HHS Public Access

Author manuscript

Semin Cell Dev Biol. Author manuscript; available in PMC 2020 June 01.

Published in final edited form as:

Semin Cell Dev Biol. 2019 June ; 90: 104–113. doi:10.1016/j.semcdb.2018.07.005.

The role of 3D genome organization in disease: from compartments to single nucleotides

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Abstract

Since the advent of the chromosome conformation capture technology, our understanding of the human genome 3D organization has grown rapidly and we now know that human interphase chromosomes are folded into multiple layers of hierarchical structures and each layer can play a critical role in transcriptional regulation. Alterations in any one of these finely-tuned layers can lead to unwanted cascade of molecular events and ultimately drive the manifestation of diseases and phenotypes. Here we discuss, starting from chromosome level organization going down to single nucleotide changes, recent studies linking diseases or phenotypes to changes in the 3D genome architecture.

Introduction

Control of gene expression is complex but vital and epigenetics play a critical role in modulating gene expression for normal cellular function. Abrogation of this epigenetic control is frequently associated with disease etiology and progression [1, 2]. Different epigenetic layers provide us context to understand the rules governing gene expression and chromatin stands out as front and center to all these layers. Chromatin is the biomolecular complex between the genomic DNA and histone proteins which allows the DNA to be folded and compacted by thousands of folds and get organized into cellular nuclei of 2–10 microns in size for human cells. Nucleosomes are the basic unit of chromatin, consisting of 146 bp of DNA wrapped around two copies of histone H2A, H2B, H3 and H4 proteins. Connecting the two nucleosomes is the linker DNA associated with histone H1 and H5 [3]. Although this initial folding and occupancy of nucleosomes along the DNA constitute a basic layer of gene regulatory system; the eukaryotic transcriptional mechanism (e.g., in human) is far from simple in nature [4]. The complexity of transcriptional machinery arises from a spatiotemporal coordination of many *cis* and *trans* acting genomic and proteomic elements that maintain the baseline gene expression profile in normal condition. Transcription is initiated by promoters, which first recruit the RNA polymerases and other

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necessary factors. Enhancers and insulators further fine tune the gene expression by recruiting transcriptional activators or suppressors in a tissue or lineage-dependent manner. Modulation of transcription through enhancers or insulators seems surprisingly difficult when the linear genomic distances between a transcription start site (TSS) and some of its distal regulatory elements are considered [5]. A simple linear map of histone modified chromatin is insufficient to fully explain cell type-and context-specific differences in gene expression and demands a detailed understanding of the three-dimensional organization of the chromosomes. To systematically elucidate the gene-regulatory programs in human or in other organisms and to decipher the complex circuit of connections between regulatory elements and genes, it is necessary to consider the chromatin in its folded state in 3D and in a tissue, lineage, and cell-type dependent manner.

The predominant method to study the 3D organization of genomes before the development of next generation sequencing (NGS)-based techniques was the fluorescence in situ hybridization (FISH) [6]. In recent years, chromosome conformation capture assays in combination with NGS technology successfully complemented imaging-based techniques to study the 3D organization of the genome. Chromosome conformation capture measures the pairwise contact frequency of genomic regions by a process that can be summarized as: crosslink, cut, label, re-ligate, shear, enrich and sequence. More specifically, chemically fixed cells with cross-links holding the spatially close DNA fragments together are subjected to chromatin digestion with a restriction enzyme (or enzymes) in order to create fragments, the ends of which are labeled with biotin and are allowed to re-ligate with the ends of nearby fragments that are cross-linked together. After shearing of the re-ligated circular DNA and potential steps for enrichment of newly created chimeric fragments (e.g., biotin pull down if ligation junctions are biotinylated) the resulting fragments are then quantified using PCR, microarrays or high throughput DNA sequencing [7–13]. Based on the number of interrogated genomic loci and enrichment of proteins, the conformation capture methods are subdivided into different categories such as 3C (one-to-one), 4C (one-to-all), 5C (many-to-many), Hi-C (all-to-all), ChIA-PET and HiChIP/PLAC-seq. In a typical 3C experiment, a pair of interacting genomic loci can be identified using PCR by targeting two known genomic loci with specific primers [14]. 4C experiments profile all interactions from a single genomic anchor point (i.e., bait region) [15–17]. Hi-C, on the other hand, measures all possible intra- and inter-chromosomal interactions in the genome in a single experiment [7]. In addition, finding genomic interactions in conjugate with different mediator proteins, especially that are associated with histones and transcription factors, several techniques have been developed adopting the chromatin immunoprecipitation (ChIP)-based enrichment methods [18–20]. Such methods provide high-resolution information about the presence of DNA-binding proteins on 3D organization of the genome and their implications on gene regulation. ChIA-PET was the first of such techniques [18], however, two other recent methods HiChIP [19] and PLAC-seq [20] overcome important limitations of ChIA-PET by reducing the input material requirement (i.e., number of cells) and increasing the sensitivity and robustness of the genome-wide interaction yield.

Since the advent of the chromosome conformation capture technology, our understanding of the human genome 3D organization has grown rapidly and we now know that human

interphase chromosomes are folded into multiple layers of hierarchical structures **(Figure 1)** and each layer can play a critical role in transcriptional regulation [21, 22]. Alterations in any one of these finely-tuned layers can lead to unwanted cascade of molecular events and ultimately drive the manifestation of diseases and phenotypes **(Figure 1, Table 1)**. Some examples include emergence or dissolution of compartments and chromosomal territories seen in several cancers (e.g. chromosomal translocations in breast cancer and prostate cancer) [23], dynamic arrangement of topologically associated domains (i.e., TADs) in some inherited diseases (e.g. F-syndrome, sex reversal) [24, 25], and formation or disappearance of chromatin-loops between enhancers and promoters in several types of cancers and other diseases (e.g. T cell acute lymphoblastic leukemia/T-ALL, asthma, heart-diseases) [26–28]. All these intricate layers of genome organization (e.g. chromatin loops, TAD arrangement, compartments) can be affected by changes in the DNA sequence or in the epigenetic landscape such as presence of single nucleotide polymorphism (SNPs) or mutations in enhancers and promoters, deletions, amplifications and translocations of genomic regions and binding of transcription factors. Such scenarios are increasingly evident from several recent studies where a strong relation is found between the changes in spatial organization of human genome due to chromosomal abrogation and the dysregulation of diseases related genes with intact DNA sequences. Our cumulative knowledge to perceive the missing links to connect human diseases with genome organization is ever increasing and summarizing the current understanding is of great importance to grasp the full picture and pave the way for the next wave of studies. There are already a number of excellent reviews that cover some of the published links between genome organization and diseases [21, 22, 29–31]. In this review, we take a top-down approach to discuss different layers of human genome organization, most of which are fully characterized only within the past decade, and we summarize the diseases or phenotypes linked to changes in the delicate fabric of the 3D genome architecture at each layer. We provide an up-to-date view of the literature and discuss potential future directions that are likely to transform the studies of 3D genome organization from discovery to translation.

Chromosomal Territories, Nuclear Compartments and Diseases

The concept of chromosomes occupying a defined space in the nucleus is as old as the study of chromosomes itself. The evidence of a territorial organization of chromosomes in animal cells was first furnished by Carl Rabl in 1885 [32] and since then numerous other studies [33–37] has backed this structural organization. Studies also demonstrated that chromosomal territories are segregated by inter-chromatin domain and according to this model the inter-chromatin domains are enriched in active genes and thus play a role in gene regulation [38]. Cremer & Cremer, 2001 [39] provided a good overview on the relationship between chromosomal territories and gene expression regulation mechanism. In general, chromosomal territories of different chromosomes exhibit several common features such as the preferential positioning in the nucleus depending on their genomic features [40], conserved nuclear localization after mitosis [41] and radial positioning that is correlated with the cell-type specific lamin association, replication timing and transcriptional activity [42, 43]. Further studies have also shown that these territories are dynamic in nature and shows a strong correlation between the frequency of chromosomal translocations and spatial

proximity among them [23, 44]. Gene transcriptional machinery at the intermingling regions is also found to be a key factor in regulating the extent and the shape of the territories in a cell type-specific manner [23].

Analysis of the Hi-C data further refined these large-scale chromosomal territories into two sets of compartments. These compartments appear as a plaid, checkerboard-like pattern when a chromosomal Hi-C matrix is first transformed to an observed/expected ratio then to a correlation matrix and can be separated into two sets of megabase-sized regions called “A” and “B” compartments by eigenvalue decomposition [7]. The regions within a given compartment are enriched for interactions within the same compartment and depleted for those that are across. Compartment A is enriched in gene density, transcriptional activity, H3K36me3, early replication and higher DNA accessibility (e.g. DNAse), which all suggest an open chromatin state for the genomic regions in this category [7]. Compartment B regions are associated with lamina-associated domains, low transcriptional activity, late replication and, hence, are enriched in heterochromatin [7]. Compartments are highly dynamic in nature and changes occur in accordance with lineage and cell-type specificity. Recent studies suggest that about 36% and 59.6% of the genome is dynamically compartmentalized during stem cell differentiation and across different primary tissues, respectively [45, 46]. Altered compartmentalization has also been reported in cancer cells. A recent study from Barutcu et. al [47] comparing normal breast cells (MCF-10A) and its cancerous counterpart MCF-7 showed a homogeneous switching of 12% compartments. Even though the normal and cancerous cells showed a similar distribution of open (A) and closed (B) compartments across the genome, a significant number of B to A compartment transitions are observed within small chromosomes (chromosome 16–22) suggesting an overall increased open conformation in small chromosomes. RNAseq analysis from the same study revealed an altered gene expression pattern across the small chromosomes in MCF-7, related to the activation of oncogenic pathways such as WNT signaling. Previous studies have also demonstrated that chromatin organization plays a major role on mutational rates in human cancer cells [48]. It has long been known that the mutation rate varies over different scales across the genome with much of this variation largely unexplained [49]. This phenomenon of regional variation in mutation rates, RViMR, has been demonstrated for base-substitutions [50], small-indels [49, 51] and transposable elements [52]. Different genomic features such as GC content, SNP density, recombination hot spots and CpG islands have been shown to contribute towards RViMR [53]. Further studies performed by Schuster-Bockler and Lehner [48] showed that arrangement of the genome into heterochromatin and euchromatin like domains plays a major influence on regional mutation rate variation in human cancer cells and can account for 55% of variations observed across different cancer types. In another relevant study, Fortin and Hansen [54] demonstrated a relationship between A/B compartments and somatic mutations in prostate cancer cell lines. The analysis reconfirmed that similar to other cancer types, prostate cancers showed an elevated somatic mutation rate in closed or B compartments than in A. Compelling evidence [55] also suggests that replication timing, which is highly correlated with the organization of compartments [56]; is an effective predictor of mutational rate and subsequent somatic copy-number variations in several cancer types. Alteration in genomic compartmentalization are now being studied in other diseases such as heart failure models in mice [57]. Along with other findings, the Hi-C

analysis also revealed an altered A/B compartmentalization with altered boundary strength upon CTCF depletion [57, 58].

Compartmentalization of the genome and different epigenetic mechanisms, which regulate the formation and stabilization these open and closed compartments play a dominant role in human disease. Few notable such epigenetic mechanisms include nucleosome remodelers [59], histone variants [60], post-translational modification of histones [61] and DNA methylation [62]. Interestingly, emerging evidence suggests that some long-noncoding RNAs (lncRNAs) act to establish genomic compartments [63, 64] and can shape the structure of chromosomes [10, 65]. A recent study by Hacisuleyman et. al. [66] showed that a lncRNA, *Firre*, which plays a key role in adipogenesis plays a role in forming a nuclear compartment with five distinct trans-chromosomal loci spatially proximal to its own loci on chromosome X and in modulating nuclear architecture across chromosomes. There is a plethora of other lncRNAs that are found to be associated with different diseases [67–70], and their roles in context to genomic architecture still needs to be explored.

Topologically Associated Domains and Diseases

In the study of human genome organization from Hi-C, topologically associated domains (TADs) emerged as a fundamental unit of the chromosomal structure since their first description in 2012 [71, 72]. These studies showed that human genome is segregated into megabase-sized topological domains and the genomic regions within a TAD have enriched intra-domain contact frequency compared to inter-domain. Although this spatial organization is found to be a general property of the interphase chromatin across different cell types in both human and mouse, further studies have suggested that TADs are not simple stable loops that are formed between two permanent genomic loci, rather they are dynamic in nature [10]. While mechanisms of TAD formation are still an open question, few studies have put forth some ideas to describe TAD formation including “strings and binders switch” [73], chromosomal supercoiling [74], block copolymer model [75] and loop extrusion model [76, 77]. Notably, the loop extrusion model is found to be generalizable at a genome-wide scale and it recapitulates experimental results in-silico by considering two important players, CTCF and cohesin, which have long been known to be critical in establishing and maintaining chromatin structure. In this model, the cis-acting loop-extruding factors such as cohesin rings form progressively larger loops until they are stalled by TAD boundary elements such as CTCF. More recently, Ganji et al provided an unambiguous evidence for Condensin-induced loop extrusion mechanism by directly visualizing the formation and processive extension of DNA loops in real time [78]. Irrespective of the TAD formation model, the importance of CTCF and cohesin in maintaining the boundaries and stable architecture of TADs are well-established [30]. CTCF, a highly conserved 11-zinc finger insulator protein that binds to a non-palindromic DNA motif and cohesin, a ring-like multiprotein complex that plays a role in DNA repair and segregation, are known to co-occupy different binding sites and CTCF has been shown to help *cohesin* in positioning to its target region [79]. Both are found to be very strongly enriched at the TAD boundaries [10, 30, 72] and depletion of either one has a significant impact on TAD organization and strength[58, 80].

The biological importance of TADs is highlighted by their potential to regulate the gene pleiotropy as suggested by studies performed on *Hox* locus [81] where TADs are found to evolve in multiple tissues with differences in enhancer-promoter interactions, which ultimately control the expression of genes at that locus. The relevance of TADs in human diseases has recently been shown also for the *Epha4* locus [24]. Epha4 is an ephrin receptor and is involved in the formation of tissue boundaries and segmentation. Distinct structural variants involving a 1.7–1.9Mb heterozygous deletion, 1.1 Mb heterozygous inversion/or 1.4 Mb heterozygous duplication, and a 900 Kb duplication around this locus are associated with different types of limb malformations in human, namely Brachydactyly, F-syndrome, and Polydactyly diseases, respectively [24]. Lupianez et. al. [24] in their detailed study showed that all the disease-related structural aberrations disrupt the native TAD boundaries associated with this locus; resulting in altered promoter - limb-enhancer interactions and thus disrupts the normal *Epha4* transcription regulation dysregulates expression profile of surrounding genes. In a separate study, Framke and Ibrahim et. al. [25] showed how genomic duplication events can affect TAD formation and lead to human disease etiology. They focused on the developmental transcription factor *Sox9* locus and this locus contains two TADs, one including the *Sox9* and the other includes potassium channel genes *Kcnj2* and *Kcnj16*. The authors found that a duplication event within *Sox9* TAD (intra-TAD duplication) leads to increased interaction propensity among the regulatory elements of *Sox9* gene and in-effect causing female-to-male sex reversal phenotype, while an inter-TAD duplication resulted in a neo-TAD with new set of regulatory elements encompassing the *Kcnj2* and *Kcnj16* genes leads to Cooks syndrome, a limb malformation disease in humans. A third inter-TAD duplication event that extended within the *Kcnj2* and *Kcnj16* TAD but not the gene body did not show any phenotype. The results demonstrated how duplication events can affect higher-order genome structure and impact of TADs on regulating gene expression profiles. Liebenberg syndrome, in which arms of a patient acquire morphological characteristic similar to legs, linked to the deletion of *H1afy* gene 300 Kb upstream of *Pitx1* gene. *Pitx1* determines hindlimb identity and deregulation of *Pitx1* in mice is associated with forelimb to hindlimb conversion. *H1afy* is a boundary element and in the normal scenario this helps to insulate the *Pitx1* TAD from the neighborhood but when deleted an enhancer from adjacent TAD interacts with *Pitx1* gene and thus misregulation of *Pitx1* takes place causing Liebenberg syndrome [82]. In autosomal-dominant Adult-onset Demyelinating Leukodystrophy (ADLD) characterized by progressive demyelination of central nervous system through overexpression of *Lamin B1* gene is also found to be linked to TAD boundary disruption. A deletion upstream of *Lamin B1* gene eliminates the TAD boundary causing an ectopic interaction between two merged TADs along with three enhancers with the *Lamin B1* gene promoter [83]. Importance of insulated neighborhood in the progression of cancer through activation of proto-oncogenes has also been studied [26, 84]. In one of such studies Hnisz and Weintraub et. al. [26] showed that in T-ALL cancer genome microdeletions are enriched at the CTCF boundary sites, thereby eliminates insulated neighborhood containing important T-ALL proto-oncogenes. With the loss of insulated neighborhood, aberrant activation of proto-oncogenes is possible via distal enhancer-promoter interactions normally located outside the neighborhood. The results from all these chromosome conformation capture studies confirm that TADs are a fundamental

regulatory unit of our genome, which when disrupted lead to dysregulation and in turn disease phenotypes.

Chromosomal Loops and Diseases

Regulatory elements such as enhancers and insulators play a crucial role in controlling the gene expression profile of a cell in a context-dependent manner. Many human genes are regulated by at least one regulatory element positioned upstream or downstream relative to the gene promoter that influences the promoters transcriptional state. The detailed analysis of high-resolution chromosome conformation capture data strongly suggests the presence of regulatory chromosomal loops as a means of communication among the local and/or distal regulatory elements with promoters to control gene expression [28, 85]. A chromatin loop by definition is two genomic loci that are physically closer in the nucleus than their intervening sequences. Early studies used the 3C technique to investigate erythroid-specific mouse *beta-globin* gene and showed that its locus control regions (LCR) situated 50Kb apart is closer to the gene in proximity in fetal liver cells but not in fetal brain cells correlated with liver specific expression of *beta-globin* [86]. A classic example of long-range gene regulation involves the *Shh* gene, expression of which is regulated by a ~1Mb away enhancer element [87]. A more recent study of a GWAS SNP related to multiple vascular diseases clearly demonstrated the problems with “nearest gene” approaches by linking this putative causal variant to a more than 600kb away gene (*EDN1*) rather than the nearest gene that harbored the SNP in its intronic region (*PHACTR1*) [88]. Another recent study using super-resolution Hi-C data in human cells again provided compelling evidence on the importance of loops in regulating the gene expression through frequently linking the enhancer-promoter regions and in maintaining the TAD boundaries [10]. The mechanism of such loop formation is now gradually accepted [78] and through several loss-of-function experimental approaches it is now inferred that transcriptional factors like GAGA [89], GATA-1 [90], STAT6 [91] and architectural proteins especially CTCF, BEAF-32, Elba and Ibf1/2 helps to mediate and maintain long-range interactions across the genome [92]. As stated earlier in the previous section; the CTCF-cohesin based extrusion mechanism of TAD formation is also a leading model to describe how the chromosome loops may form in the genome [76, 77].

With the involvement of such large number of crucial transcription factors, chromatin modulators and their role in precise regulation in gene expression profile, it is not surprising that chromosomal loops are also found to play a dominant role in different human diseases. Early studies showed that illegitimate gene looping can contribute to a rare form of blood disorder alpha-thalassemia [93]. This study identified that in the diseased condition a gain-of-function SNP creates a new enhancer-promoter link between *alpha-globin* genes, leading to altered transcription initiation and reduced *alpha-globin* expression. In a separate study by Ott et. al. [94] investigating Cystic Fibrosis related markers, the authors showed that intronic enhancers within the cystic fibrosis transmembrane conductance regulator (CFTR) gene regulate its own expression via a loop connecting the intronic-enhancer to the *CFTR* promoter. Common mutations causing cystic fibrosis is found to abolish this intronic enhancer-promoter interaction leading to a reduced expression profile of *CFTR* gene in diseased condition. Further studies also demonstrated that a plethora of human diseases

follow a similar mode of etiology where a SNP eliminates an enhancer-promoter loop, leading to mis-expression of a gene or a set of genes contributing to the development of the disease. Notable example includes Asthma [27], Inflammatory bowel disease [95], Insulin resistance, T2D, Coronary heart disease [28], autoimmune diseases [96], Cardiac rhythm disorder [97], Rett syndrome [98], Myeloproliferative disorders [99], systemic lupus erythematosus [100] and Aniridia [101]. In all of these referred human diseases, at least one enhancer-promoter interaction is lost due to a presence of a SNP either at the enhancer side or at the promoter loci causing an altered expression of the corresponding gene(s). The chromatin interaction model also offers an explanation to the effects of diseases-associated variants residing in the non-coding part of the genome. Combining chromosome conformation capture techniques with genome-wide association studies (GWAS) holds a great potential to identify new putative target genes/pathways for potentially causal SNPs/variants or revise existing connections made based on the nearest gene principle, thereby, significantly improving our understanding of human diseases including cancer.

A large number of common genetic variants in the human genome are associated with cancers and extensive studies have been performed nearly on all types of malignant cancers revealing novel target genes and pathways involved in carcinogenesis [102]. Recent studies involving capture Hi-C [103] and 3C techniques [104] in colorectal cancer risk loci also showed that most of the disease associated SNPs reside within the regulatory elements and/or enriched in transcriptional factor binding motifs in the non-coding region of the genome and exerts effects through long-range chromosomal interactions. Similar effects are also shown in cases of prostate cancer [105], breast cancer [106, 107], and in multiple other cancer systems [108]. Alteration of such 3D organization of regulatory elements has been suggested to drive the cancer development [109] and accumulating evidence suggests that chromosomal interactions also play a key role in the development of leukemia. The survival of T-ALL cells is dependent on an insulin-like growth factor receptor encoded by *IGF1R* gene and in T-ALL the *IGF1R* gene is found to be activated by an aberrant interaction between *LUNAR1* (leukemia-induced noncoding activator RNA) and the enhancer of *IGF1R* gene [110] mediated through *Notch* signaling. In a separate example, it has been shown that *AID* (*Activation-Induced cytidine Deaminase*), a B-cell specific enzyme which helps in somatic hypermutation and class-switch recombination of immunoglobulin genes in mature B-cells, also induces mutations and translocations in genes not related to immunoglobulin class [111]. Recent study by Qian et al. showed that AID targeted genes share specific features including super-enhancers and multiple target genes that are megabases apart can physically interact with each other to promote mutations and translocations that are frequently observed in B cell lymphomas [112]. Another interesting case relates to Epstein-Barr virus (EBV) infection, which is prevalent in the human population and is a primary cause of Burkitt lymphoma, Hodgkin lymphoma development trough converting resting B lymphocytes (RBLs) to proliferating lymphoblastoid cells (LCLs). The conversion of RCLs to LCLs is mediated via EBV nuclear antigen 2 (EBVNA2) which upregulates *MYC* levels in RCLs. Zhao and Zou et al. [113] showed that EBV and particularly *EBVNA2* evolved to exploit the transcriptional machinery of RCLs to its favor by depleting repressive histone marks upstream of *MYC* gene and thereby up-regulating *MYC* expression in RCLs though long-range enhancer-promoter interaction.

Future Directions

Chromosome conformation techniques have vastly improved our understanding towards the organization of the genome and further improvement in the methodology such as the ongoing development in the protocol to integrate the conjugate protein information with fewer input cells but better upshot in the resolution and throughput will be crucial to understand the biomolecular complexes that facilitate the interaction events. Acknowledging the current limitations to amalgamate the interaction data from other independent functional genomics experiments such as DNA-replication, DNA accessibility, RNA-seq, DNA-methylation and proteomics will provide us with better understanding of the structure-function relationship of disease-associated structural variants to the progress and subsequent pathology of the disease. Although common to many other sequencing-based investigations, most of our knowledge of genome organization is derived from the cell lines, which necessitates further analysis in primary cells coupled with relevant clinical information such as genotyping and copy-number variation (CNV). Studies of patient samples from large disease cohorts will be crucial in identifying the key factors controlling the integrity of the genome organization, which is likely disrupted in many diseases. Results from such studies will be of immense importance in addressing one of the long-standing challenging questions in epigenetic studies about the chromosomal changes that drive progression of cancer. Recent development in the CRISPR-Cas9 system provides an efficient molecular machinery to generate large-scale genomic deletions and other types of chromosomal rearrangements in a well-controlled biological system. The Chromatin Loop Reorganization using CRISPR-dCas9 or CLOuD9 system [114] now lets researcher form de-novo loops between specific genomic loci and provides a powerful hypothesis-driven framework to understand the role of genome organization in gene regulation, rather than depending on observational approaches. As we confront new challenges in biology, new tools such as CRISPR, CLOuD9 or other advanced synthetic biology techniques that allow us to control biochemical modifications of chromatin will be particularly important in characterizing molecular roles of factors that control several layers of the 3D genome architecture.

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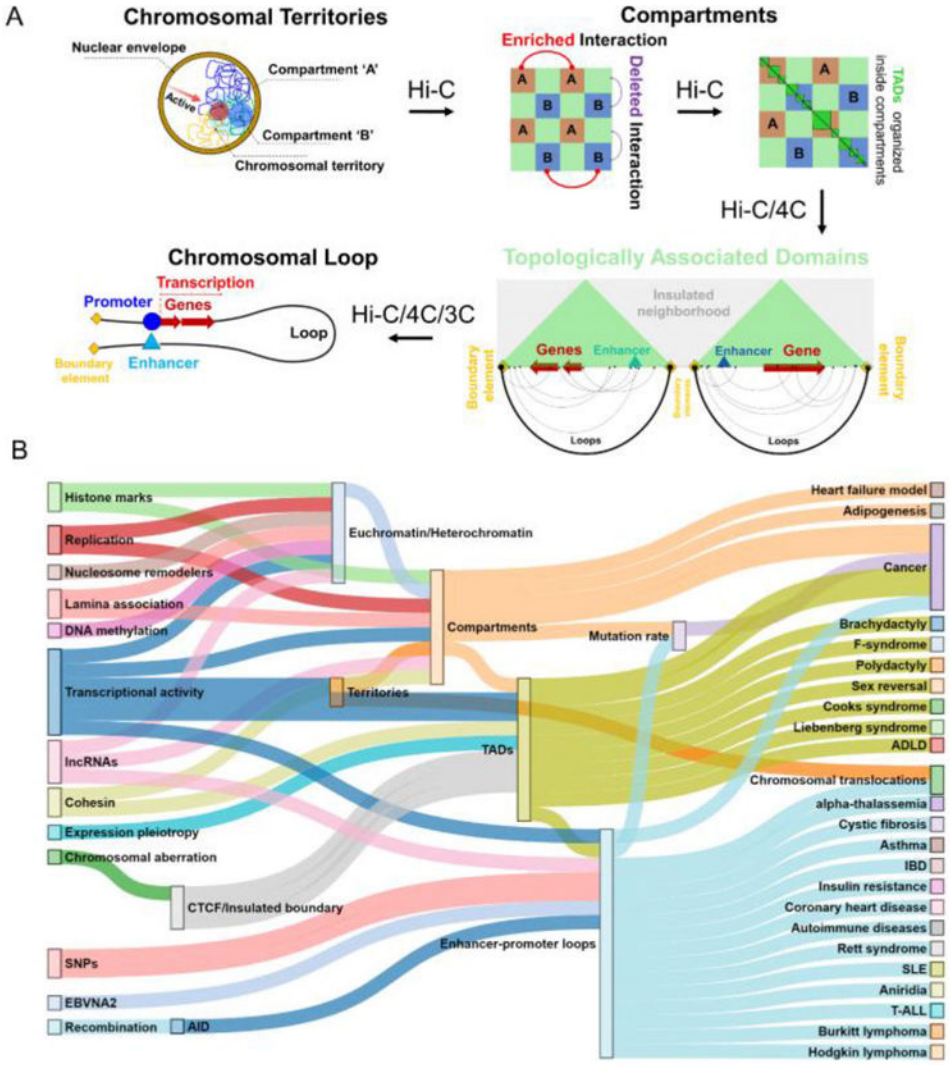

Figure 1.

Panel A shows the overall hierarchical genome organization of human genome. Panel B shows the link between different genetic and epigenetic factors (left) and different human diseases (right) mediated through changes in different layers of the genome organization (mid).

Table 1.

The features of different layers of genome organization and diseases or phenotypes that are associated with changes in the corresponding layer. The last column summarizes possible mechanism/information about each listed 3D genome feature – disease link.

Organizational Layer	Features of the Layer	Associated Diseases/Phenotypes	Mechanism/Available Information
Chromosomal Territories	1. Each chromosome in human genome occupies its own territory 2. Territories intermingle at inter-chromatin domains that are enriched in active genes 3. Territories play role in transcriptional activity and preferential positioning of loci in the nucleus	Possibly various cancers [23]	• Territories are dynamic in nature and intermingle significantly with each other • Intermingling between different chromosomes correlate with translocation frequencies in human cells
Compartments	A: 1. High gene density 2. Active histone marks 3. Early replication 4. Open chromatin 5. Low lamina association 6. High transcriptional activity 7. Low DNA methylation	Breast cancer [47]	• MCF-7 showed 12% compartment switching • Small chromosomes showed B to A transition and altered transcriptional activity • Activation of oncogenic pathways
		Regional Variation in Mutational Rate (RViMR) [48, 50, 54]	• Hetero/Euchromatin compartments play a major role and account for 55% of variation in mutational rates • Elevated somatic mutation in B compared to the A compartment in prostate cancer cell lines
	B: Opposite features compared to 'A' 1. Highly dynamic in nature and changes occur in accordance with lineage and cell-type specificity 2. Cohesin complex important for the formation and maintenance	Adipogenesis [66]	• lncRNA *Firre* plays a key role in adipogenesis • *Firre* forms a nuclear compartment with five distinct trans-chromosomal loci important for its function
		Heart failure model [57]	• Altered transcriptional activity due to change in compartments
Topologically Associated Domains	1. Enriched intra-domain contact frequency compared to inter-domain 2. Highly dynamic in nature and changes occur in accordance with lineage and cell-type specificity 3. Boundaries are enriched in motifs and binding of insulator proteins (e.g. CTCF), transcriptional activity, housekeeping genes and SINE elements 4. Considered as a regulatory unit for transcriptional activity	Brachydactyly [24]	• Deletion of *EPHA4* gene and its upstream eliminates TAD boundary • *EPHA4* associated limb-enhancer now interacts with *PAX3* genes and drives misexpression of *PAX3*
		F-syndrome [24]	• In case of inversion, the *IHH* gene and TAD boundary including the limb-enhancer of *EPHA4* gene gets inverted misplacing the enhancer close to *WNT6* gene causing misexpression • In duplication, the *WNT6*, *IHH* and TAD boundary including the limb-enhancer is duplicated, which misplaces *WNT6* gene closer to enhancer element

Organizational Layer	Features of the Layer	Associated Diseases/Phenotypes	Mechanism/Available Information
	5. CTCF and cohesin are two important factors in forming and regulating the TAD structure		causing misexpression
		Polydactyly [24]	• Downstream deletion of *EPHA4* TAD boundary causes reduction in linear distance between the limb-enhancer element and *WNT6*, *IHH* genes. This causes ectopic interactions between enhancer element and the said genes causing mis-expression
		Sex reversal [25]	• An intra-TAD duplication event. • The duplication increases the interaction propensity among regulatory elements causing ectopic expression of *SOX9* gene
		Cooks syndrome [25]	• Inter-TAD duplication event. • The duplication results in formation of a new TAD involving *KCNJ* genes and regulatory elements previously associated with *SOX9* gene. The new arrangement causes misexpression of *KCNJ* genes
		Liebenberg syndrome [82]	• *H1AFY* gene upstream of *PITX1* gene is an insulator element encoding gene and helps to insulate *PITX1* from its neighborhood • *PITX1* interacts with enhancer element from adjacent TAD upon *H1AFy* deletion causing ectopic expression of *PITX1*
		Autosomal-dominant adult-onset demyelinating leukodystrophy (ADLD) [83]	• Deletion upstream of *Lamin B1* gene eliminates the TAD boundary causing an ectopic interaction between two merged TADs along with three enhancers with *the Lamin B1* gene promoter
		T cell acute lymphoblastic leukemia (T-ALL) [26]	• Microdeletions at CTCF boundaries eliminates insulated neighborhood containing proto-oncogenes causing aberrant expression of proto-oncogenes through ectopic interactions
Chromosomal Loops	1. Proximal in three-dimensional space as compared to their linear intervening nucleotide sequences 2. Can be of two types: Structural loops (e.g. CTCF-CTCF interaction to form TADs) and Functional loops (e.g. Enhancer-promoter loops to drive the expression of a gene) 3. Cell type and lineage specific interactions	α-thalassemia [93]	• Single nucleotide polymorphism creates a new enhancer-promoter link between alpha-globin genes leading to altered transcription initiation and reduced alpha-globin expression
		Cystic Fibrosis [94]	• Cystic fibrosis transmembrane conductance regulator (*CFTR*) gene regulate its own expression via a loop connecting the intronic-enhancer to the *CFTR* promoter • Common mutations abolish this intronic enhancer-promoter interaction leading to a reduced

Organizational Layer	Features of the Layer	Associated Diseases/Phenotypes	Mechanism/Available Information
			expression profile of *CFTR* gene
		Asthma [27]	• SNPs overlap with immune cell enhancers. • CTCF binding sites are altered by SNPs • SNPs modify long-range *ORMDL3* promoter-enhancer interaction
		Inflammatory bowel disease (IBD) [95]	• Noteworthy IBD candidate genes shown to interact with enhancer region • eQTL level alters the interaction
		Insulin resistance, T2D, Coronary heart disease [28]	• *IRS1* enhancer harbours SNP causing more ectopic interaction with *IRS1* promoter
		Autoimmune diseases [96]	• SNPs associated to different autoimmune diseases physically interact with each other and regulate the associated genes with differing enhancer mechanisms
		Cardiac rhythm disorder [97]	• SNP within *SCN10A* gene interacts with *SCN5A* promoter and alters expression
		Rett syndrome [98]	• Mutation in *MECP2* gene shown to abolish 11Kb chromatin loop around *Dlx5/6* locus • *Dlx5* regulates production of enzymes that synthesize gamma-aminobutyric acid (GABA). Absence of the loop in *Dlx5/6* locus shown to alter GABAergic neuron activity in individuals
		Myeloproliferative disorders [99]	• *JAK2* gene promoter interacts with an enhancer 222kb away. The enhancer has a DHS peak and harbor a SNP. Presence of SNP within the DHS peak alters the transcription factor binding property of that region and thus causes reduced interaction with *JAK2* promoter
		Systemic lupus erythematosus (SLE) [100]	• Normal alleles control *TNFAIP3* transcription through a long-range enhancer element and subsequent stimulation of *A20* gene. • In risk allele the long-range enhancer-promoter interaction is hampered thus impaired expression of *A20* takes place
		Aniridia [101]	• Mutation in ultra-conserved *PAX6* enhancer disrupts autoregulatory feedback loop
		Colorectal, Prostate and Breast cancer [103–106]	• SNPs resides within the regulatory elements and/or enriched in transcriptional factor binding motifs in the

Organizational Layer	Features of the Layer	Associated Diseases/Phenotypes	Mechanism/Available Information
			non-coding region of the genome and exerts effects through long-range chromosomal interactions
		T cell acute lymphoblastic leukemia (T-ALL) [110]	• *IGF1R* gene is activated by an aberrant interaction between LUNAR1 and the enhancer of *IGF1R* gene through Notch signaling. IGF1R plays a key role in the survival of the cancer cells
		Induced mutations and translocations [112]	• Activation-induced cytidine deaminase (*AID*) target genes with specific features including super- enhancers and can physically interact with those genes to promote mutations and translocations
		Epstein-Barr virus (EBV) infection related cancers [113]	• EBV nuclear antigen 2 exploits the transcriptional machinery of RCLs to its own favor by depleting repressive histone marks upstream of *MYC* gene and up-regulates *MYC* expression in RCLs though long-range enhancer-promoter interaction