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REVIEW

3D genomics imposes evolution of the domain model of eukaryotic genome organization

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Abstract The hypothesis that the genome is composed of a patchwork of structural and functional domains (units) that may be either active or repressed was proposed almost 30 years ago. Here, we examine the evolution of the domain model of eukaryotic genome organization in view of the expansion of genome-scale techniques in the twenty-first century that have provided us with a wealth of information on genome organization, folding, and functioning.

Keywords Epigenetics · Chromatin · Domains · Large-scale organization

Introduction

The domain model of eukaryotic genome organization (Bodnar 1988; Goldman 1988) was proposed almost 30 years ago to account for the analysis of the generalized DNase I sensitivity of individual genes and genomic segments. The generalized sensitivity to DNase I is believed to reflect a partial distortion of higher orders of chromatin packaging and manifests itself in a randomly increased sensitivity toward DNase I over fairly long chromatin segments (up to hundreds of kilobases). In contrast, DNase I hypersensitive sites are commonly considered to be short nucleosome-free regions which are much more susceptible to DNase I than the regions of generalized sensitivity (Cockerill 2011). Although initially it was observed that active genes per se were preferentially digested by DNase I in isolated nuclei or permeabilized cells (Garel and Axel 1976; Weintraub and Groudine 1976; Weintraub et al. 1981), further studies demonstrated that relatively long genomic regions comprised of tissue-specific genes rather than individual genes resided in DNase I-sensitive or DNase I-resistant configurations in a lineage-specific fashion (Jantzen et al. 1986; Lawson et al. 1982). It was proposed that the whole genome was built from similarly organized structural-functional units (domains) that may be either active or repressed. These "chromatin domains" were defined as large genomic areas where changes of the transcriptional status and chromatin organization occurred independently of the flanking regions. The transcriptional status of domains was thought to be controlled by chromatin packaging. Each domain could be either folded (inactive) or unfolded (active) (Bodnar 1988; Goldman 1988). The differences in the pattern of chromatin domain folding could explain the differential sensitivity of these domains to DNase I (Fig. 1). Concurrently, several research teams developed the concept of the genome folded in closed loops and fixed to a proteinaceous skeletal structure both in interphase nuclei and metaphase chromosomes (Adolph et al. 1977; Benyajati and Worcel 1976; Cook et al. 1976). The sizes of these loops were reported to be in the range of 50–250 Kbs (reviewed in Razin et al. 1995). Although it was tempting to propose a direct link between these loops and the structural-functional genomic units postulated by the domain model (Fig. 1), subsequent studies did not provide substantial experimental support for this idea as profiles of DNA partitioning into loops were proved to be function-related (Ciejek et al. 1983; Cook et al.

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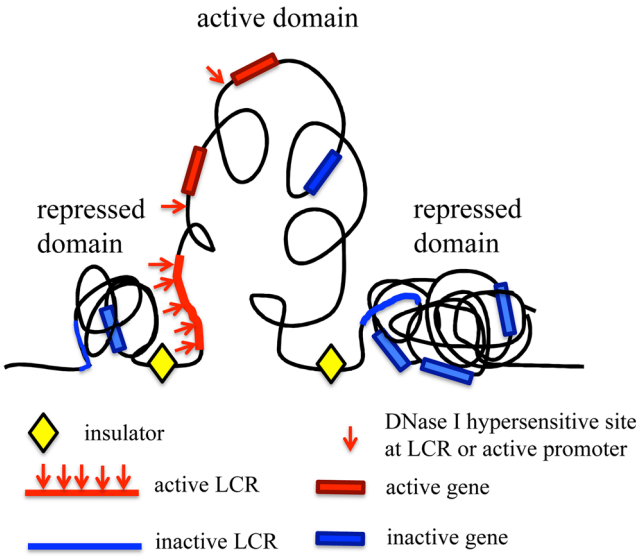

Fig. 1 Domain model of eukaryotic genome organization. Two compressed (inactive) and one unfolded (active) domains are shown. Active genes and regulatory elements (LCR) are shown in *red*. *Red arrows* show DNase I hypersensitive sites. Repressed genes and inactive regulatory elements are shown in *blue*. Insulators are shown by *yellow diamonds*

1982; Small and Vogelstein 1985), while the domain model assumed them to be invariant.

The transcriptional activity and general sensitivity of chromatin domains to DNase I was found to correlate with high levels of histone acetylation (Forsberg et al. 2000; Hebbes et al. 1988, 1994). The causal relationship between histone acetylation and generalized DNase I sensitivity of chromatin was demonstrated by Krajewski and Becker (1998). Consequently, histone acetylation was seen as a mechanism controlling the transcriptional status of chromatin domains (Eberharter and Becker 2002). Furthermore, the analysis of histone acetylation profiles allowed chromatin domains to be recognized as functional domains of the genome (Forsberg and Bresnick 2001). Henceforth, chromatin domains regardless of their loop structure are commonly considered to correspond to structural-functional units of the genome as postulated by the domain model.

The domain model of eukaryotic genome organization describes coregulation of gene clusters and switching between gene activation and repression by assuming that the domain as a whole constituted a primary unit of transcriptional regulation (Razin et al. 2007). The model postulated the existence of both domain-level regulatory elements and well-defined domain border elements and thus prompted several researchers 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 scaffold/matrix attachment regions (S/MARs) (Cockerill and Garrard 1986; Mirkovitch et al. 1984), and locus control regions (LCRs) (Forrester et al. 1987, 1990; Grosveld et al. 1987; Li et al. 1990). The domain of human beta-globin genes represents a typical example of a structural-functional genomic unit considered by the domain model. This domain harbors several globin genes along with an upstream LCR flanked by insulators. In erythroid cells, the whole domain resides in a DNase I-sensitive configuration. However, individual globin genes present within the domain are transcribed in a developmental stage-specific manner. In non-erythroid cells, the whole domain resides in a DNase I-resistant configuration. Hence, the transcription of genes within the domain is activated in two steps: Firstly, activation of the domain as a whole in erythroid lineage cells makes all genes accessible for trans-acting factors; secondly, the individual genes are activated in a developmental stage-specific fashion. Interestingly, the timing of domain replication reveals lineage-specific switches of late-replication in non-erythroid cells to early-replication in erythroid cells (Forrester et al. 1990). Beta-globin gene domains within other vertebrates are organized similarly to the human domain (reviewed in Razin et al. 2003).

The domain model of eukaryotic genome organization was based on studies performed in a limited number of animal models (beta-globin gene domains of mammals and birds, an ovalbumin gene domain of birds, a chicken lysozyme gene domain, histone and heat shock gene loci of *Drosophila*); however, the deductions were extended to the entire genome. Further studies of numerous other domains led to the evolution of the model. Indeed, organization of the alpha-globin gene clusters of birds and mammals did not fit the predictions of the domain model.

The tissue-specific alpha-globin genes are located in an area also harboring housekeeping genes. In contrast to the beta-globin genes, alpha-globin genes reside in a DNase I-sensitive chromatin domain in both erythroid and non-erythroid cells (Craddock et al. 1995; Vyas et al. 1992). The major regulatory element of the alpha-globin gene cluster is situated in an intron of a housekeeping gene located upstream to the cluster (Jarman et al. 1991; Vyas et al. 1995). Furthermore, housekeeping genes were found in a few other tissue-specific gene domains (Chong et al. 2002).

To update the domain model of eukaryotic genome organization, the concept of "strong" and "weak" genomic domains was incorporated (Dillon and Sabbatini 2000) (reviewed in Razin et al. 2003). The weak domains such as those harboring the vertebrate alpha-globin genes were first considered to be an exception to the general rule, but subsequent studies have since shown that the typical eukaryotic genome is exceedingly constituted of these domains. Indeed, even in the "classical" mammalian beta-globin gene domains, the borders of functional domains (that include genes and regulatory

modules) do not correspond to the borders of the region demonstrating lineage-dependent sensitivity to DNase I (Bulger et al. 2000; Schubeler et al. 2000). It is noteworthy that most known genes are housekeeping genes which are expressed in cells of different lineages; thus, they reside permanently in DNase I-sensitive chromatin domains. The expansion of genome-scale techniques in the twenty-first century has provided us with novel information on genome organization and folding. Below, we shall further review the evolution of the domain model of the eukaryotic genome organization alongside the progression of genome-wide data.

Structural units and domains of eukaryotic chromosomes

With the advent of genome-wide analysis techniques, the existence of chromatin domains was studied many times over, first with techniques that addressed the linear organization of the genome (ChIP-chip, ChIP-seq, transcriptome and exome analysis, genome-wide analysis of generalized DNase I sensitivity and DNaseI hypersensitive sites etc.) and then with increased precision, the 3D folding of the genome. We shall first consider the linear organization of the genome.

During the early times of the domain model, only two major types of chromatin were considered: the active and the repressed chromatin. Analysis of generalized sensitivity to DNase I was used to discriminate between genes present in active and repressed chromatin domains (Jantzen et al. 1986; Lawson et al. 1982; Weintraub and Groudine 1976; Weintraub et al. 1981). Recently, a strategy for the genome-wide analysis of generalized sensitivity to DNase I was developed (Milon et al. 2014). Using this strategy, it was demonstrated that the entire *Drosophila* genome illustrates a mosaic of interspersed DNase I-sensitive and DNase I-resistant segments (Milon et al. 2014).

Following the proposition of the histone code hypothesis (Strahl and Allis 2000), many histone marks corresponding to various chromatin states were extensively studied. Genome-wide combinatorial distribution analysis of histone modifications allowed identification of several types of distinct active and inactive chromatin regions (Filion et al. 2010; Kharchenko et al. 2011). Consequently, it became possible to distinguish several types of chromatin domains (Bickmore and van Steensel 2013). These include repressive polycomb domains (i.e., domains harboring genes inactivated in the course of cell differentiation) characterized by the presence of H3K27me3 (Tolhuis et al. 2006) and large organized chromatin K9-modifications (LOCKs) domains which may provide a cell type-heritable mechanism for phenotypic plasticity and are characterized by the presence of H3K9me2/3 (Wen et al. 2009). Domains associated with the nuclear lamina (LADs) (Guelen et al. 2008; Kind et al. 2013; Kind and van Steensel 2010; Meuleman et al. 2013) harbor mostly inactive genes.

Discrete genomic domains can be identified by analyzing the timing of replication. Extended early-replication and late-replication domains approximately correspond to active and repressed chromatin domains (Farkash-Amar and Simon 2010; Gilbert 2002). A more detailed analysis allowed the identification of four types of replication domains that colocalize with specific types of chromatin domains (Julienne et al. 2013). The Type I early-replicating domains correspond to active chromatin and are characterized by the presence of H3K4me3, H3K79me2, and H3K36me3, as well as by the presence of RNA polymerase II (RNApol II) and CTCF associated with chromatin. The intermediate- and late-replicating domains harbor different classes of repressed chromatin: facultative heterochromatin repressed by polycomb (Type 2), repressed chromatin without any distinctive histone modifications (LADs) (type 3), and classical constitutive heterochromatin containing H3K9me3 and HP1 (Type 4) (Julienne et al. 2013). The average sizes of these four types of replicative/chromatin domains vary significantly (275, 228, 325, and 718 Kb for types 1, 2, 3, and 4, respectively; Julienne et al. 2013). Of note, domains that switch their chromatin status in a lineage-specific manner, namely, active domains and domains repressed by polycomb, appear to correspond well to model domains considered at the early times of the domain model of eukaryotic genome organization.

The abovementioned domains were identified while studying linear (1D) genome organization. However, genomes are folded into a complex 3D structure and compacted over ×10,000 to fit into the nucleus. An assortment of methods based on chromosome conformation capture (3C) (Dekker et al. 2002) allowed for a genome-wide analysis of spatial proximity between various genomic regions (Belton et al. 2012; de Wit and de Laat 2012; Gibcus and Dekker 2013). Studies performed in mammals (Dixon et al. 2012; Lieberman-Aiden et al. 2009; Nora et al. 2012; Rao et al. 2014) and *Drosophila* (Hou et al. 2012; Sexton et al. 2012) demonstrated that the genome was partitioned into a set of self-interacting domains called topologically-associating domains (TADs). The average size of TADs constitutes ~100 Kb in *Drosophila* and ~1 Mb in mammals. However, TADs are often hierarchical, i.e., composed of smaller self-interacting domains separated by weak boundaries (Filippova et al. 2014; Sexton et al. 2012; Ulianov et al. 2015b; Weinreb and Raphael 2015).

Linear or three-dimensional?

Researchers engaged in genomics usually consider linear distances along DNA (i.e., in Kb). Consequently, different types of domains (either functional or structural) are also considered as linear, and they are represented as such in different genomic

browsers. However, current studies demonstrate that the genome is spatially organized in a functionally dependent way (reviewed in Dekker and Mirny 2016; Rowley and Corces 2016). In particular, remote regulatory elements may be clustered in the nuclear space or even assembled in a common regulatory complex (reviewed in Bickmore 2013; de Laat and Grosveld 2003; Pombo and Dillon 2015; Razin et al. 2013; Sexton and Cavalli 2015). Consequently, genomic domains that are split on DNA may regain their integrity due to their spatial organization within the nuclear space. Enhancers usually harbor clusters of binding sites of various transcription factors. Even if binding of transcription factors to DNA is not very strong, the presence of several recognition sequences may lead to an increase of a local concentration of transcription factors in the vicinity of an enhancer; ultimately shifting the equilibrium between binding and dissociation towards binding. In fact, this mechanism does not require the presence of binding sites for transcription factors on the same DNA chain, only their spatial proximity appears to be important. Recent data strongly support a model where spatial organization of the genome occurs via looping between remote regulatory elements and their targets (Fanucchi et al. 2013; Mifsud et al. 2015; Tang et al. 2015). The role of this organization cannot be reduced to simple activation of a single gene by a remote regulatory element. Indeed, disruption of a single spatial contact within a complex of coregulated genes results in downregulation of all genes constituting the complex. Thus, the integrity of a spatial domain as a whole is important for the transcriptional regulation of all genes involved (Fanucchi et al. 2013).

Similar to split functional domains, the split chromatin domains may reassemble in the nuclear space. This can be demonstrated by an example of the mouse beta-globin gene domain. One of the regulatory modules of this domain (HS −62) is located within the array of inactive olfactory receptor genes ~62 Kbs upstream to the beta-globin gene cluster. In erythroid cells, HS −62 and LCR have a high level of histone acetylation. However, the chromatin area that separates these two regulatory elements is not highly acetylated. In the assembled active chromatin hub (de Laat and Grosveld 2003; Tolhuis et al. 2002), this fragment is looped out and HS −62, LCR, and the transcribed beta-globin genes that harbor hyperacetylated histones become located next to each other. Another example of the assembly of a spatial structural/functional genomic domain is provided by the murine HoxD gene locus. In mice, the expression of HoxD genes is controlled by remote enhancers that are spread over an extended (800 Kb) gene desert (Montavon et al. 2011). These remote regulatory modules form lineage-specific contacts with each other and with target genes resulting in the assembly of 3D regulatory domains which the authors refer to as “regulatory archipelagos” (Montavon et al. 2011). The principle of spatial assembly of split structural/functional domains in the 3D nuclear space is shown in Fig. 2.

Splitting of a functional genomic domain into several blocks separated on a linear DNA molecule may be a consequence of integration of mobile elements and genome rearrangements (see Ulianov et al. 2015a for an extended discussion). However, it may also be advantageous as 3D combination of various regulatory elements could possibly provide additional opportunities for lineage-specific gene regulation or for the adaptation of a regulatory system to a changing environment.

Of note, the same genomic segment can acquire different 3D configurations in cells of different lineages or simply in different cells within a population (for example, depending on the stage of the cell cycle). The developmental stage-specific spatial configuration of the mouse beta-globin domain has long been described (de Laat and Grosveld 2003; Tolhuis et al. 2002). In this case, the functional relevance of various spatial configurations of the domain is quite obvious as the globin genes transcribed at each particular developmental stage are recruited to the active chromatin compartment. Similarly, different variants of Epstein-Barr virus latency programs were found to depend on the assembly of alternate spatial configurations of the viral genome (Tempera et al. 2011). The choice between these configurations is controlled by CTCF (Chen et al. 2014). In the both abovementioned cases, the alternate spatial configurations of the genomic domains were attributed to different cell populations. However,

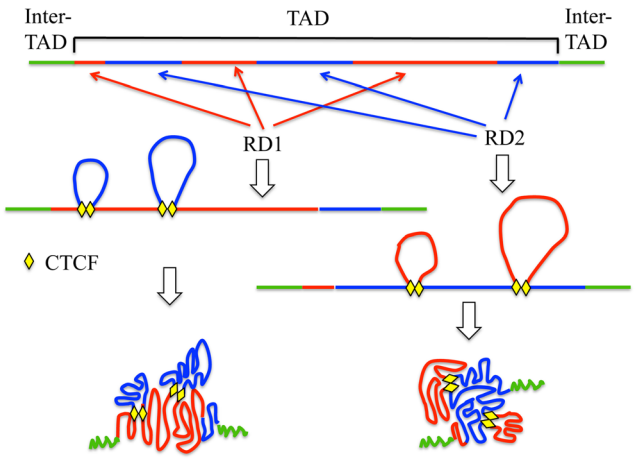

Fig. 2 A chromatin domain may not correspond to a continuous region on a linear chromatin fiber. *Upper panel*, Map of a hypothetic genomic domain composed of three genes and two enhancers. The *curve* above the map demonstrates the profile of histone acetylation that is also illustrated by the intensity of *red color* of the line representing a linear chromatin fiber. The genes and enhancers are bound to highly acetylated histones while intermediate segments of chromatin do not contain highly acetylated histones. *Lower panel*, Within the cell nucleus, all acetylated regions of the domain shown in the upper section are assembled into a single hyperacetylated spatial chromatin domain

various genomic domains, including that of chicken alpha and beta-globin genes, can adopt various spatial configurations in the same cellular populations (Philonenko et al. 2009; Ulianov et al. 2012). Evidently, this allows alternate activation of different genes by the same enhancer element. In cultured chicken erythroblasts stimulated toward terminal erythroid differentiation, the assembly of two alternate spatial regulatory complexes was observed. One of them appears to be necessary for active transcription of a subset of globin genes (Gavrilov and Razin 2008), while the other activated the *TMEM8* gene (Philonenko et al. 2009). Interestingly, these two distinct activator complexes share some regulatory elements and therefore simply cannot be assembled simultaneously in the same chromosome (Philonenko et al. 2009). Other studies performed on different experimental models strongly support the notion that spatial organization of the genome significantly differs in different cells within the same population. Likewise, in an NF-kB-regulated complex of coexpressed genes (Papantonis et al. 2010), the involved genes colocalize in the nuclear space and are essentially transcribed only in a small portion of cells in the population (Fanucchi et al. 2013). Similar observations were made when patterns of colocalization of various erythroid genes in individual cells were analyzed using 3D-FISH (Schoenfelder et al. 2010). It seems feasible that the 3D organization of the genome constantly changes thus providing a possibility for sorting and selection of optimal variants.

The question of how much the implementation of these alternative configurations correlates with changes in the structure of chromatin domains requires further study. Progress in this area of research will be determined by modeling 3D chromatin domains on the available epigenetic data. The first attempt to develop software for the prediction of 3D configurations of genomic domains based on 1D epigenetic profiles has already been reported (Zhu et al. 2016).

The current domain model suggests that histone modifications spread progressively along the chromatin fiber resulting in the expansion of various types of chromatin domains (Bannister and Kouzarides 2011; Bannister et al. 2001). However, the same mechanisms of chromatin domain expansion may also operate in the 3D space if chromatin fibers are located close enough to each other. In this case, a spherical domain of modified histones will be formed (for an extended discussion, see Razin and Gavrilov 2014). This domain may appear either continuous or discontinuous on a linear chromatin fiber, depending on the pattern of DNA packaging. The shape and size of the spatial chromatin domain will be determined by characteristics of self-assembled nucleosome conglomerates (Maeshima et al. 2016).

Topologically associating domains are the spatial units of the 3D genome

Hi-C data analysis performed on various organisms suggests that chromosomes of yeast, *Drosophila*, and mammals are organized into self-interacting chromatin domains which are referred to as topologically associating domains or TADs (Dixon et al. 2012; Hou et al. 2012; Hsieh et al. 2015; Sexton et al. 2012; Zhang et al. 2012). TADs are characterized by an increased level of intra-TAD spatial contacts and decreased level of inter-TAD spatial contacts (Lieberman-Aiden et al. 2009). TADs are usually identified with chromatin globules (Lieberman-Aiden et al. 2009), such as chromatin domains (CDs) reported by Cremer's group (Cremer and Cremer 2001; Markaki et al. 2010; Smeets et al. 2014). 3D-FISH analysis of mutual positions of genomic segments located within the same and neighboring TADs indirectly supports this interpretation (Dixon et al. 2012; Fabre et al. 2015; Nora et al. 2012). TADs are larger in mammals than in *Drosophila*. However, in both organisms, TADs are hierarchical (i.e., composed of smaller self-interacting domains) (Fraser et al. 2015; Ulianov et al. 2015b; Weinreb and Raphael 2015). Profiles of chromosomes partitioning into TADs were reported to be cell lineage-independent and, furthermore, evolutionary conserved within syntenic regions (Dixon et al. 2012; Vietri Rudan et al. 2015). However, the degree of similarity of TAD profiles between cells of different lineages is limited and does not exceed 60–80 % in mammals (Dixon et al. 2012; Fraser et al. 2015) and 40–50 % in *Drosophila* (Hou et al. 2012; Ulianov et al. 2015a). Furthermore, the internal structure of TADs is not rigid. It undergoes certain changes in the course of cell differentiation and in response to stress (Bau et al. 2011; Berlivet et al. 2013; Dixon et al. 2015; Li et al. 2015). Nonetheless, organization of chromosomes into TADs restricts spatial contacts between remote genomic regions. Most of the observed contacts occur within TADs (Gibcus and Dekker 2013; Matharu and Ahituv 2015).

It is not yet clear how two adjacent TADs are separated. Our recent data strongly suggest that, in *Drosophila*, assembly of TADs is directed by internucleosomal contacts, and TAD separation occurs due to positioning of transcriptionally active chromatin segments between TADs. These segments are less likely to establish internucleosomal interactions since they are highly acetylated (Ulianov et al. 2015a). The folding of nucleosomal arrays into secondary chromatin structures and self-association of arrays into higher-order tertiary structures is an inherent property of chromatin fibers (Blacketer et al. 2010; Hansen 2002; Maeshima et al. 2016). This follows from the ability of nucleosomes to establish contacts via interaction of positively charged tails of histones H3 and H4 with the acidic patch on the surface of a nucleosomal globule (Kalashnikova et al. 2013; Pepenella et al. 2014; Schalch et al. 2005; Sinha and Shogren-Knaak 2010) and with negatively charged DNA

(Arya and Schlick 2006). These interactions are adjusted by modifications of histone tails, in particular by acetylation (Allahverdi et al. 2011; Pepenella et al. 2014; Shogren-Knaak et al. 2006), which interferes with the establishment of internucleosomal contacts. In *Drosophila*, the TAD borders and inter-TAD regions harbor housekeeping genes and are enriched in active chromatin marks (Hou et al. 2012; Sexton et al. 2012; Ulianov et al. 2015a). We have proposed that clusters of housekeeping genes are located in spacers between TADs simply because they cannot be packed into compact structures due to a high level of histone acetylation (Ulianov et al. 2015b). This model was confirmed by computer simulations. A virtual polymer composed of 19 blocks of 500 nucleosomes capable of establishing saturating contacts ("inactive chromatin") and 50 nucleosomes incapable to establishing such contacts (active chromatin) collapsed in a series of condensed blobs (structures similar to TADs) consisting mostly of inactive nucleosomes separated by spacers of active nucleosomes. Of note, 3D configurations of this polymer observed in 12 individual simulations significantly differed. However, averaging of the results of these individual simulations allowed the acquisition of a regular pattern of compact "TADs" separated by extended "inter-TADs" (Ulianov et al. 2015b). To this end, it should be stressed that the C-methods currently used for the analysis of 3D genome organization (de Wit and de Laat 2012) give only an integrated view of millions of cells. No high-resolution single-cell TAD maps have been reported so far. The results of our computer simulations suggest that TAD profiles in individual cells may differ from each other and from the integral picture that is obtained upon analyzing a cellular population.

Stochastic factors clearly play an important role in establishing the 3D organization of the genome (Kang et al. 2011). Self-organization of chromatin fiber into TADs and spatial restoration of otherwise split functional units of the genome are likely to happen simultaneously. They contribute to a pattern that may vary significantly within different cells of a population. Indeed, the 3D global structure of X-chromosome differ significantly in individual mouse cells, as shown by modeling based on low-resolution single-cell Hi-C data (Nagano et al. 2013). The distances between individual alleles located both inside a single chromosomal territory and in different chromosomal territories also vary (Kawamura et al. 2012; Kozubek et al. 2002; Schoenfelder et al. 2010).

In mammals, average TADs are larger than in *Drosophila*, and the mechanism of their assembly appears to be more sophisticated. Specifically, the extrusion of DNA loops constrained by CTCF/cohesion complexes is likely to shape the TAD profiles (Alipour and Marko 2012; Fudenberg et al. 2015; Sanborn et al. 2015). Even so, the assembly of compact chromatin blobs within the extruded DNA loops is likely to be governed by internucleosomal interactions.

Are there functional domains in the eukaryotic genome?

The finding that many tissue-specific functional gene domains do not change their sensitivity to DNase I in a lineage-specific manner puts forward a question about a suitable definition of these domains. Intuitively, one can define a functional genomic domain as a genomic segment harboring one or several genes along with the regulatory elements controlling the expression of these genes. In principle, any housekeeping gene along with regulatory elements in a CpG island would fit this definition. However, the term "functional domain" usually designates an extended genomic region such as mammalian beta-globin gene domain or Hox gene clusters. Yet, the minimal size of this region remains undefined. The clusters of coexpressing genes which (clusters) may be fairly long (Boutanaev et al. 2002; Spellman and Rubin 2002) also fit a definition of a functional domain assuming that these genes share at least some common regulatory modules. Interestingly, in *Drosophila*, these clusters are the same size as TADs. The clusters of highly expressed genes described in human and mouse also may be related to functional domains (Caron et al. 2001; Mijalski et al. 2005). Indeed, all genes present in such domains are influenced by the same regulatory mechanism. Transgenes randomly inserted within a cluster of highly expressed genes acquire a high expression level typical of the whole cluster (Mijalski et al. 2005). Using random insertion of a construct containing a reporter gene controlled by a minimal promoter over large genomic areas, Symmons et al. demonstrated the existence of functional genomic domains, designated by the authors as "regulatory domains," harboring tissue-specific genes which impose tissue-specific expression patterns on the transgenes inserted into these domains (Symmons et al. 2014). Other authors suggested to name these large functional genomic domains "regulatory archipelagos" (Lonfat et al. 2014; Montavon et al. 2011) or "regulatory landscapes" (Spitz et al. 2003; Zuniga et al. 2004). The difference of all these domains from those that were studied 20 years ago is predominantly their size and complexity. Regulatory domains (landscapes) are not necessarily discontinuous and may overlap. The same genomic region may contain several non-related regulatory domains. The integrity of these domains composed of several split regions is only reestablished as a result of 3D folding (Lonfat et al. 2014). The identity of regulatory domains is determined by the coordinated action of numerous enhancers that may even be situated outside these domains (Marinic et al. 2013; Symmons and Spitz 2013; Symmons et al. 2014). Indeed, it is currently considered that tissue-specific gene expression is determined by enhancers rather than

promoters (Heintzman et al. 2009). There are ~400,000 enhancers in the human genome (Consortium et al. 2012), 50,000–100,000 in *Drosophila* genome (Arnold et al. 2013), and 2300 in the genome of *Caenorhabditis elegans* (Chen et al. 2013). Taking into account that the known number of human genes is ~ 25,000, the expression of an average human gene might be controlled by 10–20 enhancers, and an enhancer can control the expression of more than one gene. Thus, the number of possible combinations further increases. Certainly, gene expression of higher eukaryotes is controlled by a complex network of regulatory elements that constitutes a basis of the regulatory domains. It appears that the above regulatory domains/archipelagos are very similar if not identical to the "weak domains" discussed above (Dillon and Sabbatini 2000; Razin et al. 2003). Interestingly, a naturally occurring repositioning of a non-related gene to the tissue-specific weak domain of alpha-globin genes resulted in acquisition of the expression profile typical for the domain (Philonenko et al. 2009). This naturally occurring change of tissue specificity of a repositioned gene reinforces the observations reported by Symmons et al. (2014).

The next important question is whether there is a correlation between functional and structural units of the 3D genome. Current evidence suggests that such a correlation does exist. Indeed, coordinated changes of gene expression occur within genomic segments that colocalize with replication domains and LADs (Letourneau et al. 2014). Regulatory domains and replicating domains also appear to colocalize with TADs (Dileep et al. 2015b; Le Dily et al. 2014; Le Dily and Beato 2015; Lonfat and Duboule 2015; Nora et al. 2012; Pope et al. 2014; Remeseiro et al. 2015; Symmons et al. 2014). Furthermore, replication programs and organization of chromosomes into TADs appear to be reestablished simultaneously after mitosis (Dileep et al. 2015a). Finally, disruption of TADs, in particular fusion of the neighboring TADs, results in dramatic changes of promoter-enhancer interactions and may cause various diseases (Ibn-Salem et al. 2014; Ji et al. 2016; Lupianez et al. 2015, 2016; Petrov et al. 2006, 2008). Thus, organization of the genome into spatial domains is directly related to the functioning of the genome (Pombo and Dillon 2015; Sexton and Cavalli 2015).

Concluding remarks

There are not many concepts in modern molecular biology that have remained valid for several decades. The emergence of modern genomics, which is based on high throughput genome-wide analysis, prompted us to reconsider many hypotheses and models. Some of them have been forgotten, while others were updated and improved. We believe that the domain model of eukaryotic genome organization belongs to the latter group. The basic postulate of this model, namely, the assumption that the genome can be subdivided into structural-functional units, remains authentic. However, our knowledge about the nature of these domains has changed significantly. Fundamentally, the understanding that spatial organization of the genome plays an important role in regulation of gene expression is new. Consequently, 3D genomics and 3D chromatin domains is becoming the trend of the time.

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

Ethical approval The study was approved by the ethics committee of the Institute of Gene Biology, Moscow, Russia.

References

Adolph KW, Chang SM, Laemmli UK (1977) Role of nonhistone proteins in metaphase chromosomes structure. Cell 12:805–816. doi:10.1016/0092-8674(77)90279-3

Alipour E, Marko JF (2012) Self-organization of domain structures by DNA-loop-extruding enzymes. Nucleic Acids Res 40:11202–11212. doi:10.1093/nar/gks925

Allahverdi A, Yang R, Korolev N, Fan Y, Davey CA, Liu CF, Nordenskiold L (2011) The effects of histone H4 tail acetylations on cation-induced chromatin folding and self-association. Nucleic Acids Res 39:1680–1691. doi:10.1093/nar/gkq900

Arnold CD, Gerlach D, Stelzer C, Boryn LM, Rath M, Stark A (2013) Genome-wide quantitative enhancer activity maps identified by STARR-seq. Science 339:1074–1077. doi:10.1126/science.1232542

Arya G, Schlick T (2006) Role of histone tails in chromatin folding revealed by a mesoscopic oligonucleosome model. Proc Natl Acad Sci U S A 103:16236–16241. doi:10.1073/pnas.0604817103

Bannister AJ, Kouzarides T (2011) Regulation of chromatin by histone modifications. Cell Res 21:381–395. doi:10.1038/cr.2011.22

Bannister AJ, Zegerman P, Partridge JF, Miska EA, Thomas JO, Allshire RC, Kouzarides T (2001) Selective recognition of methylated lysine 9 on histone H3 by the HP1 chromo domain. Nature 410:120–124. doi:10.1038/35065138

Bau D et al (2011) The three-dimensional folding of the alpha-globin gene domain reveals formation of chromatin globules. Nat Struct Mol Biol 18:107–114. doi:10.1038/nsmb.1936

Belton JM, McCord RP, Gibcus JH, Naumova N, Zhan Y, Dekker J (2012) Hi-C: a comprehensive technique to capture the conformation of genomes. Methods 58:268–276. doi:10.1016/j.ymeth.2012.05.001

Benyajati C, Worcel A (1976) Isolation, characterization, and structure of the folded interphase genome of Drosophila melanogaster. Cell 9:393–407. doi:10.1016/0092-8674(76)90084-2

Berlivet S, Paquette D, Dumouchel A, Langlais D, Dostie J, Kmita M (2013) Clustering of tissue-specific sub-TADs accompanies the regulation of HoxA genes in developing limbs. PLoS Genet 9:e1004018. doi:10.1371/journal.pgen.1004018

Bickmore WA (2013) The spatial organization of the human genome. Annu Rev Genomics HumGenet 14:67–84. doi:10.1146/annurev-genom-091212-153515

Bickmore WA, van Steensel B (2013) Genome architecture: domain organization of interphase chromosomes. Cell 152:1270–1284. doi:10.1016/j.cell.2013.02.001

Blacketer MJ, Feely SJ, Shogren-Knaak MA (2010) Nucleosome interactions and stability in an ordered nucleosome array model system. J Biol Chem 285:34597–34607. doi:10.1074/jbc.M110.140061

Bodnar JW (1988) A domain model for eukaryotic DNA organization: a molecular basis for cell differentiation and chromosome evolution. J Theor Biol 132:479–507

Boutanaev AM, Kalmykova AI, Shevelyov YY, Nurminsky DI (2002) Large clusters of co-expressed genes in the Drosophila genome. Nature 420:666–669. doi:10.1038/nature01216

Bulger M et al (2000) Comparative structural and functional analysis of the olfactory receptor genes flanking the human and mouse b-globin gene clusters. Proc Natl Acad Sci U S A 97:14560–14565. doi:10.1073/pnas.97.26.14560

Caron H et al (2001) The human transcriptome map: clustering of highly expressed genes in chromosomal domains. Science 291:1289–1292. doi:10.1126/science.1056794

Chen HS, Martin KA, Lu F, Lupey LN, Mueller JM, Lieberman PM, Tempera I (2014) Epigenetic deregulation of the LMP1/LMP2 locus of Epstein-Barr virus by mutation of a single CTCF-cohesin binding site. J Virol 88:1703–1713. doi:10.1128/JVI.02209-13

Chen RA et al (2013) The landscape of RNA polymerase II transcription initiation in C. elegans reveals promoter and enhancer architectures. Genome Res 23:1339–1347. doi:10.1101/gr.153668.112

Chong S, Riggs AD, Bonifer C (2002) The chicken lysozyme chromatin domain contains a second, widely expressed gene. Nucl Acids Res 30:463–467. doi:10.1093/nar/30.2.463

Ciejek EM, Tsai M-J, O'Malley BW (1983) Actively transcribed genes are associated with the nuclear matrix. Nature 306:607–609

Cockerill PN (2011) Structure and function of active chromatin and DNase I hypersensitive sites. FEBS J 278:2182–2210. doi:10.1111/j.1742-4658.2011.08128.x

Cockerill PN, Garrard WT (1986) Chromosomal loop anchorage of the kappa immunoglobulin gene occurs next to the enhancer in a region containing topoisomerase II sites. Cell 44:273–282. doi:10.1016/0092-8674(86)90761-0

Consortium EP, Bernstein BE, Birney E, Dunham I, Green ED, Gunter C, Snyder M (2012) An integrated encyclopedia of DNA elements in the human genome. Nature 489:57–74. doi:10.1038/nature11247

Cook PR, Brazell IA, Jost E (1976) Characterization of nuclear structures containing superhelical DNA. J Cell Sci 22:303–324

Cook PR, Lang J, Hayday A, L. L, Fried M, Chiswell DJ, Wyke A (1982) Active viral genes in transformed cells lie close to the nuclear cage. EMBO J 1:447–452

Craddock CF, Vyas P, Sharpe JA, Ayyub H, Wood WG, Higgs DR (1995) Contrasting effects of alpha and beta globin regulatory elements on chromatin structure may be related to their different chromosomal environments. EMBO J 14:1718–1726

Cremer T, Cremer C (2001) Chromosome territories, nuclear architecture and gene regulation in mammalian cells. Nat Rev Genet 2:292–301. doi:10.1038/35066075

de Laat W, Grosveld F (2003) Spatial organization of gene expression: the active chromatin hub. Chromosome Res 11:447–459

de Wit E, de Laat W (2012) A decade of 3C technologies: insights into nuclear organization. Genes Dev 26:11–24. doi:10.1101/gad.179804.111

Dekker J, Mirny L (2016) The 3D genome as moderator of chromosomal communication. Cell 164:1110–1121. doi:10.1016/j.cell.2016.02.007

Dekker J, Rippe K, Dekker M, Kleckner N (2002) Capturing chromosome conformation. Science 295:1306–1311. doi:10.1126/science.1067799

Dileep V, Ay F, Sima J, Vera DL, Noble WS, Gilbert DM (2015a) Topologically associating domains and their long-range contacts are established during early G1 coincident with the establishment of the replication-timing program. Genome Res 25:1104–1113. doi:10.1101/gr.183699.114

Dileep V, Rivera-Mulia JC, Sima J, Gilbert DM (2015b) Large-scale chromatin structure-function relationships during the cell cycle and development: insights from replication timing. Cold Spring Harb Symp Quant Biol. doi:10.1101/sqb.2015.80.027284

Dillon N, Sabbatini P (2000) Functional gene expression domains: defining the functional units of eukaryotic gene regulation. BioEssays 22:657–665. doi:10.1002/1521-1878(200007)22:7<657::AID-BIES8>3.0.CO;2-2

Dixon JR et al (2015) Chromatin architecture reorganization during stem cell differentiation. Nature 518:331–336. doi:10.1038/nature14222

Dixon JR et al (2012) Topological domains in mammalian genomes identified by analysis of chromatin interactions. Nature 485:376–380. doi:10.1038/nature11082

Eberharter A, Becker PB (2002) Histone acetylation: a switch between repressive and permissive chromatin. Second in review series on chromatin dynamics. EMBO Rep 3:224–229. doi:10.1093/embo-reports/kvf053

Fabre PJ, Benke A, Joye E, Nguyen Huynh TH, Manley S, Duboule D (2015) Nanoscale spatial organization of the HoxD gene cluster in distinct transcriptional states. Proc Natl Acad Sci U S A 112:13964–13969. doi:10.1073/pnas.1517972112

Fanucchi S, Shibayama Y, Burd S, Weinberg MS, Mhlanga MM (2013) Chromosomal contact permits transcription between coregulated genes. Cell 155:606–620. doi:10.1016/j.cell.2013.09.051

Farkash-Amar S, Simon I (2010) Genome-wide analysis of the replication program in mammals. Chromosome Res 18:115–125. doi:10.1007/s10577-009-9091-5

Filion GJ et al (2010) Systematic protein location mapping reveals five principal chromatin types in Drosophila cells. Cell 143:212–224. doi:10.1016/j.cell.2010.09.009

Filippova D, Patro R, Duggal G, Kingsford C (2014) Identification of alternative topological domains in chromatin. Algorithms Mol Biol AMB 9:14. doi:10.1186/1748-7188-9-14

Forrester WC, Epner E, Driscoll MC, Enver T, Brice M, Papayannopoulou T, Groudine M (1990) A deletion of the human b-globin locus activation region causes a major alteration in chromatin structure and replication across the entire b-globin locus. Gene Dev 4:1637–1649. doi:10.1101/gad.4.10.1637

Forrester WC, Takegawa S, Papayannopoulou T, Stamatoyannopoulos G, Groudine M (1987) Evidence for a locus activating region: the formation of developmentally stable hypersensitive sites in globin expressing hybrids. Nucleic Acids Res 15:10159–10175. doi:10.1093/nar/15.24.10159

Forsberg EC, Bresnick EH (2001) Histone acetylation beyond promoters: long-range acetylation patterns in the chromatin world. BioEssays 23:820–830. doi:10.1002/bies.1117

Forsberg EC, Downs KM, Christensen HM, Im H, Nuzzi PA, Bresnick EH (2000) Developmentally dynamic histone acetylation pattern of a tissue-specific chromatin domain. Proc Natl Acad Sci U S A 97:14494–14499. doi:10.1073/pnas.97.26.14494

Fraser J et al. (2015) Hierarchical folding and reorganization of chromosomes are linked to transcriptional changes in cellular differentiation. Mol Syst Biol 11:852. doi:10.15252/msb.20156492

Fudenberg G, Imakaev M, Lu C, Goloborodko A, Abdennur N, Mirny LA (2015) Formation of chromosomal domains by loop extrusion BioRxiv doi:10.1101/024620

Garel A, Axel R (1976) Selective digestion of transcriptionally active ovalbumin genes from oviduct nuclei. Proc Natl Acad Sci U S A 73:3966–3970

Gavrilov AA, Razin SV (2008) Spatial configuration of the chicken {alpha}-globin gene domain: immature and active chromatin hubs. Nucleic Acids Res 36:4629–4640. doi:10.1093/nar/gkp838

Gibcus JH, Dekker J (2013) The hierarchy of the 3D genome. Mol Cell 49:773–782. doi:10.1016/j.molcel.2013.02.011

Gilbert DM (2002) Replication timing and transcriptional control: beyond cause and effect. Curr Opin Cell Biol 14:377–383. doi:10.1016/S0955-0674(02)00326-5

Goldman MA (1988) The chromatin domain as a unit of gene regulation. BioEssays 9:50–55. doi:10.1002/bies.950090204

Grosveld F, van Assandelt GB, Greaves DR, Kollias B (1987) Position-independent, high-level expression of the human b-globin gene in transgenic mice. Cell 51:975–985. doi:10.1016/0092-8674(87)90584-8

Guelen L et al (2008) Domain organization of human chromosomes revealed by mapping of nuclear lamina interactions. Nature 453:948–951. doi:10.1038/nature06947

Hansen JC (2002) Conformational dynamics of the chromatin fiber in solution: determinants, mechanisms, and functions. Annu Rev Biophys Biomol Struct 31:361–392. doi:10.1146/annurev.biophys.31.101101.140858

Hebbes TR, Clayton AL, Thorne AW, Crane-Robinson C (1994) Core histone hyperacetylation co-maps with generalized DNase I sensitivity in the chicken beta-globin chromosomal domain. EMBO J 13:1823–1830

Hebbes TR, Thorne AW, Crane-Robinson C (1988) A direct link between core histone acetylation and transcriptionally active chromatin. EMBO J 7:1395–1402

Heintzman ND et al (2009) Histone modifications at human enhancers reflect global cell-type-specific gene expression. Nature 459:108–112. doi:10.1038/nature07829

Hou C, Li L, Qin ZS, Corces VG (2012) Gene density, transcription, and insulators contribute to the partition of the Drosophila genome into physical domains. Mol Cell 48:471–484. doi:10.1016/j.molcel.2012.08.031

Hsieh TH, Weiner A, Lajoie B, Dekker J, Friedman N, Rando OJ (2015) Mapping nucleosome resolution chromosome folding in yeast by micro-C. Cell 162:108–119. doi:10.1016/j.cell.2015.05.048

Ibn-Salem J et al (2014) Deletions of chromosomal regulatory boundaries are associated with congenital disease. Genome Biol 15:423. doi:10.1186/s13059-014-0423-1

Jantzen K, Friton HP, Igo-Kimenes T (1986) The DNase I sensitive domain of the chicken lyzozyme gene spans 24 kb. Nucleic Acids Res 14:6085–6099. doi:10.1093/nar/14.15.6085

Jarman AP, Wood WG, Sharpe JA, Gourdon G, Ayyub H, Higgs DR (1991) Characterization of the major regulatory element upstream of the human a-globin gene cluster. Mol Cell Biol 11:4679–4689. doi:10.1128/MCB.11.9.4679

Ji X et al (2016) 3D chromosome regulatory landscape of human pluripotent cells. Cell Stem Cell 18:262–275. doi:10.1016/j.stem.2015.11.007

Julienne H, Zoufir A, Audit B, Arneodo A (2013) Human genome replication proceeds through four chromatin states. PLoS Comput Biol 9, e1003233. doi:10.1371/journal.pcbi.1003233

Kalashnikova AA, Porter-Goff ME, Muthurajan UM, Luger K, Hansen JC (2013) The role of the nucleosome acidic patch in modulating higher order chromatin structure. J R Soc Interface R Soc 10:20121022. doi:10.1098/rsif.2012.1022

Kang J, Xu B, Yao Y, Lin W, Hennessy C, Fraser P, Feng J (2011) A dynamical model reveals gene co-localizations in nucleus. PLoS Comput Biol 7:e1002094. doi:10.1371/journal.pcbi.1002094

Kawamura R, Tanabe H, Wada T, Saitoh S, Fukushima Y, Wakui K (2012) Visualization of the spatial positioning of the SNRPN, UBE3A, and GABRB3 genes in the normal human nucleus by three-color 3D fluorescence in situ hybridization. Chromosome Res 20:659–672. doi:10.1007/s10577-012-9300-5

Kellum R, Schedl P (1991) A position-effect assay for boundaries of higher-order chromosomal domains. Cell 64:941–950. doi:10.1016/0092-8674(91)90318-S

Kellum R, Schedl P (1992) A group of scs elements function as boundaries in enhancer-blocking assay. Mol Cell Biol 12:2424–2431. doi:10.1128/MCB.12.5.2424

Kharchenko PV et al (2011) Comprehensive analysis of the chromatin landscape in Drosophila melanogaster. Nature 471:480–485. doi:10.1038/nature09725

Kind J et al (2013) Single-cell dynamics of genome-nuclear lamina interactions. Cell 153:178–192. doi:10.1016/j.cell.2013.02.028

Kind J, van Steensel B (2010) Genome-nuclear lamina interactions and gene regulation. Curr Opin Cell Biol 22:320–325. doi:10.1016/j.ceb.2010.04.002

Kozubek S et al (2002) 3D Structure of the human genome: order in randomness. Chromosoma 111:321–331. doi:10.1007/s00412-002-0210-8

Krajewski WA, Becker PB (1998) Reconstitution of hyperacetylated, DNase I-sensitive chromatin characterized by high conformational flexibility of nucleosomal DNA. Proc Natl Acad Sci U S A 95:1540–1545

Lawson GM, Knoll BJ, March CJ, Woo SLC, Tsai M-J, O'Malley BW (1982) Definition of 5' and 3' structural boundaries of the chromatin domain containing the ovalbumin multigene family. J Biol Chem 257:1501–1507

Le Dily F et al (2014) Distinct structural transitions of chromatin topological domains correlate with coordinated hormone-induced gene regulation. Genes Dev 28:2151–2162. doi:10.1101/gad.241422.114

Le Dily F, Beato M (2015) TADs as modular and dynamic units for gene regulation by hormones. FEBS Lett 589:2885–2892. doi:10.1016/j.febslet.2015.05.026

Letourneau A et al (2014) Domains of genome-wide gene expression dysregulation in Down's syndrome. Nature 508:345–350. doi:10.1038/nature13200

Li L et al (2015) Widespread rearrangement of 3D chromatin organization underlies polycomb-mediated stress-induced silencing. Mol Cell 58:216–231. doi:10.1016/j.molcel.2015.02.023

Li Q, Zhou B, Powers P, Enver T, Stamatoyannopoulos G (1990) Beta-globin locus activations regions: conservation of organization, structure and function. Proc Natl Acad Sci U S A 87:8207–8211

Lieberman-Aiden E et al (2009) Comprehensive mapping of long-range interactions reveals folding principles of the human genome. Science 326:289–293. doi:10.1126/science.1181369

Lonfat N, Duboule D (2015) Structure, function and evolution of topologically associating domains (TADs) at HOX loci. FEBS Lett 589:2869–2876. doi:10.1016/j.febslet.2015.04.024

Lonfat N, Montavon T, Darbellay F, Gitto S, Duboule D (2014) Convergent evolution of complex regulatory landscapes and pleiotropy at Hox loci. Science 346:1004–1006. doi:10.1126/science.1257493

Lupianez DG et al (2015) Disruptions of topological chromatin domains cause pathogenic rewiring of gene-enhancer interactions. Cell 161:1012–1025. doi:10.1016/j.cell.2015.04.004

Lupianez DG, Spielmann M, Mundlos S (2016) Breaking TADs: how alterations of chromatin domains result in disease. Trends Genet 32:225–237. doi:10.1016/j.tig.2016.01.003

Maeshima K et al. (2016) Nucleosomal arrays self-assemble into supramolecular globular structures lacking 30-nm fibers. EMBO J doi:10.15252/embj.201592660

Marinic M, Aktas T, Ruf S, Spitz F (2013) An integrated holo-enhancer unit defines tissue and gene specificity of the Fgf8 regulatory landscape. Dev Cell 24:530–542. doi:10.1016/j.devcel.2013.01.025

Markaki Y et al (2010) Functional nuclear organization of transcription and DNA replication: a topographical marriage between chromatin domains and the interchromatin compartment. Cold Spring Harb Symp Quant Biol 75:475–492. doi:10.1101/sqb.2010.75.042

Matharu N, Ahituv N (2015) Minor loops in major folds: enhancer-promoter looping, chromatin restructuring, and their association with transcriptional regulation and disease. PLoS Genet 11: e1005640. doi:10.1371/journal.pgen.1005640

Meuleman W et al (2013) Constitutive nuclear lamina-genome interactions are highly conserved and associated with A/T-rich sequence. Genome Res 23:270–280. doi:10.1101/gr.141028.112

Mifsud B et al (2015) Mapping long-range promoter contacts in human cells with high-resolution capture Hi-C. Nat Genet 47:598–606. doi:10.1038/ng.3286

Mijalski T et al (2005) Identification of coexpressed gene clusters in a comparative analysis of transcriptome and proteome in mouse tissues. Proc Natl Acad Sci U S A 102:8621–8626. doi:10.1073/pnas.0407672102

Milon B et al (2014) Map of open and closed chromatin domains in Drosophila genome. BMC Genomics 15:988. doi:10.1186/1471-2164-15-988

Mirkovitch J, Mirault M-E, Laemmli UK (1984) Organization of the higher-order chromatin loop: specific DNA attachment sites on nuclear scaffold. Cell 39:223–232. doi:10.1016/0092-8674(84)90208-3

Montavon T et al (2011) A regulatory archipelago controls Hox genes transcription in digits. Cell 147:1132–1145. doi:10.1016/j.cell.2011.10.023

Nagano T et al (2013) Single-cell Hi-C reveals cell-to-cell variability in chromosome structure. Nature 502:59–64. doi:10.1038/nature12593

Nora EP et al (2012) Spatial partitioning of the regulatory landscape of the X-inactivation centre. Nature 485:381–385. doi:10.1038/nature11049

Papantonis A, Larkin JD, Wada Y, Ohta Y, Ihara S, Kodama T, Cook PR (2010) Active RNA polymerases: mobile or immobile molecular machines? PLoS Biol 8, e1000419. doi:10.1371/journal.pbio.1000419

Pepenella S, Murphy KJ, Hayes JJ (2014) Intra- and inter-nucleosome interactions of the core histone tail domains in higher-order chromatin structure. Chromosoma 123:3–13. doi:10.1007/s00412-013-0435-8

Petrov A, Allinne J, Pirozhkova I, Laoudj D, Lipinski M, Vassetzky YS (2008) A nuclear matrix attachment site in the 4q35 locus has an enhancer-blocking activity in vivo: implications for the facioscapulo-humeral dystrophy. Genome Res 18:39–45. doi:10.1101/gr.6620908

Petrov A, Pirozhkova I, Carnac G, Laoudj D, Lipinski M, Vassetzky YS (2006) Chromatin loop domain organization within the 4q35 locus in facioscapulohumeral dystrophy patients versus normal human myoblasts. Proc Natl Acad Sci U S A 103:6982–6987. doi:10.1073/pnas.0511235103

Philonenko ES, Klochkov DB, Borunova VV, Gavrilov AA, Razin SV, Iarovaia OV (2009) TMEM8—a non-globin gene entrapped in the globin web. Nucleic Acids Res 37:7394–7406. doi:10.1093/nar/gkp838

Pombo A, Dillon N (2015) Three-dimensional genome architecture: players and mechanisms. Nat Rev Mol Cell Biol 16:245–257. doi:10.1038/nrm3965

Pope BD et al (2014) Topologically associating domains are stable units of replication-timing regulation. Nature 515:402–405. doi:10.1038/nature13986

Rao SS et al (2014) A 3D map of the human genome at kilobase resolution reveals principles of chromatin looping. Cell 159:1665–1680. doi:10.1016/j.cell.2014.11.021

Razin SV, Farrell CM, Recillas-Targa F (2003) Genomic domains and regulatory elements operating at the domain level. Int Rev Cytol 226:63–125

Razin SV, Gavrilov AA (2014) Chromatin without the 30-nm fiber: constrained disorder instead of hierarchical folding. Epigenetics 9: 653–657. doi:10.4161/epi.28297

Razin SV, Gavrilov AA, Ioudinkova ES, Iarovaia OV (2013) Communication of genome regulatory elements in a folded chromosome. FEBS Lett 587:1840–1847. doi:10.1016/j.febslet.2013.04.027

Razin SV, Gromova II, Iarovaia OV (1995) Specificity and functional significance of DNA interaction with the nuclear matrix: New approaches to clarify the old questions. Int Rev Cytol 162B:405–448

Razin SV et al (2007) Chromatin domains and regulation of transcription. J Mol Biol 369:597–607. doi:10.1016/j.jmb.2007.04.003

Remeseiro S, Hornblad A, Spitz F (2015) Gene regulation during development in the light of topologically associating domains. Wiley Interdiscip Rev Dev Biol. doi:10.1002/wdev.218

Rowley MJ, Corces VG (2016) The three-dimensional genome: principles and roles of long-distance interactions. Curr Opin Cell Biol 40: 8–14. doi:10.1016/j.ceb.2016.01.009

Sanborn AL et al (2015) Chromatin extrusion explains key features of loop and domain formation in wild-type and engineered genomes. Proc Natl Acad Sci U S A 112:E6456–6465. doi:10.1073/pnas.1518552112

Schalch T, Duda S, Sargent DF, Richmond TJ (2005) X-ray structure of a tetranucleosome and its implications for the chromatin fibre. Nature 436:138–141. doi:10.1038/nature03686

Schoenfelder S et al (2010) Preferential associations between co-regulated genes reveal a transcriptional interactome in erythroid cells. Nat Genet 42:53–61. doi:10.1038/ng.496

Schubeler D, Francastel C, Cimbora DM, Reik A, Martin DI, Groudine M (2000) Nuclear localization and histone acetilation: a pathway for chromatin opening and transcription activation of the human b-globin locus. Gen Dev 14:940–950. doi:10.1101/gad.14.8.940

Sexton T, Cavalli G (2015) The role of chromosome domains in shaping the functional genome. Cell 160:1049–1059. doi:10.1016/j.cell.2015.02.040

Sexton T et al (2012) Three-dimensional folding and functional organization principles of the Drosophila genome. Cell 148:458–472. doi:10.1016/j.cell.2012.01.010

Shogren-Knaak M, Ishii H, Sun JM, Pazin MJ, Davie JR, Peterson CL (2006) Histone H4-K16 acetylation controls chromatin structure and protein interactions. Science 311:844–847. doi:10.1126/science.1124000

Sinha D, Shogren-Knaak MA (2010) Role of direct interactions between the histone H4 Tail and the H2A core in long range nucleosome contacts. J Biol Chem 285:16572–16581. doi:10.1074/jbc.M109.091298

Small D, Vogelstein B (1985) The anatomy of supercoiled loops in the *Drosophila* 7F locus. Nucl Acids Res 13

Smeets D et al (2014) Three-dimensional super-resolution microscopy of the inactive X chromosome territory reveals a collapse of its active nuclear compartment harboring distinct Xist RNA foci. Epigenetics Chromatin 7:8. doi:10.1186/1756-8935-7-8

Spellman PT, Rubin GM (2002) Evidence for large domains of similarly expressed genes in the Drosophila genome. J Biol 1:5. doi:10.1186/1475-4924-1-5

Spitz F, Gonzalez F, Duboule D (2003) A global control region defines a chromosomal regulatory landscape containing the HoxD cluster. Cell 113:405–417. doi:10.1016/S0092-8674(03)00310-6

Strahl BD, Allis CD (2000) The language of covalent histone modifications. Nature 403:41–45. doi:10.1038/47412

Symmons O, Spitz F (2013) From remote enhancers to gene regulation: charting the genome's regulatory landscapes. Philos Trans R Soc Lond B Biol Sci 368:20120358. doi:10.1098/rstb.2012.0358

Symmons O et al (2014) Functional and topological characteristics of mammalian regulatory domains. Genome Res 24:390–400. doi:10.1101/gr.163519.113

Tang Z et al (2015) CTCF-mediated human 3D genome architecture reveals chromatin topology for transcription. Cell 163:1611–1627. doi:10.1016/j.cell.2015.11.024

Tempera I, Klichinsky M, Lieberman PM (2011) EBV latency types adopt alternative chromatin conformations. PLoS Pathog 7: e1002180. doi:10.1371/journal.ppat.1002180

Tolhuis B, de Wit E, Muijrers I, Teunissen H, Talhout W, van Steensel B, van Lohuizen M (2006) Genome-wide profiling of PRC1 and PRC2 polycomb chromatin binding in Drosophila melanogaster. Nat Genet 38:694–699. doi:10.1038/ng1792

Tolhuis B, Palstra RJ, Splinter E, Grosveld F, de Laat W (2002) Looping and interaction between hypersensitive sites in the active beta-globin locus. Mol Cell 10:1453–1465. doi:10.1016/S1097-2765(02)00781-5

Udvardy A, Schedl P, Sander M, Hsieh T-S (1986) Topoisomerase II cleavage in chromatin. J Mol Biol 191:231–246. doi:10.1016/0022-2836(86)90260-3

Ulianov SV, Gavrilov AA, Razin SV (2012) Spatial organization of the chicken beta-globin gene domain in erythroid cells of embryonic and adult lineages. Epigenetics Chromatin 5:16. doi:10.1186/1756-8935-5-16

Ulianov SV, Gavrilov AA, Razin SV (2015a) Nuclear compartments, genome folding, and enhancer-promoter communication. Int Rev Cell Mol Biol 315:183–244. doi:10.1016/bs.ircmb.2014.11.004

Ulianov SV et al (2015b) Active chromatin and transcription play a key role in chromosome partitioning into topologically associating domains. Genome Res 26:70–84. doi:10.1101/gr.196006.115

Vietri Rudan M, Barrington C, Henderson S, Ernst C, Odom DT, Tanay A, Hadjur S (2015) Comparative Hi-C reveals that CTCF underlies evolution of chromosomal domain architecture. Cell Rep 10:1297–1309. doi:10.1016/j.celrep.2015.02.004

Vyas P, Vickers MA, Simmons DL, Ayyub H, Craddock CF, Higgs DR (1992) cis-acting sequences regulating expression of the human a-globin cluster lie within constitutively open chromatin. Cell 69:781–793. doi:10.1016/0092-8674(92)90290-S

Vyas P, Vickers MA, Picketts DJ, Higgs DR (1995) Conservation of position and sequence of a novel, widely expressed gene containing the major human alpha-globin regulatory element. Genomics 29: 679–689. doi:10.1006/geno.1995.9951

Weinreb C, Raphael BJ (2015) Identification of hierarchical chromatin domains. Bioinformatics. doi:10.1093/bioinformatics/btv485

Weintraub H, Groudine M (1976) Chromosomal subunits in active genes have an altered conformation. Science 73:848–856. doi:10.1126/science.948749

Weintraub H, Larsen A, Groudine M (1981) Alpha-Globin-gene switching during the development of chicken embryos: expression and chromosome structure. Cell 24:333–344. doi:10.1016/0092-8674(81)90323-8

Wen B, Wu H, Shinkai Y, Irizarry RA, Feinberg AP (2009) Large histone H3 lysine 9 dimethylated chromatin blocks distinguish differentiated from embryonic stem cells. Nat Genet 41:246–250. doi:10.1038/ng.297

Zhang Y et al (2012) Spatial organization of the mouse genome and its role in recurrent chromosomal translocations. Cell 148:908–21. doi:10.1016/j.cell.2012.02.002

Zhu Y et al (2016) Constructing 3D interaction maps from 1D epigenomes. Nat Commun 7:10812. doi:10.1038/ncomms10812

Zuniga A et al (2004) Mouse limb deformity mutations disrupt a global control region within the large regulatory landscape required for Gremlin expression. Genes Dev 18:1553–1564. doi:10.1101/gad.299904