Molecular Therapy
Review

3D genome alterations and editing in pathology

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The human genome is folded into a multi-level 3D structure that controls many nuclear functions including gene expression. Recently, alterations in 3D genome organization were associated with several genetic diseases and cancer. As a consequence, experimental approaches are now being developed to modify the global 3D genome organization and that of specific loci. Here, we discuss emerging experimental approaches of 3D genome editing that may prove useful in biomedicine.

INTRODUCTION

The interphase genome is folded in a highly ordered manner, essential both for DNA compaction and for the regulation of various intranuclear processes. Each chromosome occupies a restricted volume in the nucleus, a chromosome territory (CT). In mammals, large chromosomes and chromosomes with low gene density tend to localize at the nuclear periphery, whereas smaller chromosomes with high gene density are located more centrally.[1,2] CTs have a spongy internal structure and are composed of bulk chromatin masses penetrated by the channels of the "interchromatin compartment," a dynamically organized system of cavities serving for the diffusion of nucleoplasm components.[3]

Within CTs, active and repressed genomic regions are spatially segregated into A and B compartments formed by local *cis* as well as distant *cis* and *trans* interactions (Figure 1A).[4] A compartments are early-replicating gene-rich and typically highly transcribed regions enriched in active histone marks such as H3K36me3, H3K27ac, and H3K4me1–3. In contrast, B compartments contain late-replicating transcriptionally silenced regions enriched with nucleolus- and lamina-associated domains (NADs and LADs, respectively) marked with H3K9me2 and H3K9me3.[5–8] Compartment partitioning strongly correlates with the transcription profile and thus is highly cell-type specific,[9,10] whereas the degree of compartmentalization might vary significantly within a cell population.[11,12]

The compartments are further divided into subcompartments, distinguished by the patterns of different histone modifications, and further into topologically associated domains (TADs) with a high CCCTC-binding factor (CTCF)/cohesin occupancy at their borders[13,14] and representing globular structures with a remarkable cell-to-cell variability in their 3D shape and folding density.[15,16] TADs serve as "warehouses" for genes, gene loci, and their regulatory systems,[17] delimiting the areas of enhancer action (Figure 1B).[18,19] Consequently, genes within a TAD are often co-regulated[20]; this is achieved by looping between enhancers/locus control regions and promoters.[21,22] Molecular details of the loop formation mechanisms are still not fully understood, but cohesin-driven extrusion[23,24] and liquid-liquid phase separation[25,26] are the most consistent models. The CTCF-cohesin complex preferentially determines strong long-range interactions including contacts between TAD borders,[8] while the short-range enhancer-promoter and promoter-promoter interactions are also maintained through the Mediator complex and various transcription factors (TFs)[27] in cooperation with transcription machinery.[28] Complex contact patterns within TADs are manifested in non-structured hierarchical nucleosome assemblies such as clutches[29] and nanodomains.[30] Clutches are relatively small nucleosome agglomerates (about 2–20 nucleosomes/clutch) whose density and size strongly depend on the level of histone acetylation. This implies that clutches are formed by weak transient electrostatic interactions between nucleosomes. A group of clutches constitutes a nanodomain. Nanodomains are distributed throughout the nucleus, but their concentration increases near the nuclear periphery. Nanodomain structures are preserved upon CTCF and cohesin degradation, and they seem to be formed through liquid-liquid phase separation.[30,31]

In sum, TADs represent cornerstone structural and functional units of the 3D genome. These units contribute to multiple cellular processes including stem cell differentiation, limb growth and development, epidermal-mesenchymal transition, and cellular senescence.[9,19,32–34] Changes in the normal profile of TADs can lead to pathologies. Below, we highlight the role of TAD rearrangement and disruption of TAD borders in the development of severe pathologies and discuss 3D genome editing techniques.

3D ORGANIZATION OF THE GENOME AND PATHOLOGIES

Over the past several years, a number of diseases have been associated with 3D genome structure abnormalities (Figure 2). Pathological changes in loop and TAD profiles are caused by structural variations (SVs; rearrangements of 50 nucleotides or more in length: insertions, duplications, deletions, etc.), single-nucleotide polymorphisms (SNPs), large chromosomal rearrangements, viral/transposon DNA integrations, and epigenetic factors. These genome and epigenome perturbations could affect or eliminate TAD boundaries and violate

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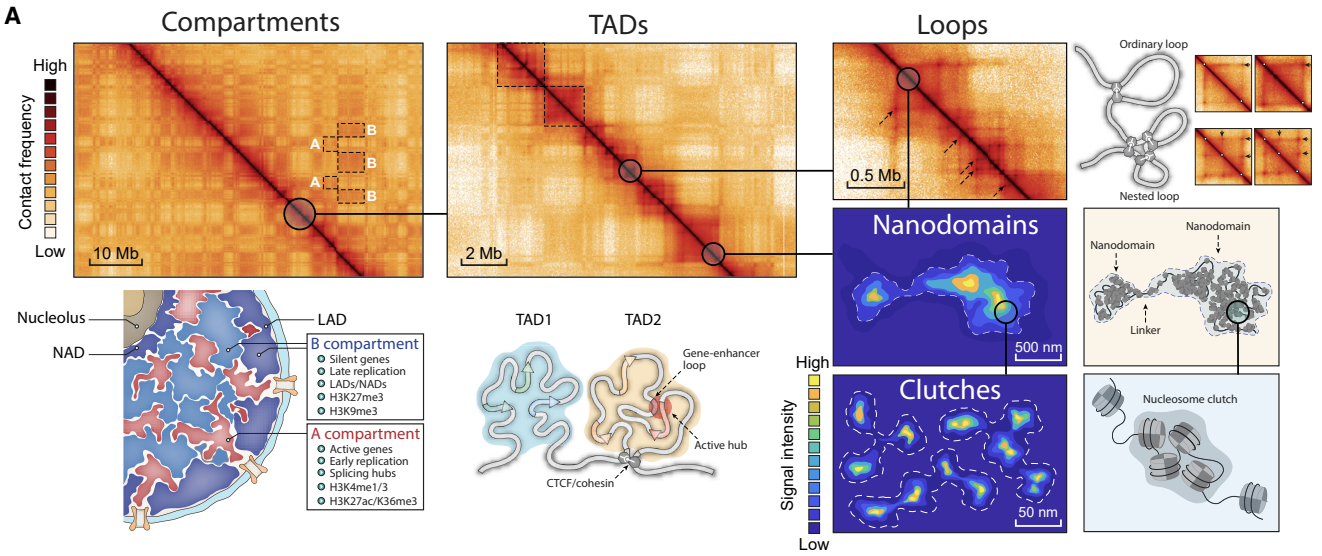

B

Structure	Size and assay	Putative functions	Editing strategy
	Identified by C-methods		
Compartments	0.5-10 Mb, Hi-C	Spatial segregation of active and inactive chromatin	Unknown
TADs	0.1-1 Mb, Hi-C, 5C	Spatial insulation of neighboring genome loci and their regulatory systems	Mutations and rearrangements of CBSs
Loops	50-500 kb, Hi-C, 4C, Capture-C	Spatial insulation of neighboring genome loci and contacts between regulatory regions	Same as for TADs + CTCF recruitment
	Identified by microscopy		
Nanodomains	0.007-0.2 mkm^2, Oligopaint-FISH	Unknown	Unknown
Clutches	100-1000 nm^2, ICH, Chrom-EMT	Unknown	Unknown

Figure 1. Different levels of 3D genome organization

(A) Top panel: contact maps of 3D genome structures; bottom panel: graphical representation of the corresponding structures. (B) Summary of different levels of 3D genome organization.

distant regulatory interactions that result in transcription dysregulation and manifest in development of pathologies.

Deletions

Deletions are among the most frequent SVs in the human genome.[35] Extended deletions eliminating CTCF-marked TAD boundaries result in aberrant activation of proto-oncogenes *TAL1* and *LMO2* by distal enhancers in T cell acute lymphoid leukemia (T-ALL).[36] A 600-kb deletion eliminating a TAD boundary results in interactions between unrelated strong enhancers and the *LMNB1* promoter, causing *LMNB1* overexpression and myelin degeneration in autosomal-dominant adult-onset demyelinating leukodystrophy (ADLD).[37] In the developing human limb bud, deletion of an entire TAD including boundaries within the 6p22.3 locus correlates with activation of the *ID4* gene by enhancers from the neighboring TAD. This results in the development of mesomelic dysplasia with hypoplastic tibia and fibula.[38]

Deletions also cause the Liebenberg syndrome, a limb malformation due to dysregulation of the *PITX1* expression: the forelimbs develop into the hindlimbs. *PITX1* controls the normal development of the hindlimbs where its expression is governed by the interaction with the *Pen* enhancer. In the forelimb buds, *PITX1* is not expressed due to spatial isolation from the *Pen* by a nearby insulator. In Liebenberg syndrome, multiple deletions eliminate this insulator, allowing the *Pen* enhancer to activate *PITX1* and leading to abnormal formation of the forelimb bones and the kneecap near the elbow.[32,39]

An SV-affecting CTCF binding site (CBS) is associated with facioscapulohumeral muscular dystrophy (FSHD). This disease is caused by

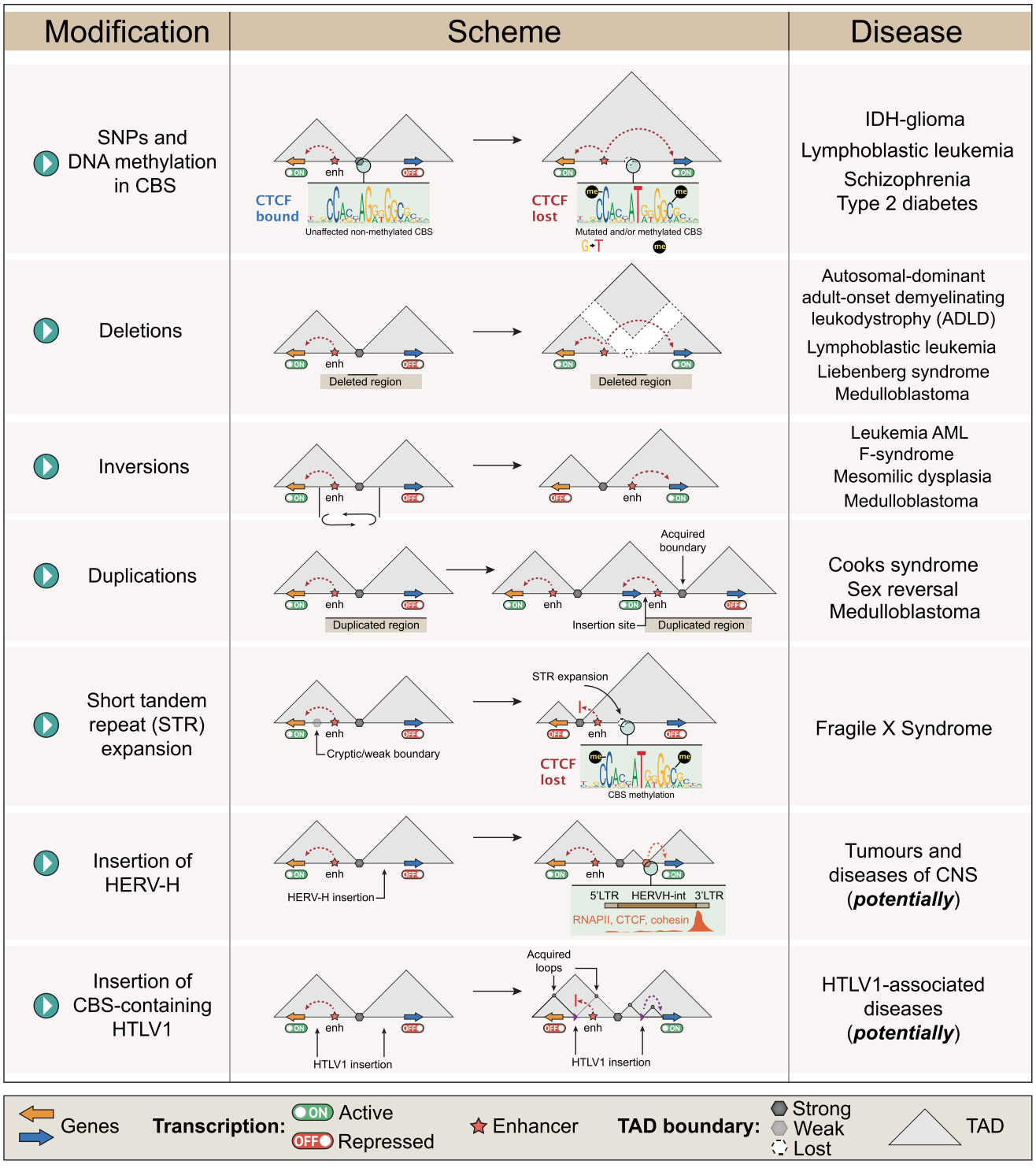

Figure 2. TAD-centric view on the 3D genome organization and disease

Note that some disorders may be causing different rearrangements: autosomal-dominant retinitis pigmentosa, F-syndrome (acropectorovertebral dysgenesis), acute myeloid leukemia, medulloblastoma, brachydactyly, polydactyly.

abnormal expression of the *DUX4* gene and potentially some other genes, including *FRG1*. In healthy muscles, enhancers regulating *DUX4* and *FRG1* are physically separated from their target genes by an FR-MAR insulator.[40–42] Massive deletion, including several copies of the *DUX4* gene and/or hypomethylation of the locus, cause redistribution of loop contacts. A decrease in the FR-MAR insulator activity leads to *FRG1* and *DUX4* upregulation and muscle pathology.[43]

Duplications

Duplications also affect the TAD profile and/or intra-TAD spatial interactions. The *SOX9/KCNJ2/KCNJ16* locus contains genes coding for the developmental regulator *SOX9* and potassium channels *KCNJ2/KCNJ16*, located in adjacent TADs. Duplications within the *SOX9* TAD result in female-to-male sex reversal. In the same locus, duplications encompassing the TAD boundary and the entire *KCNJ2* gene result in the formation of a new TAD, where *KCNJ2* is upregulated by SOX2-specific enhancers. This leads to the limb malformations with aplasia of nails and short digits known as Cooks syndrome.[44] In addition to duplications, a translocation involving the *SOX9/KCNJ2/KCNJ16* locus leads to the Snijders Blok-Campeau syndrome, which is characterized by intellectual disability, speech problems, and distinctive facial features.[45]

Inversions

Large-scale inversions lead to branchiooculofacial syndrome (BOFS), which is characterized by skin, face, and eye defects of varying severity. This pathology occurs when the normal expression of the neural crest regulator *TFAP2A* is impaired. Usually, it is a result of a partial gene deletion; however, a recent study[46] describes a patient with an 89-Mb inversion that does not affect *TFAP2A*, per se, but compromises its expression. This inversion disrupts the *TFAP2A*-containing TADs and the interaction between the *TFAP2A* gene and a group of its enhancers.

Multiple rearrangements

Numerous SVs lead to the emergence of new TADs within the *YPEL2/LINC01476* locus leading to dysregulation of gene expression in autosomal-dominant retinitis pigmentosa (adRP).[47] In some loci, large-scale SVs cause a variety of distinct pathologies. The canonical example is the *WNT6/IHH/EPHA4/PAX3* locus, where different deletions, inversions, and duplications result in limb malformation. Some of the intergenic SVs induce F syndrome (acropectorovertebral dysgenesis), a rare inherited skeletal disorder characterized by the fusion of the thumb and index finger. In particular, F syndrome is caused by an inversion, which leaves the TAD boundary intact but relocates an enhancer from the neighboring TAD to a close vicinity of the *WNT6* gene, promoting its overexpression. Other duplications and deletions affecting TAD structure within the regions cause brachydactyly and polydactyly.[48,49]

Different SVs affecting the 3D genome can lead to a phenomenon called "enhancer hijacking" where enhancers activate genes that are not their normal targets. For example, an SV in the vicinity of the *GFI1B* gene leads to *GFI1B* interaction with distal superenhancers and overexpression in medulloblastoma.[50] In acute myeloid leukemia (AML), multiple rearrangements affect more than 40 cancer-related loci.[51] These mutations are represented by translocations, deletions, and inversions; SVs lead to the formation of new loops with "hijacking" of an enhancer or a silencer in 27 of these loci.

SNPs

SNPs affect the genome topology by mutations in the binding sites of architectural proteins or tissue-specific TFs.

In the human 3p21.2 locus, the SNP rs2535629 has a strong association with schizophrenia. rs2535629 (A/G) is located within the CBS, which resides in a repressor element in the seventh intron of the *ITIH3* gene. The presence of this SV prevents CTCF binding to CBS and also changes the expression profiles of nearby genes: it downregulates *GLT8D1* and *SFMBT1* and upregulates *NEK4*. The *SFMBT1* gene product is involved in the regulation of proliferation and differentiation of nerve stem cells, the formation of dendritic spines, and the proper functioning of neural synapse. Its downregulation is associated with schizophrenia development.[52]

SNP rs7903146 (CT/TT) is present in the enhancer of the *TCF7L2* locus and promotes the formation of the gene-enhancer contact.[53] This interaction leads to an increase in the *TCF7L2* expression with a subsequent decrease in insulin secretion, possibly contributing to the development of type 2 diabetes.[54]

STR expansion and viral DNA integration

Expansion of DNA repeats and viral DNA integration can also affect the profile of spatial interactions between remote genomic elements. Short tandem repeats (STRs) alter TAD boundaries by modulating CTCF binding.[55] In several pathological models, STRs accumulate at TAD boundaries, increasing the density of CpG islands, which are often hypermethylated in pathologies. One example is the *FMR1* gene, whose repression leads to fragile X chromosome syndrome (Martin-Bell syndrome). STR accumulation at the boundary of encompassing TADs promotes DNA hypermethylation followed by a decrease of CTCF binding and significant alteration in the enhancer landscape of the locus. These lead to the loss of the TAD boundary and *FMR1* repression.[56]

Integration of a primate-specific human endogenous retrovirus subfamily H (HERV-H) transposon establishes TAD boundaries in the genome of human pluripotent stem cells.[57] In this case, active viral transcription creates a TAD boundary at the site of the HERV-H integration. This is in line with recent observations showing that active transcription constitutes a barrier for the cohesin-driven extrusion.[58] In contrast to HERV-H, insertion of the human T-lymphotropic virus HTLV-1 establishes *de novo* loops due to the presence of a CBS within the viral genome. This results in abnormal host gene transcription not only in loci proximal to the integration site but also more than 300-kb away.[59] The same was observed for the bovine leukemia virus (BLV) carrying several CBSs involved in the formation of new chromatin loops with the host chromosome loci after the provirus integration.[60]

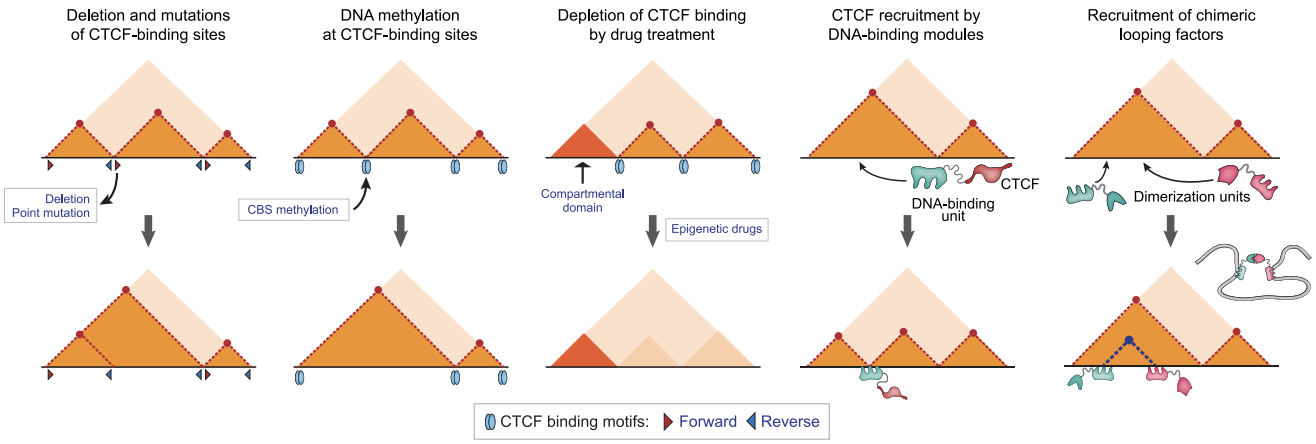

Figure 3. Strategies for 3D genome editing

Epigenetic factors

Abnormal DNA methylation is also involved in violation of the 3D genome via suppression of CTCF binding. This decreases insulation at TAD boundaries, allowing aberrant enhancer-promoter interactions that may affect disease-related genes. In human glioma, mutations in the *IDH* gene result in an increased genome-wide DNA methylation including that in the *PDGFRA* oncogene locus. Methylation of the 5′-flanking insulator serving as a TAD boundary in this locus results in a loss of CTCF binding and perturbs local interaction patterns; this drives an abnormal activation of the *PDGFRA* gene by a distal enhancer.[61] In some cases, abnormal methylation at CBSs could be driven by external factors, such as drug use. For example, cocaine addiction results in a pathogenic looping within the *IRX*A locus in brain neurons due to DNA hypomethylation at a set of CBSs.[62] Recent studies reveal that besides abnormal methylation and CBS mutations, some other mechanisms could impact chromatin looping. In T-ALL, disappearance of the TAD boundary between *MYC* and a group of enhancers in the neighbor TAD leads to *MYC* overexpression.[63] Interestingly, in this case, a loss of CTCF binding is not caused by CBS mutations or methylation and is accompanied by decreased chromatin accessibility. Thus some additional factors may influence CTCF binding. One potential candidate is *Jpx* non-coding RNA, which regulates CTCF binding at a subset of developmentally sensitive loci by competitive inhibition.[64]

Finally, at a whole-nucleus scale, contacts between non-homologous chromosomes can also lead to oncogenic chromosomal translocations. This disrupts local regulatory 3D interaction networks and alters cellular transcription programs. A few examples include interactions of chromosomes 8 and 14 or 11 and 14 in B lymphocytes leading to Burkitt or mantle cell lymphomas, respectively; chromosomes 12 and 16 in adipocytes leading to liposarcoma; and chromosomes 5 and 6 in hepatocytes leading to hepatocarcinoma.[65–69]

Together, alterations in the genome 3D organization are quite common in cancer and developmental disorders. Screening revealed that 7.3% of balanced chromosomal abnormalities disrupt TADs at known syndromic loci[70]; 14% of SVs affect TAD boundaries and lead to remarkable changes in expression of nearby genes in cancers.[71] The ability of naturally occurring SVs to affect 3D genome and cause socially significant diseases imposes the development and further clinical validation of 3D genome editing technologies applicable for treating patients.

3D GENOME EDITING

Since alterations in the chromatin contact profiles are associated with many pathologies, development of 3D genome editing methods is on the frontlines of biomedicine. The existing approaches largely rely on the use of either native architectural proteins such as CTCF or artificial looping proteins (Figure 3).

As highlighted above, CTCF is a master regulator of the mammalian interphase genome folding[72] and thus is a predominant target for 3D genome engineering. CBS deletion/insertion/inversion and point mutations are streamlined paths for the precise control of CTCF binding within genome regulatory elements and TAD boundaries/loop anchors.[73,74] In several loci, such manipulations alter loop profile and result in changes of gene expression that could be potentially used as a strategy for the cell-type-specific transcription reprogramming in patients.[75] However, genomic DNA editing possesses risks of chromosome rearrangements that should be considered while designing clinically relevant applications.[76]

In the case of *de novo* TAD formation or excessive CTCF binding, depletion of CTCF from particular sites in chromatin can be accomplished through epigenetic modifications of CBS to inhibit CTCF binding without changes in the primary DNA sequence. In this case, chimeric proteins consisting of catalytically deficient (“dead” Cas9 [dCas9]) fused with the Krüppel-associated box (KRAB), an H3K9me3-catalyzing repressor (dCas9-KRAB), or DNA-methyltransferases DNMT3A and DNMT3A3L were shown to be effective inhibitors of CTCF binding when precisely targeted to the CBS.[77,78]

Another approach being developed for clinical applications is the usage of small molecules interfering with the CTCF binding. Treatment

of cells with the anti-cancer agent curaxin (CBL0137) leads to a partial depletion of CTCF from chromatin and compromises enhancer-promoter contacts.[79] On the other hand, DNA methylation interfering with the CTCF binding could be eliminated by cell treatment with 5-aminosalicylic acid (5-ASA). In the case of AML cells, treatment with 5-ASA restores a normal pattern of CTCF-dependent insulation in chromatin and effectively suppresses the interactions between a set of enhancers and oncogenes.[51]

Other perspective molecules for the 3D genome manipulations are bromodomain and extra-terminal motif inhibitors (BETis). BET proteins are widespread transcription regulators often associated with architectural proteins. For instance, BET protein bromodomain-containing protein 4 (BRD4) binds to CTCF-associated Yin Yang 1 (YY1) factor throughout the genome including at TAD boundaries. Pan-BETi (Apa20, JQ1, IBET762) treatment weakens BRD4-YY1 binding, removes BRD4 from chromatin, and causes chromatin decondensation.[80] BETi JQ1 treatment suppresses cohesin and CTCF binding to the Kaposi's sarcoma-associated herpesvirus (KSHV) genome. This results in the loss of looping between latent and lytic control regions of the viral chromosome and virus transition to a lytic state.[81]

Together, this opens an avenue for the rational *in silico* design of inhibitors of DNA binding and competitors for the protein-protein interactions for the factors involved in the 3D genome maintenance.

Since some diseases are associated with the decrease of CTCF occupancy at particular CBSs,[82] stabilization of CTCF binding at such loci is a strategy to be considered. Several post-translational modifications are essential for the CTCF insulator and barrier activities.[83] Indeed, mutations in the CTCF region subjected to poly(ADP-ribosyl)ation by poly(ADP-ribose) polymerase 1 (PARP1) compromise cohesin enrichment at CBSs, e.g., interfere with cohesion-/CTCF-dependent loop formation.[84] In the Epstein-Barr virus genome, PARP1 acts to stabilize CTCF binding at particular sites.[85] Thus, inducible recruitment of PARP1 to certain CBSs could be a tool for the stabilization of selective CTCF binding to these sites. Other post-translational modifications of CTCF such as SUMOylation and phosphorylation[86,87] are also of interest for 3D genome manipulation in both genome-wide and locus-specific manners. Together, these examples illustrate that epigenome targeting and inducible CTCF post-translational modifications could potentially serve as a proxy for 3D genome editing.

A number of diseases are characterized by a complete loss of CBSs at critical regulatory elements due to deletions and other SVs.[88–90] In such cases, recruitment of CTCF by an unrelated DNA-binding module and restoration of the original loop profile might be a potential treatment strategy. One example is a dCas9-mediated CTCF recruitment enforced by the coupling with SunTag technology, allowing recruitment of multiple CTCF molecules to the same binding site.[91] This method has been tested in the *TFF* locus associated with breast, lung, and colon cancers[92] and demonstrated a potential utility for 3D genome engineering in multi-gene disease-associated loci.

Some pathologies are accompanied by a total loss of *CTCF* expression followed by genome-wide alterations of chromatin 3D structure. Consequently, insertion of a functional CTCF gene could restore an original loop pattern of the affected loci. For example, breast cancer could be inhibited by the increased *CTCF* expression; the *CTCF* gene is frequently deleted in this type of cancer, and this negatively affects the survival rate of patients at late stages.[93] *CTCF* gene insertion by pseudoviruses also slows down the cancer cell division and/or migration, as well as metastasis in the lungs and brain, and affects the expression of almost 130 genes.

An alternative approach for manipulation of the 3D genome architecture relies on the expression of chimeric proteins containing DNA-binding modules (zinc finger [ZF]),transcription activator-like effector [TALE], dCas9) fused with units forming homo- or heterodimers, such as dimerization domains of mammalian TFs, e.g., the self-associating domain of the Ldb1 protein.[94] These chimeric proteins form relatively stable dimers and are suitable for the formation of constant distant interactions in chromatin. These contacts can be made reversible via inducible polymerization. One example is the chromatin loop reorganization using CRISPR-dCas9 (CLOuD9) system, where PYL1 and ABI1 fused with two different dCas9 modules interact with each other in the presence of abscisic acid (ABA). Application of the CLOuD9 system to the *Oct4* locus associated with various types of cancers demonstrated that recruitment of these fusion proteins to the *Oct4* promoter and its distal enhancer induced loop formation and upregulation of *Oct4* after ABA addition.[95] A similar approach, light-activated dynamic looping (LADL), utilizes co-expression of cryptochrome 2 (CRY2) and N-truncated CRY-interacting basic-helix-loop-helix protein 1 (CIBN) fused to dCas9. Exposure of cells to 470-nm blue light induces dimerization of CRY2 and heteromerization of CRY2 and CIBN. As a result, loci targeted by dCas9-CIBN form a transient loop. In a proof-of-concept study, LADL-induced looping between *Zfp462* and the *Klf4* superenhancer was successfully used to increase the *Zfp462* expression in mouse embryonic stem cells.[96]

CONCLUDING REMARKS

Genome-wide association studies revealed a number of genomic SVs associated with the development of various diseases. Most of these SVs are located outside genes and their regulatory modules. Consequently, the mechanical links between disease-associated SVs and regulation of genome activity remained obscure. Recent results discussed here argue that many of the disease-associated SVs affect 3D genome organization. This raises the issue of the need for 3D genome editing. Several approaches for such editing have been proposed and tested on cell cultures. The question is whether any of the developed strategies have the prospect of practical application. In the case of cancer, the straightforward strategy is to kill a cancer cell if it can be recognized and targeted rather than to try to correct anything in this cell. The only possible application here is to use low-molecular-weight agents (e.g., curaxins) that affect all cells with some preference to cancer cells. More interesting opportunities for practical applications of 3D genome editing arise in cases where it is necessary to

deliver to the organism its own normal cells, which will exist alongside corrupted ones or replace them. This strategy assumes that damaged cells are taken from the patient, manipulated in the laboratory, and returned to the patient's body. In the future, this approach could be useful for the treatment of a number of diseases of the hematopoietic and endocrine organs, as well as some types of muscular dystrophies.

However, similarly to genome editing, 3D genome manipulations could have off-target effects. In particular, targeted recruitment of the full-length CTCF or any other natural architectural protein to an ectopic site requires its overexpression in a cell. This could increase the abundance of the overexpressed factor at endogenous binding sites affecting their contact profile genome-wide. Usage of truncated forms of architectural proteins lacking natural DNA-binding domains and/or weak promoters for the expression cassettes potentially solve this problem. Further, binding by an artificial module such as dCas9 may change the chromatin residence time of the recruited protein, which, in turn, may affect the looping strength and nucleosome/epigenetic profiles in a vicinity of the binding site.[97] Thus, a sophisticated design of chimeric proteins and comprehensive analysis of epigenetic and transcription profiles within the edited locus and in its neighborhood are prerequisites for the development of clinically relevant applications.

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AUTHOR CONTRIBUTIONS

S.V.R. and Y.V. conceptualized the manuscript. E.A.T., S.V.U., and A.K. wrote the draft. S.V.U. prepared the figures. All authors made corrections to the final version of the manuscript. All authors have read and agreed to the published version of the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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