uses loss-of-function point mutations in *MeCP2* within human interneurons (Xiang et al. 2020). In this system, loss of MeCP2 function was associated with an increase in E–P interactions (as measured by Hi-C) as well as widespread alterations to chromatin accessibility (as measured by ATAC-seq) (Fig. 3C). These studies demonstrate an intricate relationship between chromatin architecture and MeCP2-mediated gene regulation.

MeCP2 expression is high in mature neurons, where it regulates dendritic growth and neuron morphology, likely explaining the late-onset nature of Rett syndrome (Shahbazian et al. 2002; Zhou et al. 2006). MeCP2 duplication syndrome (MDS) also causes severe intellectual disability, indicating the importance of moderating MeCP2 levels (Van Esch et al. 2005). Cultures from MDS patients had increased synaptogenesis and dendritic complexity (Van Esch et al. 2005), likely due to its apparent role in chromatin remodeling and transcription regulation.

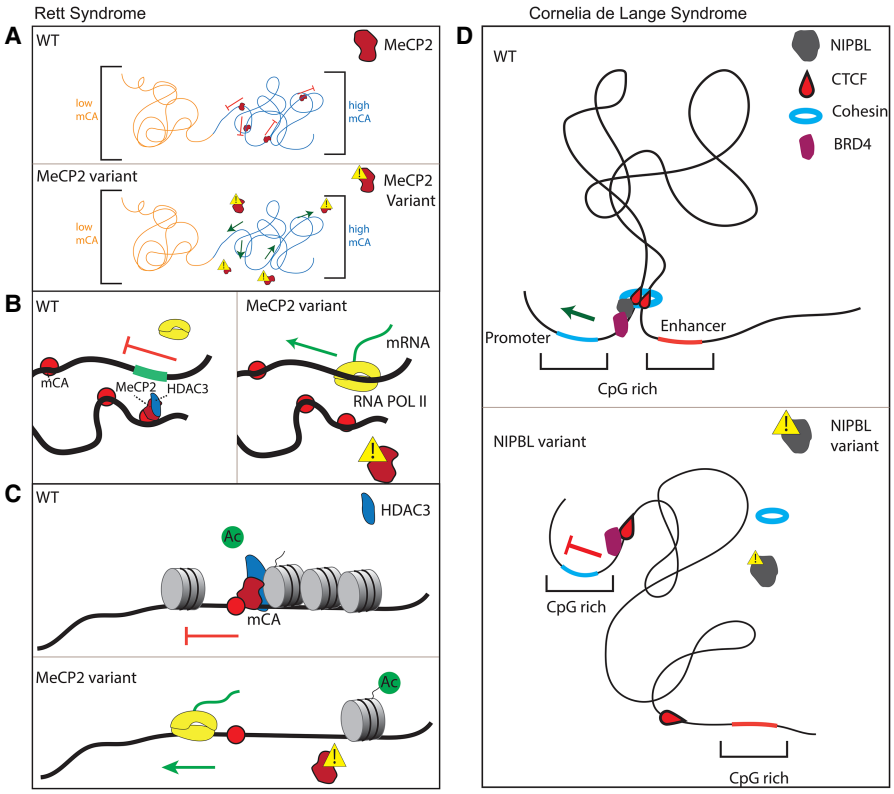



**Figure 3.** Examples of known chromatin aberrancies in neurodevelopmental disease. Rett syndrome (*left*), caused by an MeCP2 variant, is associated with differential gene repression. (*A*) MeCP2 usually represses regions with high CA methylation (blue) in the B compartment, but the variant cannot bind and repress these sites. (*B*) MeCP2 can repress RNA polymerase II initiation, even when bound within the gene body, downstream from the TSS. Interactions between the TSS and gene body could provide a means for this long-range transcriptional repression. The MeCP2 variant does not repress these genes. (C) MeCP2 binds to mCA and recruits HDAC3 (blue), which alters the accessibility of the surrounding chromatin, preventing RNA polymerase II initiation. The MeCP2 variant does not bind these sites, allowing the chromatin landscape to remain accessible. (*D*) CdLS is a cohesinopathy often due to a NIPBL variant. NIPBL (gray) loads cohesin onto chromatin, whereafter, cohesin-mediated (blue) chromatin extrusion forms loops between CTCF (red) sites. This could bring promoters and enhancers into 3D contact to facilitate transcription. NIPBL may also help with BRD4 (purple). The NIPBL variant disrupts BRD4 occupancy and cohesin loading, likely impacting extrusion, CTCF looping, and E–P regulation. Note that this is a model of events, and the various mutations associated with CdLS may impart distinct impacts to chromatin loops.

## Cornelia de Lange syndrome

CdLS, often caused by variants in *NIPBL* the cohesin loader, is the prototypical example of a class of conditions termed "cohesinopathies," resulting in an overlapping phenotype including neurodevelopment impairment, growth restriction and various congenital anomalies (Cummings and Rowley 2022). These mutations in NIBPL were found to disrupt loop extrusion in vitro (Panarotto et al. 2022). In addition to *NIPBL*, cohesinopathies have been associated with variants in each of the cohesin subunits (Kline et al. 2018; Cummings and Rowley 2022). More recently, variants in *CTCF* were also associated with a neurodevelopmental disorder that has significant overlap with the cohesinopathies (Gregor et al. 2013). Although several groups have focused on how cohesinopathies relate to abnormal genome organization, here we highlight work showing impacts on additional features of the chromatin tapestry.

A study examining chromatin dynamics in fibroblasts from patients with CdLS to that of control fibroblasts noted that NIPBL binding is strongly associated with regions of high GC

content, including CpG islands on enhancers and promoters, and that there was a substantial reduction of NIPBL binding in the CdLS patient samples. Genome-wide DNA methylation analysis identified 123 differential methylation positions in the CdLS fibroblasts, suggesting that the NIPBL variant can impact DNA methylation and chromatin looping (Garcia et al. 2021).

A second study, also using human fibroblasts from patients with CdLS, identified functional interactions between NIBPL and a chromatin remodeler (Luna-Peláez et al. 2019). This study demonstrated a direct interaction between these two proteins, overlap in their regulated genes, and at sites normally co-occupied by NIPBL and BRD4, there were reduced BRD4 levels in the CdLS patient fibroblasts (i.e., patients with impaired NIPBL function). This decrease in BRD4 occupancy in CdLS suggests that NIPBL is essential for BRD4 localization and the regulation of specific gene promoters. Variants in *BRD4* have been recently identified in individuals with a neurodevelopmental phenotype with considerable overlap with CdLS, further underscoring the functional overlap of the epigenetic processes these two genes control (Fig. 3D; Olley et al. 2018).

Overall, variants in the cohesin complex result in a multifaceted change to the epigenomic fabric. While acute depletion does not impact many genes, longer-term cohesin knockdown results in the dysregulation of a similar set of neuronal genes as found in CdLS (Weiss et al. 2021). The expression of these genes was rescued quickly after the restoration of cohesin expression (Weiss et al. 2021).

## Conclusions

The chromatin landscape is a rich tapestry where multiple features coordinate to control nuclear processes. Epigenomic features, including chromatin architecture, are not isolated entities but, rather, are inextricably intertwined. In light of this, a complete understanding of the mechanisms underlying normal and abnormal development requires a comprehensive analysis of each layer rather than a focused interrogation of one specific feature. Likely due to the cost of obtaining sufficiently resolved maps and the difficulty of analysis, 3D genome organization, in particular, has been understudied in relation to other features of the epigenome. Reduced sequencing cost, refinement of methodologies, and new bioinformatic algorithms will allow for a more detailed examination of the role of chromatin architecture in these settings. These advents will prove helpful to studies that examine the role of chromatin in both normal and abnormal neurodevelopment, as well as a wide variety of other disease states, including cancers, environmental exposures, and others (Ciafrè et al. 2020; Gong et al. 2021).

Genetic variants in key epigenetic machinery are often associated with neurodevelopmental disorders and, when studied (e.g., Rett syndrome), have been found to have disrupted 3D chromatin organization. Conversely, mutations in factors prominently known for their role in 3D chromatin organization (e.g., CdLS) affect not only chromatin loops, but also DNA methylation and the histone landscape. For the majority of human single-gene neurodevelopmental conditions, however, the effects on chromatin organization have remained unexplored, despite a great number of which result from disruption of a gene involved in epigenetic regulation. Targeted treatments in this class of conditions are relatively few in number but are emerging, and an additional understanding of the complete underlying mechanism will be important in advancing this field (Neul et al. 2023).

Moving forward, a deeper exploration into the mechanistic underpinnings of chromatin organization's impact on neural development will not only enrich our understanding of these systems and diseases, but will also illuminate novel biomarkers to aid in the diagnosis and therapeutic treatment of such diseases.

## Competing interest statement

The authors declare no competing interests.

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