Molecular Cell
Review

The Hierarchy of the 3D Genome

Johan H. Gibcus[1] and Job Dekker[1,*]
[1]Program in Systems Biology, Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, 364 Plantation Street, Worcester, MA 01605-0103, USA
*Correspondence: job.dekker@umassmed.edu
http://dx.doi.org/10.1016/j.molcel.2013.02.011

Mammalian genomes encode genetic information in their linear sequence, but appropriate expression of their genes requires chromosomes to fold into complex three-dimensional structures. Transcriptional control involves the establishment of physical connections among genes and regulatory elements, both along and between chromosomes. Recent technological innovations in probing the folding of chromosomes are providing new insights into the spatial organization of genomes and its role in gene regulation. It is emerging that folding of large complex chromosomes involves a hierarchy of structures, from chromatin loops that connect genes and enhancers to larger chromosomal domains and nuclear compartments. The larger these structures are along this hierarchy, the more stable they are within cells, while becoming more stochastic between cells. Here, we review the experimental and theoretical data on this hierarchy of structures and propose a key role for the recently discovered topologically associating domains.

Introduction

Chromosomes were discovered more than a century ago when Flemming observed the formation of stained bodies just before cell division (Flemming, 1965). Careful observations of the behavior of chromosomes during mitosis and meiosis led to the critical insight that they must be the carriers of genetic information, as articulated in the Boveri-Sutton chromosome theory of heredity at the beginning of the 20th century (Wilson, 1925). For years, biologists focused on studying the structure, dynamics, and behavior of chromosomes with the hope of learning how they contain, express, and transmit genetic information. During the 20th century, the emphasis changed with the discovery of DNA as the genetic carrier, driving new studies aimed at understanding how information is encoded in its sequence, culminating in the sequencing of the human genome in 2001 (Lander et al., 2001; Venter et al., 2001). Interestingly, during the last several years, the field has witnessed an exciting return to its beginning with the realization that in order to understand how the genome works, we need to know not only the information encoded in its sequence but also the ways this sequence is structurally and physically organized inside chromosomes.

Over the last century, improved microscopic approaches have enabled the study of chromosome organization at increasing resolution and detail (Schermelleh et al., 2010). In the last decade, the development of molecular approaches based on chromosome conformation capture (3C) technology, combined with methods of modeling and interpreting chromatin interaction data, has revolutionized the analysis of chromosome folding (Baù and Marti-Renom, 2011; Bohn and Heermann, 2010; Dekker et al., 2002; Fudenberg and Mirny, 2012; Hakim and Misteli, 2012; Kalhor et al., 2012; van Steensel and Dekker, 2010).

3C-based methods are used to probe chromosome organization by measuring the frequency of physical interactions or proximity among any pair of genomic loci. By determining the contact probability of a large set of loci, spread out along chromosomes and across cell populations, insight into the spatial organization of chromosomes can be gained (Dekker et al., 2002). 3C-based techniques are all based on formaldehyde crosslinking of chromatin, which creates a genome-wide snapshot of (long-range) interactions between any pair of genomic loci occurring in three dimensions. Chromatin is fragmented, for example by digestion, and then intramolecularly religated so that interacting loci are converted into unique DNA ligation products that are then detected using a variety of methods. The original 3C method used PCR with locus-specific primers to detect ligation products one at a time. The development of deep-sequencing platforms has enabled the detection of ligation products at increasing throughput. 3C-based methods can be combined with deep sequencing to obtain chromatin interaction maps at increasing scale (from single loci to whole genomes) and resolution (from Mb to kb). This can be done by modification of the way 3C ligation products are detected, e.g., by inverse PCR (in 4C [Simonis et al., 2006; Splinter et al., 2012; Würtele and Chartrand, 2006; Zhao et al., 2006]), by multiplexed ligation-mediated amplification (in 5C [Dostie et al., 2006]), or by introduction of a biotin mark at the ligation junction to facilitate unbiased purification of ligation junctions (Hi-C [Belton et al., 2012; Lieberman-Aiden et al., 2009]). Recent increases in sequencing throughput and reduced costs are obviating the need for such modifications to the 3C method, and comprehensive genome-wide interaction maps have already been generated by direct sequencing of ligation products generated by the classical 3C procedure (3C-seq [Rodley et al., 2009; Sexton et al., 2012]).

Observations obtained by direct imaging of chromosomes in individual cells and by probing the folding of chromosomes across cell populations with 3C-based technologies have led to the identification of two central phenomena that characterize the organization of DNA inside cells: First, widely spaced genomic loci associate with each other to form short- and long-range and intra- and interchromosomal molecular interactions or connections (Miele and Dekker, 2008; Misteli, 2007;

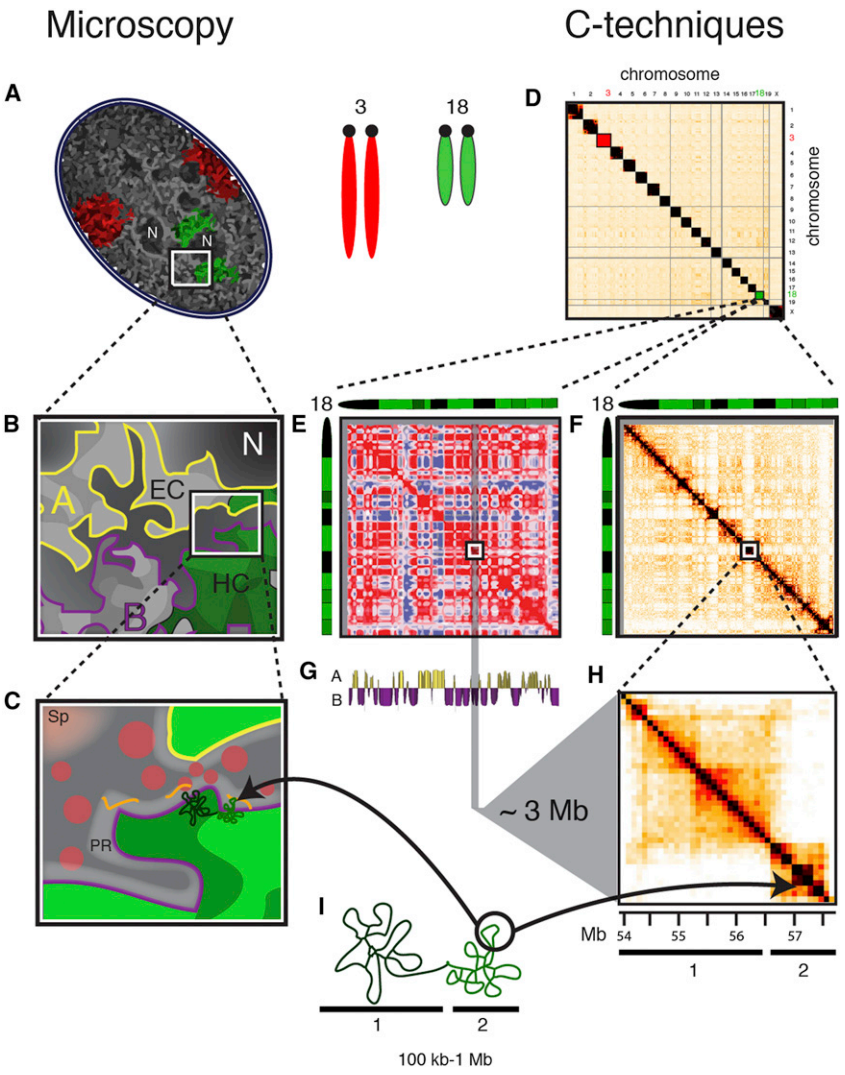

Figure 1. Large-Scale Nuclear Organization in Mammals

(A) The nucleus is composed of chromosome territories (for example, mouse chromosome 3 and 18 are depicted in red and green, respectively). DNA is organized in more or less condensed regions, as can be shown by DAPI staining (gray intensities). Nucleoli (N) are visible as dark spots (top inset).

(B) Inset shows a more detailed architecture of the nucleus with compartments (A and B), heterochromatin (HC), and euchromatin (EC) surrounding the interchromatin compartment.

(C) Zoomed-in view of chromosomal domains (hypothetical). Foci of factors interacting with looping chromatin in the perichromatin region (PR) are shown as pink circles, and RNA is shown as orange lines. The larger speckles (SP) are located in domains that are sparser in chromatin content and further away from the PR.

(D) All-by-all chromosome matrix showing the interactions within and between chromosomes.

(E) Red and blue "plaid" pattern of chromosome 18 emphasized through Pearson correlation shows the separation into two chromosomal domains (represented as red and blue).

(F) Detailed version of (D), sized equivalent to E, showing the *cis*-interaction matrix for chromosome 18. The inset indicates a ~3 Mb large B compartment.

(G) The clustering into compartments A and B after principal component analysis on the plaid pattern displayed in (E).

(H) Detailed version of the 3 Mb large B compartment from (F), revealing the organization of TADs (1 and 2).

(I) Representation of looping of chromatin as can be found at the PR (see C) or in deeper structures within TADs (see H). Nuclei were modeled to match Hi-C plots, which were adapted to scale from previously published data (Zhang et al., 2012).

van Steensel and Dekker, 2010). Second, although general principles regarding folding of chromosomes apply to all cells, the spatial organization of the genome is highly variable between cells, and no two nuclei will display the exact same set of connections (Croft et al., 1999; Orlova et al., 2012; Parada et al., 2003; Strickfaden et al., 2010). In this review, we provide an overview of the molecular processes, uncovered over the last decade, that determine the formation of three-dimensional connections between loci and how this organization affects the regulation of genes. We specifically focus on metazoa, since long-range interactions are more likely to play a role in their large genomes. We propose a way to unify the seemingly incompatible phenomena of a highly heterogeneous genome conformation between cells in a clonal cell population and the formation of reproducible gene regulatory interactions in each cell. Furthermore, we present a hierarchical view of the spatial organization of the genome in which different layers of organization display differences in real-time dynamics within cells and differential levels of stochasticity between cells.

Enduring Connections: Linked Loci Stay Together

The large size of chromosomes prevents them from freely mingling throughout the nucleus (Rosa and Everaers, 2008; Walter et al., 2003). Within the nucleus, DNA is organized into individual chromosome territories (CTs). The chromosomal arrangement into CTs was comprehensively visualized by chromosome painting (Lichter et al., 1988; Pinkel et al., 1988) (Figure 1A). CT formation was subsequently confirmed by genome-wide Hi-C analysis (Lieberman-Aiden et al., 2009; Zhang et al., 2012) that showed that loci located on the same chromosome interact far more frequently, even when separated by more than 200 Mb, than any two loci located on different chromosomes (Figure 1D). Yet, neighboring chromosomes can overlap considerably and chromatin loops from one CT can intermingle with neighboring CTs (Branco and Pombo, 2006; Misteli, 2010) (see below in the section Birds of a Feather Flock Together). Genomic linkage is clearly a very dominant factor in determining the three-dimensional connections of any gene or regulatory element.

CTs themselves are not randomly positioned in the nucleus. In both mouse and human cells, chromosomes of similar size and gene density were shown to be more likely to interact in nuclear

space: short gene dense chromosomes group together near the center of the nucleus, whereas the longer and less-gene-dense chromosomes are more often located near the nuclear periphery. This property is reflected in different propensities for interchromosomal connections (Croft et al., 1999; Lieberman-Aiden et al., 2009; Tanabe et al., 2002; Zhang et al., 2012).

Peripheral Connections

The eukaryotic nucleus is a confined organelle bounded by the double lipid bilayer membrane of the nuclear envelope (NE) (Dingwall and Laskey, 1992). Although the NE allows for communication through nuclear pore complexes, it essentially restricts genomic DNA to a confined three-dimensional space. In addition, this physical barrier provides a solid anchor point that allows for specific chromatin interactions.

The inner nuclear membrane of the NE is lined by a filamentous meshwork of lamin proteins termed the nuclear lamina (NL) (Kind and van Steensel, 2010). The NL associates with inactive, heterochromatic chromatin directly or indirectly via lamin-associated proteins (Goldman et al., 2002). This organization of chromatin-lamin association involves specific chromosomal domains. Large (0.1–10 Mb) lamin-associated domains (LADs) were identified by DamID, an approach in which lamins are fused to a bacterial dam methyltransferase, leading to methylation of lamin-associated loci (Guelen et al., 2008). It is currently unknown whether LADs are the result of an active binding process at the NL or whether they result from being "pushed out" of the nuclear center. Almost half the genome in a given cell population is composed of LADs, but not all LADs can physically be associated with the NL in each cell. Given that in different (clonal) cells some chromosomes are not located near the periphery, this implies significant cell-to-cell heterogeneity. LADs are considered heterochromatic and are characterized by low gene density and a general lack of transcription (Guelen et al., 2008). When cells differentiate, some LADs lose their association with the NL, while others may become associated with the nuclear periphery. These changes coincide with altered gene expression profiles, in which activated genes move to the nuclear interior and inactivated genes are found in new LADs (Peric-Hupkes et al., 2010). The NL thus serves as a cell-type-specific anchoring location for large stretches of the genome and constitutes a place where heterochromatic loci, scattered throughout the genome, can connect in three dimensions.

Whereas the NL associates with heterochromatin, nuclear pore complexes (NPCs) are enriched for associations with euchromatin and active genes (Brown et al., 2008; Capelson et al., 2010). The nuclear envelope must therefore be considered as a general organizing surface where not only silent loci but also actively expressed loci come together at specialized subnuclear sites.

Nucleolar Connections

Loci not only gather near the nuclear periphery, but also at other specialized subnuclear structures such as nucleoli. Nucleoli are subnuclear structures dedicated to expression by RNA polymerase I (pol I). They are formed around clustered ribosomal DNA arrays from several different chromosomes that are transcribed by RNA pol I. Interestingly, actively transcribed RNA pol III-dependent genes can also be found at the nucleoli, and enrichment for such genes has been documented in the perinucleolar regions surrounding the nucleolus (Bertrand et al., 1998; Huang et al., 1997; Thompson et al., 2003).

To directly identify the loci that associate at or near nucleoli Németh et al. purified nucleolus-associated DNA and identified nucleolus-associated domains (NADs) (Németh et al., 2010). As expected, these authors report enrichment for loci transcribed by RNA pol III and RNA pol I but in addition also identify groups of RNA pol II-dependent genes such as olfactory receptor genes. Interestingly, these RNA pol II-dependent genes are silent in the cell lines studied by Németh et al. Thus, silenced RNA pol II-dependent loci can be located both at the NL and at nucleoli.

There are indeed similarities between LADs and NADs. First, through the use of photoactivation and time-lapse fluorescence microscopy, it was shown that NADs either relocalize at the nucleoli or colocalize with the nuclear lamina after cell division (van Koningsbruggen et al., 2010). Furthermore, the size range and median sequence length of NADs (0.1–10 Mb; 749 kb) correspond to the size of LADs (0.1–10 Mb; 553 kb) (Németh et al., 2010). Thus, both the NL and nucleoli serve as structures at which heterochromatic loci located on different chromosomes come together to form specialized subnuclear structures, with additional clustering of actively transcribing RNA pol I and pol III at nucleoli (Kendall et al., 2000; Németh et al., 2010).

Birds of a Feather Flock Together

Within CTs, and in the nucleus in general, euchromatic chromatin is spatially separate from heterochromatic chromatin. It has extensively been shown that open, gene-rich areas and closed, gene-poor chromosomal domains are generally located in separate subnuclear regions (Fraser and Bickmore, 2007; Misteli, 2007; Naumova and Dekker, 2010). This functional compartmentalization can now be detected at high resolution and genome-wide scale with 3C-based methods and can be related to the association of loci with observable subnuclear structures such as transcription factories and the NL.

Hi-C data revealed the presence of subchromosomal compartments named A and B (Figures 1B, 1E, and 1G) (Lieberman-Aiden et al., 2009; Zhang et al., 2012). Loci found clustered in A compartments are generally gene rich, transcriptionally active, and DNase I hypersensitive, whereas loci found in B compartments are relatively gene poor, transcriptionally silent, and DNase I insensitive (Lieberman-Aiden et al., 2009; Zhang et al., 2012). Therefore, compartments relate to gene expression and, as such, are cell-type specific (Lieberman-Aiden et al., 2009; Zhang et al., 2012). Thus, compartments cannot be predicted solely on the basis of gene density or GC content. A and B compartments are made up of groups of large multi-Mb chromosomal domains (median size ~3 Mb in mice; see Figure 1G and the supplementary data within Dixon et al. [2012]) that are mostly located on the same chromosome but can also be on different chromosomes. The latter type of association probably occurs at the zone of intermingling between CTs (Branco and Pombo, 2006). These data reflect a general tendency for loci of similar genomic content and chromatin status to be proximal to each other, while keeping their distance from

loci with opposite status. Recently, a more detailed analysis of Hi-C data from mouse and human cells shows that there are not just two types of opposite chromatin states that associate in A and B compartments respectively, but that there is a continuum between them: chromatin domains preferentially associate with other domains of a comparable activity level (Imakaev et al., 2012).

Actively transcribed genes have been observed to colocalize at foci referred to as transcription factories (Cook, 1999; Iborra et al., 1996; Wansink et al., 1993). At these sites, groups of active genes, sometimes located on different chromosomes, are found to associate with foci enriched in RNA polymerase and other transcriptional regulators (Osborne et al., 2004). For example, work on globin genes in mouse erythroid cells shows that the transcription factor Klf1 is responsible for the colocalization of Klf1-responsive globin genes, located on different chromosomes, into transcription factories (Schoenfelder et al., 2010). A similar compartmentalization and foci formation has been found for early and late replicating regions (Ferreira et al., 1997; Ryba et al., 2010; Sadoni et al., 1999). The rather abstract A compartment identified by Hi-C probably reflects the physical association of actively transcribed genes. This has also been observed microscopically, as clustering of active genes near a variety of subnuclear foci, including transcription factories and possibly other types of structures such as nuclear speckles.

Given the many similarities between loci found in inactive B compartments, and LADs and NADs, it seems likely that B compartments reflect the clustering of loci at the NL and nucleoli. In addition, B compartments can reflect clustering of inactive chromatin at other subnuclear sites. For example, Polycomb group (PcG) proteins, transcriptional repressors that silence sets of genes through chromatin modifications and chromatin compaction, have been found to colocalize in *Drosophila* in specific nuclear foci termed PcG bodies (Saurin et al., 1998; Sparmann and van Lohuizen, 2006). In *Drosophila*, PcG proteins bind polycomb response elements (PREs) and form long-range interactions with repressed promoters; disruption of these interactions leads to a loss of colocalization with PcG bodies (Bantignies et al., 2011; Lanzuolo et al., 2007; Tolhuis et al., 2011). Although PcG domains provide an example of repressive compartmentalization in *Drosophila*, similar clustering of silent loci at subnuclear structures may occur in mammalian cells.

Topologically Associating Domains

Microscopy has revealed that CTs contain much smaller but structurally defined chromosomal domains (CDs) that are ~100 kb to several Mb in size (Cremer and Cremer, 2010). CDs have been observed at the borders of the CTs, where they project into the chromatin poor areas that separate chromosomes (Figure 1B) (Markaki et al., 2010). CDs appear as clumps of chromatin with an outer shell that contains gene-dense arrangements known as the perichromatin region (PR), which is a 100–200 nm wide area that is rich in ribonucleoprotein-containing perichromatin fibrils (Cmarko et al., 1999; Fakan and van Driel, 2007) (Figure 1C). Their small size suggests that CDs are not directly related to A and B compartments, which are typically much larger.

Recently, high-resolution Hi-C and 5C data led to the identification of small domains within larger A and B compartments in human, mouse, and *Drosophila* genomes (Dixon et al., 2012; Nora et al., 2012; Sexton et al., 2012) (Figure 1H). These domains, referred to as topologically associating domains (TADs), are characterized by pronounced long-range associations between loci located in the same domain, but less frequent interactions between loci located in adjacent domains. Thus, chromosomes are composed of a string of domains that are topologically separated from each other. TADs have a median size of 880 kb in mice, with a range of tens of kb to several Mb (Dixon et al., 2012). Interestingly, this is the same length scale as the microscopic CDs, suggesting that TADs might represent the same structures (Figures 1C, 1H, and 1I). Other structural features that were based on long-range interactions identified by 3C-based techniques and correlation analyses, such as chromatin globules (Baù et al., 2011), chroperons (Li et al., 2012), and enhancer-promoter units (Shen et al., 2012), have been described at this length scale. Although few reports have been published on the subject, we anticipate that these observed structures are descriptions of the same topological organization. This argues for a distinct, fundamental domainal organization of chromatin at the length scale of 100 kb–1 Mb. Interestingly, genes located within the same TAD tend to have coordinated dynamics of expression during differentiation, pointing to a role of TADs in coordinating the activity of groups of neighboring genes. Further evidence for a critical functional role of these domains in genome regulation is that CDs (and perhaps TADs) appear to correlate with units of DNA replication (Markaki et al., 2010).

Through the use of a modified genome-wide 3C approach in *Drosophila*, specific building blocks of 10–500 kb epigenetic domains have been described that are flanked by insulators and that seem to represent TADs (Sexton et al., 2012). Thus far, TADs or TAD-like structures have not been described in bacteria (Umbarger et al., 2011), yeast (Duan et al., 2010), or plants (Moissiard et al., 2012). It is unclear whether the larger plant chromosomes do allow for a TAD-like organization. It is also currently unknown whether TADs exist without the presence of compartments. TADs do, however, represent a feature of chromosome organization that is largely conserved across mammalian cell types (Dixon et al., 2012; Nora et al., 2012), in contrast to A and B compartments that are related to cell-type-specific gene expression. Consistently, TADs are separated by boundaries that appear to be genetically defined: deletion of a boundary region in the X chromosome inactivation center led to partial fusion and the two flanking TADs (Nora et al., 2012). Formal proof for a genetically defined boundary would require insertion of a boundary in the middle of a TAD and observation of the splitting of the TAD in two.

It is currently not clear what defines TAD boundary regions. These boundary regions are enriched in a number of features, including transcription start sites and binding sites for the CTCF protein. Finding CTCF at these boundaries is particularly intriguing given its role as an architectural protein implicated in both mediating and blocking long-range interactions (Phillips and Corces, 2009). However, the large majority of CTCF-bound sites are located within TADs, suggesting that they are not

sufficient for boundary function (Dixon et al., 2012; Nora et al., 2012). It has been proposed that CTCF-bound sites must recruit additional proteins, such as the cohesin complex (Parelho et al., 2008; Rubio et al., 2008; Wendt et al., 2008), to acquire boundary activity.

Connecting Genes and Regulatory Elements

The best-studied long-range interactions are those between genes and their distal regulatory elements, such as enhancers. Classical genetic studies of translocations and deletions have shown that distal regions, located at tens to hundreds of kilobases from a gene, affect the correct regulation of transcription, indicating that regulatory elements can exert their effect over large genomic distances (Kleinjan and van Heyningen, 2005). Several mechanisms have been proposed to explain this phenomenon of long-range gene regulation, including looping of chromatin, linking by large protein complexes, and tracking of regulatory complexes along the DNA toward the target genes, to name a few (Bulger and Groudine, 1999; Ptashne, 1986). The most widely supported looping model states that gene regulatory elements interact with promoters through direct protein interactions, while looping out intervening DNA (reviewed in Bulger and Groudine, 2011).

Much of the work on long-range interactions and DNA looping has been performed on the β-globin locus. At this locus, a strong and complex enhancer element, the locus control region (LCR), only loops to the globin promoter in cells expressing the *β-globin* gene (*HBB*), as shown by 3C and an alternative method, RNA tagging and recovery of associated proteins (RNA-TRAP) (Carter et al., 2002; Tolhuis et al., 2002). Tissue specificity of long-range looping was confirmed by Palstra et al., who showed that the LCR interacts with different globin genes during development when the locus switches from expressing fetal *γ-globin* to adult *β-globin* (Palstra et al., 2003). Many other examples of long-range interactions between genes and their cognate regulatory elements have subsequently been described, including looping in the *CFTR* locus (Gheldof et al., 2010; Ott et al., 2009), the *α-globin* locus (Vernimmen et al., 2007), the *c-MYC* locus (Wright et al., 2010), and the Th2 interleukin cluster (Spilianakis and Flavell, 2004). A recent comprehensive analysis of gene-element interactions throughout 30 Mb of the human genome showed that long-range looping between genes and distal elements is a general phenomenon (Sanyal et al., 2012). This study found abundant and tissue-specific looping interactions between gene promoters and distal enhancers and CTCF-bound elements. Genes can interact with multiple distal elements, and distal elements loop to multiple genes, suggesting complex three-dimensional interaction networks. To add further complexity, several studies have shown that distal gene regulatory elements interact not only with their target genes but also with other regulatory elements (Gheldof et al., 2010; Tolhuis et al., 2002). Clearly, gene regulation involves formation of intricate patterns of specific three-dimensional connections between and among gene promoters and sets of regulatory elements located up to hundreds of kilobases from each other in the linear genome.

While the mechanisms by which these long-range gene regulatory interactions are formed remain unclear, we do know that they are mediated by specific transcription factors and associated factors that bind these loci. By knockout of the specific transcription factors EKLF, GATA-1 or the GATA-1 interacting cofactor FOG-1, it was shown that the interaction between the *β-globin* promoter and enhancer could be disrupted or attenuated (Drissen et al., 2004; Vakoc et al., 2005). The involvement of specific, locally recruited protein complexes can explain at least in part why this class of long-range connections between distal loci is quite specific. How long-range interactions regulate gene expression is less clear. Recently, Deng et al., have shown that artificial formation of a looping interaction between the globin enhancer and the target promoter was sufficient to induce *β-globin* transcription (Deng et al., 2012). Possibly, long-range interactions help bring specific protein complexes to the gene promoter. Such complexes could help recruit RNA pol II or transcription elongation factors.

A recent high-resolution Hi-C experiment, as well as computational predictions based on correlated activity of regulatory elements and promoters across cell types, revealed that most specific long-range interactions between regulated promoters and enhancers can be found within the boundaries of TADs (Shen et al., 2012). However, this does not mean that within a TAD all promoters will loop to all enhancers, since looping interactions within these domains are still between specific pairs of loci, i.e., a specific interaction between an enhancer and its cognate target gene promoter. Many other regulatory elements located in between will also interact quite frequently due to their general proximity within the TAD, but in many cases these interactions frequencies are still below that of the specific interactions (Li et al., 2012; Sanyal et al., 2012) (Figure 2A).

The Unique Genomic Connectivity of the Single Cell

3C-based analyses present a single population-averaged view of the interaction probabilities of loci. These studies could be interpreted to mean that a reproducible and constant genome organization is present in all cells in a clonal population. In each such cell, loci connect to the same set of other loci at specific subnuclear structures, and to all their cognate distal gene regulatory elements. However, both experimental and theoretical considerations rule out such a simplistic model. First, when individual cells are studied in the microscope, a striking cell-to-cell diversity of subnuclear positioning of loci is observed (Parada et al., 2003; Walter et al., 2003). For instance, loci that are shown to be part of LADs do not associate with the nuclear periphery in each and every cell (Pickersgill et al., 2006). Similarly, pairs of loci found to be interacting in 3C-based analyses do so in only a fraction of cells (Miele et al., 2009; Noordermeer et al., 2011). Thus, every cell at a given moment in time is unique in the overall spatial arrangement of its chromosomes and the specific set of long-range chromosomal interactions. Second, comprehensive genome-wide chromatin interaction data sets can only be understood when tremendous heterogeneity is assumed in chromosome folding and positioning. Each locus is found to have some nonzero contact probability with almost every other locus in the genome, although some interactions are clearly more frequent than others. Given that each detected interaction is derived from an individual cell, one must conclude that the set of interactions at a given moment must be highly

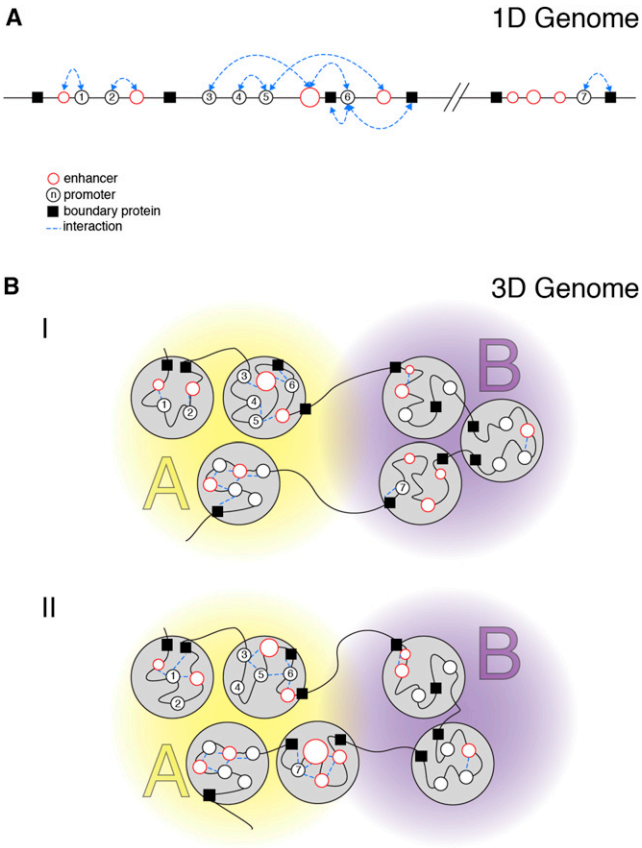

Figure 2. Genomic Interactions

Promoter (black) and enhancers (red) are represented as circles. The size of enhancers indicates the strength of their activity. Architectural boundary proteins are shown as black squares and interactions relevant to gene expression are shown as dotted blue lines.

(A) Linear representation of interactions between genomic elements.

(B) Three-dimensional representation of the genome, where interactions are largely confined to TADs (gray circles) and TADs containing elements of similar activity are arranged in compartments (A or B). Situation "I" represents the 3D organization of the linear genome depicted in (A). Situation "II" represents changing interactions (leading to altered expression) by stochastic cell-to-cell differences (for interactions with promoters 1–6) or increased enhancer activity, leading to altered promoter expression (for interactions with promoter 7) and compartment change. Note that the altered expression does not lead to a change in TAD organization.

different in each cell to allow for the detection of the large variety of interactions in the cell population (Kalhor et al., 2012; and see discussion in Zhang et al., 2012). In addition, many genes can interact with multiple distal regulatory elements, and these elements in turn interact with several genes (Sanyal et al., 2012; Shen et al., 2012). It is difficult to envision a single spatial organization that can accommodate all these long-range interactions simultaneously, and thus at any given time only some of these interactions can occur in a given cell. Yet, although a level of stochastic gene expression can be observed, individual cells within a clonal population manage to maintain a reproducible gene expression profile reflecting their tissue of origin (Liu et al., 2009). Therefore, it seems reasonable to assume that in each cell most or all genes are able to communicate with the appropriate distal gene regulatory elements and subnuclear structures, such as the lamina and transcription foci, to acquire appropriate levels of expression for maintenance of cell differentiation state. This apparent paradox can be solved when one considers that at various levels, the chromosomal connections described here, from large-scale nuclear organization to more local gene-element connections, differ in their ability to move inside cells (as also discussed in Soutoglou and Misteli, 2007). On the basis of such considerations, and as described below, we propose that TADs are the central chromosomal structures that ensure robust long-range gene regulation in every cell.

A Differential Dynamics Model of Chromosomal Connections

The chromosomes of humans and mice are very large physical objects. This severely limits their ability to move across the nucleus at time scales in the order of the length of an average cell cycle. Thus, in a given cell, once a chromosome has obtained a given position and has decondensed in early interphase, it is unlikely to move to another location. Therefore, at the level of the whole nucleus, the spatial organization of the genome is rather static. At this scale, the precise position of a chromosome varies widely between cells, making any connection or spatial proximity between two chromosomes extremely variable between cells. Yet, within a given cell, this arrangement will be highly stable over time. This directly implies that any particular interchromosomal connection is unlikely to play roles in robust gene regulation, as many cells will never be able to establish this connection in their lifetime. However, this does not rule out that these interactions contribute to gene regulation in a specific cell, and they might well contribute to known stochastic effects in gene expression (Noordermeer et al., 2011). Furthermore, this view also implies that in cases in which interchromosomal associations might play critical roles, these interactions must be established very early in G1, when chromosome positioning may still be dynamic. Alternatively, they might occur later, dependent upon yet-to-be-discovered special mechanisms that allow large-scale movement of chromosomes and chromosomal domains, as has been observed in rare cases (Chuang et al., 2006). For instance, rare rotational movements of CT assemblies have been observed that change chromatin proximity without affecting the radial position of CTs (Strickfaden et al., 2010).

Within chromosomes large domains of either active or inactive chromatin engage in a variety of interactions: some transcriptionally inactive domains (B compartments) will associate with the NL, whereas transcriptionally active domains (A compartments) will associate with other active domains at subnuclear sites such as transcription foci. The precise sets of active domains that associate with each other in any given cell will be variable, though, and because these domains are typically several megabases in size, their ability to move in a given cell will be limited. Thus, at this level of chromosome compartmentalization, interactions are again relatively stable within individual cells, whereas cell-to-cell variability is large (Figure 3). As for chromosome positioning, this implies that this level of chromosomal connections is unlikely to ensure specific gene expression in each individual cell, unless specific and yet-to-be-defined mechanisms exist to move large chromatin segments.

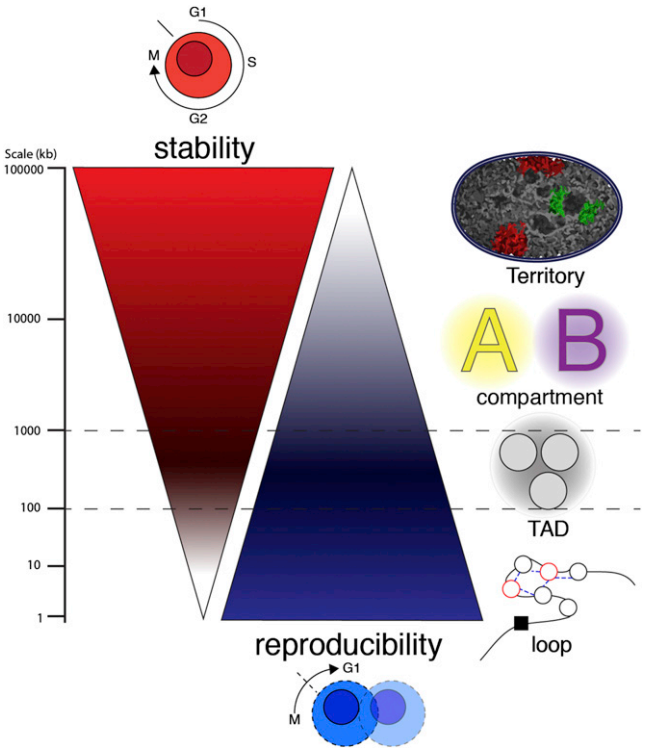

Figure 3. The Stability and Reproducibility of Chromosomal Interactions

Chromosomal territories and compartments are very stable within one cell cycle of a given cell, but they are unlikely to be reproduced from one cell cycle to the next. Conversely, interactions between loops (within TADs) will be unstable and variable within each cell cycle, but this "instability" is reproducible from one cell cycle to the next. At the junction between stability and reproducibility, TADs confine looping, while maintaining the possibility of compartmentalization.

Importantly, this is not to say that such stochastic yet stable interactions are not involved in gene regulation at all. It is possible that association with other active or inactive loci at specific nuclear sites, which occurs in each cell, is playing some role in efficiency of transcription or repression. This role is irrespective of the identity of the association partners, which is likely to differ between cells.

At the scale of up to several hundreds of kilobases, chromatin is considerably more dynamic: live-cell imaging shows that loci have a constrained radius of diffusion that is about 0.5 μm (Abney et al., 1997; Heun et al., 2001; Marshall et al., 1997). This volume corresponds to approximately 1 Mb chromatin. Thus, within the time span of a single cell cycle, any locus is likely to transiently interact by Brownian motion with other loci located within 1 Mb or so. At this length scale, interactions between loci are constrained by the formation of TADs. We propose that the observed motion represents the movement of loci within TADs and that any two loci located within a TAD are sufficiently dynamic to have an opportunity to engage in a long-range interaction. Importantly, this allows for the formation of long-range interactions between pairs of loci located within the same TAD in every cell at some point in time during a single cell cycle. At this length scale, the spatial positioning of two loci is therefore the least static within a given cell. This would ensure that specific gene-element interactions required for gene regulation can occur reproducibly, but transiently, in all cells. Consistent with our proposal that TADs are the equivalent to CDs, it was recently found that CDs are heavily oscillating within short time periods (<1 s) (Pliss et al., 2013) (creating stochastic changes exemplified in Figure 2B).

Organization of genes and regulatory elements within internally dynamic TADs also facilitates the formation of multiple long-range interactions between a gene and regulatory elements in each cell, even when these loops cannot topologically co-occur at the same time. Given that looping interactions are rather transient and loci can roam the entire volume of the TAD within a single cell cycle at short time scales, all pairwise looping interactions required for cell-type-specific gene expression could occur in each cell in the population.

The core implication of this view of genome folding is that the formation of TADs, and the mobility of loci within them, is the fundamental chromosomal feature that allows reproducible connections between genes and their distal elements in most cells. TADs provide a locally constrained volume in which genes and elements are provided ample mobility to connect and disconnect in order to facilitate gene regulation. This constrained volume also limits the number of possible interactions a gene or elements can have by insulating them from the rest of the genome. One other implication of this model is that there must be evolutionary pressure to maintain genes and their regulatory elements to remain near each other in the linear genome, within the same TAD. Our model predicts that TADs are the fundamental regulatory and structural building blocks of chromosomes that are stable between cells, but whose internal organization is highly dynamic within cells. Interestingly, smaller chromosomes such as those of yeast are at the same scale as TADs and behave in some respects like TADs: they are internally dynamic with loci exploring significant parts of the small nucleus within one cell cycle (Heun et al., 2001).

Higher-order chromosome structure and nuclear organization self-assembles using TADs as the basic building block: associations between TADs, located both in *cis* and in *trans*, form A and B compartments. In individual cells, these associations are more stable than the interactions within TADs, yet more stochastic between cells (Figure 3). Similarly, groups of A and B compartments make up chromosome territories and the zones where neighboring chromosome intermingle. Territory shape and position are rather stable, and again stochastic. Interestingly, both "stable" compartments and "reproducible" looping interactions are inherently stochastic, but at a different level and time scale. The increasingly stochastic nature of connections between and along chromosomes at larger length scales may contribute to cell-to-cell variation in gene expression. In the future, when chromatin interaction studies can be performed in single cells, some of the ideas proposed here can be directly tested. For instance, direct analysis of both the gene expression profile and genome-wide long-range chromatin interactions can provide insights into the relationship between stochastic gene expression and cell-to-cell variability of chromosome conformation.

ACKNOWLEDGMENTS

We thank the members of the Dekker lab for critically reviewing the manuscript. This work was supported by a Rubicon grant from the Netherlands Organization for Scientific Research and a Dutch Cancer Society Fellowship to J.H.G. and by grants from the National Institutes of Health, National Human Genome Research Institute (HG003143 and HG004592), and the Human Frontier Science Program and a W.M. Keck Foundation distinguished young scholar in medical research grant to J.D.

REFERENCES

Abney, J.R., Cutler, B., Fillbach, M.L., Axelrod, D., and Scalettar, B.A. (1997). Chromatin dynamics in interphase nuclei and its implications for nuclear structure. J. Cell Biol. *137*, 1459–1468.

Bantignies, F., Roure, V., Comet, I., Leblanc, B., Schuettengruber, B., Bonnet, J., Tixier, V., Mas, A., and Cavalli, G. (2011). Polycomb-dependent regulatory contacts between distant Hox loci in Drosophila. Cell *144*, 214–226.

Baù, D., and Marti-Renom, M.A. (2011). Structure determination of genomic domains by satisfaction of spatial restraints. Chromosome Res. *19*, 25–35.

Baù, D., Sanyal, A., Lajoie, B.R., Capriotti, E., Byron, M., Lawrence, J.B., Dekker, J., and Marti-Renom, M.A. (2011). The three-dimensional folding of the α-globin gene domain reveals formation of chromatin globules. Nat. Struct. Mol. Biol. *18*, 107–114.

Belton, J.-M., McCord, R.P., Gibcus, J.H., Naumova, N., Zhan, Y., and Dekker, J. (2012). Hi-C: a comprehensive technique to capture the conformation of genomes. Methods *58*, 268–276.

Bertrand, E., Houser-Scott, F., Kendall, A., Singer, R.H., and Engelke, D.R. (1998). Nucleolar localization of early tRNA processing. Genes Dev. *12*, 2463–2468.

Bohn, M., and Heermann, D.W. (2010). Diffusion-driven looping provides a consistent framework for chromatin organization. PLoS ONE *5*, e12218.

Branco, M.R., and Pombo, A. (2006). Intermingling of chromosome territories in interphase suggests role in translocations and transcription-dependent associations. PLoS Biol. *4*, e138.

Brown, C.R., Kennedy, C.J., Delmar, V.A., Forbes, D.J., and Silver, P.A. (2008). Global histone acetylation induces functional genomic reorganization at mammalian nuclear pore complexes. Genes Dev. *22*, 627–639.

Bulger, M., and Groudine, M. (1999). Looping versus linking: toward a model for long-distance gene activation. Genes Dev. *13*, 2465–2477.

Bulger, M., and Groudine, M. (2011). Functional and mechanistic diversity of distal transcription enhancers. Cell *144*, 327–339.

Capelson, M., Liang, Y., Schulte, R., Mair, W., Wagner, U., and Hetzer, M.W. (2010). Chromatin-bound nuclear pore components regulate gene expression in higher eukaryotes. Cell *140*, 372–383.

Carter, D., Chakalova, L., Osborne, C.S., Dai, Y.F., and Fraser, P. (2002). Long-range chromatin regulatory interactions in vivo. Nat. Genet. *32*, 623–626.

Chuang, C.H., Carpenter, A.E., Fuchsova, B., Johnson, T., de Lanerolle, P., and Belmont, A.S. (2006). Long-range directional movement of an interphase chromosome site. Curr. Biol. *16*, 825–831.

Cmarko, D., Verschure, P.J., Martin, T.E., Dahmus, M.E., Krause, S., Fu, X.D., van Driel, R., and Fakan, S. (1999). Ultrastructural analysis of transcription and splicing in the cell nucleus after bromo-UTP microinjection. Mol. Biol. Cell *10*, 211–223.

Cook, P.R. (1999). The organization of replication and transcription. Science *284*, 1790–1795.

Cremer, T., and Cremer, M. (2010). Chromosome territories. Cold Spring Harb. Perspect. Biol. *2*, a003889.

Croft, J.A., Bridger, J.M., Boyle, S., Perry, P., Teague, P., and Bickmore, W.A. (1999). Differences in the localization and morphology of chromosomes in the human nucleus. J. Cell Biol. *145*, 1119–1131.

Dekker, J., Rippe, K., Dekker, M., and Kleckner, N. (2002). Capturing chromosome conformation. Science *295*, 1306–1311.

Deng, W., Lee, J., Wang, H., Miller, J., Reik, A., Gregory, P.D., Dean, A., and Blobel, G.A. (2012). Controlling long-range genomic interactions at a native locus by targeted tethering of a looping factor. Cell *149*, 1233–1244.

Dingwall, C., and Laskey, R. (1992). The nuclear membrane. Science *258*, 942–947.

Dixon, J.R., Selvaraj, S., Yue, F., Kim, A., Li, Y., Shen, Y., Hu, M., Liu, J.S., and Ren, B. (2012). Topological domains in mammalian genomes identified by analysis of chromatin interactions. Nature *485*, 376–380.

Dostie, J., Richmond, T.A., Arnaout, R.A., Selzer, R.R., Lee, W.L., Honan, T.A., Rubio, E.D., Krumm, A., Lamb, J., Nusbaum, C., et al. (2006). Chromosome Conformation Capture Carbon Copy (5C): a massively parallel solution for mapping interactions between genomic elements. Genome Res. *16*, 1299–1309.

Drissen, R., Palstra, R.J., Gillemans, N., Splinter, E., Grosveld, F., Philipsen, S., and de Laat, W. (2004). The active spatial organization of the beta-globin locus requires the transcription factor EKLF. Genes Dev. *18*, 2485–2490.

Duan, Z., Andronescu, M., Schutz, K., McIlwain, S., Kim, Y.J., Lee, C., Shendure, J., Fields, S., Blau, C.A., and Noble, W.S. (2010). A three-dimensional model of the yeast genome. Nature *465*, 363–367.

Fakan, S., and van Driel, R. (2007). The perichromatin region: a functional compartment in the nucleus that determines large-scale chromatin folding. Semin. Cell Dev. Biol. *18*, 676–681.

Ferreira, J., Paolella, G., Ramos, C., and Lamond, A.I. (1997). Spatial organization of large-scale chromatin domains in the nucleus: a magnified view of single chromosome territories. J. Cell Biol. *139*, 1597–1610.

Flemming, W. (1965). Contributions to the knowledge of the cell and its vital processes. J. Cell Biol. *25*, 3–69.

Fraser, P., and Bickmore, W. (2007). Nuclear organization of the genome and the potential for gene regulation. Nature *447*, 413–417.

Fudenberg, G., and Mirny, L.A. (2012). Higher-order chromatin structure: bridging physics and biology. Curr. Opin. Genet. Dev. *22*, 115–124.

Gheldof, N., Smith, E.M., Tabuchi, T.M., Koch, C.M., Dunham, I., Stamatoyannopoulos, J.A., and Dekker, J. (2010). Cell-type-specific long-range looping interactions identify distant regulatory elements of the CFTR gene. Nucleic Acids Res. *38*, 4325–4336.

Goldman, R.D., Gruenbaum, Y., Moir, R.D., Shumaker, D.K., and Spann, T.P. (2002). Nuclear lamins: building blocks of nuclear architecture. Genes Dev. *16*, 533–547.

Guelen, L., Pagie, L., Brasset, E., Meuleman, W., Faza, M.B., Talhout, W., Eussen, B.H., de Klein, A., Wessels, L., de Laat, W., and van Steensel, B. (2008). Domain organization of human chromosomes revealed by mapping of nuclear lamina interactions. Nature *453*, 948–951.

Hakim, O., and Misteli, T. (2012). SnapShot: Chromosome confirmation capture. Cell *148*, 1068.e1–1068.e2.

Heun, P., Laroche, T., Shimada, K., Furrer, P., and Gasser, S.M. (2001). Chromosome dynamics in the yeast interphase nucleus. Science *294*, 2181–2186.

Huang, S., Deerinck, T.J., Ellisman, M.H., and Spector, D.L. (1997). The dynamic organization of the perinucleolar compartment in the cell nucleus. J. Cell Biol. *137*, 965–974.

Iborra, F.J., Pombo, A., Jackson, D.A., and Cook, P.R. (1996). Active RNA polymerases are localized within discrete transcription "factories" in human nuclei. J. Cell Sci. *109*, 1427–1436.

Imakaev, M., Fudenberg, G., McCord, R.P., Naumova, N., Goloborodko, A., Lajoie, B.R., Dekker, J., and Mirny, L.A. (2012). Iterative correction of Hi-C data reveals hallmarks of chromosome organization. Nat. Methods *9*, 999–1003.

Kalhor, R., Tjong, H., Jayathilaka, N., Alber, F., and Chen, L. (2012). Genome architectures revealed by tethered chromosome conformation capture and population-based modeling. Nat. Biotechnol. *30*, 90–98.

Kendall, A., Hull, M.W., Bertrand, E., Good, P.D., Singer, R.H., and Engelke, D.R. (2000). A CBF5 mutation that disrupts nucleolar localization of early tRNA biosynthesis in yeast also suppresses tRNA gene-mediated transcriptional silencing. Proc. Natl. Acad. Sci. USA *97*, 13108–13113.

Kind, J., and van Steensel, B. (2010). Genome-nuclear lamina interactions and gene regulation. Curr. Opin. Cell Biol. *22*, 320–325.

Kleinjan, D.A., and van Heyningen, V. (2005). Long-range control of gene expression: emerging mechanisms and disruption in disease. Am. J. Hum. Genet. *76*, 8–32.

Lander, E.S., Linton, L.M., Birren, B., Nusbaum, C., Zody, M.C., Baldwin, J., Devon, K., Dewar, K., Doyle, M., FitzHugh, W., et al.; International Human Genome Sequencing Consortium. (2001). Initial sequencing and analysis of the human genome. Nature *409*, 860–921.

Lanzuolo, C., Roure, V., Dekker, J., Bantignies, F., and Orlando, V. (2007). Polycomb response elements mediate the formation of chromosome higher-order structures in the bithorax complex. Nat. Cell Biol. *9*, 1167–1174.

Li, G., Ruan, X., Auerbach, R.K., Sandhu, K.S., Zheng, M., Wang, P., Poh, H.M., Goh, Y., Lim, J., Zhang, J., et al. (2012). Extensive promoter-centered chromatin interactions provide a topological basis for transcription regulation. Cell *148*, 84–98.

Lichter, P., Cremer, T., Borden, J., Manuelidis, L., and Ward, D.C. (1988). Delineation of individual human chromosomes in metaphase and interphase cells by in situ suppression hybridization using recombinant DNA libraries. Hum. Genet. *80*, 224–234.

Lieberman-Aiden, E., van Berkum, N.L., Williams, L., Imakaev, M., Ragoczy, T., Telling, A., Amit, I., Lajoie, B.R., Sabo, P.J., Dorschner, M.O., et al. (2009). Comprehensive mapping of long-range interactions reveals folding principles of the human genome. Science *326*, 289–293.

Liu, X., Long, F., Peng, H., Aerni, S.J., Jiang, M., Sánchez-Blanco, A., Murray, J.I., Preston, E., Mericle, B., Batzoglou, S., et al. (2009). Analysis of cell fate from single-cell gene expression profiles in C. elegans. Cell *139*, 623–633.

Markaki, Y., Gunkel, M., Schermelleh, L., Beichmanis, S., Neumann, J., Heidemann, M., Leonhardt, H., Eick, D., Cremer, C., and Cremer, T. (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.

Marshall, W.F., Straight, A., Marko, J.F., Swedlow, J., Dernburg, A., Belmont, A., Murray, A.W., Agard, D.A., and Sedat, J.W. (1997). Interphase chromosomes undergo constrained diffusional motion in living cells. Curr. Biol. *7*, 930–939.

Miele, A., and Dekker, J. (2008). Long-range chromosomal interactions and gene regulation. Mol. Biosyst. *4*, 1046–1057.

Miele, A., Bystricky, K., and Dekker, J. (2009). Yeast silent mating type loci form heterochromatic clusters through silencer protein-dependent long-range interactions. PLoS Genet. *5*, e1000478.

Misteli, T. (2007). Beyond the sequence: cellular organization of genome function. Cell *128*, 787–800.

Misteli, T. (2010). Higher-order genome organization in human disease. Cold Spring Harb. Perspect. Biol. *2*, a000794.

Moissiard, G., Cokus, S.J., Cary, J., Feng, S., Billi, A.C., Stroud, H., Husmann, D., Zhan, Y., Lajoie, B.R., McCord, R.P., et al. (2012). MORC family ATPases required for heterochromatin condensation and gene silencing. Science *336*, 1448–1451.

Naumova, N., and Dekker, J. (2010). Integrating one-dimensional and three-dimensional maps of genomes. J. Cell Sci. *123*, 1979–1988.

Németh, A., Conesa, A., Santoyo-Lopez, J., Medina, I., Montaner, D., Péterfia, B., Solovei, I., Cremer, T., Dopazo, J., and Längst, G. (2010). Initial genomics of the human nucleolus. PLoS Genet. *6*, e1000889.

Noordermeer, D., de Wit, E., Klous, P., van de Werken, H., Simonis, M., Lopez-Jones, M., Eussen, B., de Klein, A., Singer, R.H., and de Laat, W. (2011). Variegated gene expression caused by cell-specific long-range DNA interactions. Nat. Cell Biol. *13*, 944–951.

Nora, E.P., Lajoie, B.R., Schulz, E.G., Giorgetti, L., Okamoto, I., Servant, N., Piolot, T., van Berkum, N.L., Meisig, J., Sedat, J., et al. (2012). Spatial partitioning of the regulatory landscape of the X-inactivation centre. Nature *485*, 381–385.

Orlova, D.Y., Stixová, L., Kozubek, S., Gierman, H.J., Šustáčková, G., Chernyshev, A.V., Medvedev, R.N., Legartová, S., Versteeg, R., Matula, P., et al. (2012). Arrangement of nuclear structures is not transmitted through mitosis but is identical in sister cells. J. Cell. Biochem. *113*, 3313–3329.

Osborne, C.S., Chakalova, L., Brown, K.E., Carter, D., Horton, A., Debrand, E., Goyenechea, B., Mitchell, J.A., Lopes, S., Reik, W., and Fraser, P. (2004). Active genes dynamically colocalize to shared sites of ongoing transcription. Nat. Genet. *36*, 1065–1071.

Ott, C.J., Blackledge, N.P., Kerschner, J.L., Leir, S.H., Crawford, G.E., Cotton, C.U., and Harris, A. (2009). Intronic enhancers coordinate epithelial-specific looping of the active CFTR locus. Proc. Natl. Acad. Sci. USA *106*, 19934–19939.

Palstra, R.J., Tolhuis, B., Splinter, E., Nijmeijer, R., Grosveld, F., and de Laat, W. (2003). The beta-globin nuclear compartment in development and erythroid differentiation. Nat. Genet. *35*, 190–194.

Parada, L.A., Roix, J.J., and Misteli, T. (2003). An uncertainty principle in chromosome positioning. Trends Cell Biol. *13*, 393–396.

Parelho, V., Hadjur, S., Spivakov, M., Leleu, M., Sauer, S., Gregson, H.C., Jarmuz, A., Canzonetta, C., Webster, Z., Nesterova, T., et al. (2008). Cohesins functionally associate with CTCF on mammalian chromosome arms. Cell *132*, 422–433.

Peric-Hupkes, D., Meuleman, W., Pagie, L., Bruggeman, S.W., Solovei, I., Brugman, W., Gräf, S., Flicek, P., Kerkhoven, R.M., van Lohuizen, M., et al. (2010). Molecular maps of the reorganization of genome-nuclear lamina interactions during differentiation. Mol. Cell *38*, 603–613.

Phillips, J.E., and Corces, V.G. (2009). CTCF: master weaver of the genome. Cell *137*, 1194–1211.

Pickersgill, H., Kalverda, B., de Wit, E., Talhout, W., Fornerod, M., and van Steensel, B. (2006). Characterization of the Drosophila melanogaster genome at the nuclear lamina. Nat. Genet. *38*, 1005–1014.

Pinkel, D., Landegent, J., Collins, C., Fuscoe, J., Segraves, R., Lucas, J., and Gray, J. (1988). Fluorescence in situ hybridization with human chromosome-specific libraries: detection of trisomy 21 and translocations of chromosome 4. Proc. Natl. Acad. Sci. USA *85*, 9138–9142.

Pliss, A., Malyavantham, K.S., Bhattacharya, S., and Berezney, R. (2013). Chromatin dynamics in living cells: identification of oscillatory motion. J. Cell. Physiol. *228*, 609–616.

Ptashne, M. (1986). Gene regulation by proteins acting nearby and at a distance. Nature *322*, 697–701.

Rodley, C.D., Bertels, F., Jones, B., and O'Sullivan, J.M. (2009). Global identification of yeast chromosome interactions using Genome conformation capture. Fungal Genet. Biol. *46*, 879–886.

Rosa, A., and Everaers, R. (2008). Structure and dynamics of interphase chromosomes. PLoS Comput. Biol. *4*, e1000153.

Rubio, E.D., Reiss, D.J., Welcsh, P.L., Disteche, C.M., Filippova, G.N., Baliga, N.S., Aebersold, R., Ranish, J.A., and Krumm, A. (2008). CTCF physically links cohesin to chromatin. Proc. Natl. Acad. Sci. USA *105*, 8309–8314.

Ryba, T., Hiratani, I., Lu, J., Itoh, M., Kulik, M., Zhang, J., Schulz, T.C., Robins, A.J., Dalton, S., and Gilbert, D.M. (2010). Evolutionarily conserved replication timing profiles predict long-range chromatin interactions and distinguish closely related cell types. Genome Res. *20*, 761–770.

Sadoni, N., Langer, S., Fauth, C., Bernardi, G., Cremer, T., Turner, B.M., and Zink, D. (1999). Nuclear organization of mammalian genomes. Polar chromosome territories build up functionally distinct higher order compartments. J. Cell Biol. *146*, 1211–1226.

Sanyal, A., Lajoie, B.R., Jain, G., and Dekker, J. (2012). The long-range interaction landscape of gene promoters. Nature *489*, 109–113.

Saurin, A.J., Shiels, C., Williamson, J., Satijn, D.P., Otte, A.P., Sheer, D., and Freemont, P.S. (1998). The human polycomb group complex associates with pericentromeric heterochromatin to form a novel nuclear domain. J. Cell Biol. *142*, 887–898.

Schermelleh, L., Heintzmann, R., and Leonhardt, H. (2010). A guide to super-resolution fluorescence microscopy. J. Cell Biol. *190*, 165–175.

Schoenfelder, S., Sexton, T., Chakalova, L., Cope, N.F., Horton, A., Andrews, S., Kurukuti, S., Mitchell, J.A., Umlauf, D., Dimitrova, D.S., et al. (2010). Preferential associations between co-regulated genes reveal a transcriptional interactome in erythroid cells. Nat. Genet. *42*, 53–61.

Sexton, T., Yaffe, E., Kenigsberg, E., Bantignies, F., Leblanc, B., Hoichman, M., Parrinello, H., Tanay, A., and Cavalli, G. (2012). Three-dimensional folding and functional organization principles of the Drosophila genome. Cell *148*, 458–472.

Shen, Y., Yue, F., McCleary, D.F., Ye, Z., Edsall, L., Kuan, S., Wagner, U., Dixon, J., Lee, L., Lobanenkov, V.V., and Ren, B. (2012). A map of the cis-regulatory sequences in the mouse genome. Nature *488*, 116–120.

Simonis, M., Klous, P., Splinter, E., Moshkin, Y., Willemsen, R., de Wit, E., van Steensel, B., and de Laat, W. (2006). Nuclear organization of active and inactive chromatin domains uncovered by chromosome conformation capture-on-chip (4C). Nat. Genet. *38*, 1348–1354.

Soutoglou, E., and Misteli, T. (2007). Mobility and immobility of chromatin in transcription and genome stability. Curr. Opin. Genet. Dev. *17*, 435–442.

Sparmann, A., and van Lohuizen, M. (2006). Polycomb silencers control cell fate, development and cancer. Nat. Rev. Cancer *6*, 846–856.

Spilianakis, C.G., and Flavell, R.A. (2004). Long-range intrachromosomal interactions in the T helper type 2 cytokine locus. Nat. Immunol. *5*, 1017–1027.

Splinter, E., de Wit, E., van de Werken, H.J., Klous, P., and de Laat, W. (2012). Determining long-range chromatin interactions for selected genomic sites using 4C-seq technology: from fixation to computation. Methods *58*, 221–230.

Strickfaden, H., Zunhammer, A., van Koningsbruggen, S., Köhler, D., and Cremer, T. (2010). 4D chromatin dynamics in cycling cells: Theodor Boveri's hypotheses revisited. Nucleus *1*, 284–297.

Tanabe, H., Müller, S., Neusser, M., von Hase, J., Calcagno, E., Cremer, M., Solovei, I., Cremer, C., and Cremer, T. (2002). Evolutionary conservation of chromosome territory arrangements in cell nuclei from higher primates. Proc. Natl. Acad. Sci. USA *99*, 4424–4429.

Thompson, M., Haeusler, R.A., Good, P.D., and Engelke, D.R. (2003). Nucleolar clustering of dispersed tRNA genes. Science *302*, 1399–1401.

Tolhuis, B., Palstra, R.J., Splinter, E., Grosveld, F., and de Laat, W. (2002). Looping and interaction between hypersensitive sites in the active beta-globin locus. Mol. Cell *10*, 1453–1465.

Tolhuis, B., Blom, M., Kerkhoven, R.M., Pagie, L., Teunissen, H., Nieuwland, M., Simonis, M., de Laat, W., van Lohuizen, M., and van Steensel, B. (2011). Interactions among Polycomb domains are guided by chromosome architecture. PLoS Genet. *7*, e1001343.

Umbarger, M.A., Toro, E., Wright, M.A., Porreca, G.J., Baù, D., Hong, S.H., Fero, M.J., Zhu, L.J., Marti-Renom, M.A., McAdams, H.H., et al. (2011). The three-dimensional architecture of a bacterial genome and its alteration by genetic perturbation. Mol. Cell *44*, 252–264.

Vakoc, C.R., Letting, D.L., Gheldof, N., Sawado, T., Bender, M.A., Groudine, M., Weiss, M.J., Dekker, J., and Blobel, G.A. (2005). Proximity among distant regulatory elements at the beta-globin locus requires GATA-1 and FOG-1. Mol. Cell *17*, 453–462.

van Koningsbruggen, S., Gierlinski, M., Schofield, P., Martin, D., Barton, G.J., Ariyurek, Y., den Dunnen, J.T., and Lamond, A.I. (2010). High-resolution whole-genome sequencing reveals that specific chromatin domains from most human chromosomes associate with nucleoli. Mol. Biol. Cell *21*, 3735–3748.

van Steensel, B., and Dekker, J. (2010). Genomics tools for unraveling chromosome architecture. Nat. Biotechnol. *28*, 1089–1095.

Venter, J.C., Adams, M.D., Myers, E.W., Li, P.W., Mural, R.J., Sutton, G.G., Smith, H.O., Yandell, M., Evans, C.A., Holt, R.A., et al. (2001). The sequence of the human genome. Science *291*, 1304–1351.

Vernimmen, D., De Gobbi, M., Sloane-Stanley, J.A., Wood, W.G., and Higgs, D.R. (2007). Long-range chromosomal interactions regulate the timing of the transition between poised and active gene expression. EMBO J. *26*, 2041–2051.

Walter, J., Schermelleh, L., Cremer, M., Tashiro, S., and Cremer, T. (2003). Chromosome order in HeLa cells changes during mitosis and early G1, but is stably maintained during subsequent interphase stages. J. Cell Biol. *160*, 685–697.

Wansink, D.G., Schul, W., van der Kraan, I., van Steensel, B., van Driel, R., and de Jong, L. (1993). Fluorescent labeling of nascent RNA reveals transcription by RNA polymerase II in domains scattered throughout the nucleus. J. Cell Biol. *122*, 283–293.

Wendt, K.S., Yoshida, K., Itoh, T., Bando, M., Koch, B., Schirghuber, E., Tsutsumi, S., Nagae, G., Ishihara, K., Mishiro, T., et al. (2008). Cohesin mediates transcriptional insulation by CCCTC-binding factor. Nature *451*, 796–801.

Wilson, E.B. (1925). The Cell in Development and Heredity, Third Edition (New York: The Macmillan Company).

Wright, J.B., Brown, S.J., and Cole, M.D. (2010). Upregulation of c-MYC in cis through a large chromatin loop linked to a cancer risk-associated single-nucleotide polymorphism in colorectal cancer cells. Mol. Cell. Biol. *30*, 1411–1420.

Würtele, H., and Chartrand, P. (2006). Genome-wide scanning of HoxB1-associated loci in mouse ES cells using an open-ended Chromosome Conformation Capture methodology. Chromosome Res. *14*, 477–495.

Zhang, Y., McCord, R.P., Ho, Y.J., Lajoie, B.R., Hildebrand, D.G., Simon, A.C., Becker, M.S., Alt, F.W., and Dekker, J. (2012). Spatial organization of the mouse genome and its role in recurrent chromosomal translocations. Cell *148*, 908–921.

Zhao, Z., Tavoosidana, G., Sjölinder, M., Göndör, A., Mariano, P., Wang, S., Kanduri, C., Lezcano, M., Sandhu, K.S., Singh, U., et al. (2006). Circular chromosome conformation capture (4C) uncovers extensive networks of epigenetically regulated intra- and interchromosomal interactions. Nat. Genet. *38*, 1341–1347.