The Spatial Organization of the Human Genome

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Abstract

In vivo, the human genome functions as a complex, folded, three-dimensional chromatin polymer. Understanding how the human genome is spatially organized and folded inside the cell nucleus is therefore central to understanding how genes are regulated in normal development and dysregulated in disease. Established light microscopy–based approaches and more recent molecular chromosome conformation capture methods are now combining to give us unprecedented insight into this fascinating aspect of human genomics.

1. INTRODUCTION

FISH: fluorescence in situ hybridization

Chromosome conformation capture (3C): a set of molecular biology techniques, based on formaldehyde cross-linking, digestion, and ligation, that are designed to query the spatial organization of chromatin

To understand the functioning of the human genome, it is not sufficient to consider only the primary DNA sequence, or even linear maps of posttranslational histone modifications that correlate to active or inactive transcription states (32). Rather, a full knowledge of genome function in vivo requires investigation and understanding of the three-dimensional (3D) folding and spatial organization of chromosomes in the nucleus. Historically, this has been studied mainly by using fluorescence in situ hybridization (FISH) to visualize the position and organization of chromosomes, chromatin domains, and individual genes.

FISH is visually compelling but generally limited to looking at the locations of a few specific targets in a few hundred cells, although recent probe developments based on massively parallel custom oligonucleotide synthesis have expanded the scope and scale of sequences that can be analyzed in each hybridization reaction (8). Excitingly, high-throughput molecular assays that use cross-linking and intramolecular DNA ligation to infer the spatial relationships of different loci and even whole genomes have now been developed. These techniques derive from the original chromosome conformation capture (3C) method (27). Like 3D FISH, 3C approaches use formaldehyde fixation to capture spatial proximities of the genome in vivo. After restriction enzyme digestion, ligation of sequences cross-linked to the same supramolecular complexes generates chimeric DNA templates that can be amplified and identified. The relative frequencies of the chimeric DNA sequences are thought to be inversely proportional to the original spatial distances between the chromatin segments.

In the original 3C method, ligation products between two sequences are detected by polymerase chain reaction (PCR) amplification using primers specific for the two loci of interest. Expanding the scope of 3C, circular chromosome conformation capture (4C) looks out from a specific bait sequence of interest to see what other loci across the genome can be captured together with the original locus (85). This approach has been used to investigate the associations of genes with putative long-range regulatory elements (40, 62, 69).

To eliminate the need to prescribe where in the genome to start looking out from, "all-against-all" 3C methods have been developed. Of these, chromosome conformation capture carbon copy (5C) interrogates all possible contacts at high resolution within a defined genomic region of up to a few megabases (2, 30, 71, 81). Hi-C extends this to the whole genome, theoretically enabling the detection of all possible cross-linked contacts in the genome (45, 54, 105). The first Hi-C studies of metazoan genomes had quite low resolution, but increases in sequence depth have recently yielded higher-resolution maps (29).

2. RADIAL CHROMOSOME ORGANIZATION

FISH with chromosome paints has revealed that human chromosomes are not randomly organized—they have preferred positions relative to the nuclear periphery or interior (**Figure 1*a***). In many cell types, this organization is related to gene density, with gene-dense chromosomes and chromosomal regions having a preferred position in the nuclear interior (6, 7, 19, 51). Hi-C data are consistent with the spatial proximity of gene-rich human chromosomes 1, 16, 17, 19, and 22 in the center of the nucleus (45) (**Figure 1*b***).

However, beyond the congregation of gene-rich chromosomes in the center of the nucleus and the clustering of the rDNA-containing chromosomes close to nucleoli, there is no evidence that a given human chromosome consistently has a specific neighbor in the nucleus—even in a homogeneous cell population. Hi-C contacts between chromosomes in a cell population are largely stochastic (45), and FISH has revealed that although relative chromosome positions can be quite similar between the nuclei of daughter cells—a result of the shared position of sister

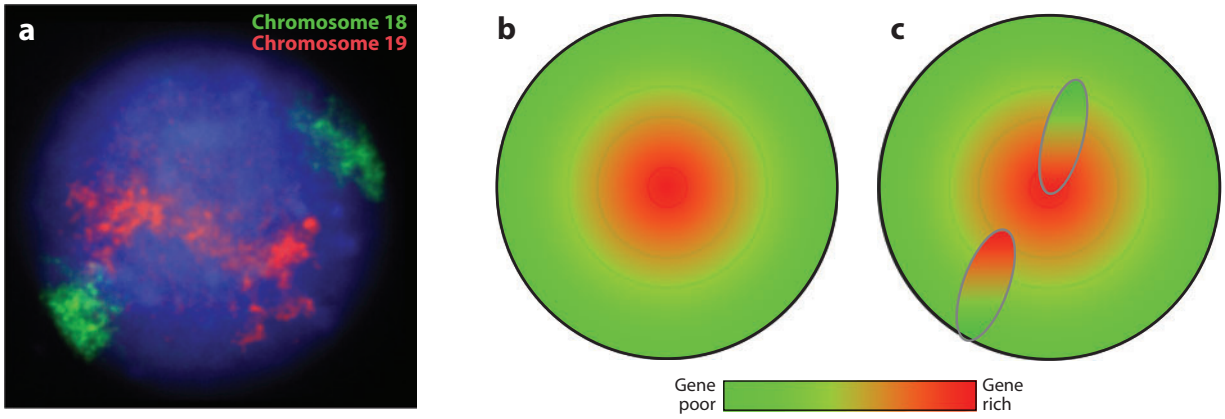

Figure 1

Radial organization of human chromosomes in the nucleus. (*a*) Fluorescence in situ hybridization (FISH) image of a human nucleus hybridized with chromosome paints for the gene-rich chromosome 19 (*red*) and the gene-poor chromosome 18 (*green*). DNA is stained with DAPI (4′,6-diamidino-2-phenylindole) (*blue*). (*b*) Schematic of radial nuclear organization, illustrating the general tendency of gene-rich chromosome domains (*red*) to congregate in the center of the nucleus. Gene-poor regions (*green*) tend to be located around the nuclear periphery. (*c*) Schematic showing the effect of a balanced chromosome translocation on nuclear organization. Translocations that join together genomic regions from gene-rich and gene-poor chromosomes result in chromosomes (*outlined in gray*) in which genes are taken out of their normal nuclear environment.

chromatids on the preceding metaphase plate—this organization is quite different from the spatial organization of the chromosomes in the mother cell nucleus (89, 97).

Clustering of gene-rich chromatin in the interior of the nucleus is not set in stone. In the nuclei of rod photoreceptors of nocturnal animals, there is a dramatic inversion of radial organization that puts inactive constitutive heterochromatin into the nuclear center and gene-rich active chromatin more toward the nuclear periphery (86), although a thin layer of apparently compact heterochromatin is still visible around the nuclear periphery in these cells (46). This inverted nuclear organization seems to be an evolutionary adaptation to assist in optimal vision under conditions of low light intensity (86) and has recently been attributed to the absence of specific lamins and proteins of the inner nuclear membrane that normally act to tether heterochromatin at the nuclear periphery (87). Movement of gene-poor human chromosomes away from the nuclear periphery has also been seen in quiescent or senescent human fibroblasts (11, 60).

2.1. Associations with the Nuclear Periphery and Nucleolus

In addition to the preferential localization of specific chromosomes at the nuclear periphery, visual assays have shown that there is also a polarized nuclear organization within chromosomes, with gene-poor regions preferentially oriented toward the nuclear periphery compared with gene-rich regions from the same chromosomes (8, 51). In many human cell types, centric and pericentric heterochromatin are also generally found at the nuclear periphery or around nucleoli (38, 98). The penchant for gene-poor chromosomes and chromatin domains to be located at the nuclear periphery is consistent with the idea that the peripheral zone of the nucleus is associated with low levels of gene expression (25).

At a more molecular scale, the parts of the genome that are preferentially associated with components of the nuclear periphery have been identified by DamID—a technique in which the bacterial DNA adenine methyltransferase (Dam) is fused to a protein on the nuclear periphery (for

DamID: a molecular biology technique that identifies regions of the genome that come into contact with a particular protein; it does not depend on the availability of a specific antibody

LAD: lamina-associated domain

Laminopathy: a group of genetic disorders caused by mutations of the nuclear periphery, including proteins of the inner nuclear membrane and the nuclear lamina

example, lamin B1), thereby leaving a novel mark (A-methylation) on the parts of the genome that come into contact with the fusion protein/nuclear periphery. In human fibroblasts, this reveals >1,000 marked domains—the so-called lamina-associated domains (LADs) (39), which collectively cover approximately 40% of the genome. Consistent with FISH experiments, LADs are generally gene poor and associated with low levels of gene expression. More direct evidence in support of a role for the nuclear periphery in suppressing gene expression comes from experiments that remodel nuclear organization by artificially tethering loci to the nuclear periphery (35, 50, 76). In these experiments, transcription of some (but not all) genes relocated to the nuclear periphery was downregulated.

The radial positions of genes are not fixed. DamID of differentiating mouse embryonic stem (ES) cells has indicated that the nuclear periphery interaction pattern is, in part, cell type specific and that loss of interaction with the periphery might unlock the potential for a gene to become activated later in differentiation (74). FISH experiments have also visualized the repositioning of some loci toward or away from the nuclear periphery during differentiation coincident with the repression or activation of nearby genes (43, 48, 99).

Surprisingly, aside from the expected rDNA loci of the acrocentric human chromosomes, the regions of the human genome that copurify with the nucleolus substantially overlap with LADs (66, 95). Indeed, microscopy has confirmed that some chromosomal regions associated with a nucleolus in a mother cell can be repositioned to the nuclear periphery in the daughter cells (95). The asymmetric distribution of a locus to the nucleolus in one daughter and to the nuclear periphery in the other daughter has also been seen in live cell analysis (91). That the nuclear and nucleolar peripheries provide two alternative locations where the same repressive genomic domains can partition is supported by the observation that late-replicating chromatin is distributed at both the nuclear periphery and around nucleoli (34, 72).

2.2. Radial Nuclear Organization, the Nuclear Periphery, and Human Disease

The mutation of genes encoding proteins of the nuclear periphery, leading to a variety of human disorders collectively termed the laminopathies, raised the possibility that the spatial organization of the genome with respect to the nuclear periphery might be associated with human disease. Indeed, there is evidence from electron and light microscopy for loss of repressive chromatin from the nuclear periphery in some of the laminopathies (21). Loss of radial chromosome organization has also been reported in the nuclei of fibroblasts from individuals with laminopathies (59).

In the dominant premature aging disease Hutchinson-Gilford progeria syndrome, the underlying genetic defect in *lamin A* leads to accumulation at the nuclear lamina of progerin—a mutant form of lamin A that cannot be correctly processed and that remains permanently farnesylated (14, 22). DamID has revealed altered genomic interactions for wild-type lamin A compared with those of progerin (49). Treatment with farnesyl transferase inhibitors prevents the accumulation of farnesylated progerin, producing a less toxic mutant lamin A protein, and this also seems to restore the normal radial nuclear positions of chromosomes in the treated fibroblasts (61).

The observation that the large domains of reduced DNA methylation (hypomethylation) seen in cancer cells are coincident with LADs has led to the suggestion that the spatial organization of the genome in relation to the nuclear periphery may be important in the gene-silencing events linked to the development of cancer (4).

2.3. Radial Organization and Chromosome Translocations

Although the extent to which the nuclear organization of the genome contributes to the molecular pathology of human genetic disorders remains unclear, radial chromosome organization itself appears to have consequences for structural abnormalities of the human genome.

Live cell imaging of individual loci (91), chromatin domains (89), and whole chromosomes (65) indicates that, apart from periods shortly before and after mitosis, chromatin has limited mobility in the cell nucleus. To generate chromosome translocations, the DNA from the two chromosomes involved must come sufficiently close together to be joined by double-strand break repair. As a consequence, the probability with which a specific chromosome translocation occurs should be influenced by the probability with which the two chromosomes concerned adopt similar positions in the nucleus. Indeed, the frequencies of specific translocations—both those occurring naturally in the human population (5) and those induced experimentally in human and mouse lymphocytes (17, 105)—are elevated for chromosomes located near one another in the nucleus. Genes involved in common translocations are also located near one another in the nuclei of cells that give rise to specific translocations (73, 77).

Chromosome territory: the portion of the nucleus that is occupied by the chromatin from a particular chromosome

However, nuclear organization is stochastic, not rigid, so chromosomes are not always in their preferred locations. Translocations can therefore occur between chromosomes that normally have very different nuclear positions. FISH has indicated that the resulting translocation chromosomes have a compromised nuclear organization, such that material from a chromosome that would normally be at the nuclear periphery (such as human chromosome 18) is dragged away from that position by fusion to a portion of a more centrally located chromosome (such as human chromosome 19) (20). Probably the most common example of this is the recurrent constitutional t(11;22)(q23;q11) translocation. Chromosome 22 is one of the most centrally located chromosomes in the human nucleus (7), and the shifted position (more toward the nuclear center) of the derivative 11 in balanced carriers of the reciprocal t(11;22) is likely influenced by its fusion to chromosome 22 material (42) (**Figure 1*c***). Similarly, the chromosome 22 material on the derivative 11 is not as centrally located as it would be on a normal chromosome 22. This altered nuclear organization is coupled to widespread deregulation of gene expression in balanced reciprocal translocation carriers, including deregulation of genes distributed along the translocated chromosomes, not just those at the translocation breakpoint. The biological consequences of this are not clear, because although t(11;22)(q23;q11) carriers are phenotypically normal, there are reports indicating that they have an increased cancer incidence. Similarly, these findings suggest that there may be unappreciated consequences for gene expression that result from the genome rearrangements—including translocations—that occur commonly in cancer cells.

Radial chromosome organization may also have important consequences during evolution. Gene-density-related radial organization is conserved in all primates studied (67). However, karyotypic rearrangements have resulted in some reshuffling of chromosomal material along evolutionary lineages, and the genes mapping on chromosomes that have been structurally rearranged between humans and chimpanzees show much more varied expression levels between these two species than do genes that are retained on completely syntenic chromosomes (57).

3. THE CHROMOSOME TERRITORY

The major feature that stands out in Hi-C analyses of all metazoan genomes is the chromosome territory—i.e., most of the captured cross-linked associations are in *cis* rather than in *trans* (45, 54, 83, 105). This is in accordance with the appearance of individual chromosome territories in FISH with chromosome paints (**Figures 1*a*** and **2*a***). Indeed, FISH has also suggested that the p and q arms of a metacentric chromosome are also quite separate entities (28). Consistent with this, 3C analyses (45, 83, 93) have suggested that the centromere attenuates associations between sequences located on the two opposite arms of a chromosome. What feature of centromeres is responsible for this has not been determined, but this attenuation does indicate that another form

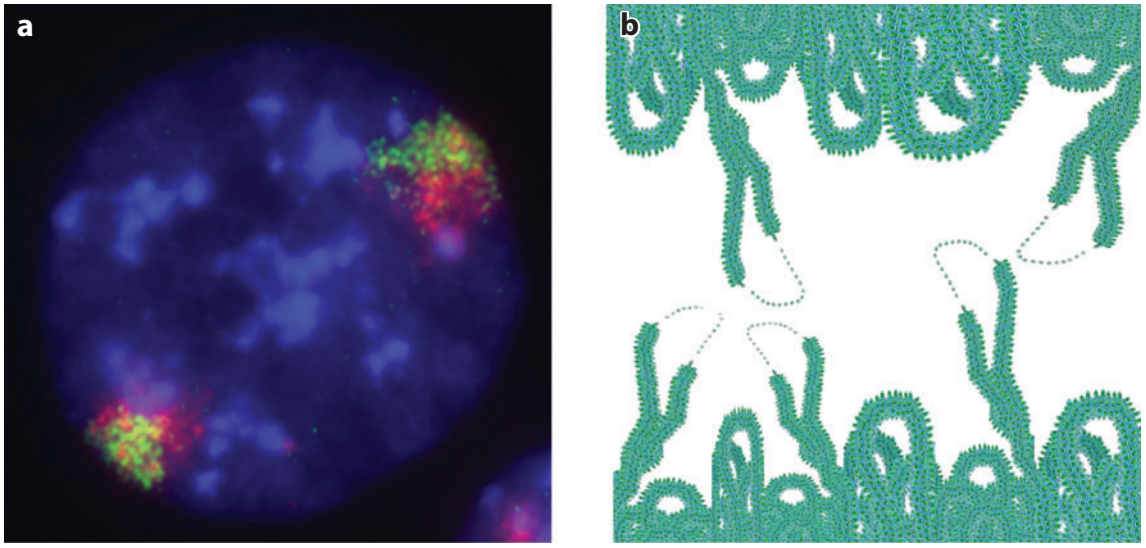

Figure 2

Spatial organization of chromosome territories. (*a*) Fluorescence in situ hybridization (FISH) image of a nucleus hybridized with a chromosome paint for a particular chromosome (*green*) and with a probe (8) that contains only the genes from that chromosome (*red*). DNA is stained with DAPI (4′,6-diamidino-2-phenylindole) (*blue*). This illustrates en masse the looping out of gene-rich areas from the chromosome territory. (*b*) Diagram illustrating how regions of the genome that loop out from chromosome territories may then have the ability to associate with one another, either in *cis* or in *trans* between chromosomes. Such associations can then be identified by chromosome conformation capture (3C) methods.

of common structural chromosome variation—pericentric inversions—can also alter long-range genome associations (93).

Although in-*cis* associations dominate most 3C studies, in-*trans* sequences are also captured. These associations tend to be between sequences from genomic regions characterized by high gene density and transcriptional activity as well as by high levels of DNase I–hypersensitive sites (DHSs), indicating the presence of active regulatory elements (40, 45, 54, 85, 102).

The explanation for this likely lies in the fact that genomic regions with these characteristics are not constrained within their own chromosome territory but rather can dynamically loop in and out of their chromosome territory (65) (**Figure 2*b***). Indeed, there is a direct relationship between the probability of capturing in-*trans* cross-links in 3C experiments and the extent of looping out visible by FISH for the individual loci concerned (45, 56). Moreover, custom FISH probes composed of high-complexity oligonucleotide pools designed to cover the exons of an entire chromosome reveal the remarkable extent to which gene-dense chromosomal regions decorate the outside of their own chromosome territories, beyond the limits of the core territory detected by FISH with traditional chromosome paints (8) (**Figure 2*a***).

DNase I–hypersensitive site (DHS): a region of chromatin that is especially sensitive to cleavage by the nuclease DNase I; the normal nucleosomal structure is disrupted at such sites, often owing to other factors bound there

This level of organization is also not fixed between cell types. When *Hox* genes are activated during ES cell differentiation, they are induced to loop out from their chromosome territories (15, 63). Coincident with this movement, 3C-type assays then begin to capture sequences from other chromosomes together with *Hox* loci (101). But the ability of a locus to move to the outside of its chromosome territory and intermingle with sequences from other chromosomes is also influenced by its linear chromosomal context. In primary erythroid cells, the human α-globin cluster is decondensed and often loops out from its own chromosome territory (12, 13). The orthologous mouse locus is embedded in a different genomic context from its human counterpart

owing to a break in conserved synteny. The condensed chromatin environment of mouse α-globin in murine erythroid cells makes infrequent associations in *trans* with other regions of the mouse genome. When the mouse α-globin locus is "humanized" by replacement with 120 kb of the human sequence, the resulting locus has the nuclear organization characteristic of mouse α-globin and not human α-globin (12).

The functional consequences of looping out remain unclear, but this level of spatial organization is thought to reflect a chromatin structure and dynamics that allow active genes the freedom of movement to explore a larger nuclear space (64). This may allow genes in such regions to interact more frequently with nuclear compartments that enhance gene expression, such as transcription factories (nuclear compartments with a high concentration of RNA polymerase II) and splicing speckles (12, 82, 90). One consequence of looping out is that activated genomic regions have a greater ability to infiltrate the territories of other chromosomes (9), which may enhance their ability to participate in chromosome translocations.

4. CHROMATIN DOMAINS

4.1. Spatial Clustering of Active Genomic Regions

Aside from the chromosome territory itself, the other consistent feature that stands out in 4C and Hi-C analyses of metazoan genome organization is that active gene-dense domains with a high DHS concentration tend to associate with one another (40, 45, 54, 83, 85, 88, 102, 105). Most of these associations are intrachromosomal. Indeed, systematic FISH analysis had demonstrated that multiple gene-rich segments scattered along a single chromosome have a tendency to cluster in the nuclei of mouse cells (84). For some of the interchromosomal associations between active regions that have been captured by 3C approaches, their spatial proximity in the nucleus has been validated by FISH.

The 4C associations between active domains are similar between different cell types, and the associations captured by the active β-globin locus in erythroid cells are with other generally transcriptionally active genomic regions rather than with regions with erythroid-specific expression (85). This suggests that clustering of active regions is not driven by genes moving to specific transcription factories that are specialized in particular transcriptional pathways (82, 90). One possibility is that the clustering of active domains reflects the congregation of such genomic regions around sites in the nucleus where mRNA splicing factors are concentrated (12, 13). This stochastic self-association of highly active genomic regions may be one of the factors that contribute to radial nuclear organization.

4.2. Clustering of Inactive Regions

As with active genomic regions, 3C-type studies also indicate some tendency for inactive genomic regions to associate with one another (54, 83, 85). However, compared with active domains, inactive chromatin domains are more constrained in their ability to interact over long genomic distances. Interactions between inactive domains are generally found within the same chromosome arm and, moreover, within restricted portions of each chromosome arm (centromere proximal or centromere distal) (102). This more constrained 3D organization probably reflects the fact that inactive regions have reduced freedom of motion compared with active domains and are found mainly inside their chromosome territories. For example, in ES cells, *Hox* clusters are kept in an inactive compact chromatin state by the Polycomb repressive complexes (see below), and the inactive *Hox* loci are found inside their host chromosome territories (15, 64). When activated, *Hox*

loci gain the ability to move outside of their own chromosome territories, and this coincides with the ability of *Hox* genes to associate with sequences from other chromosomes in 3C assays (101).

TAD: topologically associating domain

PRC: Polycomb repressive complex

4.3. Local Chromatin Domains

Recent high-resolution 5C and Hi-C studies (29, 71) have suggested that cross-linked associations are enriched locally within ~900-kb topologically associating domains (TADs). The chromatin properties of the regions that lie between TADs are not yet fully understood, but such genomic regions tend to be those enriched in sequences where the local nucleosome structure is perturbed, such as binding sites for CTCF and regions dense in housekeeping genes whose promoters will be nucleosome free and that have a high probability of looping out of their chromosome territories (see above). Such sequences might therefore be located in rather flexible chromatin structures, and so might be expected to be more promiscuous in the other regions of the genome that they can associate with compared with the sequences within TAD domains.

The genomic regions defined as being within TADs correspond quite well to the distribution of LADs along the genome and to some histone modifications, especially H3K9me3 and H3K27me3. However, TADs are also remarkably similar between functionally very different cell types, even though the underlying histone modifications change. Moreover, TADs seem to persist even when the histone-modifying activities responsible for H3K9 and H3K27 methylation are removed (71). This suggests that TADs are influenced by an inherent property of the underlying genome sequence that is yet to be determined.

However, removal of H3K27me3 does have a dramatic visible effect on chromatin domains detected by FISH. H3K27me3 is catalyzed by Polycomb repressive complex 2 (PRC2). Major targets of PRC2 in mammalian ES cells are the four paralogous *Hox* loci, which have a visibly compact chromatin conformation in ES cells and in parts of the embryo where *Hox* genes are yet to be activated. The region of visible chromatin compaction at *Hox* loci corresponds to the extent of the Polycomb-associated H3K27me3 histone modification domain. Upon gene activation in vitro and in vivo, *Hox* loci decompact coincident with the loss of Polycomb-mediated repression and H3K27me3 (16, 63). Moreover, ES cells mutant for Polycomb repressive complexes fail to keep the *Hox* loci in a compact state, and it appears that the PRC1 complex, recruited by PRC2 and H3K27me3, is responsible for this (33).

FISH has also revealed differences in chromatin compaction at different regions of the human genome. For example, the hybridization signal from the gene-rich chromosome 19 occupies a larger proportion of the nuclear space than does the signal from the equivalently sized (in megabases) but gene-poor chromosome 18 (20). Different degrees of chromatin compaction have also been inferred from the relationships of interprobe nuclear distances compared with genomic distances at G-band and R-band regions of the genome (103). This is consistent with genome-wide maps of chromatin fiber folding, established using sucrose gradient sedimentation, in human cells (37). Domains of open chromatin fiber structure correspond to the most gene-dense active R-band regions of the genome, and compact domains correspond well to G-band regions.

4.4. Replication Timing Domains

The partitioning of the human genome into compartments with differential chromatin folding and visible appearance (chromosome bands) also corresponds well with the way that the genome is replicated during the S phase of the cell cycle, with G bands being late replicating and R bands early replicating (18). High-resolution genomic maps of replication timing also show 100-kb to 1-Mb domains of early- and late-replicating DNA. Early-replicating domains generally contain active genes, whereas late-replicating domains are mostly transcriptionally silent (26, 44).

Consistent with their generally gene-poor and transcriptionally inactive state, late-replicating domains correlate well with LADs, and late-replicating domains indeed tend to concentrate at the nuclear periphery (72).

LCR: locus control region

Just as TADs seem to be hardwired in some way into properties of the underlying genome sequence, so replication timing domains appear to be rather stable fundamental units of chromosome organization. For example, a human chromosome 21 contained in mouse cells largely retains the replication timing pattern of the chromosome when in its normal context of human cells (75). Regions where the replication timing profile was altered generally corresponded to sites where the human chromosome had become rearranged structurally. Indeed, some chromosome translocations have been reported to perturb replication timing over very large chromosomal regions (10). However, altered replication timing profiles associated with translocation breakpoints in leukemia have also been found in cases where the translocation is not present. This suggests that replication timing itself can perturb genome function/chromatin structure in such a way as to predispose to chromosome structural rearrangements (78). For approximately 50% of the genome, however, the timing of DNA replication is not fixed, but rather changes during development and is linked to the transcriptional status of the region (41, 79).

5. LONG-RANGE GENE REGULATION

A striking feature of gene regulation in the mammalian genome that is not apparent in the genomes of simple eukaryotes is *cis*-regulatory elements that are distant from their target genes. Human Mendelian genetics has played an important role in highlighting this mode of gene regulation and in identifying some of the associated *cis*-acting regulatory sequences. The classic example is the locus control region (LCR) of the β-globin cluster, which was first identified through deletions associated with β-thalassemia that remove this element but that leave the β-globin genes themselves intact (31). The distance from the LCR to its target genes (a few tens of kilobases) pales in comparison with that between some other regulatory elements that have been identified through human disease-associated chromosomal translocations, inversions, and microdeletions (47). Particularly striking is the 3-Mb regulatory domain that extends 1.5 Mb both upstream and downstream of *SOX9* and is associated with campomelic dysplasia and Pierre Robin sequence (3).

It is not just structural aberrations that can disrupt the function of long-range regulatory elements. Sequence variants located in enhancers within the *SOX9* regulatory domain have also been associated with prostate cancer risk (104). Point mutations in the zone of polarizing activity regulatory sequence (ZRS), located 1 Mb upstream of *SHH*, cause preaxial polydactyly (52)—a phenotype quite distinct from those arising from deletion of the *SHH* coding region and even from deletion of the ZRS itself (80). These base changes have been found to act as gain-of-function mutations, creating binding sites for a transcription factor that activates *Shh* expression at an ectopic site in the limb (53).

This intriguing mode of transcriptional regulation has been thrown into even sharper relief by the realization that most complex disease risk and quantitative trait loci identified through genome-wide association studies fall into intergenic and noncoding regions of the human genome, many of which have other signatures of long-range enhancers such as DHSs and certain histone modifications. The abundance of these regulatory elements across the human genome was confirmed by the recent data from the Encyclopedia of DNA Elements (ENCODE) Consortium (58, 92).

5.1. Enhancer-Promoter Loops

If the human genome is considered only as a linear entity, it is hard to imagine mechanisms through which an element could regulate a target gene 1 Mb away (**Figure 3*a***). Therefore, 3D

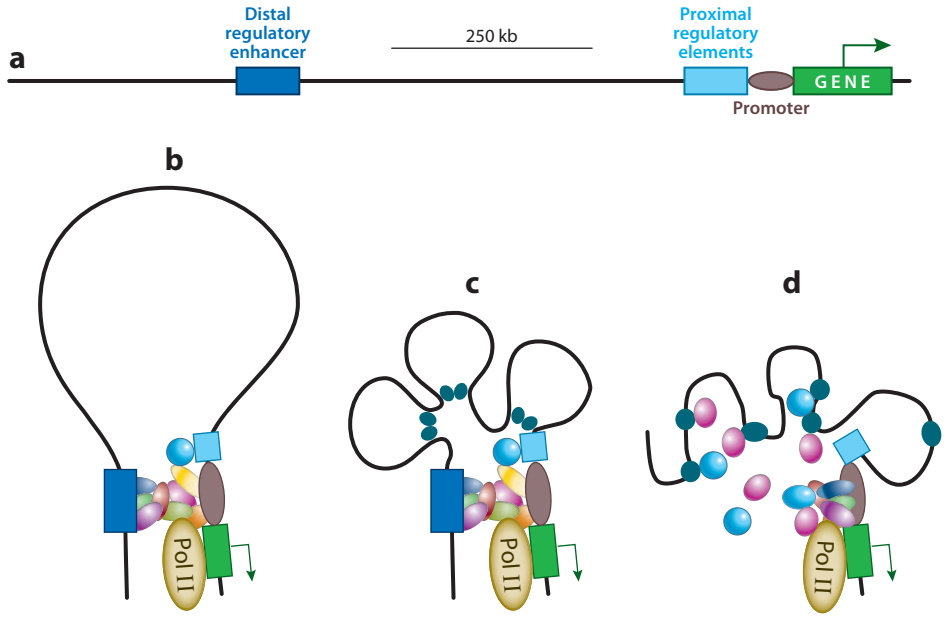

Figure 3

Chromatin conformation loops and long-range gene regulation. (*a*) Linear representation of a gene (*green box*) regulatory landscape showing the position of the gene promoter (*brown oval*), the immediately proximal regulatory elements (*light blue box*), and the distal regulatory enhancer (*dark blue box*), which may be located hundreds or thousands of kilobases from the gene promoter. (*b–d*) Models of chromatin conformation that may underlie enhancer-promoter communication. In panel *b*, factors (transcription factors, coactivators, and chromatin remodeling complexes) bound to the enhancer and promoter reorganize the intervening chromatin into a loop that juxtaposes the distal enhancer and gene promoter. In panel *c*, enhancer-promoter interactions occur by the formation of chromatin miniloops; this model may be particularly applicable to complex regulatory landscapes composed of multiple dispersed enhancer elements. In panel *d*, the regulatory elements are in a chromatin conformation that keeps them relatively close to the gene promoter but does not necessitate direct enhancer-promoter contact. Factors can diffuse locally from the enhancer to the promoter.

chromosome conformation is considered an essential component of long-distance regulation by enhancers, and the idea that chromatin loops juxtapose enhancers close to promoters in the nuclear space is a popular one (**Figure 3*b***). An example in support of this idea is the reported looping of the ZRS with *Shh* such that, by FISH, the two genomic regions appear to be colocalized in cells of the limb (1). This was seen not only on the posterior side of the limb, where *Shh* is expressed, but also on the anterior side, where it is not. Moreover, this chromosome conformation appeared to be retained when the ZRS was deleted, so the exact mechanistic relationship between this chromosome conformation and the spatial regulation of *Shh* expression in the limb remains to be determined.

Colocalization by FISH has also been seen between the *Hoxd13* gene and the global control region (GCR) located 180 kb away, which is involved in the control of *Hoxd13* expression in the late phase of limb development. In contrast to *Shh* and the ZRS, in this case enhancer gene colocalization (30% of alleles with interprobe distances of ≤200 nm) was seen only in the distal posterior part of the limb, where *Hoxd13* is expressed, and not on the anterior side or in proximal regions of the developing limb (100). In addition to visualization by light microscopy, 3C methods have been used to look for long-range regulatory elements that can be captured together with a particular gene. 4C has been used to examine sequences that can be captured by

GCR: global control region

cross-linking to *Hoxd13* in the mouse limb. In addition to the known regulatory elements such as the GCR and Prox, extensive contacts were captured to other sequences more distant from *Hoxd13* and scattered throughout the 800-kb gene desert centromeric of the *HoxD* cluster (62). In limb tissue, these elements carry histone modifications indicative of enhancer activity, and their deletion affects limb and digit development. It has therefore been suggested that this region of the genome operates as a large regulatory module within which the chromosome is folded in such a way that multiple elements are brought close to *Hoxd13* via complex chromatin looping (**Figure 3*c***). This model is similar to the complex, multilooped, and compact regulatory hub that has been described at the active LCR and β-globin cluster (23, 94).

An attractive feature of the enhancer-promoter looping model is that it ensures that an enhancer can activate the expression of only one gene at a time. However, FISH analysis of the spatial proximity of one of the regulatory elements more distant from *Hoxd13* than the GCR is did not reveal a significant level of visible colocalization in the developing limb, and the colocalization frequency was not any higher in the distal posterior limb than it was elsewhere (100). This does not seem compatible with direct promoter-enhancer contact through a chromatin loop. However, the regulatory elements were close to *Hoxd13* (200–400 nm away) in all parts of the limb examined, suggesting that the regulatory module is contained within a spatially confined chromatin domain (**Figure 3*d***). This may then provide a binding platform on which transcription factors, chromatin-remodeling complexes, and associated activities can accumulate locally to a high concentration and then diffuse the short distance to their target promoters (100). Molecular crowding in this confined domain may also drive protein-protein interactions linked to steps of transcription.

The exact spatial chromatin configuration of long-range regulatory domains remains unclear. However, the functional consequence of enhancer-promoter looping was elegantly demonstrated by the engineering of a chromatin loop at the β-globin locus in cells that lack the transcription factor (GATA1) that is important for the LCR-β-globin promoter looping in wild-type cells. Creation of an artificial chromatin loop between the LCR and β-globin in GATA1 null erythroblasts via targeted tethering (with artificial zinc fingers) resulted in activation of β-globin expression (24). The degree of enhancer-promoter looping interactions can also be modulated by genetic variation at enhancers, including by common single-nucleotide polymorphisms (96).

In several genetically well-defined cases—for example, at *Shh* and *SOX9*—long-range enhancers do not simply act on the immediately neighboring gene but rather can ignore intervening genes to act on their more distant target genes (3, 52). Many enhancers are also located within introns of other genes, which they do not regulate—for example, the ZRS is in intron 5 of the *Lmbr1* gene. So a remaining issue is how to connect identified *cis*-regulatory elements with their target promoters. The appearance of DHSs simultaneously at an enhancer and a nearby promoter across different cell types is one guide (92). The use of 3C methods to identify elements cross-linked to a promoter is also being explored as a way to scan for potential enhancer-promoter connectivities across large segments of the genome (81).

5.2. Enhancers and Chromosome Territory Organization

The observed spatial colocalization of *Shh* and the ZRS within nuclei on both expressing and nonexpressing halves of the developing limb bud suggests that enhancer-promoter chromatin looping may be insufficient for gene activation. However, on the posterior side of the limb only, the active *Shh* locus is additionally extruded from its chromosome territory (1). This suggests that spatial reorganization within the chromosome territory may play some role in enhancer function. A similar looping out from chromosome territories is induced by an ectopic β-globin LCR (69). This does not change the profile of 3C contacts that the site of genomic integration makes; rather,

the looping out from the chromosome territory enhances the frequency with which the preexisting contacts are captured (70). This is consistent with the relationship between visible chromosome territory looping out and the ability to make 3C contacts in *trans* in Hi-C analyses (45).

The ectopically integrated LCR does not affect the expression of any other mouse genes except for the endogenous mouse *Hbb-bh1* β-globin gene. When cytoplasmic *Hbb-bh1* mRNA is detected in fetal liver cells of the transgenic animals, in many cases spatial colocalization of *Hbb-bh1* with the ectopic LCR is visible in *trans* in these same cells. This experiment elegantly demonstrated that there can be functional effects on gene expression for colocalization of an enhancer and gene in *trans* but that, owing to the stochastic nature of these interactions and the constraints placed on them by their surrounding genomic context, this is unlikely to have a deterministic role in pathways of developmental gene regulation. So far, reports of endogenous enhancer-promoter colocalization in *trans* (55) have not been accompanied by genetic evidence for the functional consequences of this spatial organization (36, 68).

SUMMARY POINTS

1. The spatial organization of the human genome is neither random nor fixed—it is probabilistic.
2. The positions of chromosomes and genes in the nucleus can influence chromosome structural aberrations—e.g., translocations and inversions. The structural abnormalities of chromosomes can in turn impact nuclear organization and gene expression.
3. There are fundamental principles of spatial organization (radial organization, chromosome territories, clustering of active domains) that are largely invariant between cell types. Cell type–specific organization then plays out on top of this foundation.

FUTURE ISSUES

1. Current genome-wide methods to investigate the 3D organization of the genome require thousands to millions of cells, and the data are only the average across this cell population. An important future challenge will be to devise strategies to generate such data sets from single cells.
2. Much of our current knowledge about the spatial organization of the human genome comes from cells in culture. Analysis must be extended to primary cells.
3. Both FISH and 3C methodologies require cell fixation and so give only a static snapshot of genome organization. Ways to better investigate genome organization in living cells and to follow its dynamics need to be developed.
4. We currently have limited knowledge about the proteins bound to a particular site in the genome in a particular cell. A more complete biochemical knowledge of chromatin composition is required.
5. To what extent does genetic variation between individuals impact the spatial organization of the genome in cells?
6. How much does the spatial organization of the genome influence gene and chromosome function, or to what extent is spatial organization a consequence of function?

DISCLOSURE STATEMENT

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RELATED RESOURCES

A Catalog of Published Genome-Wide Association Studies (**http://www.genome.gov/gwastudies**): A table of published genome-wide association studies from the National Human Genome Research Institute

Encyclopedia of DNA Elements (ENCODE) (**http://genome.ucsc.edu/encode**): Data on the human and mouse genomes from the ENCODE Consortium, including data on DNA methylation, DNase I–hypersensitive sites, and histone modifications

Nuclear Protein Database (**http://npd.hgu.mrc.ac.uk**): A searchable, expertly curated database of the subnuclear location of proteins in the mammalian nucleus, together with annotated links to the functions of these proteins; it includes proteins associated with different spatial compartments of the human genome, e.g., centromere, telomere, nucleolus, and heterochromatin