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Chromosomes at Work: Organization of Chromosome Territories in the Interphase Nucleus

Andrew J. Fritz,[1] A. Rasim Barutcu,[2] Lori Martin-Buley,[1] André J. van Wijnen,[3] Sayyed K. Zaidi,[1] Anthony N. Imbalzano,[2] Jane B. Lian,[1] Janet L. Stein,[1] and Gary S. Stein[1]*

[1]*Department of Biochemistry, University of Vermont Cancer Center, University of Vermont College of Medicine, 89 Beaumont Avenue, Burlington 05405, Vermont*

[2]*Department of Cell and Developmental Biology, University of Massachusetts Medical School, 55 Lake Avenue North, Worcester 01655, Massachussetts*

[3]*Departments of Orthopedic Surgery and Biochemistry & Molecular Biology, Mayo Clinic, Rochester, Minnesota*

ABSTRACT

The organization of interphase chromosomes in chromosome territories (CTs) was first proposed more than one hundred years ago. The introduction of increasingly sophisticated microscopic and molecular techniques, now provide complementary strategies for studying CTs in greater depth than ever before. Here we provide an overview of these strategies and how they are being used to elucidate CT interactions and the role of these dynamically regulated, nuclear-structure building blocks in directly supporting nuclear function in a physiologically responsive manner. J. Cell. Biochem. 117: 9–19, 2016.

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Genomic organization is coordinated by nuclear processes that include DNA replication, chromatin remodeling, RNA transcription and splicing. In the disease state, the frequent dysfunction and compromised organization of regulatory machinery suggests a strong role of genomic organization in pathology. Although the nucleus is not subdivided by membrane-bound compartments, the regulatory machinery for transcription, replication, and repair are architecturally organized in subnuclear domains with specific functions. This compartmentalization supports a direct relationship between nuclear structure and function that is dynamically regulated in a physiologically responsive manner. Prominent domains within the nucleus, such as the nucleolus and heterochromatin, were identified long before their functions were elucidated. Other architectural components of the nucleus, including the nuclear matrix, nuclear lamina, Cajal bodies, speckles, and the Barr body were identified once advances in microscopy became available and molecular approaches were introduced [Mehta et al., 2007; Hemmerich et al., 2011; Zhu and Brangwynne, 2015].

During mitosis, the genome is organized into easily recognized X-shaped chromosomes; however, this configuration does not persist during the G1, S, and G2 phases of the cell cycle. During interphase, the genome, like the other nuclear domains, is organized in discrete bodies. Chromosomes relax into nuclear domains that are referred to as chromosome territories (CTs; Fig. 1A), [Zorn et al., 1976; Stack et al., 1977; Zorn et al., 1979; Manuelidis, 1985; Schardin et al., 1985; Cremer et al., 1981, 2006; Lichter et al., 1988; Cremer and Cremer, 2001]. This realization that genomic structure and function are dynamic and highly integrated has been driven by technologies that include fluorescence in situ hybridization (FISH, [Solovei and Cremer, 2010]) and, more recently, chromatin conformation capture (3C) techniques [de Wit and de Laat, 2012; Barutcu et al., 2015]. Here, we provide an overview of microscopic and molecular techniques that have advanced understanding of nuclear organization and a synopsis of the role of CTs in genomic organization and expression.

ORIGINS AND REESTABLISHMENT OF THE CHROMOSOME TERRITORY PARADIGM

The existence of CTs was first suggested by Theodor Boveri in 1885 and was supported by Carl Rabl in 1909 [Cremer and Cremer, 2010]. These early observations and predictions of genome

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*Correspondence to: Gary S. Stein, Department of Biochemistry, University of Vermont Cancer Center, University of Vermont College of Medicine, 89 Beaumont Avenue, Burlington 05405, Vermont. E-mail: gary.stein@uvm.edu
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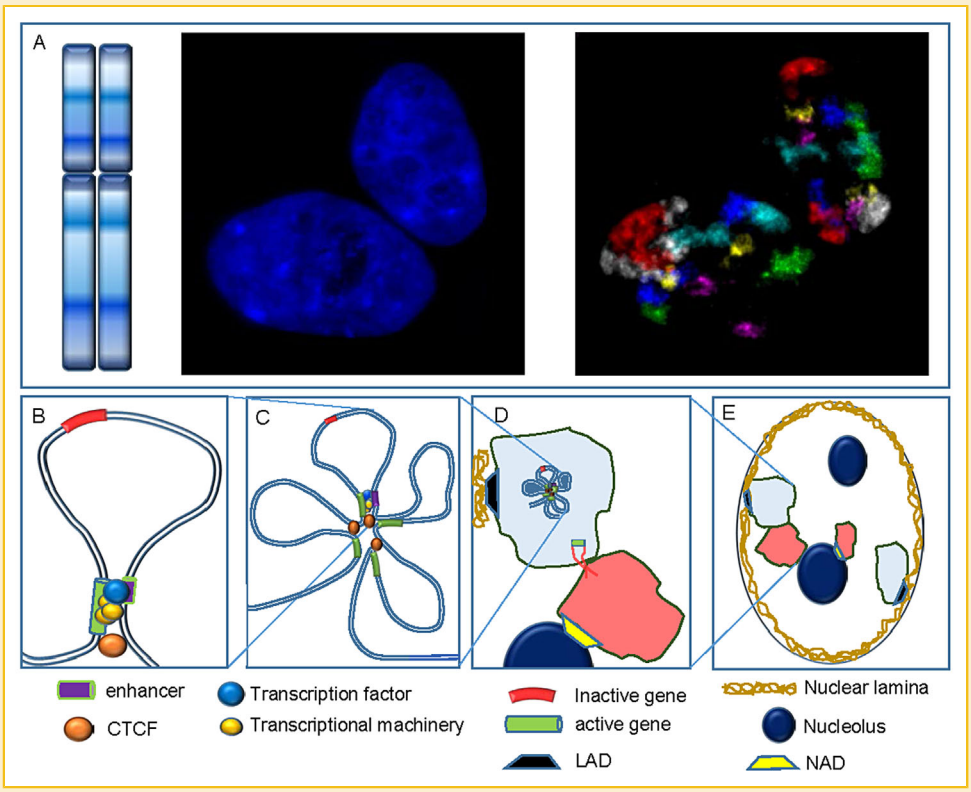

Fig. 1. A spectrum of increasingly complex chromatin conformations impacting gene expression. Whereas mitotic chromosomes are condensed into broadly recognized X-shaped entities, during interphase they relax and occupy distinct domains within the nucleus termed chromosome territories (CT). Seven CTs labeled with fluorescence in situ hybridization are shown (A). Within chromosomes, the configuration is coordinated with gene expression. Looping of regulatory elements within individual genes is essential for their expression mediated through transcription factor binding and CTCF (B). Moreover, actively expressed genes cluster in what are known as active chromatin hubs (C). Genes have also been shown to extend out of their main CT bodies in order to form interchromosomal interactions with coregulated genes (D). These interactions are mediated in part through interactions with other nuclear structures, such as the nucleoli and the nuclear lamina via lamina associated domains (LADs) or nucleolar associated domains (NADs, D–E).

compartmentalization in the interphase nucleus were superseded when electron microscopy became available and evidence of chromosome intermingling during interphase was directly observed. This led to the "spaghetti" model of the interphase nucleus that persisted for decades—in which chromatin fibers from different chromosomes are interwoven. It was not until the late 1970s that the concept of chromosome territories began to re-emerge due to compelling evidence from several lines of inquiry [Cremer and Cremer, 2010]. One such line utilized a laser microbeam to inflict DNA damage in a small region within the nucleus and then determine whether the damaged DNA was distributed throughout the genome or was localized to specific chromosomes. Investigators found that the damage was confined to just a few chromosomes, and that the reverse experiment, in which laser-UV-microirradiation was used to cause damage to a small portion of the metaphase plate produced daughter-cell nuclei with mirror-image DNA-damage visualized in interphase; a finding that strongly supports the CT model [Zorn et al., 1976, 1979]. The development of chromosome paints that consist of fluorescent-dye labeled chromosome-specific probes enabled direct visualization of individual chromosomes in interphase nuclei [Manuelidis, 1985; Schardin et al., 1985; Lichter et al., 1988]. These findings were further corroborated by experiments in which fluorescent nucleotide analogs were used to uniformly pulse-label replicating DNA. After several rounds of replication/independent assortment of chromosomes, individual CTs could be visualized in live cells [Zink and Cremer, 1998; Visser and Aten, 1999]. Within these CT, subdomains were proposed to occur via 6–12 chromatin loops of approximately 50–200 kb arranged in a rosette pattern composed of approximately 1 Mbp of DNA [Jackson and Pombo, 1998; Ma et al., 1998; Berezney, 2002]. The data obtained using chromatin capture techniques has extended this concept to include more precise characterization of the intra-chromosomal interactions defined by so-called topologically associating domains (TADs) that represent discrete domains of relatively high DNA-contact frequencies [Dekker et al., 2013; Barutcu et al., 2015]. Chromosome organization in CTs during interphase is now widely accepted in the scientific community.

FISH AND CHROMATIN CONFORMATION CAPTURE—COMPLEMENTARY APPROACHES FOR CHROMOSOME TERRITORY DELINEATION

Currently, two main techniques are used to study chromosome territory organization: fluorescence in situ hybridization (FISH) and

chromatin conformation capture. FISH is a microscopic method to visualize specific DNA or RNA sequences within the nucleus by hybridizing complementary fluorescent-dye labeled DNA or RNA probes. Chromatin conformation capture comprises several related molecular biology techniques that involve crosslinking nuclei to "capture" intra- and inter-chromosomal interactions, fragmenting DNA with restriction enzymes, ligating the interacting fragments, purifying the DNA, and finally identifying the captured sequences by deep-sequencing, microarray analysis, or PCR.

FISH has been used extensively to identify chromosomal aberrations (e.g., translocations or deletions), and characterize CTs, and other nuclear bodies and microenvironments. These analyses are enabled by an increasingly sophisticated array of 3D computational image-analysis tools, such as NEMO [Iannuccelli et al., 2010], eFISHent [Fritz et al., 2014b], and TANGO [Ollion et al., 2013]. Chromosome conformation capture strategies include a compendium of techniques that include 3C, 4C, 5C, Hi-C, and ChIA-PET [Barutcu et al., 2015]. These methods provide a global snapshot of chromosome interactions. Used together, FISH and chromosome conformation capture techniques can complement the limitations inherent to each strategy on its own; they can be used to validate each other for CT analyses. Table I summarizes the strengths and limitations of FISH microscopy and chromosome conformation capture strategies for analyzing CTs.

A SPECTRUM OF INCREASINGLY COMPLEX CHROMATIN CONFORMATIONS IMPACTING GENE EXPRESSION

The configuration of chromatin is coincident with the coordinated expression of genes under different physiological conditions, such as cell cycle, differentiation state, and disease status. This has been shown to be critical for the transcription of individual genes that rely on enhancer-promoter interactions that may cover over 100 kb of linear genomic distance (Fig. 1B) [Kadauke and Blobel, 2009; Krivega and Dean, 2012]. Examples of this include the β-globin locus [Carter et al., 2002; Tolhuis et al., 2002; Palstra et al., 2003], IFNG [Eivazova and Aune, 2004], MHC class II [Majumder and Boss, 2010], and CFTR [Gheldof et al., 2010] genes. Upon transcription, these genes and their respective enhancers are in close proximity. This conformation, termed active chromatin hubs (ACHs), is mediated through the binding of transcription factors, and is only present when these genes are expressed. In hematopoietic stem cells (HSCs), the transcription factor RunxI is required for the interaction between the CD34 gene promoter and its downstream regulatory element [Levantini et al., 2011]. Transcription factor-mediated chromatin looping was demonstrated in erythroid cells. In order to adopt an ACH formation, the Beta-globin gene and its locus of control require specific transcription factors, including EKLF1 and GATA1 [Drissen et al., 2004]. Additionally, the global chromatin organizer and transcription factor, SATB1 was also shown to form an ACH in the Rag1 and Rag2 genes in thermocyte development [Hao et al., 2015].

Physical proximity is not only essential for the expression of individual genes; multiple genes that are coexpressed may congregate into ACHs when active (Fig. 1C) [Osborne et al., 2004a; Spilianakis et al., 2005; Osborne et al., 2007]. For example, the active alpha-globin genes loop into an ACH with several nearby housekeeping genes within a 130 kb domain [Zhou et al., 2006]. These enhancer-promoter and local DNA clusters might represent what are now recognized as topologically associating domains (TADs), defined as preferential interactions that occur over approximately 100 kb to 1 Mbp genomic sequence lengths [Lieberman-Aiden et al., 2009; Dekker et al., 2013; Barutcu et al., 2015]. Although chromosomal contacts within TADs are more prevalent, interactions occur between genes that are located up to ~40 mbp apart in different TADs. In murine erythroid cells, the highly transcribed hemoglobin beta gene, Hbb-b1, and other active genes distally located in sequence, colocalize at shared transcription factories [Osborne et al., 2004b]. Clusters of active genes in open chromatin (A-type compartments) or inactive genes in closed chromatin (B-type compartments) were later established using 3C assays [Dekker, 2014; Cremer et al., 2015].

This clustered chromatin organization is created through boundaries between neighboring genes or gene clusters with different expression programs and/or nearby heterochromatic microenvironments [Giles et al., 2010]. Specifically, certain DNA sequences and protein factors may act as insulators, providing a barrier to block the inappropriate spreading of heterochromatin or contact with enhancers that belong to other genes. One such site, discovered via DNase-sensitivity analysis, is known as 52HS4; it marks the separation between the beta-globin genes and an upstream heterochromatic region [Chung et al., 1993; Chung et al., 1997]. A well-known factor involved in this insulator function is known as CCCTC-binding factor (CTCF). One function of CTCF is to prevent the inappropriate enhancer activation of genes. For example, CTCF is critical for monoallelic expression in imprinting. CTCF binds to the imprinted control region (ICR) of the maternal allele, thus preventing distal enhancers from activating Igf2. In contrast the paternal-allele ICR DNA is methylated, thereby occluding CTCF and allowing the enhancer to activate Igf2 transcription [Bell and Felsenfeld, 2000; Hark et al., 2000; Kanduri et al., 2000]. Genome-wide, CTCF and cohesin complexes have been shown to demarcate TAD boundaries [Phillips-Cremins and Corces, 2013; Seitan et al., 2013; Sofueva et al., 2013; Zuin et al., 2014] and are involved in the evolutionary conservation and alteration of TADs [Vietri Rudan et al., 2015].

Chromatin alterations can be visualized microscopically. For example, actively expressed genes project out of the CT in chromatin loops (Fig. 1D), but remain inside their respective CTs when expression is not in progress [Volpi et al., 2000; Williams et al., 2002; Chambeyron and Bickmore, 2004]. This has been demonstrated for the major histocompatibility complex on CT6 [Volpi et al., 2000], HOX genes on CT11 [Chambeyron and Bickmore, 2004], and the epidermal differentiation complex on CT1 [Mahy et al., 2002a; Williams et al., 2002]. On a broader level, it was suggested that actively expressed genes are more often found on the periphery of CT [Zirbel et al., 1993]. However, other investigations have demonstrated that both active and inactive genes are found at the CT boundary [Kurz et al., 1996; Clemson et al., 2006] and that genes are evenly distributed throughout the CT regardless of their expression

TABLE I. FISH and Chromatin Conformation Capture Techniques for Analyzing Chromosome Territories

	FISH	Chromatin conformation capture
Throughput	Slow, low throughput: microscopy and image analysis are time consuming. • FISH is ideal for analysis of individual CTs.	Hi-C is a relatively complex procedure; however, results represent the average of millions of cells and contacts across the entire genome.
Process considerations	Target sequence information required. With the most advanced equipment, it is theoretically possible to use five fluorescently labeled probes simultaneously; however, most studies query only two or three probes at a time to avoid technical difficulties.	Hi-C and ChIA-PET do not require target sequence information; both techniques provide genome-wide chromosome-contact frequency data. Other chromatin conformation capture techniques (3C, 4C, 5C) do require target sequence information.
	Conventional FISH is in fixed nuclei (data are from individual cells at the time they were prepared for microscopy). • Stage specific markers can be used to identify cells at different points in the cell cycle. • Live-cell FISH has been achieved using catalytically inactive CRISPR/cas9 labeled with EGFP and tiled sgRNAs [Chen et al., 2013].	Crosslinking fixes molecular interactions within nuclei (Data represent the average frequency of chromosome interactions in a cell population at the time nuclei were crosslinked). • Cell populations may be heterogeneous. • Values in Hi-C are relative rather than absolute. • Particular cell types and/or cell-cycle stage cells can be enriched via sorting prior to analysis. • Single-cell Hi-C averages interactions from both somatic CT homologs [Nagano et al., 2013].
	Denaturation may alter nuclear structure. • It is important to verify that nuclear structure remains intact after processing. This can be done by visualizing colocalization of EdU before and after the FISH procedure [Fritz et al., 2014b].	Chromatin decondensation in digestion and SDS steps could result in indirect ligation between fragments located hundreds of nanometers apart in nuclei [Gavrilov et al., 2013; Williamson et al., 2014]. Interactions in different regions of the genome may not be captured uniformly since different proteins have different binding affinities [Williamson et al., 2014]. • May not be a concern in relatively low-resolution Hi-C studies.
	Adequacy of hybridization efficiency is a concern. • Evaluate by labeling chromosomes in spontaneous metaphase cells using CT paints.	Repetitive sequences limit accurate mapping to chromosomes—data on interactions in these areas is typically not available.
Position information	Ideal for determining the position of individual CTs in the nucleus and/or relative to nuclear compartments. • Specificity of antibody to detect nuclear compartment should be determined.	Does not provide information on position relative to the nuclear periphery or other nuclear structures. • ChIA-PET can detect contacts mediated by protein components; however, genes may functionally and cooperatively associate with a third nuclear structure, without interacting at a molecular level—these contacts would not be detectable (e.g., associations localized to opposite sides of a nucleolus).
Data analysis	Analysis programs measure volumes of nuclei, CT or other cellular domains; overlap/colocalization volumes; distances between CT, genes and/or domains; and the degree of irregularity in shape.	Requires sophisticated analysis of next generation sequencing, but provides genome-wide specific DNA interactions. • Appropriate normalization is crucial.
Resolution	Conventional microscopy resolution is constrained by the diffraction limit of light. • Super resolution microscopy [Markaki et al., 2012; Lakadamyali and Cosma, 2015] can increase resolution up to 20 fold over conventional microscopy [Long et al., 2014].	Resolution is dependent on choice of restriction enzyme (4 or 6 bp cutter). • In Hi-C, the effective resolution for cis interactions will exceed that of trans interactions with an equal number of sequencing reads. [Belton et al., 2012]. • Capture Hi-C can increase the effective resolution [Barutcu et al., 2015].

level [Mahy et al., 2002b; Kupper et al., 2007]. Moreover, re-localization of the HOX genes to the CT periphery is not required upon their activation in different cell types [Morey et al., 2007, 2009]. More recently, with the adoption of chromatin capture techniques, it was shown that more active domains are more likely to be found on the periphery of CTs [Nagano et al., 2013], offering credence to an early hypothesis that lost favor before the use of molecular strategies.

The presence of active domains at CT boundaries suggests the possibility that specific coregulated genes may interact at CT interfaces. Interchromosomal interactions have been detected between Myc and Igh [Osborne et al., 2007] and human erythroid-specific genes [Brown et al., 2008]. Interestingly, Spilianakis and colleagues also found that, not only do the regulatory regions of the Locus Control Region (LCR) and TH2 interact on chromosome 11, but they also form an interchromosomal cluster with the IFN-γ gene on chromosome 10 [Spilianakis et al., 2005].

Colocalization has also been shown to preserve gene inactivity. For example, downregulation of the lamin b receptor mediates clustering of olfactory genes at heterochromatic foci [Clowney et al., 2012]. The localization of heterochromatin at specific nuclear microenvironments such as the nuclear periphery, perinucleolar regions [Nemeth et al., 2010; van Koningsbruggen et al., 2010], and within polycomb bodies in drosophila [Tolhuis et al., 2011] results in the clustering of inactive genes [Guelen et al., 2008].

CHROMOSOME TERRITORY NEIGHBORHOODS, A NETWORK OF INTERACTING CHROMOSOMES

In addition to specific interchromosomal interactions between genes, heterologous CT-neighborhood arrangements have been established [Nagele et al., 1995; Bolzer et al., 2005]. This suggests that the colocalization of genes between chromosomes may be established not only by genes projecting out of their CT on chromatin

loops, but also through repositioning of entire CTs. Preferential interchromosomal associations measured between pairwise heterologous CTs suggest an overall nonrandom organization of CTs with respect to each other [Kuroda et al., 2004; Bolzer et al., 2005; Mayer et al., 2005; Brianna Caddle et al., 2007; Khalil et al., 2007; Marella et al., 2009a,c; Zeitz et al., 2009]. This nonrandom organization of chromosomes in neighborhoods is relevant for the establishment of cellular identity. As cells differentiate, entire CTs are repositioned [Kuroda et al., 2004; Marella et al., 2009c] and individual genes within CTs exhibit altered interchromosomal interactions; the pluripotency-related gene Nanog is an example [Apostolou et al., 2013]. Subsequently, unique CT interaction profiles are found in different cell types and tissue lineages [Tanabe et al., 2002b; Parada et al., 2004; Mayer et al., 2005; Marella et al., 2009a; Zeitz et al., 2009]. These profiles, in turn, suggest that genomic organization could explain the high frequency of particular translocations prevalent in different cancer types (discussed below).

Although seminal, microscopic studies of interchromosomal organization have limitations. First, the intranuclear locations of CTs are probable rather than absolute, and therefore efforts to map CTs are challenging. One such limitation is in the determination of the CT positions. The majority of studies that utilize FISH to determine CT organization have primarily focused on their gravity centers, thus interactions at the interfaces of CTs are not captured. This is significant because studies that have focused on CT-border interactions reveal high variability in the distance between CT-centers of interacting chromosomes [Fritz et al., 2014b; Pliss et al., 2014]. Furthermore, due to technical limitations, most studies have analyzed only three chromosomes per nucleus. Recent investigations, however, using a variation on FISH known as reFISH to sequentially label up to nine CT, have determined specific CT neighborhoods for each individual homolog within the nucleus [Fritz et al., 2014b; Pliss et al., 2014]. Regardless, technical limitations in microscopic studies restrict their ability to provide a full understanding of the complex nature of nuclear organization. Hi-C, on the contrary, can provide a more comprehensive view of intra- and inter-chromosomal interactions. However, the data represent a snapshot in time and the average of millions of interactions within a population. In a heterogeneous population, Hi-C cannot differentiate stable CT interactions in a subpopulation of cells from a series of dynamic interactions that occur in most cells at different times. The probable, yet variable, nature of interchromosomal CT interactions is likely due to the fact that the genome as a whole is far more complex than any particular gene family or network of gene regulation.

RADIAL NUCLEAR POSITIONING, CENTERING THE GENOME

Genome organization is a fundamental epigenetic mediator of gene expression [Berezney, 2002; Stein, 2003, 2008; Misteli, 2004; Berezney et al., 2005; Meaburn and Misteli, 2007; Misteli, 2007; Kumaran et al., 2008; Cremer and Cremer, 2010; Bickmore, 2013]. It is generally accepted that the three dimensional positions of CTs are nonrandom relative to the nuclear periphery. Initial studies indicated that CT positioning correlates with gene activity, with higher gene activity levels associated with more interior CT positioning [Boyle et al., 2001; Kreth et al., 2004]. The inactive-X CT is a clear example; it is located closer to the nuclear periphery than its active counterpart [Dyer et al., 1989]. Later studies suggested that gene density influences the nonrandom radial organization of somatic CTs [Boyle et al., 2001]. Because gene-dense chromosomes generally exhibit higher levels of transcription than gene-poor chromosomes [Versteeg et al., 2003], gene activity may be the underlying basis for the radial positioning of the genome. Interestingly, this paradigm holds true not just for entire CTs, but also for individual genes and gene complexes within chromosomes; expressed alleles are generally found further from the nuclear periphery than alleles that are not expressed [Dietzel et al., 2004; Pickersgill et al., 2006; Fraser and Bickmore, 2007; Finlan et al., 2008; Reddy et al., 2008; Takizawa et al., 2008; Fedorova and Zink, 2009; Therizols et al., 2014]. This could result from the preferential localization of tightly packed chromatin at the nuclear periphery [Busch, 1966; Belmont et al., 1993]. While tethering to the nuclear lamina results in repression of transcription [Amendola and van Steensel, 2014], some genes do not display this phenomenon when recruited to the nuclear periphery [Kumaran and Spector, 2008]. It is important to note that transcription can and does occur near the nuclear periphery and conversely, inactive genes are sometimes located in the nuclear interior [Misteli, 2013; Cremer et al., 2015].

Another possible principle governing nuclear organization is born out by studies suggesting that chromosome size is the major determinant of the nonrandom radial positioning of CTs. These investigations have found that longer chromosomes are located closer to the nuclear periphery [Sun et al., 2000]. Efforts to reconcile the findings from these studies point to cell cycle and nuclear shape as possible effectors. For example, CT are repositioned in cycling fibroblasts compared to cells in G0 [Mehta et al., 2010]. Other studies have attributed differences in nuclear shape between cell types as a key determinant of whether chromosome size or gene densities drive radial CT positioning [Bolzer et al., 2005]. CT size–radial position relationships are more prevalent in ellipsoidal fibroblast nuclei, whereas studies in more spherical lymphocyte nuclei find a higher correlation between gene density and radial arrangement which is conserved across evolution [Tanabe et al., 2002a, 2005].

INTERACTIONS BETWEEN CHROMOSOME TERRITORIES AND OTHER NUCLEAR COMPARTMENTS

A more mechanistic perspective examines nonrandom organization of CTs established through functional interactions with other nuclear structures. For example, the nuclear lamina that lines the nuclear envelope have been proposed to have a major role in organizing the genome [Filesi et al., 2005; Goldman et al., 2005]. Lamina-associated domains (LADs) within chromosomes tether CTs to the nuclear periphery (Fig. 1D–E) and are found with greater frequency in CTs that are peripherally positioned [Bridger et al., 2007; Guelen et al., 2008]. Consistent with observations about gene activity and CT radial positioning, LADs are typically found in repressed heterochromatin

microenvironments. The condensation of DNA into tight heterochromatin substantially hinders access to DNA by nuclear machinery for transcription, replication, and DNA/chromosome modification. Some studies have suggested that recruitment to a peripheral heterochromatic microenvironment may have functional implications for replication [Li et al., 2001], transcription [Finlan et al., 2008; Kumaran et al., 2008; Reddy et al., 2008] and genome stability [Oza and Peterson, 2010]. These ideas are supported by the observation that disruption of the nuclear lamina in patients with progeria results in perturbation of telomere length [Gonzalez-Suarez et al., 2009], radial CT positioning [Mehta et al., 2011], and the condensation and compartmentalization of peripheral heterochromatin [Goldman et al., 2004; Nikolova et al., 2004; Taimen et al., 2009].

While the nuclear lamina is thought to exert its largest influence on CTs near the nuclear periphery, other nuclear bodies and microenvironments mediate genomic organization further inside the nucleus. For example, the interchromosomal interactions between the human erythroid-specific genes colocalize at nuclear speckles [Brown et al., 2008]. However, one of the most extensively studied prominent domains impacting nuclear organization is the nucleolus. They are formed by the congregation of the acrocentric chromosomes 13, 14, 15, 21, and 22, specifically their nucleolar organizer regions (NORs), which are comprised of tandem arrays of ribosomal RNA (rRNA) genes [Carmo-Fonseca et al., 2000; Prieto and McStay, 2005]. Although some NORs remain associated with components of the RNA polymerase I transcriptional machinery through mitosis (so-called competent NORs), others are not "bookmarked" and rRNA transcription factors assemble and disassemble with each cell cycle [Smirnov et al., 2006; Schlesinger et al., 2009]. During interphase, competent NORs tend to be located closer to nucleoli than their non-competent counterparts [Kalmarova et al., 2007]; however, both types of NOR-bearing CTs are frequently associated with nucleoli [Sullivan et al., 2001; Kalmarova et al., 2007]. Detailed biochemical and DNA-sequence analysis of nucleoli indicates that not only the acrocentric NOR-bearing chromosomes interact with nucleoli, but all chromosomes have nucleolar-associating domains (NADs). These NADs share sequence similarity with LADs and localize to the perinucleolar heterochromatin (Fig. 1D–E) [Nemeth et al., 2010].

Despite their substantial impact on the nuclear landscape, the number of nucleoli in any given nucleus varies from one to five. As a result, NOR-bearing CTs display heterogeneous nucleolar-interaction profiles within a population of cells. In nuclei with multiple nucleoli, a dominant nucleolus has more contributing NOR-bearing CTs than the others. Furthermore, even nuclei with the same number of nucleoli may exhibit different patterns of association with NOR-bearing CT. For example, a different complement of NOR-bearing CT are found to associate with each nucleolus in cells that contain only two nucleoli. This association of CTs with nucleoli at the nuclear interior, may help to explain the correlation between short sequence length and interior radial positioning [Bolzer et al., 2005; Raska et al., 2006; Heride et al., 2010]. The fact that shorter NOR-bearing CT are preferentially located further from the nuclear periphery than their longer NOR-bearing counterparts [Pliss et al., 2014] suggests that CT size outweighs the influence of nucleolar association in determining radial position.

CHROMOSOME TERRITORY ORGANIZATION IN THE CELL CYCLE

As cell exits mitosis, chromosomes de-compact and gene expression resumes—ostensibly recapitulating the program of the parent cells to maintain their cellular lineage. This is accomplished in part through a process known as bookmarking, in which key transcription factors are retained on chromosomes through mitosis [Delcuve et al., 2008]. Following mitotic de-compaction, bookmarking enables the rapid resumption of cell-specific gene expression or continued genes repression in subsequent generations [Kadauke and Blobel, 2013]. An interesting example of this is the residency of transcription factors at rDNA. In mesenchymal stem cells (MSCs), which are not lineage committed, Myc bookmarking of rDNA promotes rRNA expression in progeny cells. This bookmarking could enable immediate post-mitosis resumption of gene expression that is needed to fuel the rapid cell cycle progression characteristic of MSCs. However, in cells derived from MSCs, that have a longer cell cycle, various transcription factors act to reduce rRNA expression; these include Runx1 in osteoblasts, MyoD in myoblasts, and C/EBP in adipocytes [Zaidi et al., 2010].

Following mitosis, in early G1, in vivo-labeled chromosome domains are mobile relative to the nucleus and each other [Csink and Henikoff, 1998; Lucas and Cervantes, 2002; Walter et al., 2003] whereas during the rest of the cell cycle, the large-scale arrangement of chromatin in the cell nucleus exhibits a degree of stability [Shelby et al., 1996; Abney et al., 1997; Zink and Cremer, 1998; Bornfleth et al., 1999; Chubb et al., 2002; Lucas and Cervantes, 2002; Gerlich et al., 2003]. This observation suggests that the breakdown of the nuclear envelope and its reassembly at the end of mitosis could provide an opportunity for remodeling of nuclear organization in subsequent generations. Outside of mitosis, overall CT morphologies were demonstrated to be maintained for up to 4 h in the cell cycle [Edelmann et al., 2001; Muller et al., 2010]; however, alterations were detected in internal CT organization [Muller et al., 2010]. CT morphology was found to be altered, however, in studies that examined CTs in G1 compared to S-phase nuclei [Sehgal et al., 2014]. Along with this altered CT morphology, cell-type specific alterations in the interchromosomal organization of specific CT were demonstrated [Fritz et al., 2014a].

On a smaller scale, chromatin remodeling has been shown to control progression through the cell cycle by regulating transcription of essential cell-cycle genes [Cao et al., 1997; Vignali et al., 2000] and DNA replication. For example, during S phase, loosening of chromatin structure enables the replication machinery to access the DNA [Raynaud et al., 2014]. At a global level, as cells progress from early S to mitosis, chromatin becomes increasingly condensed and less sensitive to DNase I [Pfeffer et al., 1991]. Using Hi-C on synchronized HeLa cells, it was demonstrated that the intrachromosomal TADs within specific CTs are largely, but not entirely, maintained across the cell cycle [Naumova et al., 2013]. This work also determined that TADs are broken down in mitosis and are re-established in early G1 [Naumova et al., 2013]. However, the degree to which the organization within specific TADs is altered during interphase from early G1 to late G2 remains unclear.

CHROMOSOME TERRITORY ORGANIZATION IN CANCER

Changes in nuclear structure have long been used as a major diagnostic tool to detect cancer [Zink et al., 2004; Zaidi et al., 2007]. Cancer nuclei are generally larger and more irregularly shaped than nuclei in healthy cells [Zink et al., 2004]; nuclear compartmentalization is also altered. For example, some cancers are characterized by an increase in the numbers and sizes of nucleoli, possibly as a result of an increased requirement for protein synthesis [Derenzini et al., 2000; Maggi and Weber, 2005]. Partial loss of heterochromatin compartmentalization is another characteristic change [Zink et al., 2004]. Although it is known that nuclear architecture is altered in cancer, less is known about the concomitant changes in CT organization. Studies of nuclear organization in cancer are complicated by the fact that comparisons are often made between cells from different origins and the karyotypes of each cancer cell type are highly variable—karyotype heterogeneity within a population of cells further complicates analyses. Even in cells with similar karyotypes, cell-to-cell variability in CT organization has been demonstrated using microscopy and single-cell Hi-C [Nagano et al., 2013]. Finally, even single-cell Hi-C, cannot currently distinguish the data from individual somatic chromosomes; data represent an average of both CT homologs [Nagano et al., 2013]. When determining the organization of CT this is of particular importance. For example, in chromosomes that are involved in translocations, altered contact frequencies could be found within the translocated CT, the non-translocated CT, or distributed between both homologs of the CT.

Interchromosomal interactions have been suggested to play a role in cancer progression. Cell-type differences in CT organization may present an explanation for the high frequency of specific translocations that occur in the progression of several different cancers. Increased translocation frequency has been determined between specific CTs that are in closer proximity [Parada et al., 2002; Roix et al., 2003; Brianna Caddle et al., 2007; Soutoglou et al., 2007; Folle, 2008] and have higher degrees of intermingling [Branco and Pombo, 2006]. Within cancer nuclei, it was demonstrated that translocated CT regions retain the preferential interaction partners they had before the translocation [Parada et al., 2002]. A wide-scale alteration in interactions between non-translocated CTs was determined in MCF10A cells versus their metastatic counterpart MCF10CA1a cells [Fritz et al., 2014b]. Interestingly, the inactive X CT was found to have fewer interchromosomal interactions in MCF10A, but not in MCF10CA1a cells. This increase in interchromosomal interactions was coincident with increased CT volume and global gene expression within the X chromosome [Fritz et al., 2014b]. Changes in CT interaction profiles extend to the gene level in cancer; it was shown that the specific genes that participate in interchromosomal interactions are different in breast cancer versus normal breast cells [Zeitz et al., 2013].

CONCLUSION

Although progress has been made in understanding how CTs influence and/or respond to nuclear organization, further work to fully elucidate the rules that govern the functional relationships between genomic organization, and genetic and epigenetic regulation in the context of nuclear microenvironments is needed. Due to the complexity of genome-wide regulatory networks, high-throughput biochemical techniques will be vital for illuminating these functional relationships. The dynamic nature of structural and functional relationships across the cell cycle (e.g., from mitotic bookmarking of genes with their respective regulatory proteins to domain assembly during interphase) as well as their perturbation in disease also require further study. Architectural signatures that reveal specificity of localization and/or colocalization for regulatory domains that may be altered could represent targets for the detection and treatment of disease states. Microscopy remains an important approach for high-throughput genome-wide studies; used in combination with emerging molecular chromatin conformation capture techniques, and ever more sophisticated and powerful analysis methods, it is likely that ongoing investigation will yield ever more precise and detailed information on the role of CT organization in biology.

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