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The genome in space and time: Does form always follow function?:

How does the spatial and temporal organization of a eukaryotic genome reflect and influence its functions?

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Abstract

Recent systematic studies using newly developed genomic approaches have revealed common mechanisms and principles that underpin the spatial organization of eukaryotic genomes and allow them to respond and adapt to diverse functional demands. Genomes harbor, interpret, and propagate genetic and epigenetic information, and the three-dimensional (3D) organization of genomes in the nucleus should be intrinsically linked to their biological functions. However, our understanding of the mechanisms underlying both the topological organization of genomes and the various nuclear processes is still largely incomplete. In this essay, we focus on the functional relevance as well as the biophysical properties of common organizational themes in genomes (e.g. looping, clustering, compartmentalization, and dynamics), and examine the interconnection between genome structure and function from this angle. Present evidence supports the idea that, in general, genome architecture reflects and influences genome function, and is relatively stable. However, the answer as to whether genome architecture is a hallmark of cell identity remains elusive.

Keywords

chromatin; chromosome conformation capture (3C); genome; genome architecture; genome function; three-dimensional (3D) organization

Introduction

“Form follows function”, a principle coined by the American architect Louis Sullivan and first introduced to the field of biology by Kosak and Groudine [1], is associated with modern architectural design, underscoring the idea that the shape of a building or object should be based primarily upon its intended function or purpose. In biology, this principle is reflected in the close relationship between a specific biological structure and its purpose. However, owing to the complex nature of biological phenomena, sometimes it is very challenging to reveal the precise relationship between form and function of an organelle. Nevertheless, by applying this principle, Kosak and Groudine [1] have proposed a conceptual framework for approaching this question.

It is widely accepted that, in the eukaryotic nucleus, the DNA double helix wraps around histone proteins in repeating units of nucleosomes to form chromatin, which in turn is folded

into multi-level higher-order structures [2]. Advances in microscopy techniques during the last five decades (Box 1; reviewed in [3–5]), in particular those based on fluorescence in situ hybridization (FISH, [6]) and live-cell imaging of in vivo tagged genomic loci [7], have made it increasingly clear that eukaryotic genomes are non-randomly organized inside the nucleus (reviewed in [8–12]). To date, at least four aspects of the non-randomness of the 3D organization of eukaryotic genomes have been revealed. First, individual chromosomes occupy distinct chromosome territories (CTs), with only a limited degree of intermingling between CTs [13]. Second, the eukaryotic genome is partitioned into cytologically and functionally distinct euchromatin and heterochromatin [2]. The heterochromatin is characterized by its transcriptionally repressed state and highly condensed structure, whereas euchromatin is transcriptionally active and less condensed [2]. Third, individual chromosomes, genes, and genomic elements show preferences for nuclear positioning, which may correlate with genomic properties (gene density, GC content, and chromosome size) as well as with genomic functions (transcriptional activity and replication timing) (reviewed in [13, 14]). Fourth, the nucleus itself is geometrically compartmentalized in mammalian cells and contains a multitude of morphologically and molecularly distinct sub-structures, such as nuclear bodies, indicating that nuclear activities are also spatially organized [8, 15].

Box 1

Overview of the technologies for characterizing genome architecture

To date, there are two types of tools for dissecting the 3D organization of genomes: microscopy-based imaging technologies and biochemical tools.

Imaging technologies are based on either electron microscopy (reviewed in [3, 5]) or light microscopy (reviewed in [4, 5]). Electron microscopy techniques include transmission electron microscopy (TEM) and cryo-electron microscopy (Cryo-EM). Electron microscopy approaches have been typically used in cell-free systems. Light microscopy-based techniques used in the fields of biology are usually based on fluorescence labeling, such as DNA FISH [6] and live-cell imaging [7]. The resolution of conventional light microscopy is very limited. Hence, several super-resolution fluorescence microscopy approaches have been developed recently (reviewed in [4]). Fluorescence microscopy approaches can be applied to visualize the chromosome structure in the nuclei of single cells both in situ and in live cells. However, at present, on a genomic and cell population scale, the main obstacle in the application of microscopy technologies is still their limitations in resolution and throughput.

Biochemical methods can be classified into two groups (reviewed in [24–27]). One group of techniques, including ChIP (chromatin immunoprecipitation), DamID (DNA adenine methyltransferase identification), and sedimentation fractionation, probe physical contacts between genomic loci of interest and nuclear landmarks such as the nuclear envelope or nucleolus, which can yield important information about the position of genomic loci in nuclear space. The other group of molecular tools, including RNA-TRAP (RNA tagging and recovery of associated proteins) and 3C (chromosome conformation capture)-based methods, are able to measure the relative spatial proximity between individual genomic loci, which provides insight into the local or global folding of chromosomes, and into the relative positioning of individual chromosomes in relationship to one another.

3C derivatives include 3C, ChIP-loop assay, 4C (circular 3C or 3C on chip), e4C (enhanced ChIP-4C), 5C (3C carbon copy), CHIA-PET (chromatin interaction analysis by paired end tag sequencing), Hi-C (high throughput 3C), and TCC (tethered 3C) (reviewed in [24–27]). 3C and ChIP-loop specifically detect individual chromatin

interactions between a given pair of genomic loci of interest (“one vs. one”); 4C and e4C are able to identify all the interactions associated with the genomic locus of interest at the genome-wide level (“one vs. all”); 5C is a multiplex version of 3C and can detect interactions between multiple loci in a high-throughput way (“many vs. many”); CHIA-PET is able to detect all the interactions mediated by the protein of interest (protein-mediated “all vs. all”); and finally, Hi-C and TCC are developed for mapping all the chromatin interactions occurred in a nucleus (“all vs. all”).

By measuring spatial proximity, these biochemical techniques, in particular, the 3C-based methods are able to offer detailed molecular views of chromosome structure beyond the resolution and throughput limits of microscopy. However, the probabilistic nature of the results yielded from 3C-based technologies limits the application of these technologies for describing the variability between individual cells. Also, 3C-based methods can only capture a snapshot of the genome architecture. Therefore, to accurately and quantitatively describe the temporal and spatial organization of genomes at high resolution and high throughput, it is necessary to combine both the microscopy- based imaging technologies and the genome-wide 3C-methods.

Systematic studies with novel genomic tools have demonstrated that the 3D organization of eukaryotic genomes is highly relevant for their function [16–20]. During the last decade, several biochemical techniques, in particular the DamID [21, 22] and 3C-based methods [23], were developed to allow quantitative characterization of the spatial organization of genomes at unprecedented resolution and throughput (Box 1). Combining these new techniques with next generation sequencing technologies has allowed systematic characterization of genome 3D organization on different genomic scales (reviewed in [24–27]). During the last three years, 3D models for the genomes of species ranging from yeast to human have been constructed by comprehensively mapping long-range chromosomal interactions using the 3C-based high throughput methods (Table 1) [16–20, 28, 29]. These systematic studies have revealed some common structural features (loops, clusters, domains, and CTs), biological properties (polymer behavior and non-randomness), and mechanisms (looping, clustering, and compartmentalization) of 3D organization.

However, it appears that the spatial arrangement of genomes has not evolved solely for the purpose of executing nuclear functions. It was recently revealed that, in order to adapt to their lifestyle, the genomes of rod photoreceptor cells in nocturnal mammals are organized in a fundamentally different way to the conventional genome architecture found in nearly all other eukaryotic cells [30]. In these rod photoreceptor cells, heterochromatin localizes in the nuclear center and euchromatin in the nuclear periphery in order to maximize light transmission. This provides the first evidence that the arrangement of a genome reflects the need of a cell to accomplish a biological function (e.g. the genome as an optical system in the case of rod cells) other than genome function per se. This observation also indicates that

similar genomic functions based on the same genetic blueprint (i.e. the DNA sequence) can be accomplished with completely different 3D organizations.

Here, in light of a flood of new information about the 3D architecture of genomes, we look further into the relationship between shape and function of eukaryotic genomes by scrutinizing the four fundamental and widespread phenomena (looping, clustering, compartmentalization, and dynamics) observed in the 3D organization of genomes and examining their respective functional relevance and stochastic properties.

Looping, clustering, and compartmentalization are mechanisms widely adapted in the functional organization of genomes, but are they necessarily driven by functional demands?

Chromatin loops, clusters of genes, genomic elements, or individual chromosomes, chromosome domains, and subnuclear compartments are common structural features of eukaryotic genomes. Although it is clear that these structural features are of strong functional relevance, the extent to which their respective formation reflects the demand of specific genomic functions remains elusive.

Looping is both a physical property of chromatin fiber and a functional phenomenon

Chromatin loops are likely the building elements for higher-order chromosome structure and arrangement. On the one hand, the random (for some loops, see below) and dynamic nature of chromatin loops might contribute heavily to the plasticity of genome architecture. On the other hand, looping is likely one of the most commonly used mechanisms that genomes use to execute functional activities.

Chromatin looping is a phenomenon intrinsic to the polymer property of chromatin fiber, yet the formation of some chromatin loops is driven by genomic activities

Chromatin loops can be conceptually classified into two categories, one of which is merely a physical property of chromatin fibers, whose formation is not intended for a specific function, and the other whose formation is function-facilitated.

The first type of chromatin loops, which can be termed “structure-only loops”, is intrinsic to chromatin fibers and form autonomously. A chromatin fiber resembles a polymer chain, which simultaneously possesses two opposing properties: flexibility and stiffness [31, 32]. It is not hard to imagine that increased stiffness and less flexibility of the fiber correlate with a higher degree of compaction. For instance, when using the parameter “persistence length” (which is defined as the length of a polymer over which correlations in the direction of its two ends are lost) to measure stiffness, the persistence length for naked DNA is about 50 nm [32], whereas for yeast chromosomes it is 170–220 nm [31]. Hence, according to the rule of thermodynamic stability, chromatin fibers will randomly form loops even in the absence of a functionally mandated purpose [32, 33], and we propose to term this type of chromatin loops as “structure-only loops”. The frequency of the formation of structure-only loops is likely inversely proportional to the level of compaction, i.e. less compacted chromatin loops more frequently [32, 33]. Although not quite clear yet, it is reasonable to imagine that this type of looping can occur at each level of chromosome structure (i.e. double helix, nucleosome, 30 nm fiber, or higher-order structure) [33, 34]. It is also unclear whether the formation of a structure-only loop requires protein tethering. Nevertheless, the formation, size, and stability of a structure-only loop in vivo are likely determined by the local chromatin composition (e.g. the number and position of nucleosomes, epigenetic modifications, and *trans*-factors bound to the local chromatin) and its nucleoplasmic microenvironment (e.g. the crowded macromolecules). Notably, however, although it is easy to image the virtual existence of

structure-only loops based on the polymer properties of chromatin fiber, in reality, for any given chromatin loop detected by present biological methods, it is hard to claim that the particular loop has no functional implication (e.g. it might result in unintended functional consequences).

The second type of chromatin loop is function-oriented, and the formation of this type of loop is facilitated by, or even dependent on, specific functional activities. For example, recent studies of several different genomic loci have demonstrated that each individual locus forms distinct loops that differ in size and position in different biological states [35–40]. Consequently, the 3D architecture of each of these loci is dependent on the biological state [35–40]. The formation of functionally relevant loops is likely mediated by proteins [37–47]. However, it is not clear whether protein tethering initiates the formation of a new loop or simply increases the stability or frequency of an existing loop.

Chromatin looping plays important roles in various genomic activities

Chromatin looping has three direct consequences: bringing the two anchoring sites into close proximity, looping out the intervening region, and alternating the orientation of some DNA-based nuclear processes such as DNA replication (Fig. 1). In addition, the formation of loops might affect the activity of enzymes that use DNA as a substrate. All of these effects of looping could have important biological relevance. For instance, in DNA replication, the formation, size, and distribution of replication loops in the lagging-strand of the replication fork have at least three important roles: (1) ensuring that both the leading- and lagging-strand DNA polymerases synthesize new strands in the same direction; (2) controlling the choice of replication initiation sites; and (3) coordinating the speed of DNA replication of the leading- and lagging-strand [48, 49]. Also, the formation of a chromatin loop between the promoter and the 3' end of a gene during transcription is important for coordinating transcription with RNA processing and for facilitating transcription re-initiation [50]. Moreover, chromatin looping may serve as a mechanism to ensure specificity of gene regulation. It was frequently observed that there are dramatic differences in transcription and/or epigenetic states between genes located at different positions relative to a chromatin loop (upstream, downstream, or within a loop) [43, 45].

The most studied and well-established functional properties of chromatin looping relate to bringing genes/genomic elements into spatial proximity. For example, spatial proximity mediated by looping influences partner selection in DNA recombination [37, 51, 52] and, unintendedly, in pathological chromosomal translocation events [20, 53, 54]. The important roles of looping in establishing and maintaining the formation of gene clusters and nuclear compartments during long-distance gene regulation and co-regulation are discussed in detail below.

Clustering and compartmentalization: Is the formation of various kinds of spatially defined nuclear or chromosomal neighborhoods function-oriented or do these neighborhoods function as physical constraints for genomic activities?

Clustering and compartmentalization are two related but not identical phenomena. Compartmentalization, a featured principle for genome 3D organization, refers to the partitioning of the nuclear space and individual chromosomes into distinct geographic or topological areas, domains or compartments (such areas, domains, or compartments may simply be called nuclear neighborhoods). Clustering, defined as the process that brings together different parts of the genome into spatial proximity, also serves as a mechanism for

eukaryotic cells to adapt genome structure to its function. Clustering results in co-localization of linearly distant parts of the genome to allow their participation in a common genomic event so that they may be co-regulated or share similar nuclear resources. While clustering results in co-localization of clustered partners in a nuclear neighborhood (compartment or domain), it is unclear whether clustering promotes the de novo formation of a nuclear neighborhood or simply brings the clustered partners into an existing neighborhood.

The nucleoplasm is functionally heterogeneous

It is widely accepted that the nucleoplasm is functionally heterogeneous, and this is highlighted by the fact that the nuclear positioning of some genes is important to their expression regulation (reviewed in [8, 14]). For example, in budding yeast, the nuclear periphery generally functions as a repressive environment [8, 14], with the exception of nuclear pore complexes that associate with gene loci containing active euchromatin despite their peripheral location [55].

Clustering may occur at preferred nuclear positions, a phenomenon that may also reflect nuclear heterogeneity. For example, recent Hi-C experiments have revealed that a subset of chromosomes, characterized by small size or high gene density, tend to cluster in the interior of human nuclei [17, 18, 56]. While the functional implications of this phenomenon are unclear, it likely arises from biophysical properties (both specific and entropic) of the chromosomes [57]. Another example is the clustering of repetitive DNA sequences, a widespread phenomenon that is conserved from yeast to human and is believed to both maintain these repetitive sequences and sequester silencing factors from the rest of the genome. In both budding and fission yeast, centromeres are clustered and anchored to the spindle pole body (SPB), serving as a major driving force for chromosome organization, whereas telomeres cluster at the nuclear periphery [28, 58–60]; in both human and murine lymphocytes, most centromeres are clustered at the nuclear periphery, whereas the distribution of telomere clusters differs between mouse and human cells: in mouse, most clusters were detected at the nuclear periphery, while, in human, most clusters were located in the nuclear interior [61].

Individual chromosomes are spatially partitioned into functionally distinct domains; is the formation of these domains determined by function?

It has been demonstrated that each individual chromosome is partitioned into functionally distinct domains that correlate with different epigenetic states and protein components [62, 63], a phenomenon that might be related to the non-random distribution of genes along the individual chromatin fibers [1]. However, it is unclear how these linear domains are organized in the nuclear space. Observations from several recent studies have shed light on this question. First, in fly, mouse [embryonic stem cells (mESCs), lineage-committed neural precursor cells, terminally differentiated astrocytes, and MEF cells], and cultured human lung fibroblasts, genomes are partitioned into large, discrete nuclear lamina associated domains (LADs) that are characterized by low levels of gene expression and enrichment of repressive epigenetic marks [21, 22, 64], consistent with the observations from a study using high-resolution microscopy [65]. During lineage commitment and terminal differentiation of mESCs, many genes specifying cellular identity disassociate from the nuclear lamina and migrate to the interior of the nucleus. It is surprising that the unit for this movement is individual genes or linear gene clusters instead of individual LADs [22]. This observation indicates that LADs might be structurally rather than functionally oriented, since their formation is largely independent of cell type and developmental state [22]. Second, in mouse and human cells, the genome can be partitioned into two compartments: active and inactive (compartments A and B, respectively), with compartment A enriching for the presence and

expression of genes, DNase hypersensitivity, and activating histone modifications and compartment B displaying the opposite behavior [16–18, 20]. Obviously, the formation of compartments A and B in the genome is driven by function and, as can be predicted, the contents of these two compartments should be cell-type specific. Third, the genomes of metazoa such as flies, mice, and humans, are spatially partitioned into topological domains [also referred to as physical domains or topologically associating domains (TADs)] [16, 19, 66]. In the fly genome, it was suggested that physical domains are indeed epigenomic domains [19]. In mouse and human cells, the topological domains or TADs are stable across different cell types and conserved across species, indicating that topological domains, as a pervasive structural feature, are an inherent property of the mammalian genomes [16, 66]. It is unclear whether the notable difference between the physical domains identified in the fly embryo genome and the topological domains of mouse and human genomes reflect differences between species (between fly and mammalian genomes).

Nuclear activities are spatially compartmentalized; are nuclear compartments determined according to function?

It has been demonstrated and widely accepted that most nuclear events do not occur ubiquitously throughout the nucleus but are limited to discrete nuclear suborganelles or compartments [8]. In addition to the distinct CTs, the nucleoplasm also consists of many functionally specialized compartments, such as various nuclear bodies [15], replication [67, 68] and transcription factories [69, 70], and DNA repair foci [71]. These nuclear suborganelles compartmentalize the nuclear space, create distinct microenvironments, and host specific genomic activities. For example, in budding yeast, we and others have observed that tRNA genes (transcribed by RNAPIII), which are dispersed throughout the genome, form two clusters: one inside the nucleolus [28, 72] and the other in association with the centromeres [28].

Although the mechanisms underlying the biogenesis and maintenance of the various nuclear bodies remain largely unknown, notably, the formation of some of them is independent of genomic activity [15]. In contrast to nuclear bodies, the formation of transcription factories is likely to be strictly dependent on transcriptional activity [69], and this likely also holds true for DNA replication factories (i.e. dependent on replication activity). However, despite the relative stability of nuclear bodies, their distribution in the nucleus seems random and the size and number of a given type of nuclear body are variable [15]. Similarly, the distribution of replication and transcription factories also appears to be randomly dispersed throughout the nucleus. It is unclear how the distribution of each of these nuclear suborganelles is related to the nuclear positioning of the respective chromatin elements that nucleate their formation.

Transcriptional regulation and co-regulation across distance: How are the partners in a spatial gene cluster chosen? When and how do they come into spatial proximity during transcription?

Although the precise mechanisms for gene regulation/coregulation by distant-acting regulatory elements remain largely elusive [73–76], recent observations underscore several aspects of the interplay between genome structure and long-range transcription regulation/co-regulation. First, chromosome higher-order structure and transcription might be relatively independent of one another. Several observations support this notion: (1) blocking elongation of RNAPII did not affect the contacts of active genes [77]; (2) activation of estrogen receptor alpha (ER α) target genes did not induce or require their nuclear co-localization [78]; (3) glucocorticoid receptor (GR) binding led to diverse transcriptional responses in the same spatial clusters of distal GR-responsive elements without dramatic nuclear reorganization [79]; (4) deletion of the gene encoding Xist resulted in partial

refolding of the inactive X chromosome into a conformation resembling the active X without affecting gene silencing [38]; and (5) the recently identified topological domains or TADs in human and mouse genomes are stable across different cell types [16, 66]. Second, spatial proximity matters. Distant regulatory elements and their target promoters or multiple co-regulated genes can be brought into proximity through chromatin looping, which is an important strategy for gene regulation in higher eukaryotes [73]. It was recently observed that more than 70% of RNA polymerase II (RNPII) associated promoters were engaged in long-range promoter-enhancer interactions in human cells [47]. Genes regulated by a common transcription factor might be co-localized and co-regulated [41, 43, 80]. However, it is noteworthy that close proximity is likely a necessary but insufficient prerequisite for productive promoter-enhancer interactions. Other factors, such as promoter-enhancer compatibility, chromatin accessibility, and availability of the right combination of transcription factors and co-factors all seem necessary [38, 81]. This may serve as an important mechanism to ensure the specificity of promoter-enhancer interactions, considering that there are many randomly formed structure-only loops. For instance, the human β -globin locus control region (LCR), when site-specifically integrated into a gene-dense region on mouse chromosome 8, contacts many different genes including those that share a set of common regulatory proteins, but does not significantly affect the expression of most of them [81]. Third, chromosomal higher-order structure serves as an important constraint for the action of both *cis*-elements and *trans*-factors [16, 63, 66, 82–84]. For example, interactions among PcG target genes are thought to be constrained by overall chromosome architecture [84]. Moreover, recent observations revealed a connection between the physical domains or TADs in the human and mouse genomes and long-range transcriptional control [16, 66]. In the mouse genome, coordinately regulated loci were found to be more frequently located within the same TAD, indicating TADs might provide a structural framework for the formation of *cis*-regulatory neighborhoods in 3D space [66].

Collectively, the observations presented above and the others not listed here due to space limitations may lead to two alternative-models (Fig. 2) to describe the two closely-related fundamental questions regarding long-range transcriptional regulation/co-regulation: how are the spatially co-localized partners chosen and when are they brought into close proximity? In the “function-deterministic” model (Fig. 2A), *cis*-regulatory elements (e.g. enhancer in Fig. 2) and their targets are brought into spatial proximity to form a cluster (e.g. in a transcription factory) by tissue-specific transcription factors through looping during transcription initiation. The activation of productive transcription depends on both promoter-enhancer compatibility and the availability of the right combination of *trans*-factors. In contrast, in the “pre-existing architecture” model (Fig. 2B), the *cis*-regulatory elements and their targets are colocalized in a preformed chromosome spatial neighborhood (e.g. TAD) independent of transcription activity. Each model is consistent with some experimental observations, but neither can explain all of them. Although it is possible that there are indeed multiple distinct mechanisms governing long-range transcriptional gene regulation/co-regulation in a genome, new insights are necessary before we can draw clear conclusions.

Eukaryotic genomes are dynamic steady-state structures

Despite randomness in the spatial organization of eukaryotic genomes and the constant influence of various nuclear activities on genome architecture, growing evidence supports the idea that eukaryotic genomes are dynamic steady-state structures.

Genomes in motion: Dynamics reflects both randomness and nuclear activities

Genomes are dynamic entities, and this property poses a major challenge to deciphering the rules and principles of their 3D organization [10, 85]. The dynamic nature of genome architecture can be classified into two categories based on biological origin: intrinsic or

genomic activity-related. The former is a consequence of biophysical properties of the individual components of a genome. The nucleus is crowded with macromolecules, discrete nuclear bodies, DNA replication and transcription factories, and CTs, and each follows the rule of diffusive motion, albeit in a constrained manner [10]. Adding to the complexity, not only do the individual CTs display constrained Brownian motion as an entity, but also individual gene loci, chromatin segments or domains show constrained random walk phenomena [10]. Although the molecular causes of such movement remain largely unknown, recent studies suggest that chromatin remodeling complexes can increase large-scale chromatin movement by enhancing the flexibility of the chromatin fiber [86]. In addition, it has been suggested that interactions between chromosomes as well as interactions between chromosomes and nuclear landmarks (e.g. lamina) are stochastic [26, 27].

A second type of dynamic behavior is functionally related. Each living cell is in a meta-stable state that responds almost instantaneously to diverse internal and external signals, including those associated with the cell cycle, development, or cues from the environment. Hence, genome architecture must be adaptable and responsive – in other words, functionally dynamic. This dynamic response also shows many different facets, including: (1) the DNA/RNA-nucleated stochastic formation of transcription and replication factories, nuclear bodies, and other sub-nuclear foci; (2) the nuclear relocation of individual genes, genomic loci, domains or even an entire chromosome during development [21, 22, 64, 87]; and (3) the local and global conformational changes of chromosomes caused by DNA-inherent genomic activities such as transcription and DNA replication. Although it remains largely unknown yet how genomic activities such as transcription lead to direct chromosomal movement (e.g. relocation of genomic loci), current evidence suggests that nuclear actin and myosin play important roles for the first two types of function-induced dynamics [88].

Are genomes stable structures?

As described above, the three fundamental mechanisms (looping, clustering, and compartmentalization) that define the 3D organization of the eukaryotic genome are characterized by both their functional relevance and stochastic nature (Table 2). Meanwhile, the dynamic aspects of genome architecture can be partially attributed to functional activities (Table 2), indicating that both order and plasticity are intrinsic to genome architecture. Hence, the outstanding question is whether the genome of a cell adopts a preferred 3D architecture that can be described by present technologies. Based on observations from recent systematic studies, the answer is likely yes. First, observations from studies using high-throughput 3C-based methods are highly reproducible not only between biological replicates but also between experiments conducted by different research groups, indicating that – at least at a population level – the architecture of a genome is relatively stable and describable. Recently, Kalhor et al. [17] observed that on average only 21% of chromatin interactions are shared between any two structures (based on a population consisting of 10,000 structures). This observation might be attributed to several possibilities, including the architectural heterogeneity between single cells or between cells at different stages of the cell cycle, insufficient complexity of chromatin interactions as a result of insufficient sequence depth, and finally the low resolution of the structure models. In another recent study, Zhang et al. [20] compared the architectures of mouse genomes in G1-arrested wild-type and Ataxia-Telangiectasia mutated (ATM)-deficient pro-B cells, and the authors observed structural changes of the genome at only 8% of chromatin domains. Notably, the resolution of the 3D models of the mouse genomes described in this study is about five to ten times higher than those of previous studies [20]. Second, 3D models of genome architecture based on chromatin interaction profiles recapitulate known features of genome organization revealed by other technologies [16–20, 28, 29]. Third, 3D models

demonstrate that common principles and rules governing the 3D organization of eukaryotic genomes do exist [16–20, 28, 29]. Fourth, both of the distinct types of domain structures of mouse and human genomes, topological domains (TADs) and LADs, are stable across different cell types and/or during differentiation [16, 22, 66]. Collectively, these observations and many other lines of evidence indicate that eukaryotic genomes are dynamic steady-state structures. The next question of interest is how eukaryotic cells manage to keep the genome structure steady state despite its highly dynamic nature. In this regard, it is reasonable to postulate the existence of genome organizers, which are able to hold the global or large-scale local chromosomal shapes and therefore keep the 3D architecture of a genome in a relatively stable frame, acting like knots in a fisherman's net. Recent evidence indicates that several proteins, including CTCF, cohesin, condensin, SATB1, and nuclear lamins, are good candidates for genome organizers [89–93]. With respect to the role of *cis*-elements in the 3D organization of genomes, classical boundary elements, housekeeping genes, transfer RNAs, and short interspersed element (SINE) retrotransposons have been suggested to play an important role in establishing the topological domains of human and mouse genomes [16, 66].

Does the shape of the eukaryotic genome harbor epigenetic information?

It is known that various types of epigenetic information, such as DNA methylation, histone modification, histone variants, and non-coding RNAs (ncRNAs), are carried in the chromosome structure, but most studies have been focused at the nucleosome level, thus revealing information in only a single dimension [94, 95]. While accumulating evidence argues for a relatively stable topological organization of genomes, it is attractive to consider the possibility that genome architecture carries epigenetic information. Epigenetic information features two characteristics: inheritability and tissue/cell-type-specificity. Hence, it is important to examine whether genome architecture also possesses these same two properties. At the level of CT arrangement in the nucleus, the relative radial position of CTs is stably maintained from mid G1 to late G2/early prophase during the cell cycle in both Hela cells and normal rat kidney (NRK) cells [96, 97]. Moreover, in NRK cells, it was demonstrated that global chromosomal arrangements are transmitted through mitosis [96], although the observation in Hela cells is completely opposite [97]. Intriguingly, a connection has been established between global CT arrangement and coordinate gene regulation during *in vitro* differentiation of murine hematopoietic progenitors [11, 87]. At the level of chromosomal domains, it was observed that the physical domains of fly genomes correlate well with various epigenomic patterns, indicating that the physical domains are actually epigenetic domains [19]. In mouse and human, although the topological domains/TADs are independent of epigenetic domains, cell type- or differentiation, state-specific chromatin interactions do occur within these domains [16, 66]. Similar observations of the behavior of LADs during *in vitro* differentiation of mESCs were also reported [22]. Therefore, within the context of these recent results, it remains largely elusive whether genome architecture carries epigenetic information. To address this question, a detailed comparison of genome architectures at different time points during the course of cell cycle or development is necessary, and will reveal whether genomes are tissue-/cell type-specific.

Conclusion and perspective

The recent systematic characterization of the spatial organization of genomes has revealed some general principles and rules, which provides a first glimpse into the interconnection between the structure and function of eukaryotic genomes. For example, recent observations indicate that the partitioning of metazoan genomes into topological/physical domains might provide a structural framework for gene regulatory networks [16, 19, 66], which extends the notion that coordinate gene regulation is linked to genomic organization [11, 87]. It will be of great interest to ascertain whether or not the spatial arrangement of genes, including their

distribution in topological domains and interactions between individual topological domains, determines cell-specific patterns of expression. This will require precise mapping of the temporal and spatial (four-dimensional) organization of genomes at unprecedented resolution, as well as systematic approaches for integrating structural information with a variety of functional parameters [98]. The results will shed new light on the question of whether genome architecture is cell type-specific and can serve as a hallmark of cell identity.

Meanwhile, details regarding chromosome folding in vivo and the influence of DNA-based genomic activities remain to be established. Many factors, including core histones, linker histones, histone chaperones, histone variants, chromatin remodeling factors, DNA methylation and histone modifications, regulate the formation of nucleosome structure (i.e. the “beads-on-a-string” structure) (Fig. 3) [2, 94]. However, how and whether further compaction into the 30-nm chromatin fiber or the more condensed “chromonema” fiber occurs in vivo remains debatable [99]. Although recent observations [100] have indeed shown that there is an intimate link between in vivo chromosome folding and DNA-based genomic activities (e.g. transcription and replication), it remains largely unclear how the local chromatin dynamically transits between the different levels of its higher-order structure during the various nuclear processes (Fig. 3). Answers to these questions will greatly enhance our understanding of the interconnection between the structure and function of eukaryotic genomes.

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Abbreviations

CT	chromosome territory
LAD	lamina associated domain
mESC	mouse embryonic stem cell
TAD	topologically associating domain

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Figure 1.

The direct effects of looping. **A:** Looping brings the two chromosomal elements H and P into close proximity and loops out the region between H and P. **B:** Looping results in change in direction. Before looping, the black molecule in the upper strand and the red molecule in the lower strand of the chromatin move in opposite directions. After looping of the lower strand they are now moving in the same direction. Note, the biological relevance of this type of effect of looping is observed during the process of DNA replication. During replication, since DNA synthesis is always in the 5' to 3' direction, the anti-parallel nature of duplex DNA allows the leading-strand polymerase to advance in a continuous fashion, but forces the lagging-strand polymerase to synthesize in the opposite direction. By forming a DNA loop in the lagging-strand of the replication fork, the lagging-strand DNA polymerase is re-oriented so that it advances in parallel with the leading-strand polymerase. See [48, 49] for details.

Figure 2.

Two models for the structure–function relationships during gene regulation by distant-acting enhancers. **A:** In the “function-deterministic” model, tissue-specific transcription factors (red circle) cooperate with the other co-factors (colored ovals) to bring the green enhancer, the two genes (black rectangle), and their respective promoters (red and blue curves) into close proximity (co-localized) through looping. However, in this cluster, only the gene with the red promoter is activated by the green enhancer because the red transcription factor is specific to the red promoter. At another developmental stage, when the blue tissue-specific transcription factor (specific to the blue promoter, blue triangle) becomes available, it mediates the formation of a similar cluster (due to the shared co-factors), but only the gene with the blue promoter is activated by the green enhancer. **B:** In the “pre-existing architecture” model, two genes and the green enhancer are co-localized in a pre-formed nuclear compartment (e.g. TAD). At a different developmental stage, the availability of the red or blue transcription factor activates the red or blue promoter, respectively.

Figure 3. Mechanisms and transacting-factors contributing to the multilevel folding and un-folding of eukaryotic genomes. Packaging of DNA into nucleosome (“beads on a string”) and higher order structures requires the coordinated assembly and post-translational modification of histones and nuclear factors. The precise contributions of individual mechanisms to the 3D structure of chromosomes are largely unknown.

Table 1

3D models of whole genomes

Genomes	Cell type/strain	Functional implication	Reference
Budding yeast	BY4741	clustering of tRNA genes; clustering of early firing origins	[28]
Fission yeast	–	clustering of functionally related genes	[29]
<i>Drosophila</i>	embryos	genomes partitioned into physical domains; physical domain correlating with epigenomic domain; domains being organized hierarchically	[19]
Mouse	Bcl-2 transgenic Abelson Murine Leukemia Virus (A-MuLV) transformed pro-B cell lines; mESCs; mouse cortex	genomes partitioned into active and inactive compartments; influencing the choice of translocation partners; genomes partitioned into topological domains; topological domains are stable across different cell types and conserved between mouse and human	[16, 20]
Human	lymphoblastoid GM06990 and GM12878; erythroleukemia K562; hESCs; IMR90 fibroblast cells	genomes partitioned into active and inactive compartments; the distribution of chromosomal alterations in cancer spatially related to three-dimensional genomic architecture; genomes partitioned into topological domains; topological domains are stable across different cell types and conserved between mouse and human	[16, 17, 18]

Table 2

Double faces of the common themes in 3D organization of the eukaryotic genomes

Theme	Functional implication		
	Influencing	Fitting	Randomness
Looping	+	+	+
Clustering	+	+	+
Compartmentalization	+	+	+/-
Dynamics	+	+	+