Chapter 2
Eukaryotic Genome in Three Dimensions

Sergey V. Razin, Alexey A. Gavrilov, and Sergey V. Ulianov

Abstract Modern ideas regarding the three-dimensional organization of the genome and its role in controlling gene expression are largely based on the results of research performed using the proximity ligation protocol. It has been demonstrated that genome folding is much less regular than was previously assumed. On the other hand, the genome was found partitioned into semi-independent structural-functional units commonly referred to as topologically associating domains (TADs). TAD borders restrict the areas of enhancer action via interfering with establishment of long-distance enhancer-promoter contacts. Within TADs, spatial juxtaposing of promoters to various enhancers or silencers results in the assembly of activating or repressing chromatin hubs that constitute an important part of epigenetic mechanisms regulating gene expression in higher eukaryotes. Within the cell nucleus, the spatial organization of the genome is tightly connected with functional compartmentalization of the nucleus. Recent evidence suggests that liquid phase separation plays an important role in establishing both the 3D genome organization and nuclear compartmentalization. In this chapter, we review the present state and outline the most important trends for future research in the area of 3D genomics.

Introduction

Studies of the 3D genome organization have become a trend in modern genomics. One may say that modern genomics has acquired a third dimension. As is often the case in science, a new stage in the study of genome organization and functioning was predetermined by the development of appropriate research tools. One biochemical protocol that had a major impact on the development of 3D genomics is the chromosome conformation capture protocol (Dekker et al. 2002). The main steps of this protocol are presented in Fig. 2.1. The key step of this procedure is

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I. Iourov et al. (eds.), *Human Interphase Chromosomes*,
https://doi.org/10.1007/978-3-030-62532-0_2

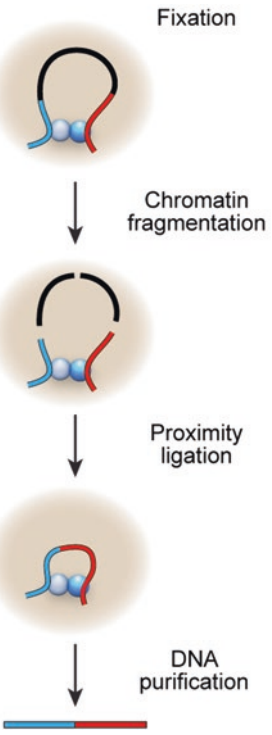

Fig. 2.1 Main steps of the chromosome conformation capture protocol. Restriction enzymes are used to cut chromatin in intact nuclei isolated from formaldehyde-fixed cells. DNA fragments located in close proximity to each other are ligated with the T4 DNA ligase. qPCR or next-generation sequencing are used for the analysis of DNA chimeras obtained

introduction of breaks into DNA within a fixed nucleus, followed by cross-ligation of closely located ends of broken DNA. Joining of DNA fragments located far from each other on the DNA chain but close in physical space creates chimeric DNA sequences containing information about the spatial proximity of the corresponding segments of genomic DNA. Analysis of the pools of chimeric fragments allows reconstructing the spatial organization of the genome based on the sets of captured pairwise interactions. This procedure was first successfully used to demonstrate that all remote enhancers of mouse beta-globin genes along with the promoters of genes, which are actually expressed, are organized into a common active chromatin hub (Tolhuis et al. 2002; de Laat and Grosveld 2003). This work highlighted the importance of 3D genome organization for the regulation of transcription. It has long been assumed that, to activate a gene, an enhancer should be in direct contact with this gene ((Bondarenko et al. 2003; West and Fraser 2005; Vernimmen and Bickmore 2015) and references herein). Taking into account that most enhancers are located far from the target gene, the ideal solution is to loop out the intervening segment of DNA, and 3C analysis has demonstrated that such situations are indeed quite common (Tolhuis et al. 2002; de Laat and Grosveld 2003; Gavrilov and Razin 2008; Philonenko et al. 2009; Vernimmen et al. 2007; Vernimmen et al. 2009). The

number of enhancers in mammalian and Drosophila cells exceeds at least ten times the number of genes (Arnold et al. 2013; Consortium et al. 2012). The possibility of gene activation by different combinations of enhancers likely increases the regulatory capacity of the eukaryotic cell transcription control system. Disclosure of the functionally dependent mouse beta-globin gene domain 3D organization (Tolhuis et al. 2002; de Laat and Grosveld 2003) demonstrated for the first time how one gene or group of genes can be simultaneously activated by one or several enhancers.

The original 3C procedure allowed studying interactions between various regions within individual genomic loci. Eventually, various derivative procedures were developed collectively known as C-methods (reviewed in de Wit and de Laat (2012)). Most of these procedures, such as 4C (van de Werken et al. 2012), Hi-C (Lieberman-Aiden et al. 2009), and ChIA-PET (Fullwood et al. 2009), allowed performing genome-wide analysis. Application of these experimental protocols has provided deep insights into the role of 3D genome organization in transcription control (Denker and de Laat 2016; Dekker and Mirny 2016; Valton and Dekker 2016; Krijger and de Laat 2016). Of special importance, the genome was found to be partitioned into semi-independent self-interacting domains termed topologically associating domains or TADs (Nora et al. 2012; Dixon et al. 2012; Sexton et al. 2012). TADs appear to restrict the areas of enhancer action and thus can be considered as structural-functional units of the eukaryotic genome (Symmons et al. 2014, 2016). Disruption of TAD borders results in development of various genetic diseases (Lupianez et al. 2015, 2016; Krumm and Duan 2018; Franke et al. 2016). In normal situations, the patterns of enhancer-promoter spatial interactions change in the course of cell differentiation accordingly to activation and/or repression of particular genes. However, most of these changes occur within TADs while the TAD borders remain relatively stable (Dixon et al. 2016; Fraser et al. 2015). Nevertheless, a certain fraction of TAD boundaries is changed in the course of cell differentiation (Bonev and Cavalli 2016). To obtain further insights into mechanisms of eukaryotic genome functioning, it is highly important to disclose the nature of both TADs and TAD borders. This task is complicated by the fact that in virtually all eukaryotic cells studied, the contact chromatin domains are hierarchical (i.e., within larger domains, it is possible to annotate several levels of smaller and more dense nested domains) (Phillips-Cremins et al. 2013; Luzhin et al. 2019; Weinreb and Raphael 2016). It is not always obvious the domain of which level should be considered as TADs. Some authors claim that TADs can be discriminated only based on their functionality (i.e., as functional units of the genome rather than the units of a particular level of hierarchical genome folding) (Zhan et al. 2017). In this review, we shall discuss mechanisms of TAD formation and the impact of TADs on genome functioning.

Hierarchical Model of DNA Packaging in Chromatin

In most textbooks, it is possible to read that, in eukaryotic cells, genomic DNA is sequentially folded into 10 nm chromatin fiber (nucleosomal chain), into 30 nm chromatin fiber (which is frequently represented as a solenoid or zigzag), and then into loops of 30 nm fiber or several levels of "supersolenoid" structures (Fig. 2.2). Remarkably, this model of chromatin folding into regular structures was proposed approximately 30 years ago (Getzenberg et al. 1991) and is poorly supported by recent data. On the contrary, it is becoming increasingly evident that the only regular level of genomic DNA folding is wrapping of DNA around the octamers of nucleosome histones, resulting in formation of 10 nm fibers (Fussner et al. 2012). The latter then aggregate to form more or less compact chromatin masses (Maeshima et al. 2014a, b, 2016). Aggregation of chromatin fibers is promoted under conditions of macromolecular crowding (Hancock 2008) typical for nucleoplasm. Although at a medium scale thus formed chromatin masses appear irregular, at larger scales, they are subdivided into self-interacting domains that are commonly interpreted as chromatin globules. Such chromatin globules were observed in a high-resolution microscopic study of cell nuclei hybridized to chromosome- or locus-specific probes (Markaki et al. 2012; Smeets et al. 2014). Furthermore, the same structures

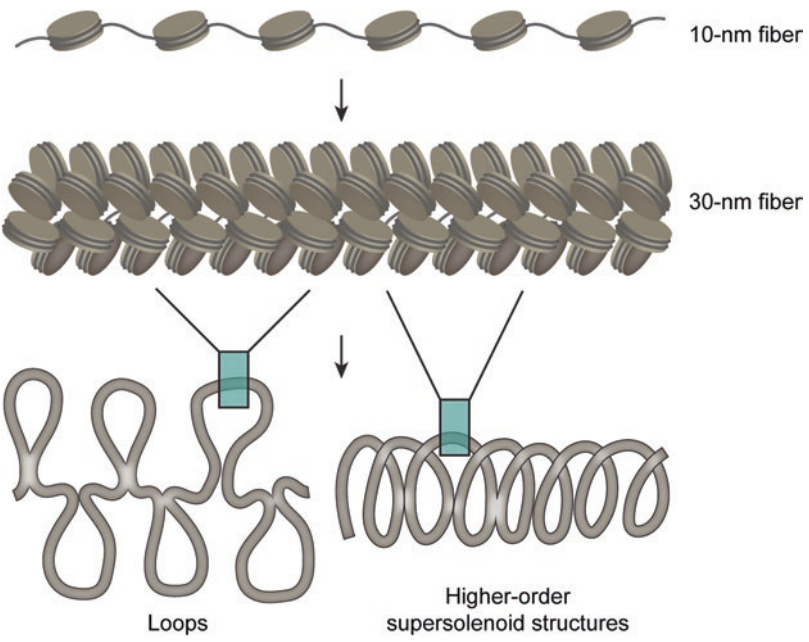

Fig. 2.2 A classical view of hierarchical folding of DNA in the nucleus. 10 nm nucleosome fiber folds into 30 nm fiber of variable architecture, which then forms hierarchical loops and supersolenoid structures

(1 Mb chromatin clusters) appear to correspond to early replicating chromatin domains (Markaki et al. 2010). In a recent study by the Cavalli laboratory, it was directly shown that TADs correspond to chromatin globules that can be visualized using FISH with TAD- and locus-specific probes (Szabo et al. 2018). Within the entire chromatin domain, TADs containing mostly active and mostly repressed chromatin are spatially segregated into the so-called A and B chromatin compartments, which likely correspond to euchromatin and heterochromatin (Lieberman-Aiden et al. 2009; Gibcus and Dekker 2013; Eagen 2018).

Most of the current knowledge about higher levels of DNA packaging in chromatin is based on the results of Hi-C analysis. The contact chromatin domains were observed in different taxa including mammals (Dixon et al. 2012; Nora et al. 2012), insects (Sexton et al. 2012), and birds (Ulianov et al. 2017). Of note, in Drosophila, TADs have a size in the range of 100 Kb (Sexton et al. 2012; Hou et al. 2012), while mammalian TADs are ten times larger (Dixon et al. 2012, 2016). Some contact domains can also be revealed in the genomes of plants and lower eukaryotes (Wang et al. 2015; Hsieh et al. 2015; Eser et al. 2017; Nikolaou 2017). However, they are substantially different from the TADs of mammals and Drosophila both in size and in the levels of insulation and genome coverage.

Interpretation of Hi-C maps strongly depends on resolution of the analysis. At 1 Mb resolution, only segregation of active and inactive chromatin can be registered (Lieberman-Aiden et al. 2009). 20–100 Kb resolution revealed TADs (Dixon et al. 2012, 2016; Gibcus and Dekker 2013). Finally, 1 Kb resolution maps demonstrated that TADs comprise two types of self-interacting domains, namely, looped domains and ordinary domains (Rao et al. 2014). The distinctive feature of looped domains in Hi-C maps is a spot at the top of a triangle reflecting a spatial proximity of loop bases (Fig. 2.3). In mammalian cells, chromatin loops originate due to enhancer-promoter interaction (Jin et al. 2013; Sahlen et al. 2015; Ghavi-Helm et al. 2014) or

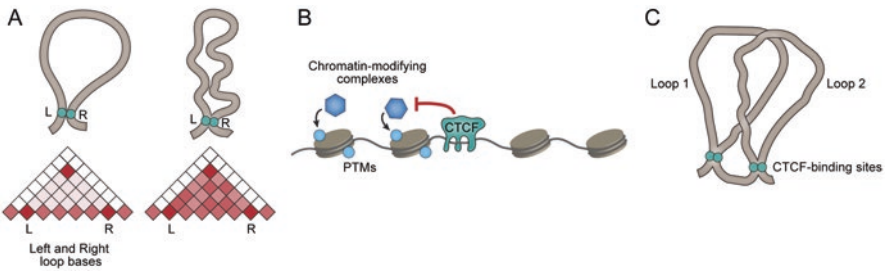

Fig. 2.3 Potential role of CTCF in defining chromatin spatial organization and epigenetic state. **(a)** Chromatin loop is manifested as a filled triangle in the Hi-C heat map only if numerous interactions between loop internal regions occur. **(b)** In a "traffic jam" model, DNA-bound CTCF restricts the spreading of histone posttranslation modifications along the chromatin fiber, preventing binding of chromatin-modifying complexes to nucleosomes located downstream of the CTCF-binding site. **(c)** Point-to-point interactions between CTCF-binding sites are unable to insulate extended loops from each other in the 3D nuclear space

because of interactions between CTCF-binding sites (Sanborn et al. 2015). The nature of ordinary chromatin domains is less clear. It has been proposed that these domains originate due to clustering and spatial segregation of active and inactive genomic regions. Accordingly, it was proposed to call them "compartmental domains" (Rowley and Corces 2018). The mechanisms underlying the spatial segregation of chromatin compartments (or compartmental domains) are still unclear. A current model postulates that proteins enriched in different chromatin types trigger phase separation, resulting in their spatial segregation (Nuebler et al. 2018; Rada-Iglesias et al. 2018).

Functional Domains of the Eukaryotic Genome

The eukaryotic genome has long been proposed to be a mosaic of semi-independent structural-functional domains (Bodnar 1988; Goldman 1988). The original model was inspired by the results of analysis of DNaseI sensitivity of individual genes and genomic segments (Weintraub and Groudine 1976; Weintraub et al. 1981; Lawson et al. 1982; Jantzen et al. 1986). It was proposed that the entire genome is built from similarly organized structural-functional units (domains) that may be either active or repressed. The transcriptional status of the domain was thought to be controlled at the level of chromatin packaging. The model stimulated research aimed to identify regulatory elements controlling the chromatin status of genomic domains. These studies resulted in identification of domain bordering elements (insulators) (Kellum and Schedl 1991, 1992; Udvardy et al. 1986), nuclear matrix attachment regions (MARs) (Cockerill and Garrard 1986), and locus control regions (LCRs) (Forrester et al. 1987, 1990; Grosveld et al. 1987; Li et al. 1990). Although in its initial form the domain model of eukaryotic genome organization cannot account for a number of recent observations, it can be upgraded taking into account the 3D genome organization (Razin and Vassetzky 2017). Considering the necessity of juxtaposition of enhancers and promoters, one may conclude that any self-interacting chromatin domain would impose certain restrictions on enhancer action. Indeed, it has been demonstrated that, in most cases, the areas of enhancers' action are restricted to the so-called insulating neighborhoods (Sun et al. 2019), regulatory archipelagos (Montavon et al. 2011), regulatory landscapes (Spitz et al. 2003; Zuniga et al. 2004), or regulatory domains (Symmons et al. 2014). These functional genomic blocks are large (100 Kb to 1 Mb) segments of the genome within which non-related genes demonstrate similar tissue specificity of expression. Being integrated in such a domain, a reporter gene under control of a minimal promoter demonstrates a tissue-specific expression profile typical for the domain as a whole (Ruf et al. 2011; Symmons et al. 2014). Although there is still some discrepancy in the results of different authors, they all agree that insulated areas colocalize with self-interacting chromatin domains identified by Hi-C analysis, either with TADs (Montavon et al. 2011; Symmons et al. 2014) or looped domains (sub-TADs) (Sun et al. 2019).

Interestingly, TADs harboring superenhancers are preferentially insulated by boundaries possessing a particularly high insulation score (Gong et al. 2018).

Partitioning of the genome into semi-independent structural-functional domains appears important for two reasons. First, it minimizes the possibility of an off-target activity of any given enhancer. To this end, it is of note that genomic rearrangements affecting TAD boundaries frequently result in compromising gene regulation networks and development of diseases (Lupianez et al. 2015; Franke et al. 2016; Valton and Dekker 2016; Ibn-Salem et al. 2014; Vicente-Garcia et al. 2017). Second, partitioning of the genome into TADs restricts the area the enhancer should explode to find a target promoter. Correspondingly, the time necessary to establish enhancer-promoter communication is reduced (Symmons et al. 2016). Lack of rigidity in the TAD structure is of importance in this context. Alternative configurations of the chromatin fiber continuously interchange within a TAD (Tiana et al. 2016). This interchange is likely to provide additional possibilities for cell adaptation to a changing environment (Razin et al. 2013). The functional relevance of genome partitioning into TADs is likely to explain the apparent conservation of this organization in the genomes of related species (Dixon et al. 2012) as well as the fact that TADs are stable against rearrangements during evolution (Krefting et al. 2018; Lazar et al. 2018). Interestingly, paralog gene pairs are enriched for colocalization in the same TAD and frequently share common enhancer elements (Ibn-Salem et al. 2017).

Besides constituting the insulation neighborhoods for transcription regulation, the TADs also contribute to the control of replication because they correspond to units of replication timing (replication domains) (Pope et al. 2014). Interestingly, after being disrupted in mitosis (Naumova et al. 2013), TADs are re-established in G1 phase of the cell cycle at about the same time with the establishment of the replication-timing program (Dileep et al. 2015a, b). It may be that exactly at the level of chromatin packaging, the link between active transcription and early replication is established.

TAD Assembly and Insulation

Taking into consideration the fact that TADs restrict the areas of enhancer action, it is particularly important to understand how they are assembled and why they are insulated. Comparison of Hi-C maps with genome-wide distribution of various epigenetic marks demonstrated that, in mammals, TAD boundaries are enriched in CTCF-binding sites and active genes (Dixon et al. 2012). Also, cohesin was found enriched at TAD boundaries (Hansen et al. 2017). Deletion of CTCF-binding sites at TAD boundaries resulted in a full or partial loss of TAD insulation (Narendra et al. 2015, 2016; Lupianez et al. 2015; Sanborn et al. 2015). The same effect was observed upon targeted degradation of CTCF in living cells (Nora et al. 2017). CTCF has long been implicated in mediation of enhancer-blocking activity of

insulators (Chung et al. 1997). In addition, it mediates formation of DNA/chromatin loops (Vietri Rudan and Hadjur 2015; Holwerda and de Laat 2012). It should be mentioned, however, that by itself, formation of a chromatin loop is not sufficient for TAD assembly. Within a loop, only the bases are permanently located in a spatial proximity. On a Hi-C heat map, a DNA loop can be recognized as a high interaction signal between bases that looks like a spot at the top of a triangle. However, to "fill" the triangle, it is necessary to ensure mutual interaction of internal parts of the loop (Fig. 2.3a). It is also not clear how deposition of CTCF at TAD boundaries can prevent spatial interactions between internal regions of different TADs. Although CTCF is a large protein (~130 kDa), the octamer of histones constituting the nucleosomal core has approximately the same summary weight, and the 1 Mb mammalian TAD is composed of ~5000 nucleosomes. It is easy to speculate about a mechanism by which deposition of CTCF can interfere with spreading if signals travel along a linear chromatin fiber. Here, a traffic jam model fits perfectly (Fig. 2.3b). However, it is difficult to see how spatial interactions between internal regions of large TADs can be prevented by CTCF (Fig. 2.3c). In fact, it is easier to consider a possibility that TAD is held together by some internal links (see below). However, preferential deposition of CTCF as well as cohesin at mammalian TAD boundaries is an established fact (Sofueva et al. 2013; Nora et al. 2012; Dixon et al. 2012; Zuin et al. 2014; Wutz et al. 2017), and there should be a reason for this deposition.

The model explaining the roles of CTCF and cohesion in TAD formation was suggested by two research teams (Fudenberg et al. 2016; Sanborn et al. 2015). According to the model, cohesin mediates DNA loop extrusion. The process of extrusion may start anywhere in the genome but cannot pass CTCF-binding sites present in a certain orientation. The last supposition was based on the observation that CTCF-binding motive has a direction and that CTCF-binding motives present at TAD boundaries (and bases of sub-TAD loops) usually have convergent orientation (Sanborn et al. 2015; Vietri Rudan et al. 2015; de Wit et al. 2015). Of note, the model considers TAD as a population phenomenon. In each individual cell, only a loop or a set of loops exist within the area that is considered as a TAD. However, all Hi-C maps that have been discussed so far were obtained when cell populations were studied. That is typical for a normal biochemical experiment. In a typical Hi-C protocol, one starts with 1–10 millions of cells. The loop extrusion model assumes that filled triangles (TADs) seen on population Hi-C maps represent superimposition of signals reflecting mainly interaction of bases of a variety of loops extruded in individual cells. This model has been supported by in silico modeling (Fudenberg et al. 2016). Also, it has been demonstrated that depletion or degrading of cohesin results in partial or full disruption of TADs (Sofueva et al. 2013; Rao et al. 2017), whereas depletion of cohesin unloading factor WAPL results in generation of longer chromatin loops (Wutz et al. 2017; Haarhuis et al. 2017) as predicted by the DNA loop extrusion model. The main challenge of the model is that the ability of cohesin to extrude DNA loops was not directly demonstrated. At the same time, it is known that cohesin possesses ATPase activity (Hirano 2005) and is able to move along

DNA both in vitro (Stigler et al. 2016; Kanke et al. 2016) and in vivo (Busslinger et al. 2017). Of note, this movement is restricted by CTCF (Davidson et al. 2016; Busslinger et al. 2017). Recently published results of Casellas's lab demonstrated that loop domains are formed by a process that requires cohesin ATPases (Vian et al. 2018). Finally, a condensin complex that is closely related to cohesin was found able to extrude DNA loops (Ganji et al. 2018). Taken together, these observations strongly support a supposition that cohesin may act as a DNA loop extrusion motor in the interphase nucleus.

It should be stressed that the DNA loop extrusion model (Fudenberg et al. 2016; Sanborn et al. 2015) considers TAD as a population phenomenon. The single-cell Hi-C studies performed so far have not provided a definitive answer to the question of whether there are TADs in individual mammalian cells due to a low resolution of Hi-C maps (Nagano et al. 2013; Flyamer et al. 2017). On the other hand, compact, and at first approximation globular, domains can be visualized in nuclei by FISH with TAD-specific probes (Bintu et al. 2018; Szabo et al. 2018). It is thus likely that there should be another mechanism that ensures compactization of entire TADs or extruded loops. It has been proposed that entropic forces primarily drive the formation of compact contact domains in a polymer confined to a limited space (Vasquez et al. 2016). This supposition made based on results of computational simulations is indirectly supported by the fact that contact domains occur in one or another form in the genomes of various organisms, including bacteria (Le et al. 2013), and special cell types, such as spermatozoa, which contain protamines in place of histones in their nuclei (Battulin et al. 2015). However, organization of nucleosomal fiber into compact domains may be also promoted by electrostatic interaction between nucleosomal particles. The ability of nucleosomal fibers to form various conglomerates is well documented. The conglomerates are stabilized by interactions between positively charged N-terminal tails of histones H3 and H4 and a negatively charged acidic patch on the surface of a nucleosomal globule (Kalashnikova et al. 2013; Pepenella et al. 2014). The same interactions facilitate the formation of 30-nm nucleosome fibers at low fiber concentrations, when between-fiber contacts are unlikely (Luger et al. 1997; Sinha and Shogren-Knaak 2010).

The main concern regarding the model of TAD assembly by condensation of nucleosomal fibers is to explain why individual TADs are separated. To this end, it should be mentioned that, in Drosophila, CTCF loops do not play a major role in 3D genome organization (Rowley et al. 2017). We and others reported that, in Drosophila cells, TAD boundaries harbor transcribed genes and are enriched in histone modifications typical for active chromatin (Ulianov et al. 2016; Sexton et al. 2012; Hou et al. 2012). Histone acetylation, which is typical of active chromatin, decreases the histone charge and prevents internucleosome interactions (Shogren-Knaak et al. 2006; Allahverdi et al. 2011). We argued that these processes may be sufficient to prevent assembly of active chromatin regions into compact domains (Ulianov et al. 2016). Thus, the distribution of active and inactive genes along a DNA molecule may determine the profile of chromosome organization in TADs. To test this idea,

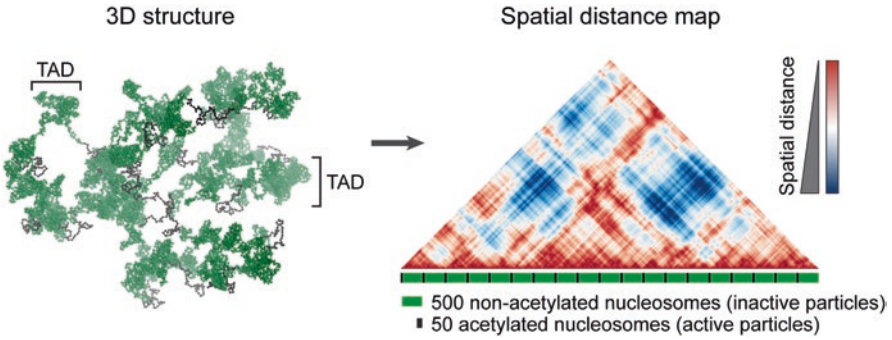

Fig. 2.4 Model heteropolymer built up from long blocks of inactive particles (non-acetylated nucleosomes interacting with each other) interspersed with short blocks of active particles (acetylated nucleosomes unable to interact with other nucleosomes) recapitulates some structural properties of chromatin. Polymer simulations demonstrate that blocks of inactive particles fold into globules manifested as TADs in spatial distance maps of the polymer. The results of a typical simulation are presented

we performed computer modelling of self-folding of a virtual polymer that consists of alternating nucleosome blocks of two types reproducing the properties of active and inactive chromatin regions (Fig. 2.4) (Ulianov et al. 2016). The particles of inactive block (500 particles in each block) were allowed to establish a limited number of relatively unstable contacts with the particles of the same type from the same or other inactive blocks. The particles of active blocks (50 particles in each block) were not allowed to establish contacts with each other or with particles from inactive blocks. The self-folding of polymer simulated using dissipative particle dynamics algorithm resulted in formation of globular structures roughly colocalizing with inactive blocks separated by unfolded active blocks (Ulianov et al. 2016). Of course, in each individual simulation, the folding of polymer was not fully regular. In some cases, conglomerates of inactive nucleosomes fused to produce superconglomerates; in other cases, nucleosomes of one inactive block formed more than one conglomerate with less compact spacers between the conglomerates (Fig. 2.4). However, averaging of the results of 12 simulations allowed generation of a Hi-C map containing contact domains (TADs) that coincided with inactive nucleosome blocks and were separated by spacers of active nucleosomes (Ulianov et al. 2016). Other simulations have demonstrated that short patches of "active chromatin" inserted into "inactive chromatin" blocks tend to be extruded on a surface of inactive block (Gavrilov et al. 2016). Insertion of larger stretches of "active chromatin" resulted rather in splitting of inactive blocks. This observation was in agreement with experimental observations that activation of transcription of tissue-specific genes located within TADs correlates with decompacting of the corresponding region, which, in some cases, resulted in TAD splitting (Ulianov et al. 2016).

It should be mentioned that DNA loop extrusion and nucleosome condensation are not mutually exclusive. Thus, nucleosome condensation may contribute to the compaction of extruded chromatin loops in mammalian cells. There is yet another group of models postulating that TAD formation is mediated by architectural proteins that form intra-TAD links, thus pulling together remote segments of a chromatin fiber. To explain the existence of isolated TADs, the models assume a multiplicity of architectural protein groups, each ensuring the formation of a particular TAD (Barbieri et al. 2012, 2013; Pombo and Nicodemi 2014). The models are supported by computer simulations but seem implausible biologically because there are 100 times fewer architectural protein types than TADs even in Drosophila, which is known to have several architectural proteins in addition to CTCF (Zolotarev et al. 2016).

3D Organization of the Genome in the Context of Nuclear Compartmentalization

The current model of the global genome organization within the eukaryotic cell nucleus was formulated long before the development of Hi-C and other C-methods. Initially, this model was based exclusively on the results of microscopic studies. Territorial organization of interphase chromosomes and the existence of an interchromatin domain (ICD) that spans chromosomal territories are the main points of the model (Cremer and Cremer 2001, 2010, 2018; Cremer et al. 2017, 2018). The interchromatin domain is the place where various membraneless nuclear bodies such as nucleoli, splicing speckles, Cajal bodies, paraspeckles, histone locus bodies, and PML bodies are assembled (for a review, see Mao et al. (2011); Ulianov et al. (2015); Stanek and Fox (2017)). The initial version of the model placed ICD between chromosomal territories (Cremer et al. 1993; Zirbel et al. 1993). With the increase of resolution of microscopic methods, it became evident that the ICD also penetrates chromosomal territories (Cremer and Cremer 2010, 2018). Chromosome territories themselves are composed of chromatin domains and chromatin domain clusters that likely correspond to TADs and contact domains of higher order. Interestingly, internal parts of these domains appear to contain mostly inactive chromatin, whereas active genes are preferentially located at the perichromatin layer (Cremer and Cremer 2018; Cremer et al. 2018). Although individual chromosomes constitute rather separated entities within the cell nucleus, interchromosomal contacts could still be found at various reaction centers such as transcription factories, PML bodies, and splicing speckles. Such contacts were first observed using FISH to visualize various genes in combination with immunostaining to observe functional nuclear compartments (Wang et al. 2004; Sun et al. 2003; Shopland et al. 2003; Szczerbal and Bridger 2010; Moen et al. 2004) and then reanalyzed using genome-wide C-methods (Wang et al. 2016; Schoenfelder et al. 2010; Quinodoz et al. 2018).

It should be mentioned that biochemical protocols based on a proximity ligation (C-methods) allow for identification of only particularly close spatial contacts. Recruitment of several genomic regions to the same compartment is difficult, if not impossible, to detect using C-methods. Development of alternative experimental protocols based on barcoding of DNA fragments present within the same, even quite large, fixed chromatin complex (Quinodoz et al. 2018) solved the problem. Using such an experimental procedure termed "SPRITE" (split-pool recognition of interactions by tag extension), Quinodoz et al. have identified two hubs of interchromosomal interactions that are arranged around the nucleolus (repressed hub) and nuclear speckles (active hub) (Quinodoz et al. 2018). Another genome-wide protocol that enables measuring distances between various genes and nuclear compartments is TSA-Seq (Chen et al. 2018). The procedure utilizes the tyramide amplification cascade (Wang et al. 1999) to biotinylate DNA in the vicinity of sites to which horseradish peroxidase (HRP) catalyzes the formation of tyramide-biotin free radicals recruited using an appropriate cascade of antibodies. Biotinylated DNA is then pulled down on streptavidin and sequenced. Using TSA-Seq, Belmont and coauthors confirmed clustering of active genes close to nuclear speckles. In agreement with a number of previous reports (Shevelyov and Nurminsky 2012; van Steensel and Belmont 2017), the repressed genes were found more in proximity to the nuclear lamina (Chen et al. 2018).

Taking together, the above observations argue that 3D organization of the genome and functional compartmentalization of the cell nucleus are mutually dependent. 3D organization is not simply a sum of enhancer-promoter and CTCF loops. It relies on a number of factors present in non-disturbed nuclei. Various fractionation procedures compromise this complex organization and drastically affect the results of analysis based on capturing pairwise interactions of remote DNA fragments (Gavrilov et al. 2013). Juxtaposition of remote genomic elements is not only ensured by interaction of proteins bound to these elements but rather represents a result of specific folding of a large genomic segment supported by numerous interactions outside the juxtaposed regions (Razin et al. 2013). These interactions include repositioning of various genomic segments to the vicinity of functional nuclear compartments. On the other hand, the folded genome as a whole provides a structural basis for nuclear compartmentalization (Misteli 2007; Schneider and Grosschedl 2007; Lanctot et al. 2007; Razin et al. 2013). The ICD where all these compartments are assembled is formed by exclusion from the areas occupied by chromatin. Segregation of interphase chromosomes resulting in the existence of chromosomal territories appears to be ensured by basic physical properties of charged polymers (Rosa and Everaers 2008; Mateos-Langerak et al. 2009; Bohn and Heermann 2010; Tark-Dame et al. 2011). It is less clear what supports the existence of channeled compartment within chromosomal territories. The simplest supposition is that repulsion between surfaces of TADs is of primary importance. The key point to be taken into account is that the surface of TADs should be more charged than the internal regions. Recent results of the Cremer team demonstrate that active chromatin is located at the surface of 1 Mb chromatin domains (TADs) (Cremer and Cremer

2018; Cremer et al. 2018) and thus lines the ICD channels. This finding is corroborated by the results of in silico modeling of TAD assembly (Gavrilov et al. 2016). High levels of histone acetylation typical for active chromatin (Shogren-Knaak et al. 2006; Allahverdi et al. 2011) should make the perichromatin layer more negatively charged compared to the internal part of chromatin domains/TADs. Thus, the perichromatin layer should stabilize and insulate inactive chromatin domains/TADs via generating electrostatic repulsion between them. This layer may prevent intermingling of TADs and ensure existence of intrachromosomal channels. The basic landscape for nuclear compartmentalization is thus directed only by physical laws (Rosa and Everaers 2008; Cook and Marenduzzo 2009; Dorier and Stasiak 2009; Kim and Szleifer 2014). Once established after mitosis, the territorial organization of interphase chromosomes becomes stabilized by interaction of certain chromosomal regions with the nuclear lamina (Guelen et al. 2008; Pickersgill et al. 2006) and nucleolus (Nemeth et al. 2010; van Koningsbruggen et al. 2010). Nucleoli are assembled at particular genomic loci harboring arrays of rRNA genes. The same is true for histone locus bodies. Transcription factories are likely to assemble stochastically by aggregation of closely located transcription complexes (Razin et al. 2011). Still, spatial positioning of the involved transcribed genes will predetermine their location. Typically for biological systems, this organization is highly dynamic. This dynamism applies to the both folding of interphase chromosomes and assembly of nuclear compartments. Live imaging studies have demonstrated that both chromosome territories and individual domains within chromosomal territories undergo constant movement (Marshall et al. 1997a, b, Marshall 2002; Levi et al. 2005; Pliss et al. 2013). The typical configuration of an interphase chromosome or shorter genomic segments represents an equilibrium of a number of possible configurations (Nagano et al. 2013; Stevens et al. 2017). The nature of functional nuclear compartments has been a matter of long-term discussions. The current model suggests that these compartments are liquid droplets formed by phase separation. They can fuse or separate into smaller droplets depending on external conditions. Although each type of compartments is rich in a particular set of proteins, the sets of proteins present in different compartments may overlap, and proteins present within compartments rapidly exchange with those proteins present in nucleoplasm. Furthermore, while speckles were reported to be positionally stable within hours (Misteli et al. 1997; Kruhlak et al. 2000), Cajal bodies and PML bodies appear to diffuse within the ICD as freely as an artificially created inert object of the same dimensions (Gorisch et al. 2004). An apparent order within the cell nucleus is thus likely to emerge out of a disorder due to a shaky equilibrium of different forces including a depletion attraction force (Cho and Kim 2012; Marenduzzo et al. 2006; Hancock 2004b; Rippe 2007). Apparently, the interplay between various functional processes that occur in the nucleus in any given moment directs both the chromosome folding and spatial compartmentalization of the nucleus (Rippe 2007; Kim and Szleifer 2014; Hancock 2004a; Razin et al. 2013; Golov et al. 2015; Sengupta 2018; Shah et al. 2018). Consequently, the cell nucleus should be considered as an integrated system, the properties of which emerge due to the interaction of

numerous components and cannot be fully explained or predicted based on the properties of individual components. Further progress in understanding mechanisms of eukaryotic genome functioning will depend on reconsideration of all pull of existing data in terms of systems biology.

Acknowledgments This work was supported by the Russian Science Foundation (grant 19-14-00016) and by the Interdisciplinary Scientific and Educational School of Moscow University «Molecular Technologies of the Living Systems and Synthetic Biology».

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