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A View of Interphase Chromosomes

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Metaphase chromosomes are dynamically modified in interphase. This review focuses on how these structures can be modified, and explores the functional mechanisms and significance of these changes. Current analyses of genes often focus on relatively short stretches of DNA and consider chromatin conformations that incorporate only a few kilobases of DNA. In interphase nuclei, however, orderly transcription and replication can involve highly folded chromosomal domains containing hundreds of kilobases of DNA. Specific "junk" DNA sequences within selected chromosome domains may participate in more complex levels of chromosome folding, and may index different genetic compartments for orderly transcription and replication. Three-dimensional chromosome positions within the nucleus may also contribute to phenotypic expression. Entire chromosomes are maintained as discrete, reasonably compact entities in the nucleus, and heterochromatic coiled domains of several thousand kilobases can acquire unique three-dimensional positions in differentiated cell types. Some aspects of neoplasia may relate to alterations in chromosome structure at several higher levels of organization.

EUKARYOTIC DNA EXPRESSES AND REPRODUCES ITSELF only in the context of an interphase nucleus. It is therefore biologically most meaningful to understand chromosome organization in this state. Until recently, however, only very general features of euchromatic DNA (extended chromatin) and heterochromatic DNA (more condensed chromatin) could be distinguished in interphase nuclei, and each type of chromatin appeared ultrastructurally homogeneous. Furthermore, nuclear chromatin patterns bore no obvious relation to individual mitotic chromosomes, the recognizable genomic structures studied for over 100 years. For this reason the chromatin of mitotic chromosomes was often considered to be randomly and diffusely dispersed throughout the interphase nucleus. Recent advances in molecular biology combined with high-resolution in situ hybridization have made it possible to visualize individual genes (*1, 2*), selected chromosome domains (*3–8*), and entire single chromosomes (*9, 10*) in interphase nuclei. As will be discussed below, these and other studies demonstrate that (i) even in genetically active regions chromatin can be highly folded and confined to discrete, spatially limited nuclear domains; (ii) whole individual chromosomes are organized as finite morphological entities in interphase; and (iii) at least some chromosomal domains are nonrandomly arranged in a cell type–specific manner.

The term chromatin is imprecise because it does not identify specific levels of folding or address functional regions that can encompass hundreds of kilobases of DNA. In this article the term chromatin is used to designate the lower levels of folding, where nucleosome fibers (DNA wrapped around histones) are wound into 30-nm-wide solenoid fibers (*11*). Each full turn of the solenoid accounts for only ~1.2 kb of DNA, which is less than the sequence length of many transcriptional open reading frames. Functional single genes span 30 kb to more than 1 megabase (Mb) of linear DNA. The most fundamental higher structural level of chromosome folding considered below encompasses small functional genetic units of ~30 kb (a loop domain). Additional higher levels of folding correspond to (i) larger transcriptional and replication units that define band-like chromosome domains (of 0.3 to >3 Mb) and (ii) constitutive heterochromatic coiled domains of ~9 Mb. The highest and most complex level of genome organization manifests itself as the massive regions of dense heterochromatin and more extended euchromatin that morphologically characterize each interphase nucleus. A uniformly heterochromatic region within the nucleus can include coil size domains from several different chromosomes (*6, 7*).

How are these structural domains distinguished in molecular

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terms? Noncoding DNA, often referred to as "junk," constitutes more than 90% of the mammalian genome and includes a variety of sequence classes, such as satellite DNA, long interspersed repeated elements, and smaller DNA sequence motifs. Because a direct function for this DNA is not readily apparent, it is often disregarded. However, a substantial proportion of this excess DNA may specify genetic and structural partitions and may also provide essential recognition features that are important for orderly gene function (*3*). These classes of noncoding DNA are nonrandomly organized in chromosomes of different mammalian species, and the domains they define have similar characteristics with respect to transcriptional capacity, temporal order of replication, and higher order folding in interphase. Indeed, excess DNA may be essential for the efficient "compartmentalization" of genes at several hierarchical levels of organization. The term compartment here refers to a structural domain determined by specific DNA sequence features and protein–nucleic acid interactions. Each hierarchical level of gene compartmentalization may incrementally affect its function. Because DNA sequences define individual chromosomal regions more precisely than currently identified proteins, the role of DNA sequence motifs in chromosome conformation is inevitably accentuated in this review.

Although structure is often viewed as subservient to function, interphase chromosome structure itself may have a role in harmonizing global nuclear processes. Such processes affect only a subset of domains on many different chromosomes. During transcription, many active genetic regions are differentially accessible, or in an "open" chromatin configuration, as evidenced by their nuclease sensitivity. Thus the molecular and physical structure of a domain can affect its ability to interact with diffusible cytoplasmic factors and trans-activating proteins. These structural differences are relevant for developmental changes. In general, the amount of DNA is constant in differentiated cells of each species, yet nuclei vary greatly in the amount and distribution of stainable chromatin. At successive stages in the course of differentiation of the same cell type the appearance of chromatin changes. Erythropoesis exemplifies progressive changes leading to a diminution in nuclear size and a progressive increase in heterochromatin, where later-stage normoblasts are small (~5 μm in diameter), almost entirely heterochromatic, and transcriptionally inactive. Neuronal development exemplifies the opposite process in postmitotic cells. The migratory neuroblast nucleus, which is small and heterochromatic, can become huge (>20 μm) and almost completely euchromatic as it differentiates, consistent with the well-known high transcriptional complexity of brain. Thus the coordinated expression of selected gene sets in different cell types may depend on acquired structural patterns affecting many chromosomal domains. In this sense the development of complex organisms ultimately rests on mechanisms that flexibly alter chromosome conformation in numerous, but specific, genetic regions. Furthermore, an ~5-μm resting lymphocyte nucleus (more than 25 times smaller than the volume of a very large neuron), even when stimulated, does not approach the size of a large neuronal nucleus. These limitations would suggest that at least some acquired structural conformations are propagated during terminal differentiation. Structure may also influence the time of replication of selected genetic domains. As discussed below, actively transcribed housekeeping gene regions, which are uniformly accessible, are early replicating. Several disease-related events [such as site-specific viral insertion, chromosome breakage, selective DNA loss (*12*), and translocation] may also depend on local chromosome structure and sequence. At least some of these events probably take place during interphase on actively transcribed and replicating DNA.

Each somatic daughter cell of an organism is born with the segregation of replicated mitotic chromosomes. Chromosomes are most highly condensed during the brief metaphase period of cell division. This essentially complete heterochromatic state is associated with negligible gene transcription and affects the entire genome. Thus all daughter cells begin with an overall chromosome structure that signifies gene inactivity, and the structure must be dynamically modified in interphase to carry out essential cellular functions. However, some regions of the genome appear to maintain a condensed metaphase-like configuration in interphase. It is therefore useful to relate dynamic changes in interphase to this common, albeit complex, structure. Metaphase chromosomes also provide a useful reference point for cytogeneticists. Each mammalian chromosome has its equivalent counterpart in other mammalian species, and, remarkably, chromosomal banding characteristics as well as gene order appear to be highly conserved in mammalian evolution (*13*). Thus, wherever possible, chromosomal domains, identified by their molecular properties, are related to the landmarks of differentially stained cytogenetic bands.

Transitions from Metaphase to Interphase

In intact cells each metaphase chromosome contains two dense, parallel, ~0.7-μm-wide sister chromatid fibers. In metaphase, chromatin compaction is extreme, and it is difficult to analyze all the multiple and complex folding levels of chromatin in this condensed state. Nonetheless, numerous morphological studies of nondisrupted chromosomes and cells are consistent with the proposal (*14*) that each mammalian metaphase chromatid is formed by the tight helical coiling of a long, ~0.25-μm-wide fiber (*15*). The universal nature of such fibers is illustrated by *Saccharomyces pombe* mutants that are defective for topoisomerase II, a highly conserved protein that can cut and untangle chromatin (*16*). When topoisomerase II is inactivated, *S. pombe* chromosomes are unable to complete the metaphase condensation process, and they display many prophase-like elongated fibers (*17*). Such sister chromatids are unable to separate and contract to form tightly coiled metaphase chromosomes. When a mitotic cell reenters interphase the process of mitotic condensation is reversed. At least some tightly coiled regions must unfold so that transcription and replication can begin again. A three-dimensional model of a reversibly coiled and more extended chromatid fiber with these fundamental dimensions is shown in Fig. 1A. Presumably the more extended structure is a prerequisite for interphase transcription.

Metabolic energy appears to be required for metaphase chromosome condensation. Phosphorylation of specific proteins by protein kinases is required for mitosis. For example, phosphorylation-dependent activation of a histone $p34^{cdc2}$ kinase occurs early in mitosis (*18*), and injection of antibodies to the $p34^{cdc2}$ homolog into *Xenopus* oocytes or human HeLa cells blocks cell division, but not DNA synthesis (*19*). Another family of peptides are modified by extensive threonine phosphorylation during the G_2 to M transition of the cell cycle (*20*). These and possibly other energy-requiring modifications result in a highly condensed mitotic chromosome coil poised to spring back into a more extended interphase state. Phosphatases presumably are involved in reversing this condensation process, although many complex biochemical processes may be involved.

Only some genes are required for cellular maintenance and, as discussed below, some chromosome regions can remain in highly condensed metaphase-like coils in an interphase nucleus. Thus mechanisms are needed to select specific chromosome regions for decondensation at the start of interphase. Because all chromatin is uniformly heterochromatic in metaphase, specific molecular signals that can direct appropriate local decondensation must be in place at

the end of mitosis. One mechanism for nonuniform decondensation may involve selective binding of proteins that can prevent local decondensation. For example, specific proteins selectively bind centromeric regions of chromosomes, are carried on the chromosome from metaphase into interphase (*21*), and decorate large heterochromatic interphase domains that are replete with noncoding satellite DNA (*22*). Another mechanism may exploit chromosomal proteins that are transiently released into the cytoplasm during mitotic condensation. Such proteins may preferentially bind chromosome regions that contain DNA with special conformations or sequence motifs. Histone H1 depletion combined with H3 and H4 hyperacetylation (*23*) in such regions may also facilitate selective decondensation.

Despite the structural similarity of all chromosomes in their most condensed metaphase state, different spatial domains or bands within each chromosome can be distinguished by Giemsa staining (G bands). Metaphase and prometaphase bands are readily recognizable, whereas interphase band organization is not as easily visible. However, the fundamental organization of the metaphase chromosome is carried through into interphase. Although Giemsa bands per se cannot be visualized in interphase cells, equivalent band-like domains of 0.3 to >7 Mb can be identified with molecular probes. These large domains characteristically contain different types of noncoding DNA, and in some cases also bind different sets of proteins. Differential properties of transcription, time of replication during S, and condensation are associated with each of these domains as discussed below.

Constitutive Heterochromatin

Constitutive heterochromatin is relatively resistant to decondensation in interphase. Constitutively heterochromatic bands (C bands) stain darkly after heat treatment and are localized at or adjacent to centromeric regions in most eukaryotes. Occasionally C-band heterochromatin is also found on chromosome arms, such as the telomeric region of the human Y chromosome. These regions characteristically are transcriptionally silent and replicate very late in S phase in most interphase cells. C bands are almost entirely occupied by noncoding, tandemly repeated satellite DNA sequences (*24*). Conserved terminal telomeric repeats can also be detected in centromeric C band regions, including those in metacentric chromosomes (*25*). It is possible that C bands may also hide a few specific genes that would be deleterious if expressed in most cells. Recent studies with pulse-field gel electrophoresis show that a single C band can contain as much as 9 Mb of satellite DNA in linear length (*26*). This size roughly corresponds to the calculated amount of DNA contained in one complete turn of a condensed metaphase coil (Fig. 1A) (*15*).

All C bands remain in a condensed, metaphase-like state in nonreplicating interphase cells. Multiple factors probably determine this compact interphase structure. However, studies with transgenic mice may provide some insight into basic mechanisms that are responsible for this structure. When large (~11 Mb) arrays of tandem repeats are inserted in transgenic mice at a single locus (*27*), the transgenic locus acquires all the structural characteristics of

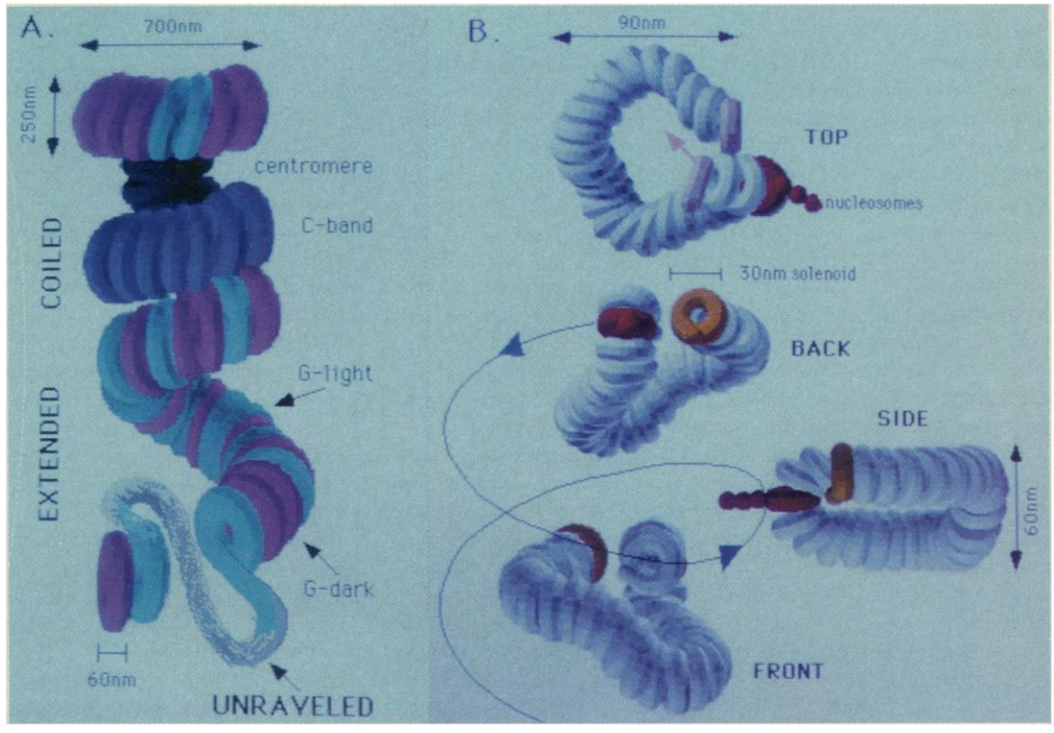

Fig. 1. Scaled three-dimensional model of dynamically contracted and decondensed domains in a spatially cohesive interphase chromosome fiber. (**A**) Highly coiled heterochromatic regions can extend to form a 250-nm-wide interphase chromatid fiber. This fiber is formed by stacks of relatively G-light (turquoise) and G-dark (violet) 300-kb radial arrays, modeled in their most condensed state. Entire G-light radial arrays may, however, puff out or further unravel to occupy a more diffuse spatial region. Unraveled fibers are defined by chromatin loops in various states of condensation. C bands (blue) generally remain highly coiled during G_1. (**B**) Scaled orthographic views of condensed 90 × 120 × 60 nm chromatin loops. These 30-kb loops are the helically wound subunits within each ~300-kb radial array. The portion labeled top is of a 30-kb loop with each ~1.2-kb DNA solenoid turn (gray) shown. An arbitrary start solenoid (pink) is shown for this loop and also for the adjacent loop, which continues in the direction of the arrow. Twenty-five turns of the solenoid define each regularly folded loop, and ten 30-kb loops with the depicted dimensions can be helically wound in each turn of a 250-nm-wide radial array (*15*). Representative en face views (labeled back, front, and side) of condensed 30-kb loops, with the position of starting solenoids in orange for orientation. Only some 30-kb loops attach, by way of nucleosomal fibers emanating from one interior solenoid (red), to core matrix components, which align down the center of the helical axis indicated by arrowheads. During replication and transcription the helical path of the radial array can reversibly widen and lengthen to yield expanded and unraveled fibers (A). Additionally, loops that are not attached to core matrix components may further unfold and puff out circumferentially. Unraveling can encompass one or several radial arrays corresponding to morphological G-light or G-dark bands. These bands are molecularly defined by their DNA signatures, coordinate replication, and other characteristics such as protein binding (see text). The proposed model qualitatively accounts for DNA contraction in metaphase (*15*) as well as interphase structural observations to date.

constitutive heterochromatin. Even in mature neurons that are largely euchromatic, these regions remain condensed and ultrastructurally coiled (*28*). Therefore specific chromosome recognition features are imparted by these large DNA sequence arrays. It is not known whether centromeric proteins associate with these transgenic arrays. Regardless, this heterochromatic configuration in interphase effectively removes these domains from transcriptional searches and may set up a barrier for larger trans-activating factors. Although C bands are most often considered in the context of mitosis, they may also act in interphase as organizing centers for heterochromatin (*28*).

Demethylation leads to C-band decondensation. C bands are extended under special circumstances. In mouse satellite DNA, seven of eight CpG sites are methylated (*29*). In murine C bands, each of which contains an average of 7.5 Mb of the 234-bp tandem satellite repeat (*15*), the overall concentration of methylation is 3%. Consequently, antibodies to 5-methyl C concentrate in mouse centromeres and also decorate C bands of other species (*30*). After demethylation with 5-azacytidine, C bands become structurally extended (*31, 32*) and can acquire an earlier replication pattern (*33*). Thus, DNA sequence attributes can effect both uncoiling and altered physiological properties. Although methylation is not utilized by all eukaryotes, methylation appears to shadow gene inactivation in higher eukaryotes with large genomes (*34*). DNA methylation is not necessary for mitotic condensation and is probably not a characteristic of all highly contracted interphase domains. However, once a chromosome region is marked by methylation it apparently resists interphase decondensation. It is not known exactly how methylation prohibits decondensation, but methylation may be one of several biochemical signals recognized by proteins active in the initial phases of interphase decondensation. The methylated state can prevent the binding of some transcription factors, possibly because methylated stretches of DNA are already bound to a methyl-CpG binding protein (*34*). Such obstructive modifications may also locally prevent the access and action of other molecules such as phosphatases, which are involved in chromosome decondensation. Demethylation may also have a more direct effect on DNA helix structure, because DNA binding dyes induce similar decondensation effects (*35*).

Thus a very large, highly folded chromosomal domain can be both structurally and functionally converted by demethylation, and this mechanism can be used to reversibly specify other large chromosome domains during differentiation. In essence, methylation can solidify additive molecular characteristics that prohibit G_1 interphase decondensation in large domains. Gene inactivity and late replication patterns in methylated chromosome regions are likely to be a consequence of this chromosome conformation.

C bands unravel during replication. During replication, constitutive heterochromatin can also transiently lose its heterochromatic attributes. In most interphase nuclei, C-band regions remain remarkably compact or coiled despite swelling and disruptive preparative procedures (*10*). However, C bands unraveled beyond the extended fiber level (Fig. 1A) can be detected by in situ hybridization in a small proportion of cells (*3, 8, 36*). Because in situ hybridization signals and pulse-labeled replicating domains can be simultaneously detected with high resolution (*7, 15*), it was possible to directly connect C-band unraveling to the process of replication. During replication, a subset of all hybridizing C bands that simultaneously incorporate bromodeoxyuridine (BrdU) display a complex network of longer and thinner strands. These unraveled domains are spatially more diffuse than nonreplicating regions (*37*). Some recently replicated C bands, however, cannot be morphologically distinguished from their nonreplicated counterparts. Therefore unraveling associated with replication is a transient process, and replicated DNA rapidly returns to a more condensed configuration. In accord with this, several thousand postmitotic neurons hybridized to C-band probes (*6, 7, 38*) fail to show this degree of unraveling. This dynamic structural change during replication is also apparent in defined individual chromosome arms (*37*).

During replication, unraveled fibers (Fig. 1A) must locally unfold to the nucleosomal fiber or naked DNA level (*39*). Unwinding factors that act locally during replication (*16, 40*) are probably required for this degree of structural unraveling. Protein phosphorylation in S and G_2 phases (*18–20, 40*) as well as other energy-dependent mechanisms may also be involved in rapid unfolding and subsequent refolding of chromatin into a more compact structure. Nonetheless, unraveled chromosome domains, although focally quite decondensed, are confined within discrete regions of the nucleus and maintain a three-dimensional relationship to their individual interphase chromosome "territory" (vide infra). Presumably, unraveled chromatin fibers rapidly recondense as replication proceeds, and replicated, extended sister chromatid fibers can interdigitate with each other to reform typical 0.25- to 0.4-μm-wide interphase fibers during S and G_2, as previously detailed (*15*).

In summary, C bands with characteristic DNA sequence motifs exemplify the high degree of condensation and complex levels of folding that can be maintained in interphase. This structure is not a subtle alteration limited to a short stretch of chromatin. Entire C-band regions are also dynamically and coherently converted to an extended structure by demethylation, and an even higher degree of unraveling may transiently occur during DNA replication. Similar dynamic functional modifications of these higher order structures also appear to apply to other chromosome regions.

Smaller Compartments on Chromosome Arms

G-dark and G-light bands: DNA sequence signatures. In acid-fixed mammalian metaphase preparations, chromosome arms are differentially sensitive to trypsin. Trypsin-resistant arm regions are known as G-dark bands; those that are more susceptible appear light. In metaphase chromosomes G-light bands can also show more nuclease sensitivity than G-dark bands (*13*). This demonstrates that even in a completely heterochromatic structure there can be subtle differences that may relate to functional capacity. In interphase as well, only relatively subtle conformational differences may distinguish different functional compartments on chromosome arms.

G-light and G-dark bands also have distinctive molecular characteristics, some of which have evolved during the course of vertebrate evolution (*13*). It is known that human G-light bands contain high concentrations of short interspersed Alu repeats that are GC-rich. These GC-rich sequences, and their counterparts in other species, can have a Z-DNA conformation. In contrast, G-dark bands preferentially concentrate the AT-rich L1 family of long interspersed repeats. Both of these repeat families are virtually excluded from the very heterochromatic C-bands (*3, 5, 15, 26, 41, 42*). Several repeat sequences, such as minisatellites, are known to be specific for an individual G-dark band on a chromosome arm (*7*). Other relatively infrequent genomic repeats, such as selected endogeneous retroviral elements (*26*), may impart additional unique recognition features or DNA signatures that can specify individual as well as functionally related domains on different chromosomes. Specific proteins may also contribute to G-band signatures. HMG-I nonhistone proteins, which bind AT-rich stretches of DNA and a transcriptional octamer DNA motif, preferentially localize in G-dark bands (*43*).

G-light chromosome bands correspond quite closely to early replicating regions in lymphocytes. All the housekeeping genes studied to date are early replicating (*13, 44*) and probably reside in G-light domains. Housekeeping genes are actively transcribed in

almost all cells. These genes are clustered in long unmethylated stretches of DNA and contain CpG islands at their 5′ ends (*45*). In contrast, G-dark DNA, including L1 DNA, replicates later in S, but not as late as C-band DNA (*15, 44, 46*). This differential replication pattern is most clearly resolved with antibodies to BrdU (*7, 15, 47*). Tissue-specific genes are transcribed only in selected cell types. These genes tend to be late replicating and reside in G-dark bands (*46*). At least a portion of these genetic domains need not be decondensed in many cell types. In essence, G-light domains are functionally more euchromatic than most G-dark regions in many interphase nuclei. However, as previously noted for C-band DNA, replication characteristics can be altered. In cells of different lineage, for example, the G-dark human 140-kb β-globin gene locus was either early or late replicating. Furthermore, only the early replicating β-globin locus showed nuclease sensitivity (*48*), a feature of many actively transcribing genes in an open chromatin conformation. Thus a G-dark domain can acquire G-light replication and transcriptional characteristics. In this context, the established landmarks of metaphase Giemsa banding do not adequately indicate molecular and structural modifications that may occur within these domains during differentiation.

Functional consequences of chromosome arm compartmentalization. Replication, which occurs at the nucleosome or naked DNA level (*39*), can erase essentially all nonhistone proteins and potentially leave the genome as a tabula rasa. In principle, DNA sequence motifs, including junk DNA signatures which define selected individual as well as related chromosomal domains, can provide the most basic genomic strategy for recognizing discrete gene clusters that are to be selectively modified for subsequent cell type–specific expression. Methylation can be effected during replication, and local environmental and cytoplasmic factors can stake their claim for cell-specific expression in progeny cells. Relatively infrequent molecular complexes that bind nascent DNA can command or influence many genes in a locale more efficiently when chromatin is refolded. In progeny cells, clusters of genes with suitable acquired secondary features can also be modified together. For example, the widely spaced Zn^{+2} finger protein Suvar(3)7 may collectively spread a heterochromatic conformation into euchromatic regions containing hundreds of kilobases of DNA during differentiation (*49*).

In a more general way, chromosome arm compartments, with their specific molecular characteristics, provide an indexing system. Diffusible trans-acting transcriptional factors may examine categorical gene arrays from many different chromosomes. In interphase, only selected domains are generally accessible. In most nuclei, early replicating G-light housekeeping genes are probably uniformly accessible to transcriptional factors, and are in an open, nuclease-sensitive domain. Thus these selected chapters of the genome can always be read. G-light motifs are essential for cell survival. Whereas constitutive heterochromatin is almost completely forbidden, G-dark domains with tissue-specific genes may be restricted until a certain maturity is acquired, as in terminal differentiation. Conversion of the large G-dark β-globin locus into an accessible (nuclease-sensitive) domain in only some types of cells (*48*) provides an example of this concept. G-dark domains that maintain a relatively heterochromatic configuration may also act structurally to terminate transcription progressing through G-light domains. Thus this segmental organization can be advantageous for the development of complex organisms. However, G-light bands contain considerably more DNA than is accounted for by housekeeping genes, and at least some tissue-specific genes reside in G-light domains (*13*). If transcription or regulation within a domain is functionally imprecise, complex nuclear post-transcriptional processing mechanisms may add the required controls for specificity. The G-light β-globin locus in chicken, for example, is inappropriately transcribed in brain nuclei, but β-globin expression appears to be controlled by other nuclear regulatory mechanisms (*50*).

Band size compartments also have relevance for disease processes involving chromosome breakage and recombination. Since G-light and G-dark bands are replicated at different times, junctions between replicated and nonreplicated segments in S may be structurally fragile. During unraveling, these junctions would be susceptible to physical stress. This may be the basis for the observation that some fragile sites map to junctions between G-light and G-dark bands (*51*). Unraveled replicating regions should also be more prone to radiation-induced damage, and it is well known that radiation preferentially affects dividing cells. GC-rich G-light bands on chromosome arms that are conformationally more relaxed or unfolded during transcription would also be more susceptible to breakage, recombination, and translocation than either G-dark or C bands. Indeed, many oncogenes involved in primary neoplasias are early replicating and map to G-light regions (*44, 52*). Transposed genes can also structurally convert and rearrange flanking DNA in their new neighborhood (*53*). These secondary rearrangements are an additional source of structural instability and are relevant for the well-known progressive chromosomal changes seen in many malignant epithelial cells.

The size and structure of G bands. G bands contain variable lengths of DNA. Approximately 2,000 G bands of various thicknesses can be morphologically resolved in extended prometaphase chromosomes (*54*). Therefore an average G band would contain ~1.5 Mb of DNA. Light microscopy does not allow resolution of domains of less than 0.2 μm (*55*), and some G bands may contain only ~300 kb of DNA. Pulsed-field gel electrophoresis studies with rare-cutting restriction enzymes have shown that Alu, L1, and other repeats can cluster on distinct DNA fragments that range from 45 kb to as much as 3 Mb, with an average length of ~250 kb (*26*). This size range is consistent with the morphological variability of G bands, but indicates the presence of DNA lengths that may define domains smaller than the finest G bands yet resolved morphologically. In interphase, G-dark L1 repeats hybridize as punctate single or coalesced signals that reside within the general confines of extended ~0.25-μm-wide interphase fibers in euchromatic regions of several cell types (*5, 7*), and corresponding HMG I proteins show a similar pattern of distribution in interphase cells (*43*). Volumes of a single 0.5 to 1 Mb G dark telomeric locus are also reasonably small and discrete in aldehyde-fixed tissue (*7*). Although early replicating G-light domains of known DNA length have not been measured precisely in interphase cells preserved in aldehyde, it is likely that such regions are variably condensed, and particularly sensitive to minor changes, such as the ionic environment. In two-probe hybridization studies of swollen unsynchronized cells, individual interphase chromatid arms can resemble their linear prometaphase counterparts, but appear to be more expanded than corresponding C-band regions (*10*). Nonetheless, clones that span G-light domains decorate reasonably compact, cohesive regions, rather than linear stretches of extended chromatin (*2*). In aldehyde-fixed cells, furthermore, early replicating DNA that is not transiently unraveled is also confined to extended interphase fibers that are ~0.25 to 0.4-μm wide (*56*). Transcriptionally active nucleolus organizing regions delineated with silver similarly show compact ~0.25-μm-wide fibers (*57*), and ribosomal DNA probes decorate comparable fibers within the nucleolus (*22*).

G bands are consistent in size with one or several adjacent ~300-kb "radial arrays" (*15*) (Fig. 1). Stacks of more or less extended radial arrays are based on the observation of many ~0.25-μm-wide interphase fibers in euchromatic regions of mammalian nuclei (*14, 15*) and the delineation of entire individual chromosomes confined to discrete spatial "territories" in the nucleus

(*9, 10*). The proposed model is based on the assumption that the majority of genomic DNA, which is noncoding, will be folded in a reasonably regular way. Since radial arrays are helically continuous, G-band compartments and transition zones between them (*26*) can be flexibly defined by several molecular signals or very subtle structural conformations that are not rigidly periodic. Several G-light domains may have a "puffed" conformation, analogous to some, but not all, transcriptionally active polytene chromosome regions and similarly respond to acute stress or diffusible factors. Puffed G-light radial arrays can extend circumferentially and longitudinally to accommodate rapidly accumulating transcriptional products. Microdomains within G-light radial arrays can also further unravel to the nucleosomal or naked DNA level during active transcription. In contrast, most G-dark arrays probably do not puff or extensively unravel during transcription. However, details of chromatin folding that encompass domains of 30 to 120 kb of DNA have not been well visualized in intact preparations.

Regular Loops with Varying Matrix Attachment Sites

How do 30-nm solenoids relate to radial arrays of the interphase chromosome? Much of what is known about the folding of long solenoid fibers relies on experimental molecular and morphological analyses of disrupted or swollen preparations. Considerable evidence from these diverse studies indicates that solenoid fibers form discrete loops that are attached at their base to a complex scaffold or nuclear matrix (*58*). This matrix contains heterogeneous nuclear RNA (*59*), topoisomerase II, and a variety of other proteins, some of which are tissue-specific (*60*). On the average, loops are 60 kb in size. However, matrix attachment sites are probably irregular. Some large genes may have relatively few attachment sites, with "functional" loop sizes of ≥150 kb, whereas other small genes may reside within loops of ~30 kb. Matrix attachment sites may also be nonuniform in different G-band compartments. Indeed, it has been proposed that tissue-specific genes with L1 repeats may be more closely associated with the matrix than housekeeping genes (*61*). Thus matrix attachment characteristics of a domain, in addition to DNA sequence motifs, modifications such as methylation and histone acetylation, and protein binding characteristics, may additively contribute to subtle conformational differences reflected in higher orders of folding and collective accessibility.

Solenoid fibers can be folded into regular loop-like structures of 30 kb (Fig. 1B) to form helically wound radial arrays generating the 0.25-μm-wide interphase fiber. This sequential folding also accounts for DNA compaction in interphase and metaphase (*15*). In interphase, radial arrays may dynamically extend and contract. Furthermore, because core matrix elements probably attach to nucleosomal DNA on the interior aspect of only some 30-kb loops, those loops that are not attached to the matrix may unfold to form larger loops (of 60 kb or more). These extended loops may be structurally kinked or more completely puffed out. In at least some actively transcribing GC-rich housekeeping gene regions, solenoid fibers may also focally unravel to the nucleosomal or naked DNA level. The microscopic discrimination of single-copy genes separated by 130 kb in interphase (*1*) may reflect these types of radial array relaxation and loop unfolding, although swelling, acid extraction, and spreading conditions may also artifactually increase in vivo distances to two or three times the actual size. Unraveling of loops appears to be physiologically reversible, as completely euchromatic chromatin can become more condensed shortly after microinjection of $p34^{cdc2}$. The ultrastructural depiction of newly condensed loop-like structures, as well as larger condensed domains (*62*), are consistent with the above model. More completely condensed 0.25-μm-wide interphase fibers are seen in the presence of polyanions (*3, 14, 57*). However, in such condensed fibers there exists sufficient room in and around loops to accommodate transcriptional complexes (*15*). This is relevant when considering that specific G-dark gene regions with frequent sites of matrix attachment are known to be transcriptionally competent (*61*).

Natural Chromosomal Contexts

Transgenic studies emphasize the importance of the natural chromosomal context for correct gene expression. Exons driven by artificial promoters are inappropriately expressed in comparison to gene-sized genomic constructs of 35 kb or more that are tissue specific (*63*). Some transgenic position effects (*64*) probably derive from even larger neighborhoods of band or even coil size. Changes in gene position have also been shown to be accompanied by changes in both replication time and transcriptional activity (*65*), further indicating the influence of larger chromosomal domains. Indeed, the condensation state of a whole chromosome can generally relate to its overall transcriptional and replication characteristics. It has long been appreciated that the extra X chromosome in female cells is generally condensed (Barr body) in interphase. This example of facultative heterochromatin brings out several relevant points that deserve mention before consideration of higher order nuclear structure as resolved by in situ hybridization. First, X chromosome condensation signifies general but not absolute transcriptional silence. Some genes on the inactive X can be transcribed despite the overall condensation of this chromosome (*66*). Specific molecular signals within chromatin complexes, diffusing from the cytoplasm, or from the extracellular environment may override and effectively utilize genes that are associated with this overall heterochromatic chromosome. Selected domains may be structurally extended into a more euchromatic configuration (*15*). Alternatively, some morphologically condensed domains may be transcriptionally competent and require only slight extension for their function. In fact, small heterochromatic nuclei of metabolically quiescent cells appear to maintain low but sufficient levels of transcription from many condensed domains. Second, the inactivated X chromosome or Barr body is a morphologically discrete entity in interphase nuclei. Many other chromosomes have recently been delineated by in situ hybridization and, remarkably, each chromosome is morphologically discrete and confined to an individual spatial territory in the nucleus (*9, 10*). Although chromosomes can be conformationally modified in interphase, they still maintain their overall integrity. It is proper, therefore, to speak of each interphase chromosome in a structurally unified way, rather than chromatin. Third, in fibroblasts, single and aberrantly multiple Barr bodies are invariably positioned adjacent to the nuclear membrane (*32*). This indicates that chromosome positions in interphase can be nonrandom. On the other hand, individual polytene chromosomes show no nuclear position preferences (*67*) and also do not condense into large heterochromatic bodies in an interphase nucleus.

Higher Order Nuclear Compartmentalization

Euchromatic and heterochromatic compartments within each interphase nucleus provide an additional level of gene organization. Nuclei display vastly different and cell type–specific patterns of condensation and nuclear shape that are conserved in mammalian evolution. For example, polymorphonuclear leukocyte nuclei are visibly different from nuclei of gastrointestinal epithelial cells in

many species. Different types of neuroectodermal cells also have characteristic chromatin patterns that are conserved in evolution (*6, 7, 57*). Such morphologies ultimately are based on the relative condensation and position of individual chromosomes in different cell types. However, nuclear morphology can be dynamically modified, and both the chromatin pattern and nuclear shape can rapidly change when a cell is exposed to a different environment. This phenomenon is commonly observed in tissue culture. An endothelial cell nucleus, for example, is highly condensed in vivo, but in the less complex environment of monolayer culture its nuclear size increases and at least a portion of the chromatin expands to a more euchromatic state. Such cultured cells can be phenotypically altered in the absence of genetic changes. These classic examples of interphase chromosome structure indicate dynamic alterations can subserve different functional requirements. In situ experiments highlight a few key changes that can occur in interphase chromosome organization.

In mitosis, centromeres are collected together at the spindle. C bands rapidly disperse as the nuclear envelope reforms at the end of anaphase in cultured mammalian cells (*22*). The molecular signals for this reorganization are entirely unknown, but this process probably precedes site-specific decondensation of metaphase chromosomes. In fact, most centromeres remain closely associated with the nuclear membrane in cultured cells (*5, 21*), although they may be rearranged in G_2 to M phases of the cell cycle (*22*). Complete chromosome arms that in some species are entirely G dark can also take up residence on the nuclear membrane (*37*), and facultative heterochromatin may collectively organize on the nuclear membrane in some cell types. On the other hand, C bands largely collect on centrally placed nucleoli in large interphase mammalian neurons, and only a few individual centromeres remain on the nuclear membrane (*6, 7*). The Barr body also associates with perinucleolar C bands in some large neurons (*38*). Because all interphase cells examined to date show morphologically discrete single chromosomes (*9, 10*) rather than spatially unrestricted or unfolded chromatin, entire chromosomes should be positioned differently in various diploid cell types.

At least some chromosomal repositioning can take place during G_1. Changes in heterochromatic position have been documented in living neurons (*68*). Furthermore, in developmental studies of post-mitotic Purkinje neurons, centromeres move from the nuclear periphery into a central perinucleolar position. This repositioning occurs during synaptogenesis (*22*), and environmental or membrane adhesion signals may initiate this process. In pathological states such as focal epilepsy, X chromosome centromeres move away from their normal position in heterochromatic aggregates and take up stable residences in an interior euchromatic compartment (*38*). The involved centromeres are closely adjacent to the mapped locus for synapsin (*69*), a protein that would be needed during chronic seizure activity. Such stabilized changes in neuronal X chromosome positions may also imprint seizure-generating cells (*38*). In cells treated with 5-azacytidine, there is also extension and repositioning of the X centromere in interphase (*32*). These types of observations indicate that euchromatic and heterochromatic nuclear compartments can be reversibly and dynamically defined in interphase.

There are, however, several general rules of interphase chromosome organization. In almost all mammalian cells, chromosome homologs are spatially separated from each other, albeit at different relative distances (*7–10, 36, 38*). Furthermore, terminally differentiated cells show different fundamental nuclear patterns for different chromosome regions. This is most readily appreciable in adult brain cells where centromeres and nucleolus-organizing regions are characteristically positioned (*6, 7, 57*). These positions are highly stable for each cell type and are conserved in evolution. Specific neuronal subsets, such as large cerebellar Purkinje neurons and small granule neurons, exhibit different arrangements from each other and from glial cells (*6*). Studies of single C bands also confirm differences in homolog positions in different neuronal populations. Whereas each homologous human chromosome 1 C band is widely separated in many large cortical neurons, with one signal on the nuclear membrane and one on the nucleolus (*7*), these two C-band signals are very close to each other in internal granule neurons and do not have an obvious connection with the nuclear membrane (*70*). Telomeric regions may also show positional differences in cells of different lineage. At least some telomeric domains are found in the euchromatic interior compartment of many different types of brain cells, whereas the same telomeric regions in cells of different lineage can be membrane associated (*7, 71*). These telomeric positions are likely to reflect, or contribute to, the use of these domains by each cell type.

At the other extreme, chromosome positions in tumor cells are more idiosyncratic. Nucleolus organizing regions (*57*) and C bands (*36*) can be variably positioned in glioblastomas, and entire chromosomes seem to have few rules governing their nuclear position in such cells (*10*). These more random chromosome positions may facilitate biologically licentious functions. Nonetheless, individual chromosomes, as well as pathologically broken chromosome arms, form spatially discrete entities, and chromosome homologs are always separated, even in these malignant cells (*10*).

Summary

Genes are strategically positioned and hierarchically segregated in the interphase nucleus. Sequential compartmentalization of genes within domains of different size, such as genetic loop domains (30 to 300 kb), chromosomal bands (0.3 to >3 Mb), constitutive heterochromatic coils (~9 Mb), and the nucleus as a whole, may additively influence phenotypic expression. Each of these compartments has characteristic molecular and structural features. It is probably insufficient to consider that only lower orders of chromatin folding affect processes such as transcription, replication, differentiation, and malignancy. Studies of dynamic changes in experimentally manipulatable systems are likely to unify several molecular and structural motifs. Specific molecular probes have been invaluable in revealing definitive chromosome structures in interphase, but more detailed analyses of specific chromosome domains and chromosome positions are required. Dynamic developmental changes in interphase chromosome structure and position are only beginning to be addressed, but the nucleus need not remain as inscrutable as it once was.

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72. I thank my colleagues for their helpful suggestions and E. Zelazny for printing Fig. 1. Supported by NIH grant CA15044.