Published in final edited form as:

Genes Chromosomes Cancer. 2019 July ; 58(7): 407–426. doi:10.1002/gcc.22732.

Chromosome territories and the global regulation of the genome

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Abstract

Spatial positioning is a fundamental principle governing nuclear processes. Chromatin is organized as a hierarchy from nucleosomes to Mbp chromatin domains (CD) or topologically associating domains (TADs) to higher level compartments culminating in chromosome territories (CT). Microscopic and sequencing techniques have substantiated chromatin organization as a critical factor regulating gene expression. For example, enhancers loop back to interact with their target genes almost exclusively within TADs, distally located coregulated genes reposition into common transcription factories upon activation, and Mbp CDs exhibit dynamic motion and configurational changes in vivo. A longstanding question in the nucleus field is whether an interactive nuclear matrix provides a direct link between structure and function. The findings of nonrandom radial positioning of CT within the nucleus suggest the possibility of preferential interaction patterns among populations of CT. Sequential labeling up to 10 CT followed by application of computer imaging and geometric graph mining algorithms revealed cell-type specific interchromosomal networks (ICN) of CT that are altered during the cell cycle, differentiation, and cancer progression. It is proposed that the ICN correlate with the global level of genome regulation. These approaches also demonstrated that the large scale 3-D topology of CT is specific for each CT. The cell-type specific proximity of certain chromosomal regions in normal cells may explain the propensity of distinct translocations in cancer subtypes. Understanding how genes are dysregulated upon disruption of the normal “wiring” of the nucleus by translocations, deletions, and amplifications that are hallmarks of cancer, should enable more targeted therapeutic strategies.

1 | INTRODUCTION

Spatial positioning has emerged as a fundamental principle governing nuclear processes and, together with the field of genomics, has led to a paradigm shift in the study of gene regulation.[1–17] Rather than studying individual genes and their regulation, the emphasis is

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now on understanding the regulation and coordination of up to 1000s of genes at any given time. An even more daunting challenge is deciphering how these large numbers of genes are spatially arranged, expressed, and regulated within the three-dimensional (3-D) context of the cell nucleus.

Key to this understanding is the realization that the chromatin in the cell nucleus is arranged as a hierarchy (Figure 1A) ranging from the DNA double helix organized into chromatin to the arrangement of chromatin into increasingly higher levels of organization culminating with the entire chromosome as a 3-D entity termed the chromosome territory (CT).[1,2,18,19] In this manner, the CT acts as an epigenetic feedback system where events and modifications occurring at all levels of chromatin organization affect the global expression and regulation of the genome. For example, at the molecular level, histones are dynamically regulated through a diverse array of modifications defining the histone code and leading to alterations in chromatin organization and function.[20–23,25,28,30,31] Targeted alterations in DNA methylation along with a variety of other factors, such as chromatin remodelers, have further provided the foundation for studying chromatin as an integral factor driving the epigenetic regulation of the genome.[22,26,28–30] Analyses of these epigenetic markers enable the distinction of euchromatin or “open” chromatin—where active genes are predominantly located—from the gene poor heterochromatic or “closed” chromatin regions.[22,30,31] In higher order chromatin domains (CD) up to entire chromosome territories (CT), nuclear architecture coupled with genome organization have been implicated in the regulation of genomic functions such as DNA replication, transcription, and RNA processing.[1–4,8,12–15,17,18,23,24,27,32–40] With this in mind, our review is focused on the 3-D architecture of CT and their potential role in the global orchestration of genomic expression and regulation within the functional milieu of the cell nucleus.

1.1 | The Concept of CT

It is now well established that in the nucleus the chromosomes are present as discrete entities. Carl Rabl in 1885[41] first suggested a territorial organization of interphase chromosomes in animal cell nuclei. Later, Theodor Boveri coined the term *chromosome territory* (CT) during his study of roundworm blastomere stage.[42,43] Despite its early discovery, the existence of CT was not well accepted, and in the 1950s to 1970s, it was generally believed that the chromatin intermingled in the cell nucleus “*like a bowl of spaghetti*”. The idea was still tested, and the first substantial experimental evidence was provided in the 1970s. In these experiments, squashed Chinese hamster ovary cells were treated with acetic acid, air dried, and subjected to Giemsa staining. Upon visualizing these nuclei, large patches of chromatin were observed and interpreted as CT.[44] While some of these treatments may have artificially “induced” territorial-like arrangements of the chromatin, other evidence avoided this caveat. Using a laser micro beam, DNA damage was inflicted to a small region in the nucleus. The cells were then immediately pulse labeled with ^{3}H-thymidine, which marked sites of DNA repair. Observing the cells during mitosis revealed that the labeled DNA was restricted to a region on one of the chromosomes rather than being present in smaller amounts on several chromosomes, which would be expected if the chromatin was freely intermingling.[45,46]

Development of in situ hybridization and chromosome-specific painting probes enabled the first direct visualization of CT in the nuclei of fixed cells.[47–49] Later techniques were developed to visualize CT in live cells. This was achieved by incorporating fluorescent nucleotide analogs into replicating DNA in cells, followed by several cell divisions. Through independent assortment after several generations, individually labeled CT could be seen in the live cells.[50–52] While these studies indicated that the intranuclear position of CT are relatively stable throughout the interphase, further investigations in living cells revealed that discrete replication labeled CD in the Mbp size range undergo significant oscillatory motion and frequent shape transitions between condensed and open configurations.[52–55] Remarkably, chromatin dynamics in live cells appears to correlate with gene activity. Chromatin enriched in active genes had higher levels of oscillatory motion and configurational changes than gene poor chromatin regions.[53,54] Furthermore, chromatin motion is significantly reduced by inhibitors of RNA synthesis, such as actinomycin D.[53,54]

1.2 | Interactions of CT Within the Nuclear Milieu

Behind the complex 3-D structural organization of the cell nucleus is a striking degree of functional order (Figure 1B) wherein specific genomic functions such as DNA replication, transcription, and RNA splicing are compartmentalized within discrete sites along with a host of nuclear bodies such as the PML, Cajal, and histone loci.[1,6,8,39,56–63] While our understanding of the biogenesis and assembly of various nuclear bodies are still in their infancy, insight has been gained from physiochemical considerations. For example, it has been proposed that nuclear bodies form when proteins bind and cross-link the chromatin as polymer segments thereby collapsing the chromatin into globule-like configurations. Another proposed mechanism is that proteins binding to the chromatin induce liquid-liquid phase separations whereby “liquid-like” nuclear bodies form around the chromatin globules.[64,65]

A longstanding question in the field is whether the plethora of functional domains in the cell nucleus are present as separated entities or, alternatively, are they linked into an interactive network as studies into the nuclear matrix suggest (Figure 1B).[4,12,37,39,66–72] The nuclear matrix was first identified by treating cells with high salt and nucleases.[73] Under these conditions, the nucleus retains its overall architectural features such as the nuclear lamina, nucleoli, and a fibrogranular matrix network.[35,73–75] Detailed electron microscopic studies revealed a close resemblance of this fibogranular matrix network to nonchromatin structures visible under certain staining conditions in the interchromatinic regions of intact cells.[35,68,71,72,74–76] The retention of functionally related properties associated with the isolated nuclear matrix (eg, DNA replication, repair, transcription, RNA splicing, steroid hormone receptors, viral associations, protein kinases, oncogenes, tumor suppressors, and many other regulatory factors) is consistent with the nuclear matrix as a structural framework for nuclear functions. In this view, the in vivo matrix is a milieu in the nucleus where domains of chromatin and ribonucleoproteins dynamically interact to form higher order nuclear architecture undergirding genomic functions.[1,35,68,70,71,75,76] Indeed most of these functional associations are maintained in a structural organization that is indistinguishable from those in the nuclei of untreated cells. This is most strikingly illustrated in studies of DNA replication and transcription sites that remain bound to the isolated nuclear matrix.

[39,71,77] Moreover, DNA replication and transcriptional activities are still present in these preparations and function on the matrix-attached DNA fragments.[39,71,77,90]

Despite this progress, it is important to realize that isolated nuclear matrix systems are likely static and highly stabilized versions of a dynamic nuclear architecture in living cells.[13] Moreover, the precise relationships of the isolated matrices to in vivo nuclear architecture and function remain to be defined. In one previously stated view, the “in vivo nuclear matrix” is considered as the milieu in the nucleus where domains of chromatin and ribonucleoprotein complexes dynamically interact to form modules of higher order architecture and function.[1,13,35,68–71,75,76] To further extend this model, interactions among individual modules may occur resulting in many local network-like connections dispersed throughout the nucleus.[13,69] Isolation conditions for the nuclear matrix may then result in one stable network termed the nuclear matrix. In this view, a continuous nuclear matrix structure is not present in the in vivo nucleus. Instead, higher order discontinuous architectural elements may provide the basis for nuclear matrix isolation. More research on many levels is needed to resolve these issues.

The architectural role of the nuclear matrix likely extends to the CT themselves. Previous studies have indicated the association of telomeres,[78,80,81] centromeres,[82,83] chromatin loops and the attachment sites for chromatin loops termed S/MARs (matrix or scaffold attachment regions)[79,84–93] on the nuclear matrix. S/MARs DNA binding elements have been identified in silico[94,95] and recently analyzed on a genomic scale, which should open additional doors to the understanding of these critical but enigmatic regions of the genome.[96,97] Moreover, experiments on whole cells to isolate nuclear matrix demonstrated that despite the removal of up to 90% of the chromosomal histones, the chromosomal DNA in the remaining nuclear matrix structures are still folded into territorial arrays resembling intact CT.[98] Subsequent disruption and extraction of nuclear matrix proteins, led to a corresponding disruption of territorial organization.[98]

Several proteins associated with isolated nuclear matrix have been identified as potential factors involved at chromatin loop S/MAR attachment sites and/or higher order CD. These include topoisomerase II,[99–102] CTCF,[103] cohesin,[104–106] matrin 3 (also called as P-130 nuclear scaffold protein[107,108]), HnRNP-U (also called as SAF-A, for Scaffold/Matrix attachment region factor[109–113]), SAF-B,[114,115] ARBP (Attachment Region Binding Protein[116]), SAT-B,[117–119] and nuclear lamins.[120,121] Recent proteomic approaches have further confirmed the S/MAR binding activities of SAF-A, matrin 3, and nuclear lamins.[122–124] CTCF and cohesin have been identified as key players in the arrangement of chromatin into higher order domains termed Topologically Associated Domains (TADs), which mediate long range intrachromosomal and interchromosomal interactions within the nucleus.[15,106,125–134] Recently, HnRNP-U (SAF-A) has been demonstrated to be integral to maintaining genome organization at TADs and higher levels of chromatin.[113]

Matrin 3 is an acidic ~120 kDa nuclear matrix protein[35,135] that is among the major proteins of isolated nuclear matrix based on two-dimensional (2-D) gel separations.[136] It has both DNA and RNA binding properties and is involved in a wide range of processes ranging from cell survival to association at S/MAR chromatin loop anchor sites to a variety of functions

involved in RNA metabolism including RNA splicing, mRNA assembly/stabilization, RNA export/retention, and viral RNA regulation.[107,108,123,137–145] Consistent with these findings, yeast two hybrid screening identified interactions with a constellation of proteins involved in DNA replication/repair, transcription, and RNA splicing.[66] A recent transcriptome and interactome analysis revealed over 100 proteins that bind matrin 3 (prominently including proteins associated with RNA processing/splicing[146]). Deletion of a RNA recognition motif (RRM1) or Zn finger motifs (ZnF1 or ZnF2) diminished the binding of a subset of matrin 3 interacting proteins and altered its intranuclear organization.[124] This study also uncovered a crucial role of the RRM2 motif of matrin 3 for regulating interactions with other nuclear proteins and maintaining its characteristic intranuclear localization.

Detailed visualization studies including computer-assisted analysis demonstrated that matrin 3 forms a network-like structure in the nucleus, which permeates the interior of CT and is in close proximity to functional domains containing active sites of transcription, RNA polymerase II, RNA splicing, and other functionally related factors.[4,8,12,66] While the potential role of matrin 3 as a major component of an overall scaffolding network in the cell nucleus remains to be resolved, it is interesting to note that deletion mutations in the RRM2 domain resulted in major alterations of the network-like organization of matrin 3 into large nuclear body-like structures and protein-protein interactions.[124] Moreover, colocalization studies demonstrated a similar network organization and close 3-D proximity of the S/MAR binding protein SAF-A (hnRNP-U) with matrin 3 including penetration into the interior of CT.[8,12,66] Based on these findings and similar properties of these two proteins, it was proposed that SAF-A and matrin 3 are major components of a nuclear scaffolding structure that serve as dynamic assembly centers for the organization of functional neighborhoods of genomic function in the cell nucleus.[4,12] The potential physiological roles that matrin 3 plays in nuclear organization and function are further emphasized by the recent findings connecting mutations in the matrin 3 gene (MATR3) to the diseases ALS, distal myopathy, and left ventricular outflow tract obstruction (LVOTO), a common congenital heart defect.[124,141,146–149]

Within the nuclear matrix, the nuclear lamina—the network of peripheral proteins surrounding the inner nuclear membrane—has also been posited as a major factor in the organization of the genome.[61,150–156] The major proteins of the nuclear lamina are lamins A, B, and C. These bind to a myriad of other proteins involved in many nuclear functions. Among these proteins are histone H2A or H2B dimers, actin, retinoblastoma protein, components of the RNA-polymerase-II-dependent transcription, and replication complexes.[150,151] Lamins also bind to integral membrane proteins such as emerin, lamin-B receptor (LBR), and MAN1.[157] Through these many interactions, it is proposed that specific regions within the genome are tethered to the nuclear periphery.[152,153,158] Lamins are also MAR binding proteins and are generally believed to be involved in the binding of the characteristic heterochromatin along the nuclear periphery[120,121] and domains of chromatin loops termed LADs, which are enriched in inactive genes and serve a regulatory role in gene repression along the nuclear periphery.[11,153,158,159]

The important role of nuclear lamin proteins in genomic organization and function is further highlighted by the linkage of mutant forms of the lamins to disease states termed

laminopathies.[154–156,160] Over 400 mutations throughout the *LMNA* gene, which codes for both lamins A and C, have been linked to various forms of laminopathies such as muscle dystrophies, cardiomyopathies, and partial lipodystrophies.[154] Laminopathies typically lead to nuclear blebbing and striking alterations in both nuclear shape and chromatin morphology in the nuclear interior.[161,162] These alterations are consistent with a fundamental role of the nuclear lamina in genomic organization and function.

In considering one mode in which the lamins on the nuclear periphery might influence chromatin in the nuclear interior, a recent study demonstrated a specific binding site in the C-terminal tail of lamin A for matrin 3.[163] This was corroborated by pull-down experiments and the close proximity of a small portion of matrin 3 with the nuclear lamins on the nuclear periphery by immunofluorescence. A cell line, containing a C-terminal mutant lamin A that leads to characteristic forms of distal myopathy and lacks the matrin 3 binding domain, was devoid of close proximity with matrin 3 along the nuclear periphery.[163] It is tempting to speculate that this particular laminopathy involves disruption of a normal linkage between lamin A and matrin 3 on the nuclear periphery. This in turn may be linked to the elaborate network structure that matrin 3 forms in the nuclear interior including localization within the interior of CT.[66]

While substantial findings indicate that nuclear lamin A is also present throughout the nuclear interior, its exact role in this noncanonical location remains unknown.[164,165] In this regard, striking increases were reported in overall chromatin motion in a lamin A deficient double null cell line. Appropriate experiments suggested that this increase was a result of changes in lamin A interactions within the nuclear interior where it might serve as part of a scaffolding system for chromatin.[166] Recently, it was determined that lamin A is responsible for the viscoelasticity of the nuclear interior.[167] It would be interesting to determine if this interior lamin A population is influencing chromatin motion through its association with matrin 3 and/or other components of the fibrogranular internal nuclear matrix structure. Compatible with a scaffolding role of interior lamin A, increases in the intranuclear movement of PML bodies was also reported in a lamin A double null cell line.[168]

Chromatin loops within LADs may also play a role in the overall anchoring of CT within the nuclear interior through association with the nuclear lamina. Early studies of DNA attached to the nuclear matrix suggested that while the bulk of the DNA loop attachment sites are located within the nuclear matrix interior, a small but reproducible proportion (<20%) are present within nuclear lamin preparations.[90] While removal of the internal components of the nuclear matrix leads to the disruption of the characteristic territorial organization of CT in the cell nucleus, the DNA remains tightly packaged inside the cell nucleus.[98] Because the nuclear lamina was maintained in those preparations, it is conceivable that the DNA is anchored in the nucleus via the persistence of LAD DNA loops. Further studies are necessary to resolve this issue and the potential architectural role of the nuclear lamin-associated LADs and chromatin loops in CT organization.

The third major architectural component of the isolated nuclear matrix is the residual nucleolar structure.[35,74,75] A large body of studies demonstrate the association of a subset of CT termed the nucleolar organizing region (NOR)- chromosomes (CT13, 14, 15, 21, 22)

with the nucleolus.[169] The NOR-CT contain the amplified arrays of rDNA genes, which provide the basis for ribosomal RNA production and underlies the formation of the nucleolus as a ribosome assembly factory.[169–171] The nucleolus is also associated with a population of chromatin loop domains termed nucleolar-associated domains, which like the LADs are enriched in inactive genes and are believed to be involved in the repressive state of the nucleolar-associated heterochromatin.[172,173]

1.3 | The Hierarchy of Chromatin Organization and Function

The organization of chromatin and the impact of nuclear structure on the regulation of the cell have fascinated biologists because the nucleus was first visualized by microscopy. The eukaryotic genome is hierarchically organized across a spectrum of levels (see Figure 1A). At the lowest scale, chromatin is packaged by histone proteins into repeating structures known as nucleosomes. These consist of 147 base pairs of DNA, core histone subunits, and tails that extend out and are subject to posttranslational modifications such as acetylation, phosphorylation, and methylation. At this level, nucleosomes on the DNA have been described as “beads on a string” 10 nm fibers.[174,175] Upon methylation and the addition of histone H1, chromatin is believed to be condensed into a 30 nm fiber. However, the exact structure of the 30 nm fiber and whether it exists in vivo is still controversial and unresolved.[176]

The next level of organization involves the folding of the chromatin into loops anchored to the nuclear matrix or chromosome scaffold[1,19,177] Chromatin loops were first identified in human cells as DNA supercoiled domains with an average size of ~ 200 kbp.[178] Electron microscopic analysis further revealed that the DNA halos, which appear following relaxation of the supercoiled domains,[88] consisted of tightly packed and highly convoluted DNA loops anchored to the chromosome scaffold and nuclear matrix, respectively.[75,179,180] Following the initial finding that newly replicating DNA is associated with the nuclear matrix,[181] it was demonstrated that replication occurs in a bidirectional movement of the DNA loops through fixed replication complexes present at the base of nuclear matrix attached DNA loops. In this way, postreplicated DNA accumulates progressively in the distal portions of the loops at a rate consistent with the bidirectional replication fork rate.[88,182–184] Analysis of gene positioning along the DNA loops further indicated that inactive genes positioned in the distal regions of DNA loops are repositioned near the nuclear matrix attached sites for the DNA loops in correlation with gene activation.[185–187] This suggests the intriguing idea of a dynamic chromatin loop program corresponding to the overall transcriptional program of a cell.[75,76] Consistent with these results, biochemical and genomic approaches have revealed that S/MAR attachment sites preferentially associate with actively transcribed genes.[96,188]

Further studies of DNA replication sites using 3-D microscopy and quantitative computer image analysis resulted in an important breakthrough in our understanding of higher order CD beyond the chromatin loops. Appropriate pulse-chase experiments revealed that replication is organized at discrete replication sites (RS) in the nucleus with each RS containing an average of ~1 Mbp DNA.[58,189] Based on the average lifetime of each RS or replication factory, this corresponded to ~ 5 to 6 replicons or chromatin loops with an average size of ~ 180 kbp.[58] These results were consistent with earlier biochemical findings

demonstrating the organization of DNA polymerases and primase into large megacomplexes bound to the isolated nuclear matrix from regenerating rat liver.[190] Chase studies extending hours to days revealed that the originally labeled RS persist as similarly sized CD throughout the cell cycle and into subsequent cell generations.[58,189] Similar CD have also been visualized several generations after longterm labeling of DNA.[52] These Mbp CDs are, therefore, a fundamental unit for both the higher order assembly of chromatin and it replication at replication factories.[1] More recent studies of higher order CD using the high throughput chromosome conformation capture (Hi-C) approach (described below) have led to the identification of similarly sized domains termed TADs.[127,128,132] It is now generally accepted that TADs and Mbp CDs represent the same higher order CD identified by two different approaches.[125,128]

The findings of discrete transcription factories where multiple genes can be transcribed in close proximity may represent another functional aspect of the Mbp CDs.[10,191–194] Genes from different regions of individual CDs could loop out to form a cluster of active genes under common transcriptional regulation within the transcription factory.[8,12]

Understanding the arrangement of chromatin beyond the Mbp CDs is a challenging area of research. In one direction, studies of the arrangement of RS and transcription sites (TS) in 3-D led to the finding of discrete higher order zones of replication and transcription that appeared as separate network structures in the cell nucleus.[1,36] These higher order zones of replication and transcription are maintained following extraction of whole cells for nuclear matrix further implicating a critical role of this overall network structure in the structural compartmentalization of these two fundamental genomic processes.[39]

In recent years, a compendium of chromosome conformation capture (3C) approaches have provided biochemical methods for determining genome organization.[133,195] These techniques rely on the ligation of DNA fragments that are in close proximity in fixed nuclei. The advancement of next generation sequencing technologies has allowed for high throughput unbiased investigation of genome organization in the technique termed Hi-C[132,133] (Figure 2). In brief, Hi-C involves the 3C technique followed by adapter ligation and results in a library of chimeric DNA fragments, each end of which are subsequently mapped to the genome. The pairwise frequency of interactions between regions of the genome is then represented as a matrix. The diagonal of this matrix denotes local interactions, whereas distal interactions are represented farther off the diagonal (see Figure 2).

Using these techniques, investigators have confirmed and extended most of the findings that were based upon microscopy and increased the resolution at which these fundamental principles of nuclear organization are measured (Figure 2). For example, the previously discussed microscopic visualization experiments that identified ~ 1 Mbp CDs,[58,189] were confirmed by Hi-C to be TADs and can be seen in interaction matrices as increased local frequencies of contact[125,128,132] (ie, looping interactions, see Figure 2).TADs are largely invariable across cell types and conserved across evolution.[125] Identification of replication domain boundaries intersect with boundaries of TADs.[196] Thus TADs define modules of early or late replicating CD and are dynamic domains that correlate with genomic

function[197] just as proposed for the Mbp CDs analyzed by microscopic and computer imaging approaches.[58,189]

Hi-C experiments have also resolved the genome into two major compartments, A and B, representing euchromatic vs heterochromatic microenvironments, respectively.[198] While constitutive heterochromatin is typically composed of repetitive DNA and is involved in structures such as centromeres and telomeres, facultative heterochromatin is the result of gene silencing via histone deacetylation.[199] Thus, facultative heterochromatin is a more dynamic and reversible structure that can decondense and become transcriptionally active.[199]

In Hi-C matrices, compartments are seen as blocks of interaction that are off-diagonal and therefore represent coalescing TAD regions of heterochromatin or euchromatin (Figure 2). These compartments are enriched in histone marks indicative of their chromatin state. As expected, A compartments are generally more gene rich and have higher levels of expression than B. This demarcation of chromatin states has become more complex because heterochromatin has been subdivided into four or five distinct states that range from constitutive to facultative and are marked by different epigenetic modifications.[200] Similarly, using a higher resolution Hi-C technique, the A and B compartments can be subdivided into distinct subcompartments.[201] More recently, however, single cell Hi-C approaches have determined that rather than distinct subcompartments TADs exist within a spectrum of compartments that range from A to B.[202]

Heterochromatin is preferentially located at the nuclear periphery.[203,204] As such inactive TADs associated with B compartments are preferentially localized at the nuclear periphery, while active TADs in A compartments are generally more interior.[201] A role for the nuclear lamins in this localization is supported by the finding that lamin A knock-out mice lose this peripheral organization of heterochromatin.[205] This is also the case in cells of progeria patients with mutated lamin A.[206] In fact, the nuclear compartmentalization is lost in late passages of cells with progeria.[207] While still under investigation, the specific association of lamin proteins with heterochromatin likely occurs through the interactions of lamins with the lamin receptor LBR which in turn binds to the Heterochromatin Protein 1 (HP1),[208] which plays a vital role in heterochromatin formation.[209–211] The gene repressive microenvironment of heterochromatin has functional implications for the regulation of replication, transcription, and DNA repair. Interior “nucleoplasmic” lamin A likely also plays a role in these processes and should also be considered.[165]

1.4 | Organization of CT

Following the establishment of CT as the basis for the organization of the genome in the cell nucleus, investigators focused on understanding how the individual territories were arranged within the 3-D architecture of the cell nucleus. As a first step, the relative radial arrangement of chromosomes was examined with respect to the nuclear periphery and center. A fundamental question was whether CT are randomly arranged or whether there was a level of nonrandomness in their positions within the cell nucleus. These studies clearly established a high level of order in the radial positioning of individual CT in the cell nucleus.[9,11,18,212–217,219]

Because the transcriptionally inactive heterochromatin in the nucleus is preferentially localized along the nuclear periphery, the relationship of peripheral position to the gene density of individual chromosomes was analyzed in detail. In an initial study, the inactive X (Xi) CT had a more peripheral location within the nucleus than its active (Xa) counterpart.[212,213,219]

The relationship between radial positioning and gene activity can also be demonstrated for the somatic CT because gene density generally correlates with gene activity.[218] Thus CT that are gene poor may be positioned more peripheral than those that are gene rich because they are less transcriptionally active. For this reason, several studies have concluded that gene density is the major contributing factor to the radial positioning of CT.[212,213,219] For example, the gene poor CT18 is more peripheral than the gene rich CT19.[9,212,220] This paradigm can be extended to individual genes or gene complexes within the chromosomes. Individual genes that are expressed are typically found more interior than those that are not.[10,221–227] Interestingly, aberrant localization to the nuclear periphery by tethering regions to the nuclear lamina results in the inhibition of transcription.[223] Moreover, the radial positioning of CT in the same cell type are conserved throughout primate evolution.[214]

Studies demonstrating a close correlation between gene density and radial positioning were performed predominantly in the relatively spherical nuclei of lymphocytes. Analysis of radial positioning in flatter cells such as fibroblasts, however, have shown a more complex situation where chromosome size becomes an important factor. In these cases, the radial positioning generally correlates with chromosome size and not gene density.[228–232,246]

It was recently reported that the radial positions of a subset of seven chromosomes were not altered during the cell cycle but had limited alterations between fibroblast and epithelial cell types.[229] In addition, five of nine CT examined showed significant differences in radial position between normal breast epithelial cells and a corresponding malignant cell line.[230] Even more striking, it was reported that the radial positioning of seven CT in undifferentiated human keratinocytes strongly correlated with gene density, while the positioning in the corresponding CT from late differentiating keratinocytes were highly altered with no relationship to gene density or chromosome size.[233] These findings are consistent with the probabilistic radial positioning code proposed by Cremer and associates[213] and support the view that the probabilistic positioning code is cell-type-specific and can undergo alterations during both malignant progression and cell differentiation where massive changes in the genomic expression programs generally occur.

Several factors other than gene density or chromosome size have been implicated in the radial arrangement of CT. Interactions with other nuclear compartments are proposed as one mechanism by which radial positioning is established. For example, the interaction with nucleoli is implicated in the radial positioning of the nucleolar NOR-CT.[169,228,234] The nuclear lamina has also been demonstrated to be important for the radial organization of CT.[235] Specifically, cells that express the aberrant truncated lamin-A protein (progerin) have an altered radial arrangement of CT.[236]

In addition to interactions with other nuclear compartments, several protein factors or protein modifications may have a role in the radial positioning of CT. Knocking down the actin family member Arp6,[237] a histone variant H2A.Z,[237] myosin,[235] or inhibiting histone deacetylation[238] all lead to abnormal radial positioning of CT.

1.5 | Interactions Between CT

The discovery of nonrandom radial positioning of CT suggested the intriguing possibility that there might be nonrandom interactions patterns among the population of CT. Initial studies using multifluorescence in situ hybridization (FISH) techniques were consistent with this view and indicated nonrandom albeit probabilistic interchromosomal associations.[228,239,240] Alterations in these associations in different cell and tissue types,[66,231,241,242] during cell differentiation[243] and in cancer[215,231,244] further supported a relationship to genomic function.

One limitation of most of these studies, however, is that they evaluated interchromosomal associations based on the distances between the centers of gravity of the CT. Thus specific interactions at the borders or interfaces between neighboring CT are not captured. Studies measuring CT-interactions based on distances between their nearest border sites, revealed that the corresponding distances based on CT centers are highly variable and often do not correspond to the positive associations measured by nearest border-to-border interactions.[230,245] Furthermore, due to technical limitations of multi-FISH labeling and analysis, most of these studies analyzed up to only three chromosome pairs per nucleus.

To circumvent these difficulties, a 3-D re-FISH approach[228] was adapted to simultaneously analyze large subsets of CT in individual nuclei. Figure 3 depicts typical CT multicolor labeled images from re-FISH experiments with MCF10 normal human breast and malignant breast cancer cells (Figure 3A,B), undifferentiated and differentiated human keratinocytes (Figure 3C,D), WI38 normal human fibroblasts (Figure 3E), and the NOR-CT in WI38 cells (Figure 3F). An integrated computer imaging program was then applied to segment the individual CT and determine a large battery of measurements among the total population of labeled CT in the individual nuclei.[229,230,233,245,246] These measurements included the 3-D nearest neighbor distances between the borders of all labeled CT. The latter computations resulted in a listing of the 3-D nearest neighbor distances for every possible combination of chromosome pairs. In studies involving nine CT pairs, a total of 153 pairwise CT interactions were determined.[230] From this data, the percentages of pairwise associations (interactions) were calculated using a threshold distance of ≤4 pixels, which corresponded to ≤0.28 μm or the approximate resolution limitation of the fluorescence microscopy used for collecting the images.[229,230,233,246]

Using these techniques, major differences were reported in the total pairwise interchromosomal interaction profiles between normal luminal MCF10A epithelial cells and the corresponding malignant MCF10Ca1a breast cells derived from the MCF10 normal cells.[230] Nineteen of the 36 heterologous CT pairs changed by >10% association, and these alterations were even more pronounced (26 of 36 CT pairs) in profiles showing multiple pairwise interactions. Analysis of the total number of heterologous CT interacting with each CT demonstrated a similar average of ~ 3.5 interactions of the possible 16 heterologs in both

10A and Ca1a cells with the exception of CTX where both Xi and Xa had much lower values in both cell lines.[230] Assuming these interactions are representative of the entire genome, each CT interacts on average with 9 to 10 other heterologs at any given moment or nearly one-fourth of the genome. These findings predict a high level of interactions among the individual CT in the genome. Similar results in CT from human WI38 fibroblasts support this conclusion.[229]

Major differences in the pairwise interactive CT profiles for WI38 and MCF10A, point to cell-type specificity in interchromosomal interactions.[229] In addition, striking changes in the CT interactive profiles involving multiple interactions (>2) were detected between undifferentiated vs highly differentiated human keratinocytes.[233] These overall findings of major alterations in the interchromosomal interactions relating to cell type, malignant progression, and epidermal cell differentiation are consistent with the view that the probabilistic patterns of CT interchromosomal interactions in the genome are dynamic and correlate with corresponding alterations in the global gene expression programs.

The interchromosomal positioning and interactions of the subset of human NOR chromosomes (CT13, 14, 15, 21, 22)—which participate in the formation of nucleoli and contribute rDNA for transcription and ribosome biogenesis—were investigated in WI38 human diploid fibroblast cells.[245] The radial positioning of NOR-CT in the nucleus correlated with their size wherein the smaller NOR-CT (CT 21, 22) were more interior than the larger ones (CT 13–15). High levels of pairwise CT interactions ranging from 52% (CT13–21) to 82% (CT15–21) were detected as well as a triplet arrangement of CT 15-21-22 (72%). In cells with multiple nucleoli, one of the nucleoli (termed “dominant”) always associated with a higher number of NOR-bearing CT. Also, certain CT pairs more frequently contributed to the same nucleolus than to others.[245] This nonrandom pattern suggested that a large number of NOR-chromosomes are poised in close proximity during the postmitotic nuclear recovery and through their NORs may contribute to the formation of the same nucleolus.

1.6 | Dynamics of Interchromosomal Networks

The nonrandom profiles of pairwise interchromosomal associations, which demonstrate cell-type specificity and are altered during the cell cycle, malignant transformation, and keratinocyte differentiation, raise a fundamental question: *Is there an overall arrangement of the individual CT into a global interactive network that might in turn drive genomic function and regulation*? A major limitation in addressing this issue is that the demonstrated pairwise CT interactions, while preferred, are also probabilistic. This is most evident when one examines individual nuclei labeled with a series of CT. No two overall patterns are precisely alike and many appear very different. Thus, if a preferred overall pattern of CT associations exists in the nucleus, it will by nature be probabilistic and difficult to identify. Another difficulty is the enormous number of potential CT interactions in any given nucleus. With nine sets of labeled CT, there are 153 potential pairwise interactions among the nine homologous pairs.[230] There is also the problem of properly distinguishing the two copies of each CT and their individual interchromosomal associations.

To solve these problems, computational geometry algorithms were specifically developed to determine the possible presence of probabilistic interchromosomal arrangements of an entire population of labeled CT. In an investigation involving eight chromosome pairs in WI38 lung fibroblast cells, a geometric graph mining approach termed the *Generalized Median Graph* or GMG[247] was applied to determine the probabilistic best fit for the spatial arrangement of the entire set of eight CT pairs (see Figure 3E for multicolor CT FISH image).[246] The GMG has been widely used to model the best representation for a set of structural associations in computer vision and pattern recognition research. For every image set under analysis, each of the 16 CT in the eight labeled chromosomes is segmented, masked, and represented as a simplified graph called an "association graph" containing 16 nodes corresponding to each CT. The individual association graphs are first compared with an initially unknown GMG to obtain its parameterization. A nonconvex optimization procedure is then applied to determine the GMG, which is the "best fit" match graph to all the individual association graphs. While this network of CT associations was probabilistic, other computational algorithms determined that the association graphs for each image set in the analysis contained a minimum of 40% of the connecting associations found in the final GMG interchromosomal network (ICN).[246]

One limitation of the GMG method was that it identified each homologous CT of a pair based on a slight difference in the apparent volumes of each homologous CT rather than their interchromosomal association differences. This problem was solved with the introduction of a geometric technique termed the *Chromatic Median* or CM, which uses combinatorial optimization to infer the common chromosome interaction pattern or network for the overall cell population.[248] While the GMG used integer linear programming and rounding techniques, the CM is more accurate and robust. It is based on a number of newer techniques, such as semidefinite programming, multilevel rounding, geometric peeling, and adaptive sampling.[249,250] The CM technique results in much better approximation ratios and yields near optimal solutions in all tested random or real data sets.[248] Figure 4 illustrates the major steps of the CM to generate the most probable network of CT interchromosomal interactions.

Using the CM graph mining algorithm, a network was reported of 20 interchromosomal associations among a subset of nine labeled chromosomes in both normal human epithelial breast cells (MCF10A) and a malignant breast cancer line (MCF10CA1a) derived from MCF10A.[230] The ICN was nearly completely altered in the malignant CA1a with only one of twenty connections in common (Figure 5A,B). Similarly, the preferred probabilistic ICN were nearly completely different between human WI38 lung fibroblasts and MCF10 human breast epithelial cells, strikingly altered during the cell cycle in MCF10 cells[229] as well as undergoing major alterations (~ 70% differences in interchromosomal connections) following differentiation of human keratinocytes (Figure 5C,D).[233] An ICN was also determined among the entire population of NOR-CT.[245] Because NOR-CT are also found in other CT ICNs, they are likely part of an overall genomic network.

In conclusion, CT investigations combining the tools of re-FISH, computer imaging algorithms, and geometric graph mining approaches have revealed that large subsets of CT form ICNs with cell-type specificity. Alterations in these interactive networks during

malignant cancer progression, cell differentiation, and the cell cycle illustrate their dynamic nature and suggest their possible role in the corresponding genomic expression programs of cells. Moreover, the discovery of ICNs in several different cell lines under different conditions and involving over 50% of the total human chromosomes provide support for a probabilistic “chromosome positional code” wherein the overall interactive networks are different between cell types and may contribute to the global regulation of gene expression.[231,246] It is further speculated that the preferred network defined by this positional code is maintained epigenetically and facilitates specific genomic expression programs characteristic of the particular cell and its differentiation state. The probabilistic nature of the overall interactive network could in turn provide the flexibility for alterations and contribute to corresponding changes in the overall genomic program.

1.7 | Interchromosomal Interactions and Translocations

A characteristic feature of cancer cells are the large number of chromosomal rearrangements. More than 600 recurrent balanced chromosomal rearrangements have been documented in human cancers.[251] This strikingly illustrates the key role that interchromosomal interactions and translocations likely play in tumorigenesis. In order for translocations to occur, the CT involved in the translocations should be in close proximity.[252,253] Indeed, the close associations of certain CT may be characteristic of the normal cell/tissue types that progress into tumorigenesis. For example, in a T-cell lymphoma mouse cell line that has a reoccurring 12:14 translocation, a close proximity of CT 12 and 14 were found in both the lymphoma cells and normal splenocytes.[215] In differentiated adipocytes, CT 12 and 16 are closely associated enabling the (12, 16) translocation that is characteristic of human liposarcoma.[243] Importantly, this association is not observed in preadipocytes indicating that a dynamic rearrangement of interacting CT during adipocyte differentiation plays a crucial role in human liposarcoma tumorigenesis.[243] Tissue specificity of interchromosomal interactions and translocations have also been reported.[242] Proximity of CT 12 and 15 but not CT 5 and 6 were observed in mouse lymphocytes where 12/15 translocations occur in lymphomas.[242] Conversely, close proximity of CT 5 and 6 but not CT 12 and 15 were detected in mouse hepatocytes where 5/6 translocations are common in hepatomas.[242]

Studies have also documented the proximity of genes involved at the breakpoints for translocations. A t(9;22) translocation involving the *ABL* and *BCR* genes is a recurrent primary abnormality in chronic myeloid leukemia (CML) and adult acute lymphoblastic leukemia (ALL). A generally accepted hypothesis is that the physical proximity of the involved chromosomal regions may be one important factor in the genesis of these phenomena. In landmark studies, a close proximity was discovered in hematopoietic cells belonging to different cell lineages for the ABL and BCR genes of t(9;22)[32,254] and the PML and RAR genes of t(15;17).[32] A high level of proximity of the BCR (CT 22) and ABL (CT 9) genes involved in this translocation was also detected in B-lymphocytes.[255] It was, therefore, concluded that this characteristic translocation is mediated by the close association of the participating CT in the normal cell type that give rise to these leukemia cells.[255] Similarly, *MYC*, *BCL*, and immunoglobulin loci, which are recurrently translocated

in various B-cell lymphomas, are preferentially positioned in close spatial proximity relative to each other in normal B cells.[256]

The findings that translocations are more prevalent between sites of higher interaction frequency were confirmed via the Hi-C approach.[257] As anchor sites on chromatin loops are fragile sites for double strand breaks,[258,259] double strand breaks are enriched at TAD boundaries.[258,259] Overall, these studies implicate cell- and tissue type-specific interchromosomal interactions and higher order chromatin organization as the epigenetic basis for the generation of characteristic chromosome translocations during cancer progression.

1.8 | Gene Positioning and Dynamics Within CT

An earlier report concluded that active genes are found at the CT boundaries.[260] Other studies concluded that genes are distributed throughout the CT regardless of their level of expression.[261,262] More recently single-cell Hi-C studies suggested that domains of active gene expression are enriched at the interface of CT.[263,264] These findings, however, were based on a very limited number of individual cells and will require further analysis. Several studies have demonstrated that upon very active expression, genes are found within chromatin loops that project out of the CT. This has been reported for the major histocompatibility complex on CT6,[24] HOX genes on CT11,[23] and the epidermal differentiation complex on CT1.[265] In a groundbreaking study, it was discovered that during murine erythroid differentiation, several genes involved in hemoglobin synthesis (eg, Hbb-b, Eraf, and Uros) are repositioned in close proximity to the same transcription factories for co-regulated gene expression.[266] This occurs despite the large Mbp distances between these genes on CT7.

Repositioning of actively transcribed genes on different chromosomes to the same transcription factory, which must be mediated by specific interchromosomal interactions, has also been reported between Myc and Igh[267] and olfactory genes.[268] Another study found that several housekeeping genes colocalize into an active hub.[269] Repositioning of genes to sites of high proximity may also play a role in gene silencing. Clusters of inactive genes were detected at the nuclear periphery,[159,172,173] perinucleolar regions,[172,173] and within polycomb bodies in Drosophila.[270]

The repositioning of distally located genes into common transcriptional factories is indicative of a major role that the 3-D topology of CT and their interchromosomal interactions may play in regulating gene expression. Consistent with this view, studies have found that enhancers loop back to interact with their target genes.[271] These contacts have been observed across a myriad of physiological conditions and can occur over vast distances of the DNA sequence with some enhancers even looping back over megabases.[272] For example, upon transcription, the β-globin locus, α-globin gene cluster, T_H2, IFNG, MHC class II, CF, IgH and their respective enhancers are in close physical proximity.[271,273–277] The interactions between the beta-globin and its locus of control were shown to require specific transcription factors, including EKLF1 and GATA1.[276,278] It was further reported that not only do the regulatory regions of the locus control region (LCR) and T_H2 interact on CT11, but they also form an interchromosomal cluster with the IFN-γ gene on CT10.[279]

A subset of these enhancers called *super-enhancers* (SEs) are clustered together and occupied by a large number of factors that function in a synergistic cooperative manner to increase expression of their cognate genes.[280] SEs have been implicated in cell-type specificity[281,282] and driving expression of oncogenes in cancer.[281,283] The advancement of chromosome capture techniques has allowed for unbiased and global determination of enhancer-promoter interactions within the context of the global genomic organization.[284] Investigations into the higher order chromatin organization around enhancers has provided the insight that the concentrated activity around SEs induce phase separation of nearby chromatin[64] and that enhancers-promoter interactions are generally constrained within the TADs.[128,285,286]

1.9 | 3-D Topology of Chromosomes and Gene Regulation

It is widely assumed that the 3-D arrangement of CT and the spatial positioning of genes within the CT are linked to genomic function and regulation.[9,18,19,287,288] While elucidating the 3-D arrangement of individual CT and their relationships to gene activity is a challenging endeavor,[214,216,228,242,289,290] progress has been made in understanding the overall shape and 3-D organization of chromatin within individual CT.[239,291–299] CT display a wide range of 3-D shapes from regular ellipsoid-like to highly irregular.[300,301] Properties that could influence the global organization of individual CT include heterochromatin/euchromatin levels and arrangements,[302] gene density,[301,303] RIDGES/ANTI-RIDGES,[296] and overall levels of gene activity.[304,305] A higher degree of irregularity in CT shape is found with increasing gene density.[301] For example, despite being similar in sequence length, the gene-rich CT 17 is less compact and much more irregular in shape than the gene-poor CT 18.[301] The potential influence of gene activity on shape irregularity is demonstrated in female cells, where one homolog of the X chromosome is inactivated (Xi) and more regular in comparison to its active counterpart Xa.[304,305]

Gene-rich CT have a greater proportion of regions that are sensitive to micrococcal nuclease than gene-poor CT and thus have more open chromatin fibers.[302] Moreover, the CT regions that are most sensitive to micrococcal nuclease were among the most gene dense.[302] By mapping the transcriptome, it was shown that these gene dense regions were more active in gene expression (RIDGES) than those that are gene poor (ANTI-RIDGES).[295,306] Chromatin within RIDGES is much less compact and more irregular in shape than the chromatin in ANTI-RIDGES.[295,296]

If there are distinct differences among CT in overall shape, how is the chromatin arranged three dimensionally at the global level of the entire CT? Limited studies using multi-FISH and computer analysis have revealed distinct 3-D organization and specificity for relatively short regions (<5 Mbp) within CT.[296,299,307] A morphometric geometrical approach and statistical shape theory for 3-D reconstruction and visualization of the mean positions of five consecutive probes on a 3.7 Mbp region of chromosome X, provided evidence for a nonrandom organization that differed between Xa and Xi.[307] Similarly, a nonrandom organization in a 4.3 Mbp region of CT14 in mice was detected[299] and significant differences in organization in RIDGE and anti-RIDGE regions were demonstrated for chromosomes 1 and 11 in six different cell lines.[296] More recently, integrated yeast 3C data

were used to model 3-D chromatin structures based on a Bayesian inference framework.[308] This approach, however, is designed to model chromatin structure at a level ≤ 1Mbp.

The organization of larger regions has been until recently largely limited to investigations of chromatin folding by the method of polymer modeling and mean squared distances (MSDs)[309,310] with only one previous study analyzing entire human chromosomes (CT 4, 5, and 19).[310] These investigations have led to general models of chromatin loops and higher level folding that are of potentially great significance,[17,309–313] but do not directly address the precise organization of chromatin within individual CT. In this regard, studies involving both FISH and chromosome capture (3C and Hi-C) techniques have been performed to fit chromosomes or regions of chromosomes to the proposed polymer models.[312,314,315]

While the Hi-C approach has been instrumental for understanding higher order CD of 1 to 10 Mbp across the entire chromosome and defining specific sequences within these domains such as the TADs,[125,128,316,317] a multi-FISH approach is necessary for analyzing sequences separated by the much larger genomic distances that range up to the full length of the chromosome (80–240 Mbp). As valuable as 3C, Hi-C and other chromatin conformation capture techniques are for elucidation of the 3-D organization of CT,[318,319] the findings of these approaches may not always correlate precisely with the actual spatial distances between DNA sites in the 3-D microenvironment of the cell nucleus.[318] A more reliable approach would be to perform both multi-FISH and chromatin capture approaches.

In earlier whole chromosome studies using microscopy, CT 4, 5, and 19 were shown to behave according to a “random walk or giant loop” based on MSD analysis.[310,313] These investigations demonstrated a large increase in MSD within 2 Mbp followed by a much more gradual increase in MSD extending the length of each chromosome.[310] A more recent study combined the tools of 3-D microscopy, multi-FISH, computer imaging, and computational geometric analysis to analyze six labeled regions spanning each CT in the G1 and S phase of WI38 normal diploid human fibroblasts.[320] The six CT investigated (1, 4, 12, 17, 18, X) are representative of both the range of gene densities and CT size in the genome. The average distance between labeled DNA probes ranged from ~15 to 60 Mbp. At these large Mbp distances between probes, MSD analysis along with studies of the folding ratios (FRs) across the CT demonstrated that each CT had a specific folding pattern with some limited alterations across the cell cycle.[320] Random simulation plots of all the CT except CT 17 were very different from the actual CT determinations and uniformly showed little or no changes in MSD or FRs across the entire simulated CT. Thus, when measured at large genomic separations, each CT displays a different profile of genomic to spatial distances that are nonrandom. An exception was CT 17 whose MSD/FR plots were similar to its random simulations with only a small portion displaying nonrandom folding.[320]

A classic data-mining algorithm termed the k-means[321–324] was then applied to determine the best fit probabilistic 3-D topology of the labeled probes across each CT[320] Consistent with the MSD analysis, the 3-D topological models derived from this data mining approach were specific for each CT (Figure 6) and had a high degree of nonrandomness compared with models generated from random simulations. CT 17, however, was again characterized by a high level of randomness.[320] Alterations were detected in G1 vs S phase and it was

concluded that each CT except CT 17 had a specific probabilistic 3-D topology at the global level spanning the whole chromosome (Figure 6). This analysis further revealed that at least one of the telomeres of each chromosome was located at or near the CT periphery. The most striking examples of this were the telomere regions of 17-q during S phase and Xi-p, which were positioned on projections extending from the main CT body.[320] Interestingly, the labeled q-telomeric region of CT 17 contains the gene for tubulin cofactor D, which is a cell cycle regulated protein, and plays a role in cell division.[325] Similarly, the pseudoautosomal region on chromosome X, which is homologous to a region on the Y chromosome and escapes X-inactivation,[326] was found to be the most peripheral in the inactive X in comparison with all other regions in Xi or its Xa counterpart.[320]

In conclusion, recent advancements in chromosome capture techniques such as Hi-C are enabling analysis of the intricacies of chromatin looping and folding and the identification of specific DNA interactions within CD ≤10 Mbp.[9,125,128,316,319] Several physical models have been proposed to explain the organization of chromatin at even higher levels of organization but their application to resolving the global 3-D topology of individual chromosomes has been limited.[309] By combining multi-FISH 3-D imaging with computational and pattern recognition algorithms, progress has been made in understanding both specificity and uniqueness in the overall global folding of each chromosome as well as some cell cycle-related alterations.[320] Chromosome specific differences in structural organization and changes during the cell cycle may provide topological signatures that contribute to the global expression programs of individual chromosomes.

Overall, studies of CT organization and their alterations have potentially important implications for understanding cancer progression and deciphering optimal treatment strategies. Cancer is characterized by compromised global interactions and regulation of the genome.[327] A major challenge in cancer screening is the determination of which patients require aggressive treatment or only to be monitored. Determining whether particular alterations in epigenetics and/or chromatin organization are indicative of poor outcomes could aid in defining tumors that are particularly deleterious. Furthermore, understanding how genes are dysregulated upon disruption of the normal “wiring” of the nucleus by translocations, deletions, and amplifications that are hallmarks of cancer should also enable more targeted therapeutic strategies.

Funding information

Division of Information and Intelligent Systems, Grant/Award Numbers: IIIS1422591, IIS-0713489, IIS-1115220; NIH Clinical Center, Grant/Award Numbers: GM072131, GM-23922; University at Buffalo Foundation, Grant/Award Number: 9351115726; National Cancer Institute, Grant/Award Number: F32-CA220935; University at Buffalo; National Science Foundation; National Institutes of Health

ABBREVIATIONS:

CT	Chromosome territory
S/MARs	Matrix or scaffold attachment regions
CTCF	CCCTC-Binding Factor

MATR3	Matrin 3
LBR	Lamin-B receptor
LADs	Lamin associated domains
NOR	Nucleolar organizer region
NADs	Nucleolar-associated domains
RS	Replication sites
TS	Transcription site
CD	Chromatin domain
TAD	Topologically associating domain
3C	Chromosome conformation capture
Hi-C	High throughput chromosome conformation capture
HP1	Heterochromatin protein 1
SEs	Superenhancers
RIDGES	regions of increased gene expression
MSD	Mean squared distance
FRs	Folding ratios
GMG	Generalized Median Graph
CM	Chromatic Median

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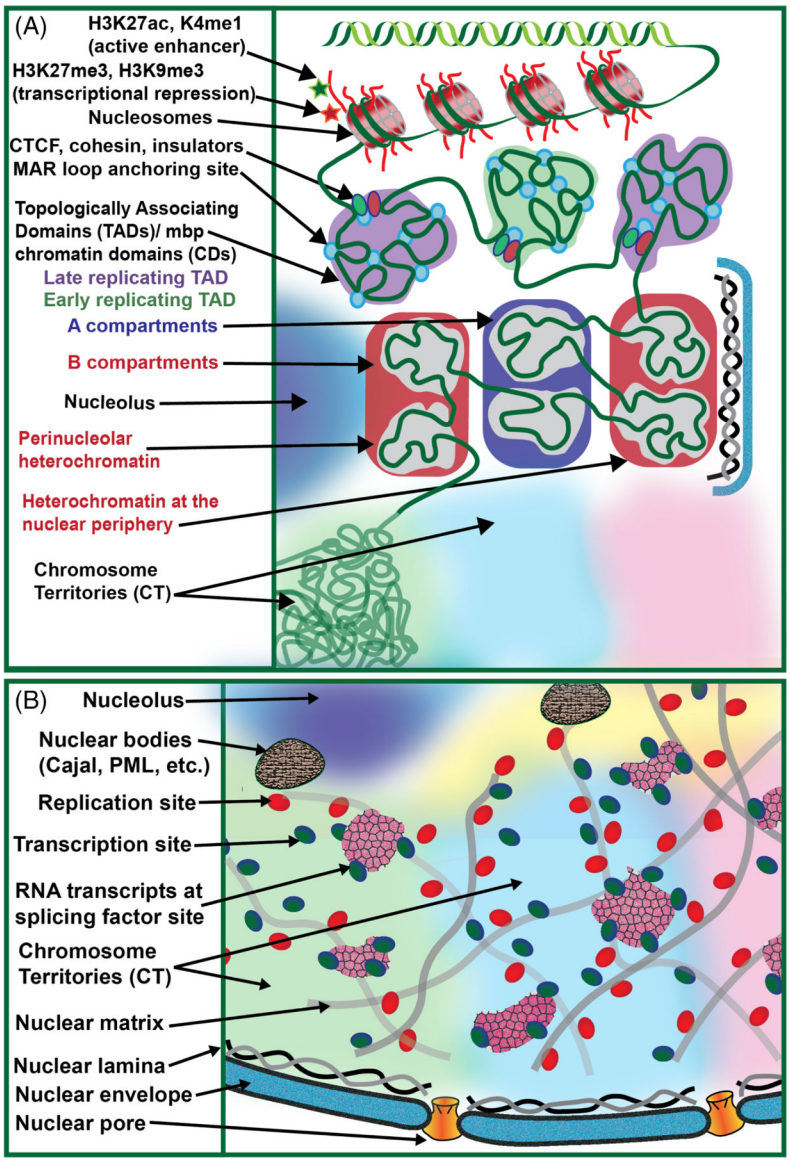

FIGURE 1.

Higher order chromatin organization and functional nuclear architecture. A, Hierarchal levels of chromatin organization are shown including: nucleosomes, chromatin fibers, chromatin loops, mbp CDs (Chromatin Domains)/TADs (Topologically Associating Domains), A and B compartments, and CT (chromosome territories). B, The functional nuclear architecture is depicted. Replication sites and nuclear speckles are associated with the nuclear matrix. Transcription sites associate with the nuclear matrix or nuclear speckles. Other nuclear bodies and compartments are also shown such as the nuclear envelope, nuclear lamina, nuclear pore complex, and the nucleolus

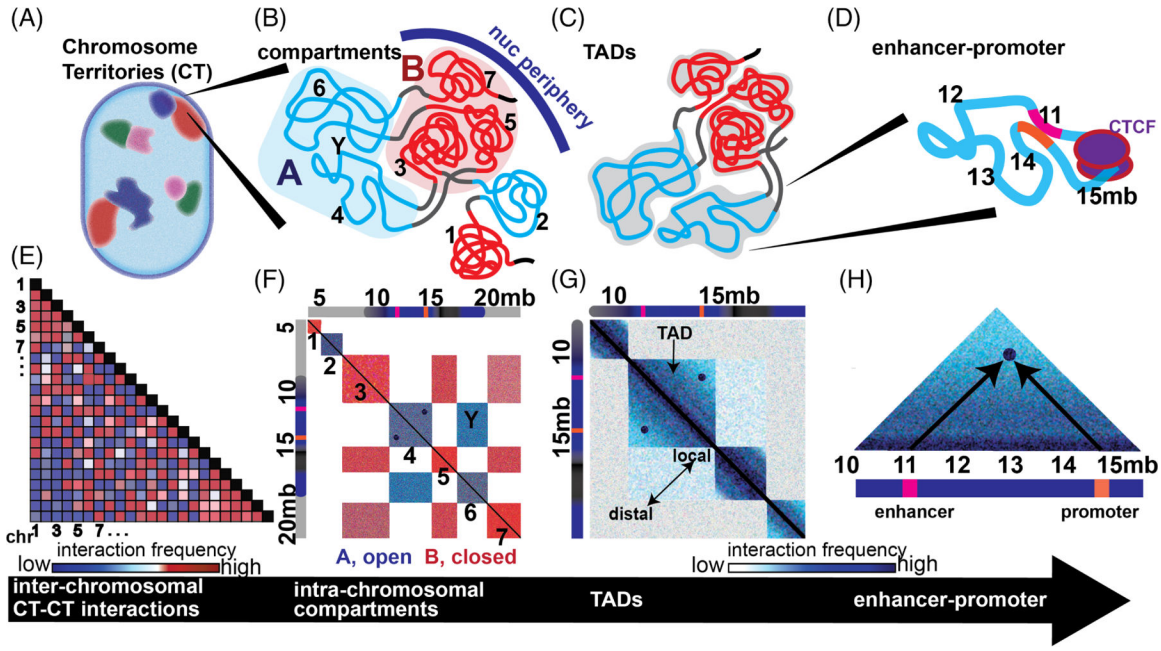

FIGURE 2.

The hierarchy of higher order chromatin organization based on high throughput chromatin conformation capture (Hi-C). Diagrams are shown of higher order chromatin organization in a hierarchy from the whole CT to A and B compartments to topologically associating domains (TADs)/mbp chromatin domains (CDs) to specific enhancer-promoter interactions A-D, with corresponding schematics of Hi-C visualizations representing interchromosomal interactions, A and B compartmentalization, TADs/CDs, and intra-TAD interactions E-H. The Hi-C technique enables determination of pairwise interaction frequencies between regions of chromatin across the genome (represented as a matrix). A,E, At the highest level, total pairwise interaction frequencies between chromosomes can be determined. A simulated matrix of pairwise CT–CT interactions is shown (red = high frequency, blue = low, and white = intermediate). BF, Within CT, chromatin is organized into open, A, euchromatic or closed, B, heterochromatic, compartments. These compartments are in turn composed of TADs exhibiting a triangular pattern of higher frequency interactions along the diagonal. Seven TADs numbered from 1 to 7 are colored red or blue with respect to whether they are contained within A (blue) or B (red) compartments. An interaction between two TADs within a compartment is demarcated with a Y. C,G, A section of this matrix encompassing TADs 3 to 6 is displayed enlarged (darker blue - high frequency, light blue - low). Local interactions are closer to the diagonal while distal interactions are farther from the diagonal. D,H. Enhancer-promoter interactions are generally contained within TADs. A simulated enhancer-promoter interaction is displayed within TAD number 4. This TAD is enlarged to demonstrate the enhancer at ~11 mbp (pink) interacting with the promoter at ~15 mbp (orange)

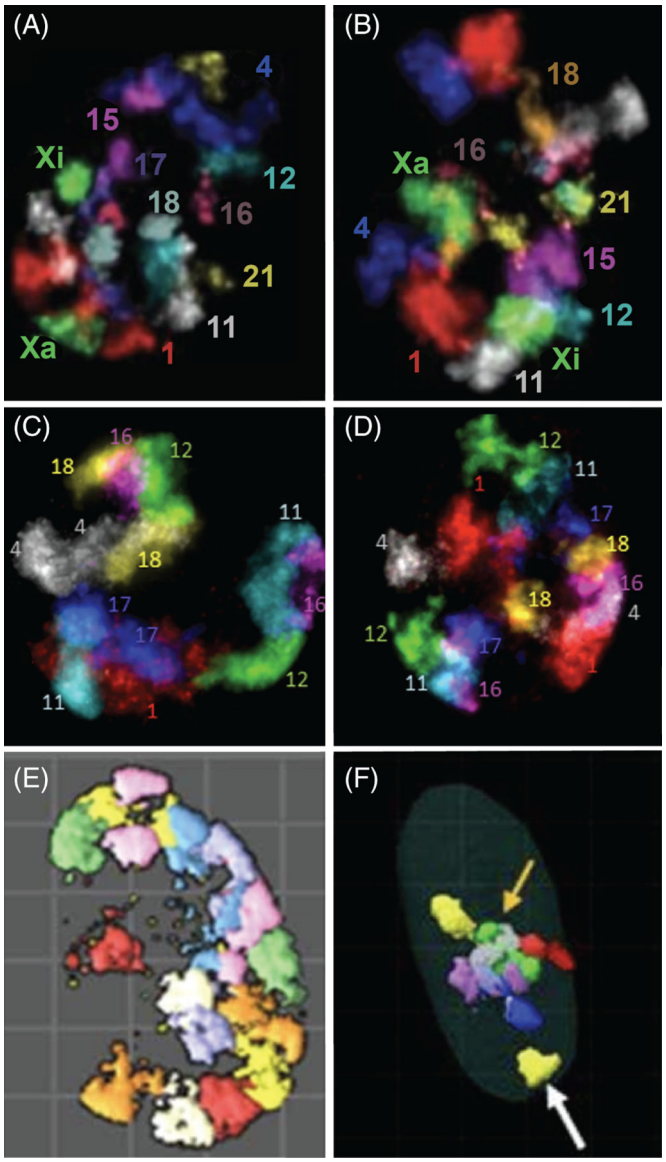

FIGURE 3.

CT multicolor images from re-FISH experiments. Typical CT multicolor labeled images from re-FISH experiments are depicted in nine CT pairs (1, 4, 11, 12, 15, 16, 18, 21, X) from MCF10 normal human breast A, and malignant breast cancer cells B[230]; seven CT pairs (1, 4, 11, 12, 16–18) from undifferentiated C, and differentiated D human keratinocytes[233]; eight CT pairs (1–4, 6–9) from WI38 normal human fibroblasts E 247; and the NOR-CT pairs (13–15, 21, 22) in WI38 cells F.[245] Note the NOR-CT distal to any visible nucleolus denoted by a white arrow and the dominant nucleolus with the many associated NOR-CT denoted by an orange arrow in F.[245] A to D are 2-D projection images while E and F are 3-D surface renderings. An integrated computer imaging program was then applied to segment the individual CT images followed by a large battery of measurements among the total population of labeled CT in the individual nuclei[229,230,233,245,246]

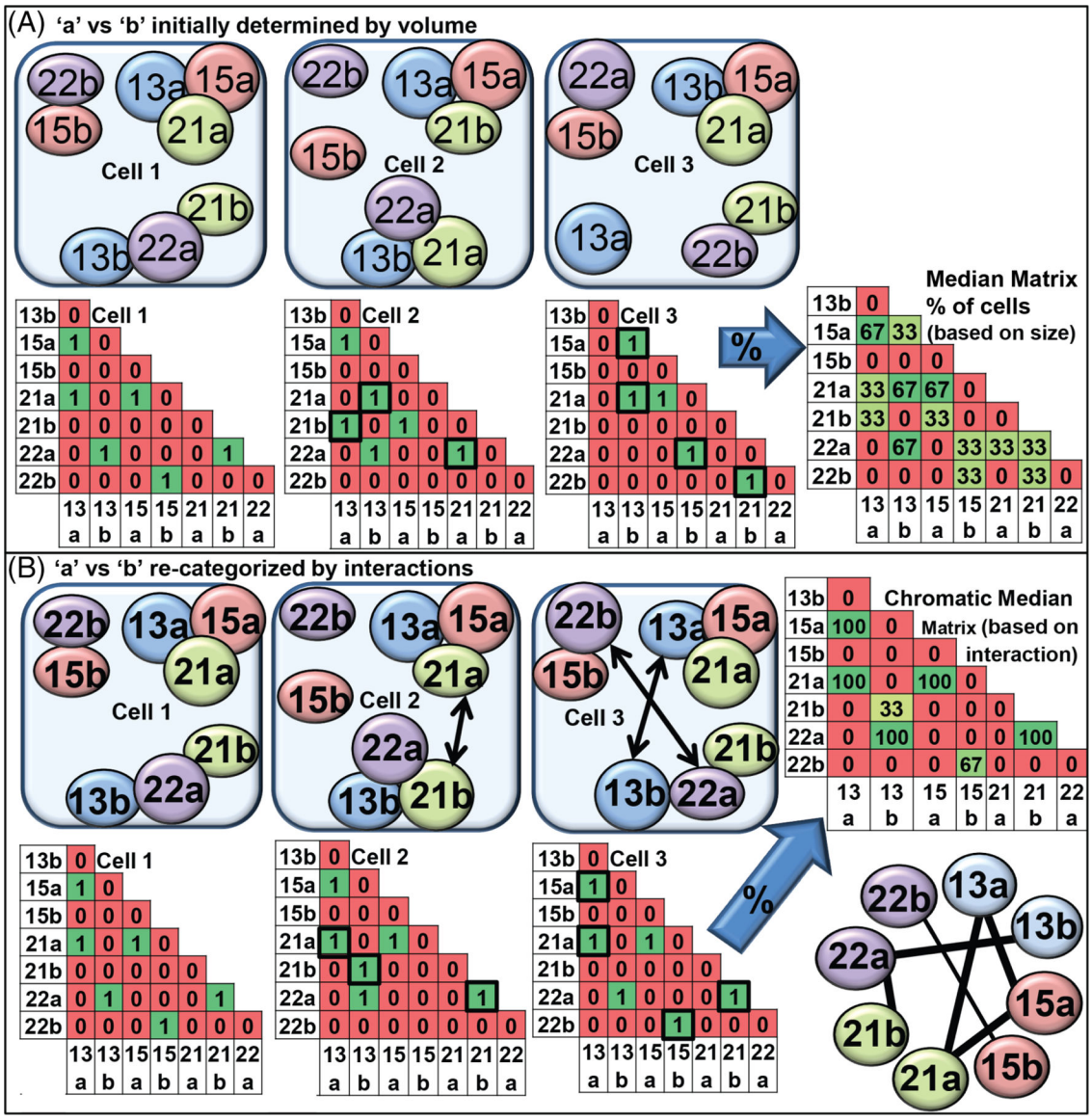

FIGURE 4.

Diagram illustrating application of the chromatic median (CM) analysis to determine the best fit probabilistic arrangement of a set of CT into an interchromosomal network (ICM). A, Three schematic drawings of CT associations in nuclei are shown with the larger homolog defined as copy 'a' and the smaller one as copy 'b'. The associations are represented in binary matrices wherein a 1 indicates an interaction and a 0 the absence of an interaction; B, the chromatic median program determines which homolog is "copy a" vs "copy b" based upon which other CT are associated and switches "a" for "b" to match the best fit model for the population. The percent of cells with an interaction at any given position within the matrix is calculated. Using a threshold, a chromatic median graph enriches for those connections, which are greater than randomizations of the input matrices[245]

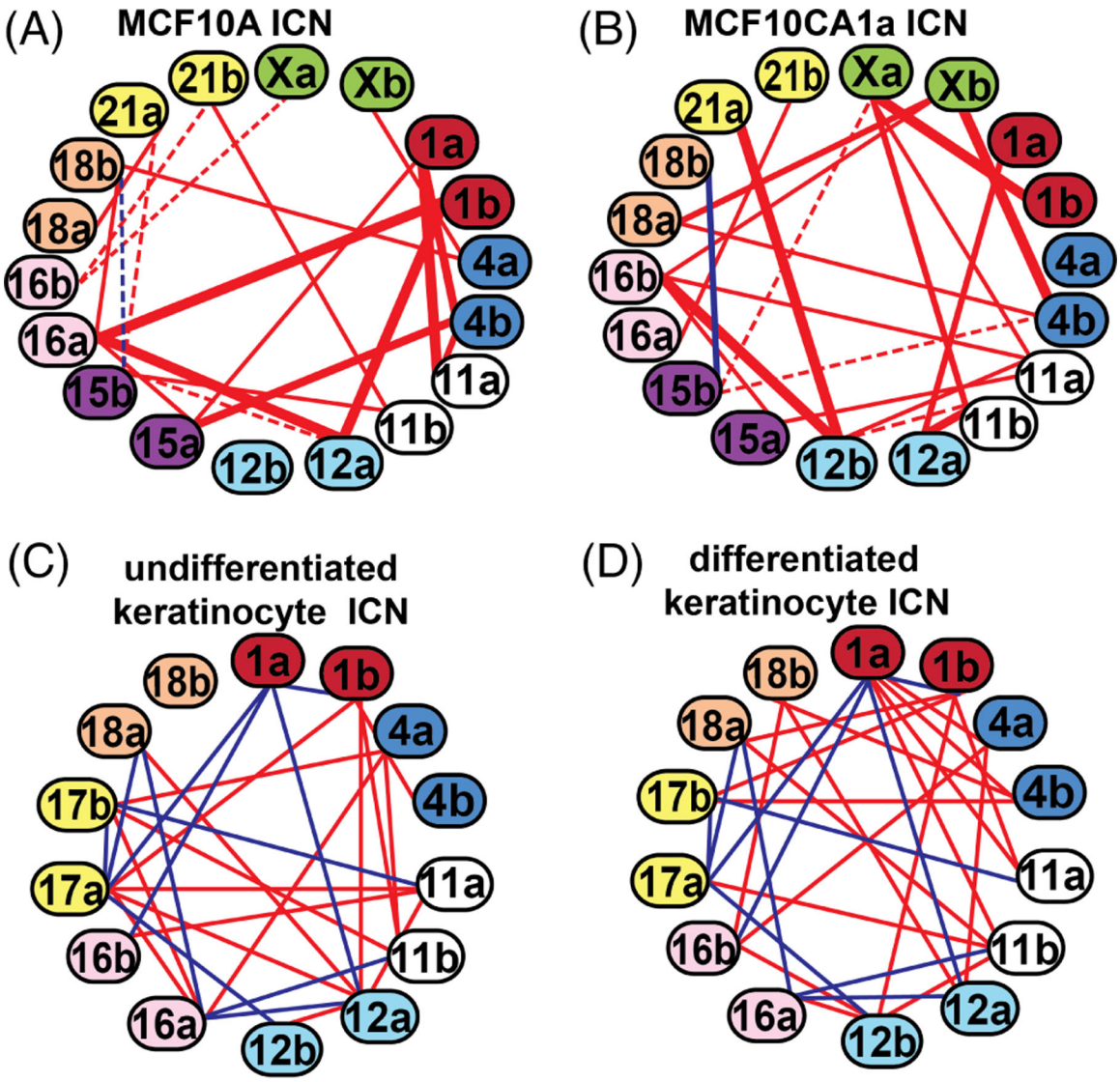

FIGURE 5.

Global nonrandom interchromosomal network (ICN) models. Chromatic median (CM) analysis of the interchromosomal association data generated from re-FISH experiments involving subsets of CT have enabled the generation of the most probable interactive network for all the CT in a particular subset. The general method involved is summarized in Figure 3. The connections between CT pairs depicted in the model were all well above random simulation values.[229,230] ICNs of CT 1, 4, 11, 12, 15, 16, 18, 21, X in MCF10A normal human luminal breast cells A, and MCF10CA1a malignant breast cells derived from the MCF10A cells B. There are massive differences in the ICNs of 10A and CA1a. Nineteen of the twenty preferred nonrandom pairwise associations were unique to both 10A and CA1a (red lines) with only one in common (CT 15b–18b, blue line). Thick solid connections scored the highest in pairwise association levels, thin solid lines are at moderate levels of association, while dashed connections are among the lowest of those above randomization. ICNs of CT 1, 4, 11, 12, 16, 17, 18 in undifferentiated human keratinocytes C, and differentiated keratinocytes derived from the same undifferentiated cells D. Red connecting

lines are unique to each cell type while blue lines depict common interchromosomal interactions common between undifferentiated and differentiated keratinocytes. Approximately two-thirds of the pairwise associations were unique to each cell differentiation state

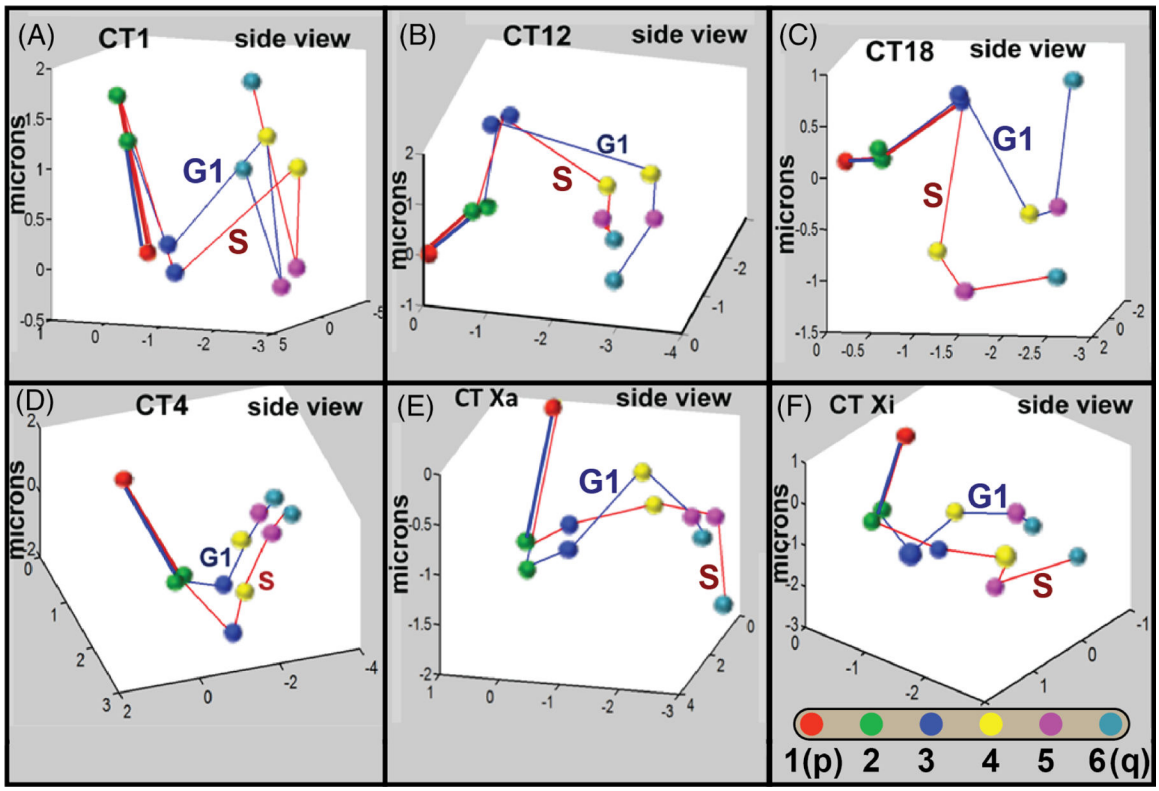

FIGURE 6.

Large scale 3-D topology of CT. The large scale 3-D topology of six CT ranging in size and gene density (CT 1, 4, 12, 17, 18, X) was determined in the G1 and S-phases of human WI-38 diploid fibroblasts. As a first step, multicolor FISH was performed using six BAC probes extending across each chromosome. In-house computational geometric algorithms were applied to measure the 3-D distances between every combination of probes and to elucidate data-mined structural patterns. These findings demonstrated a high degree of nonrandom arrangement of individual CT that were specific for each CT and displayed distinct changes during the cell cycle.[320] A classic clustering and pattern recognition algorithm (k means) was then applied to determine the best fit probabilistic arrangement (topology) in the 3-D positioning of the six BAC probe positions within each CT.[320] The analysis successfully defined a single most probable arrangement and specificity in the 3-D topology of each CT. Comparisons with random simulations demonstrated that all the CT except CT 17 showed significant levels of nonrandomness in the preferred 3-D models with some significant differences between the G1 and S-phases. Side views of these 3-D models are shown in panels A-F with connecting blue lines for CT in G1 and red lines for CT in S-phase. Position 1 and the trajectory to position 2 are overlaid in order to compare topology between individual CT in G1 and S phase. The positional six color scheme from the p(1) to q(6) ends of each CT are shown in panel F