

A Unified Model of Eukaryotic Chromosomes

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"Is all good structure in a winding stair . . ." — George Herbert

A revised model of DNA packaging into chromosomes is presented. Its features are consistent with observed structural dimensions and the molecular periodicities related to transcription, replication and matrix attachment domains. The transitions between euchromatic, heterochromatic and metaphase states are ex-

plained simply. Molecular and physical properties of chromosomal bands, and their correlation with specific DNA sequence motifs are discussed.

Key terms: Chromatin, chromosome structure, matrix, replication, Giemsa bands, repeated DNA

The higher order molecular and physical structure of chromosomes is still poorly understood. The winding of the DNA double helix around histones to form 10 nm thick nucleosomes or "beads on a string," and the subsequent coiling of nucleosomes into solenoids of ~ 30 nm \times 10 nm is by now well-established. Each solenoid turn however encompasses only ~ 1.2 kb of DNA, and is too small to embrace complex transcriptional and replication domains that may span several hundred kilobases of DNA. These larger "functional" units of DNA are likely to be arranged into hierarchical structures that can be recognized and conveniently utilized in an orderly fashion. On a purely structural level, the delineation of metaphase chromosome bands by Giemsa staining suggests that such hierarchies exist; Giemsa bands encompass megabase (Mb) stretches of DNA and are not meaningless artifacts; for example, Giemsa-light bands are preferentially digested by trypsin, indicating structural and/or molecular components are specifically organized in these compartments, and overall binding patterns may be conserved in evolution (27). Furthermore, domains of similar large size, that may correspond to Giemsa bands, are involved in sister chromatid exchanges, translocations and replication. Sam Latt made many original contributions to the molecular organization of the genome, and one of his eminent contributions was the delineation of sister chromatid exchanges (32). In his honor, we present a physical model of chromosome structure that encompasses units of this size.

We here build an updated model that incorporates the longer features of molecular gene organization that have become apparent in the last 10 years. Such features include units that range in size from very long

linear lengths of DNA, as defined by chromosome banding techniques, pulse-field gel electrophoresis (PFGE), and high-resolution non-isotopic in-situ hybridization, through smaller DNA lengths of 30–120 kb that periodically attach to matrix proteins and replication complexes. To date, no model of chromosome structure adequately addresses the folding or compaction of these longer DNA lengths in both interphase and metaphase chromosomes of known dimensions. Since these dimensional constraints are essential to any realistic model of chromosome folding, we first address the actual sizes of chromosome domains in both interphase and metaphase chromosomes. The structure of interphase chromosomes is pivotal, since interphase cells carry out key functions such as transcription and replication, and also prepare for the rapid transition through metaphase. The model we present can effectively compress the diploid human genome into a nuclear volume of 50–60 μm^3 , and still leave adequate room in a small nucleus of 5 μm in diameter (65.6 μm^3) for several nucleoli, protein, and RNA species that are essential for the maintenance of biological function.

RESULTS AND DISCUSSION

Dimensions of Interphase and Metaphase Chromosomes With Reference to Different Functional States

In the model we include the following essential structural dimensions and orders of organization: 1) the sequential compaction of DNA into nucleosomes and solenoids (21) of known dimension. This is schematically summarized in Fig. 1 for the non-cogcentric; 2) the extended interphase chromosome fiber, which has an average diameter of ~ 240 nm, and 3) the maximally

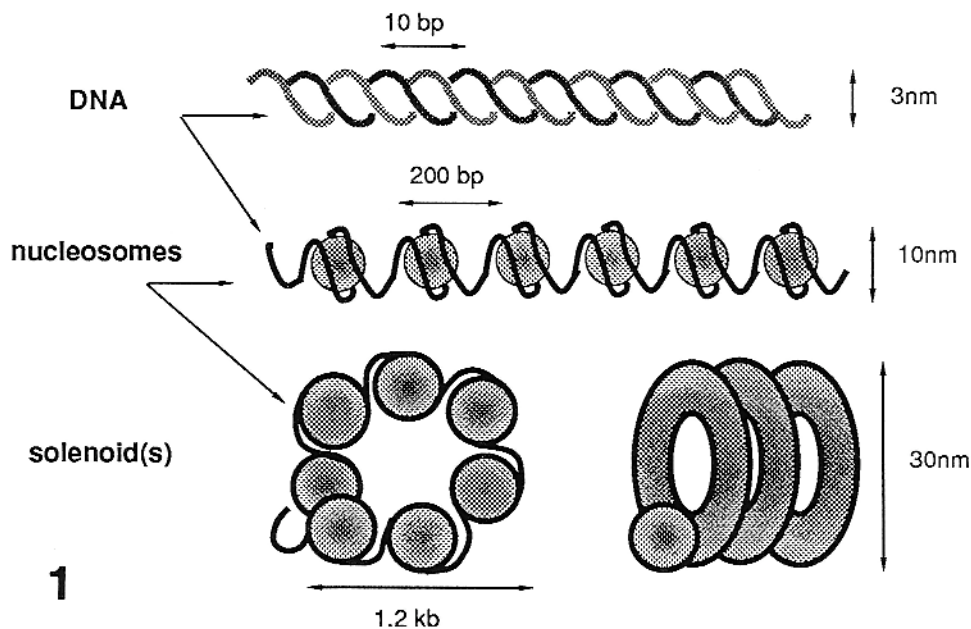


FIG. 1. Generally accepted model of the folding of DNA through the solenoid level. DNA double helix, with 10 bp per turn is 3 nm wide and is shown at larger scale than the next two levels of folding. DNA is wrapped around cores of histones yielding an essentially solid 10-nm-wide fiber. H1 histone in the intervening region is not depicted, and in transcriptionally active chromatin H1 may be depleted (e.g. refs 19,67); these H1 depleted sites are hypersensitive to nucleases, and yield ~200 bp DNA fragments or multimers of this length.

Nucleosome fibers twist in a helix to form solenoids. One turn of the solenoid is shown from the front (~1.2 kb DNA per turn), with an overall width of 30 nm. From the side these solenoid turns will be the thickness of the nucleosome fiber (10 nm), and multiple solenoids will form a fiber, as shown in the slightly oblique view above. The DNA fiber in the solenoid diagram only signifies continuity between nucleosomes, not the actual course of DNA.

contracted metaphase chromosome which measures ~700 nm in width. The latter two, and especially the interphase chromosome, require some comment.

The organization of DNA in the interphase nucleus is especially poorly understood, and has not been the subject of extensive study, despite its importance for most biologically meaningful functions of the cell. Organized interphase chromosome structures may be destroyed by bulk chromatin isolation procedures which have been used so successfully for visualizing nucleosome and solenoid features. An apparent lack of organization above the chromatin (solenoid) level in interphase may also partially derive from the limited recognition of larger chromosomal (DNA) structures by thin section electron microscopy. It is often overlooked that in ~600 nm thin sections, which will display nucleosome fibers (~10 nm thick) and solenoid fibers (30 nm thick), only fortuitous section angles will reveal long curving structures that are >200 nm wide. Furthermore, chromosomal (DNA) elements may be difficult to distinguish from other RNA and protein complexes in the nucleus. Many people still consider that chromosomal DNA in interphase is spaghetti-like, and virtually endless, with little apparent higher order organization. Such a view is exemplified by the model presented by Comings (12) where there are few spatial restrictions on the boundaries of individual chromosomes; in this type of model, solenoid fibers, and their

even lengthier substructures, nucleosome fibers, are thought to meander throughout the nucleus with little constraint. This view is untenable in the face of more recent in-situ evidence indicating chromosomal domains, and even entire chromosomes are visible in the interphase nucleus as spatially limited structures (vide infra). On purely theoretical grounds, it is functionally more efficient for complex enzyme machinery to utilize solenoid fibers that are folded (spatially collected) with a higher level of organization in actively transcribing or replicating interphase cells.

The nuclear membrane has also been considered an essential anchor for genetic organization (3). However, three-dimensional nuclear structure is independently conserved when the nuclear membrane and most nuclear proteins (with the exception of histones and sparse, tightly-bound matrix proteins) are removed (41). In such a situation, nuclei become rounder, probably as a consequence of loss of cytoplasmic cages of intermediate filaments, but more importantly, a plethora of reasonably regular, dense interphase chromosome fibers that are ~200 nm wide become prominent throughout the nucleoplasm by electron microscopy. Although extraction of large amounts of nuclear RNA and protein complexes may lead to a somewhat collapsed state of these interphase chromosomes, it is remarkable that three-dimensional DNA-rich structures are preserved with only ~5% of the DNA bound to

matrix proteins (20). At the very least, solenoids must be folded into structures of this width (or slightly larger) in interphase. We assume that this folding pattern is generalized.

High voltage electron microscopy of thick sections, and scanning electron microscopy of cracked whole interphase nuclei, also show fibers consistent with a width of 240 nm*; furthermore, corresponding interphase chromosomal fibers that are ~250 nm wide can be readily visualized in three-dimensional preparations of whole live cells or thick sections stained with DNA specific fluorochromes (64). Such interphase fibers are prominent in euchromatic, diploid neurons (Fig. 2A). These 240 nm-wide extended fibers clearly represent a general level of organization above the solenoid fiber. Studies of known chromosome regions using a variety of staining and in-situ methodologies have corroborated these dimensions, and in some cases, indicate their functional capacities.

First, it is notable that decoration of proteins tightly associated with actively transcribed regions, such as the active ribosomal cistrons, delineate fibers with a diameter of 200–250 nm in a variety of whole aldehyde-fixed cells (41). In-situ hybridization of rDNA probes to three-dimensionally preserved cells shows similar, compact DNA fibers of reasonably regular width, albeit at the lower resolution of light microscopy (43). Therefore transcription does not necessitate large changes in these structures, nor, indeed, a spaghetti like extension of transcribed DNA domains at distant locales. However, local extension of small linear lengths of DNA to a nucleosomal or naked DNA level may occur. Any model of interphase chromosomes should account for the overall width and appearance of these transcribed fibers in a functionally compatible manner. Moreover, in serial optical sections, some active ribosomal cistrons can show an overall loose helicity (41). This further degree of folding (coiling) is discussed below.

Unlike these rDNA transcribed regions, constitutive heterochromatic domains in interphase, such as those that define paracentromeric regions on human chromosomes, are generally wider than 240 nm. In-situ hybridization of specific tandem repetitive repeats that define such regions in individual chromosomes, delineate interphase domains that are generally 700–800 nm in diameter (13); measurements of these regions in aldehyde-fixed cell preparations evaluated at both the light and electron microscopic level are similar (4,47).

Antibodies to the paracentromeric regions, known to be constitutively heterochromatic, also delineate similarly large compact domains, and do not entail DNA denaturation (see, e.g., 43,51). The dimensions of these larger interphase domains approaches the 700 nm width of highly contracted metaphase chromosomes. We suggest that, in interphase, these special heterochromatic regions are almost always in a relatively contracted state, resembling their configuration in metaphase. In our model, we consider these regions represent relatively tight coils of the 240 nm fiber. This condensed motif in constitutive heterochromatin parallels a general lack of transcriptional activity in these domains in the interphase nucleus. Such regions can contain as much as 9Mbp (megabase pairs) of linear DNA as determined by recent PFGE studies (9). Interestingly, these contracted domains are the last regions to be replicated prior to mitosis.

In interphase therefore, chromosomes can assume a euchromatic fiber configuration (240 nm wide) through a metaphase-like coiled configuration (≥ 700 nm wide). It is not unreasonable to assume that these two configurations represent a continuum, with local differences along each chromosome arm. In accord with this, different overall patterns of chromosome condensation are observed in different cell types. Facultative heterochromatin reflects the source of these differences. Like constitutive heterochromatin, facultative heterochromatin is generally thought to be largely inactive with respect to transcription, and selected regions of a chromosome arm can probably assume a metaphase-like contraction, or coiling. This facultative condensation permits large amounts of DNA to be fit into a relatively small nucleus that characteristically does not require extensive transcriptional activity for maintenance of the cell. As an example of this, Fig. 2A shows a small diploid glial cell nucleus. In contrast to the adjacent neuron, this glial cell contains tightly packed, more condensed chromosomes. It is generally acknowledged that glial cells are considerably less transcriptionally active than larger neurons, and the concept that more condensed or heterochromatic interphase fibers are likely to contain relatively inactive transcriptional domains is supported by this example, as well as by data from numerous other biological specimens. Fig. 3 shows an idealized depiction of both more extended (euchromatic) and more contracted (heterochromatic) interphase chromosomes. This hypothetical scheme is based on what we know to date about mammalian interphase chromosomes from aldehyde fixed specimen in three-dimensional in-situ hybridization studies (4,37,42,45–47).

In striking contrast to the spaghetti-like view of interphase chromosomes, in situ hybridization to specific chromosomes in interphase has unequivocally demonstrated that individual chromosomes are spatially confined in the interphase nucleus. In mouse-human hybrid cell lines, each human chromosome occupies a discrete "territory" in interphase (45,58,62); in alde-

*Dehydrated and embedded (or critical point dried) specimen often show an artifactual decrease of linear measurements of ~20% of their in vivo counterparts. For example, the dense intraperiod lines of peripheral myelin are separated by 12nm using conventional transmission microscopy, whereas X-ray diffraction studies of fresh myelin shows a separation of 17–18 nm (57). Thus the observed measurement of ~200 nm wide fibers is likely to be ~240 nm prior to aldehyde fixation and dehydration, i.e. we assume a shrinkage artifact of ~16% in such preparations.

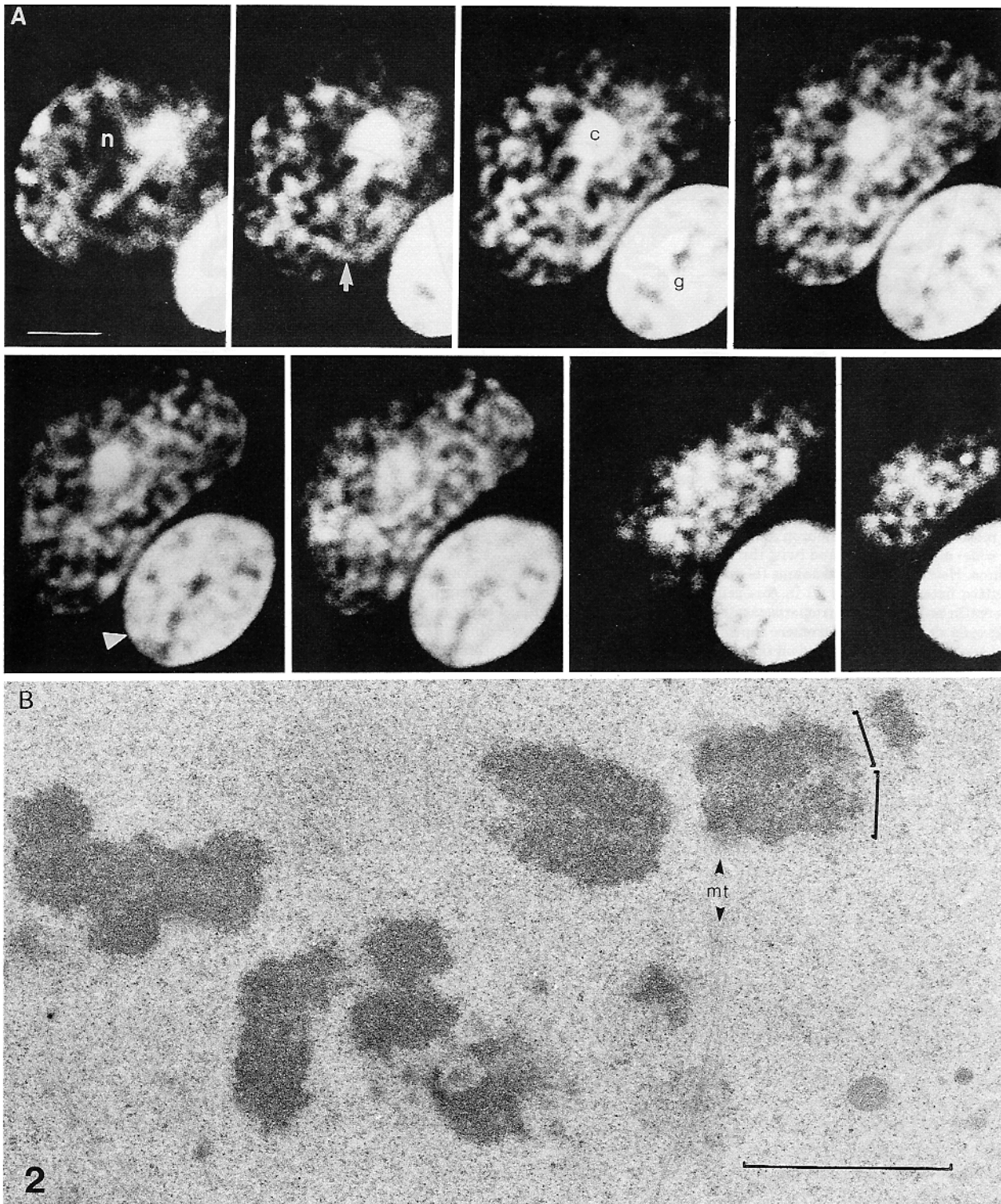


FIG. 2. A) Mouse neuron labeled with propidium iodide to delineate DNA in aldehyde fixed vibratome section. Many fibers that are ~ 250 -nm-wide are seen (e.g., arrow) in confocal fluorescent optical sections of 200 nm in the Z-axis (left to right). Neuronal nucleolus (n), and larger fluorescent region (c) on the nucleolus and known to contain constitutive heterochromatin (42,47) are marked. Note adjacent glial nucleus (g) is smaller and contains more heterochromatic domains and thicker contracted fibers (arrowhead). Mammalian glial cells nor-

mally have an extremely low level of replication. Bar is $2\mu\text{m}$. B) Thin section of mitotic cell. One contracted metaphase chromosome is roughly perpendicular to the plane of section as judged by the microtubules (mt) that are entering the centromeric constriction; each sister chromatid arm is denoted by brackets. There are no chromatin poor regions or holes ≥ 200 nm in any chromosome, regardless of orientation. Bar is $2\mu\text{m}$.

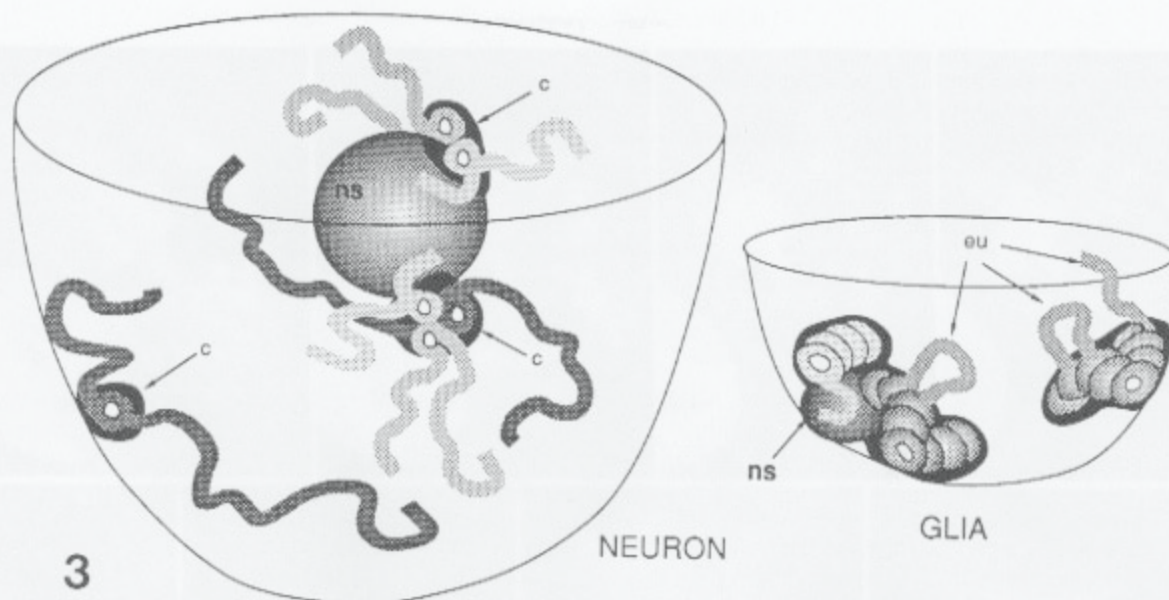


FIG. 3. Idealized representation of three chromosome homologs in the nucleus of a hemisected euchromatic large neuron and a small heterochromatic glial cell. The arms of each interphase chromosome are more extended in the neuron and these euchromatic fibers (~ 240 nm wide) may gently coil and twist through a relatively large nuclear volume. Heterochromatic domains (black) are largely defined by constitutive heterochromatin (c) in this cell type. Constitutive heterochromatin around the centromeric constriction is depicted by 240 nm wide coils around the centromere (open circles). In larger neurons, most centromeres are collected on the surface of the central nucleolus

(ns), although a few of these domains are on the membrane. Two chromosomes (homologs are of corresponding gray scale) contain ribosomal genes and thus invade the nucleolus. In the smaller diploid glial nucleus, only a few chromosome regions extend into 240 nm fibers that are euchromatic (eu). The rest of the chromosome arm is thought to be more contracted (coiled). This facultative heterochromatin joins constitutive heterochromatin (on or adjacent to the nuclear membrane) to define more complex heterochromatic aggregates (black). Data for the separation of homologs, and domain positions in these cell types are presented elsewhere (37,41,42,47).

hyde fixed cells, decorated chromosomal fibers in each human chromosome territory vary between 240 to 800 nm in thickness (45). Fibers of 240 nm are detected within spatially limited domains, and these may be more or less loosely coiled in three-dimensional space. The territorial features of individual chromosomes in interphase are more universally substantiated in non-hybrid cells using library probes specific for whole individual chromosomes (14,36,37,59). It should be noted, however, that in hypotonically swollen nuclei, chromosome territories distal to the centromere may appear more diffuse than they actually are in vivo.

Although there is some increase in overall nuclear size with replication, we have as yet been unable to detect large width changes in euchromatic interphase fibers during replication. For this reason we suggest a replication model that does not appreciably increase the width of interphase chromosome fibers until G_2 , or possibly the onset of mitosis. Our replication model does, however, increase the length of the interphase chromosome by ~ 2 -fold. To date, in three-dimensionally intact preparations, the length of an individual interphase chromosome has not been determined. In contrast, length measurements for prometaphase and metaphase chromosomes are reasonably reliable and uniform in different cell types. The length of human chromosome 1 in prometaphase is $\cong 10 \mu\text{m}$ in acid-fixed

preparations [see Fig. 6]. We assume that acid fixed extended prometaphase preparations may reasonably be used to estimate the *minimal* diploid interphase chromosome length. Since each chromosome is not completely or uniformly extended along its entire length in most interphase cells, i.e. it contains relatively heterochromatic and euchromatic domains, we would doubt the overall length of a chromosome would achieve its *maximal* theoretical value; in our model the maximal value for an extended 240 nm fiber is $\sim 7.5 \times$ the length of a contracted metaphase chromosome.

Fig. 2B shows a typical thin section of an aldehyde fixed metaphase chromosome cut roughly in cross section, as judged by the orientation of microtubules about to enter the centromeric constriction. Metaphase chromosomes are known to be transcriptionally inactive. Each sister chromatid generally measures ~ 600 nm in width; without the artifacts of thin section visualization, and dehydration, each sister chromatid may be reasonably considered as ~ 700 nm thick. Both in serial thin and thick sections stained with DNA specific dyes, no well-oriented "holes," or DNA-poor regions of significant size are observed within metaphase chromosomes (unpublished data), and the surface of these structures is smooth. In our model of highly contacted metaphase chromosomes we therefore fill all regions of the metaphase chromosome with chromatin, and

thereby also achieve a higher degree of DNA compaction (vide infra). The length of a completely contracted (coiled) human chromosome 1 in metaphase is 7–8 μm . This corresponds to 270–300Mb of DNA in each sister chromatid, or 9–10% of the haploid genome. We use the size, DNA content, and Giemsa-banding pattern of this chromosome as one test for the accuracy of our model. It should be emphasized, however, that our model suggests an *average* for all structures, and does not imply rigidly defined regular boundaries. Actual biological boundaries may be determined by the presence of absence of special DNA sequences and protein motifs [cf. section on "Bands and Subbands," page 19].

The transition from a 240 nm diameter extended interphase fiber to a condensed 700-nm-diameter metaphase structure should be extremely simple, as this transition occurs within a relatively short time (<30 minutes). More extended replicated interphase fibers rapidly contract in metaphase, and after cell division, the diploid chromosome complement rapidly extends. Similarly, in diploid interphase nuclei, the dynamic recruitment of a complex set of inactive domains into their transcriptionally viable counterparts, as for example during heat-shock, or during differentiation, must be rapid and efficient. We have suggested above that this structural conversion is almost identical to the one used during mitosis, i.e. it entails a simple, reversible coiling between more extended (240nm) and more condensed (700nm) arrays. In both diploid and tetraploid (replicated) cells, these extended and contracted configurations represent a spectrum of structural features that are present in varying degree in each cell type, and in different physiological states. The structural dimensions calculated represent the extreme of each state (extended or contracted). Actual dimensions are likely to include intermediate, transitional forms.

Lengths of DNA With Meaningful Biological Properties

The central problem, then, is to arrange DNA (packed in solenoids) in a reasonably regular manner that: 1) fits these dimensions, 2) packages sufficiently large amounts of DNA, and 3) also leaves adequate space for molecular processes such as transcription and replication. First, it should be noted that coding regions (open reading frames) may be as small as a few kilobases, but promoter and control regions for a gene can be >10 kb distant from the open reading frame. Some transcribed genetic regions may also be quite large, equivalent to >40 kb (e.g. ribosomal genes, HLA genes). Thus a solenoid turn with 1.2 kb of DNA is a relatively small unit in terms of the long-range organization of transcriptional domains; for processing, it is more economical to collect distant regulatory regions with their related coding sequences into small spatial domains. More recent studies indicate that DNA lengths of 30–120kb have significant structural and functional relevance.

Matrix studies on interphase nuclei have produced a great deal of evidence for supramolecular chromatin structures with periodicities in the 30–120kb range in typical eukaryotic cells. An excellent and herculean review of the evidence has been presented (55), and only a few salient references are cited here. One useful concept is that of an 60 kb "loop" of DNA. Many investigators have analyzed the components and structure of the nuclear matrix (also known as a scaffold), which is operationally defined as the small proportion of nuclear non-histone proteins (and a large proportion of hnRNA [20]) that is tightly bound to chromatin. Together the data indicate reasonably regular DNA interactions with the matrix at every ~60 kb. This value is fairly typical for the anchoring of DNA to structural proteins, although units of 30- up to 120 kb have been reported (16,55). A variety of structural, extraction, and digestion studies have also indicated that specific proteins such as topoisomerase II and selected DNA sequences are also tightly organized at the base of each 60 kb DNA loop. Since actively transcribed genes, such as histone and other coding DNAs, can be tightly attached to this matrix (10,15), transcriptional activity in regions close to these protein attachment sites (as well as at more distant, non-attached DNA sites) must be possible. Newly synthesized DNA is also apparently attached to an internal network of the nuclear matrix (56), and increased amounts of DNA polymerase α -DNA primase complex is bound to the matrix during the DNA synthetic S phase (11). Furthermore, there are $\sim 10^5$ replication origins in human or mouse DNA, equivalent to one replication origin per 60 kb of diploid DNA.

The concept of a loop has wide appeal, as extended oocyte lampbrush chromosomes display visible loops, and highly divergent organisms (such as bacteria) also possess characteristic molecular DNA loops that are anchored to protein every ~40 kb (35). Neither bacterial nor eukaryotic DNA loops have been modeled three-dimensionally with any precision. It is reasonable to assume that the majority of eukaryotic DNA in each loop is already packed into solenoids. Can such loops be accommodated in interphase chromosome fibers of the above dimensions (~240 through 700nm diameter) with sufficient compaction to fit into a small nucleus?

A recent detailed model (55), has been presented which achieves sufficient DNA compaction for a metaphase chromosome, but does not adequately explain euchromatic interphase fibers of 240 nm diameter. In this model, chromosome fibers are 840 nm wide. This model does, however, propose an attractive sliding mechanism for DNA replication, which we incorporate into our current model. Rattner and Lin (60) have combined the general radial loop model of Laemmli and coworkers (18,49), with the 200 nm interphase/coiled metaphase structure suggested by Sedat and Manueldis (64). However, no detailed numeric or spatial calculations were given. Our previous model (64) ad-

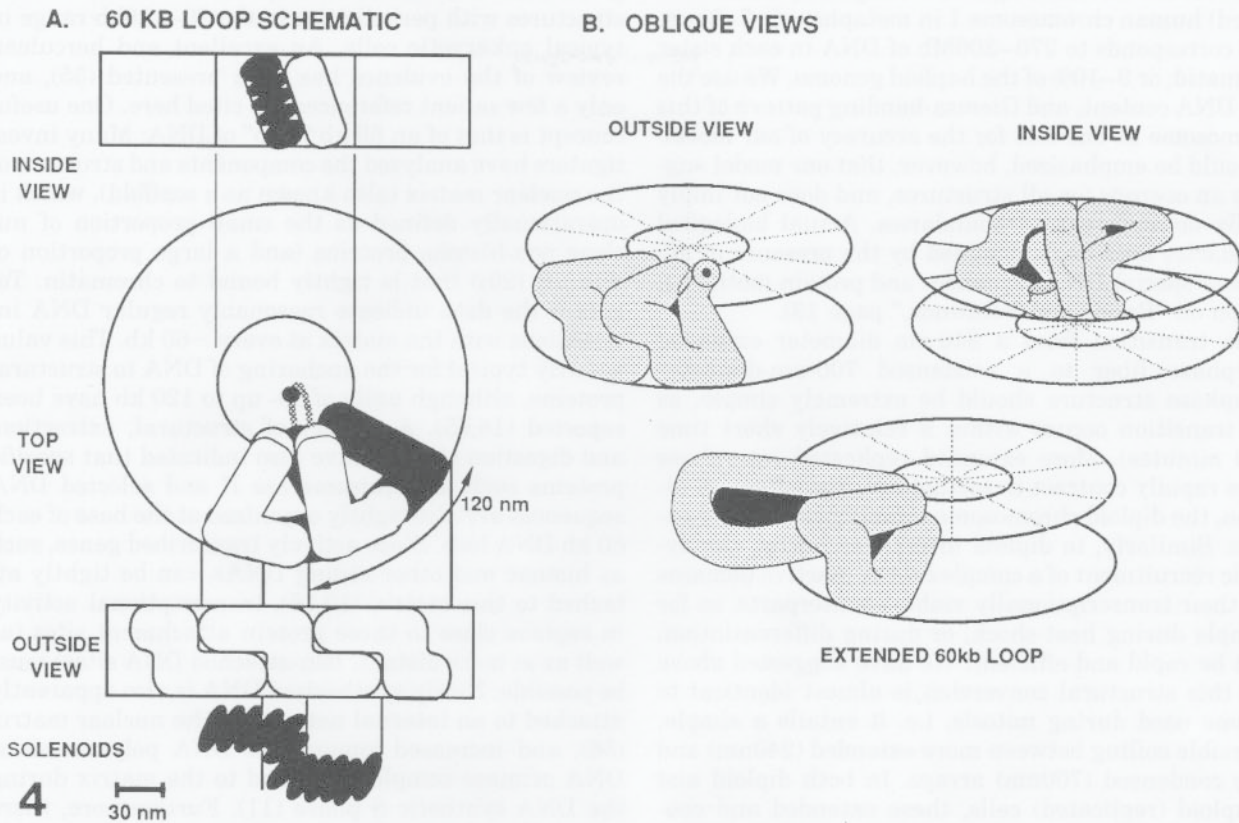
RADIAL ARRAY

FIG. 4. **A)** Scale drawing of 2 radial subunits in a radial array. For each subunit the extending upper arm is represented in white and the lower returning arm in black (top view). Solenoid turns for one subunit are modeled for both the inside and outside surface view. Each subunit connects to the next radial subunit. Two subunits form an average 60kb radial loop, with a single matrix anchoring site in the center of the array. The matrix complex is depicted by a solid circle, and may be attached to every other radial subunit via a more extended nucleosome-DNA fiber (gray line). Each 300kb radial array is formed by 10 radial subunits to form a repeating helix (see Fig. 6, top). **B)** Appearance of a 60kb loop of solenoids from the outside and inside

of the array (oblique views). The black region denotes space within each radial subunit. In the bottom view, two radial subunits (a 60kb loop) are extended laterally as a less kinked solenoid. This loop is seen just behind a single folded radial subunit. Recent evidence suggest that topoisomerase II may be removed during transcription (65); extension of selected loops may occur during transcription, and may be facilitated by this mechanism. With lateral extension, the diameter of the radial array is increased by ~ 100 nm, and there is increased space within the loop. Small locales of naked DNA on the extended solenoid may be used for transcription.

dressed the ~ 200 -nm-wide interphase fiber but, even with updated measurements, allows insufficient DNA compaction into individual metaphase chromosomes. In addition to compaction of DNA within defined dimensions, we here required that 60 kb loops be arranged in a fashion that is consistent with sequential transcription in, and orderly replication of larger genetic domains containing >500 kb of DNA.

The Radial Subunit

We explored several models, including supertwisted loops, for the parsimonious folding of solenoids containing 60 kb of DNA into a fiber of 240 nm in diameter. One model adequately fit sufficient DNA, effectively utilized the entire radius of the observed interphase chromosome fiber, and was consistent with regular, directionally continuous "loops" of solenoids containing

~ 60 kb of DNA. This simple model is represented in Figure 4. Unlike our previous model (64), it does not postulate an extra coil of solenoids, and fits considerably larger amounts of DNA into a fiber that approximates the width of an extended interphase fiber. A solenoid fiber, constituted by a total of 25 solenoid turns (representing 30 kb of DNA), extends and returns to the center of a "radial array." This folded solenoid defines each radial subunit. The returning arm of solenoids lies just beneath and 15 nm lateral to its origin. The lower arm then ascends 60 nm (interiorly) to begin the next radial subunit. Twelve solenoids can fit on the outer surface of each subunit, 5–6 solenoids fit on each of its extending and returning arms, and 4–5 solenoids connect this subunit to the next 30 kb subunit. Thus a sector of two radial subunits (a loop) can accommodate a maximum of 58 solenoids or 69.6

kb of DNA. We assume that the average value for each loop sector is ~60 kb (Table 1). Ten radial subunits form a single 240-nm-diameter radial array, depicted as a disk in Figure 4. Thus each radial array contains ~300 kb of DNA.

The outer surface of each radial subunit contains bends (Fig. 4); these are plausible in view of the various configurations of DNA (22,24,29,61) and known enzymes, such as topoisomerases and gyrases, that can induce twisting. Furthermore, two bends on the outer radial surface define a reasonable space in the center of each 30 kb subunit (represented in black in Figure 4B). This space can accommodate transcriptional complexes and short segments of naked unfolded DNA that may be transiently needed for transcription. This subunit space is accessible to the external surface of the radial array. Furthermore, at the center of each radial array there is additional space (Fig. 4A). This space is ~65 nm (or more in longitudinally-extended fibers, *vide infra*), and can accommodate matrix proteins, topoisomerase II, hnRNA and replication complexes. (For size reference, the complex ribosomal machinery is ~30 nm). On the average, we estimate that one matrix anchoring site is present at every alternate 30 kb subunit (one 60 kb loop sector), although this may vary in selected genetic regions, i.e. some subunits may be attached every 30 kb, whereas others may be anchored at every 90 kb or 120 kb. Selected anchoring-associated proteins (for transcription and replication) that are diagrammatically depicted in Figure 4A may actually attach to a small extended region of nucleosomal or naked DNA fibers rather than to a solenoid. Topoisomerase II has been shown to follow the distribution of DNA in interphase chromosome fibers (17), and the above model is compatible with this observation. Although we have modeled anchoring proteins and transcriptional complexes near the center of each radial array, it should be noted that matrix preparations are three-dimensionally complex (20); not all components of the matrix need necessarily attach directly to chromatin or reside in the central space. The space around contiguous chromatin surfaces in this model can accommodate a complex three-dimensional matrix.

Radial Arrays Form Extended Interphase Fibers

The offset of each radial subunit with respect to its neighbor allows for the creation of a continuous fiber that is on the average ~240 nm in diameter. Radial arrays of 300 kb proceed without clear structural demarcation into an adjacent radial array which lies 60 nm beneath the first array. In fact, groups of these arrays may be rich in certain DNA sequences [see section called "Bands and Subbands" page 19], and may also contain selected proteins that will enable us to identify them as bands, or definable domains of large size. A fiber constructed of many radial arrays, equivalent to an extended chromosome fiber, is simulated in Figure 5A, where each radial array is schematically depicted as one disk in a sequential continuous stack; a

more realistic appearance would be a curving helix, much like a flexible spiral staircase [see Fig. 6]. If a 60 kb sector is extended laterally for transcription (Fig. 4B), as it may be in some actively transcribing regions, the overall diameter of the radial array will increase by only 100 nm. This change would be barely appreciable in gently curving three-dimensional interphase fibers, especially since only a small proportion of the genome is actively involved in transcription. Additionally, a slight longitudinal stretching of the helix formed by the radial arrays (Fig. 6, top) would leave even more room for the inclusion of complex molecules, such as long RNA transcripts. In such longitudinally-extended regions, fibers could appear to be 90–120 nm in diameter, as is sometimes seen by electron microscopy (see dimensions in Table 1). The picture is compatible with the appearance of chromosomes in larger mammalian neurons that are largely euchromatic (extended) and do not replicate (Fig. 2A). In principle, in three-dimensional preparations, two single copy unique sequences of 1–2 kb separated by 20–50 kb, could be discriminated from each other in more extended euchromatic domains by high resolution *in-situ* hybridization. Sequences at a distance of 135 kb have already been resolved experimentally in interphase nuclei (33). A gene mapping resolution of similar small magnitude might also be appreciable in selected regions of more extended prometaphase preparations, such as the GC rich distal region of the p arm of human chromosome 1. It is notable that some RNA transcripts may follow the course of a chromosome (34), and it remains to be determined if there are special proteins or other recognition complexes on the outer surface of radial arrays that may help to guide nascent mRNAs toward the cytoplasm.

In interphase, some extended fibers may show small curves or twists. Selected regions of each chromosome may also assume a heterochromatic or coiled appearance (*vide supra*). A more condensed or coiled configuration of radial arrays is also shown in Figure 5A. Three-dimensional reconstructions of individual paracentromeric domains delineated by *in-situ* hybridization are consistent with the local coiling of chromosome fibers in constitutive heterochromatin (47). Elegant three-dimensional studies of matrix complexes which may roughly mimic the course of radial arrays (*vide supra*), also show coiled as well as more extended domains in interphase after most of the DNA is digested (20). A transition along this continuum may accompany dynamic changes in interphase chromosome structure associated with different functional states. In some diploid interphase cells, such as glial cells, this feature is taken to an extreme, where most of the non-replicated interphase chromosome fibers appear to be tightly coiled, much as they are in metaphase (Figs. 3 and 5). It can be readily appreciated that mapping of closely spaced genes in such coiled, heterochromatic regions of the interphase nucleus (or in contracted metaphase preparations) will be less favorable than in the more extended regions described above.

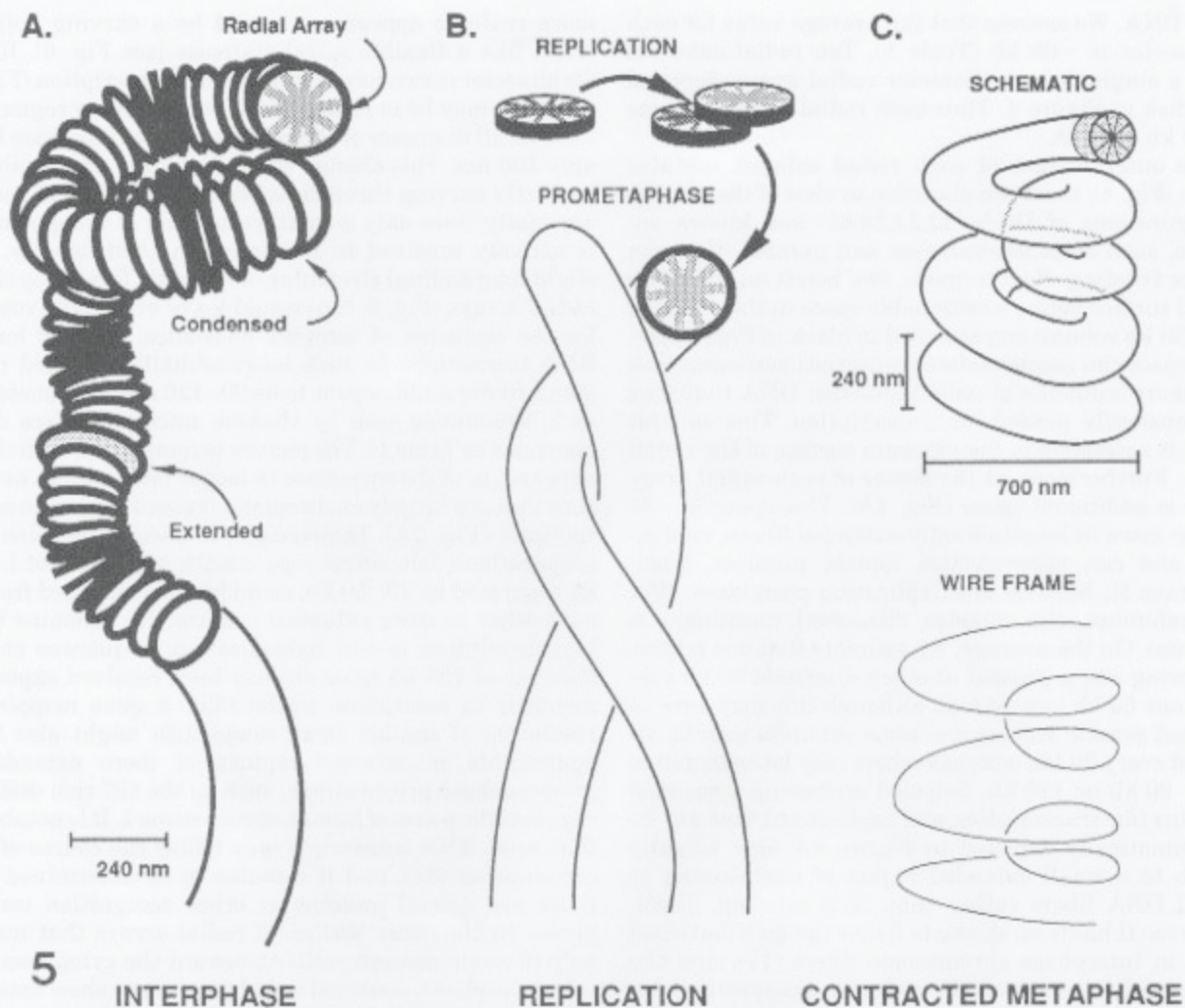


FIG. 5. Predicted patterns of DNA folding from interphase to metaphase. **A)** Radial arrays (depicted as disks, rather than helices for simplicity) in diploid interphase nuclei form more coiled or condensed regions as well as more extended regions that will appear as fibers. One array with laterally extended loops is depicted in gray; vertical extension may also occur with transcription (see Fig. 6, top). **B)** Model of replication, where replicated radial arrays interdigitate

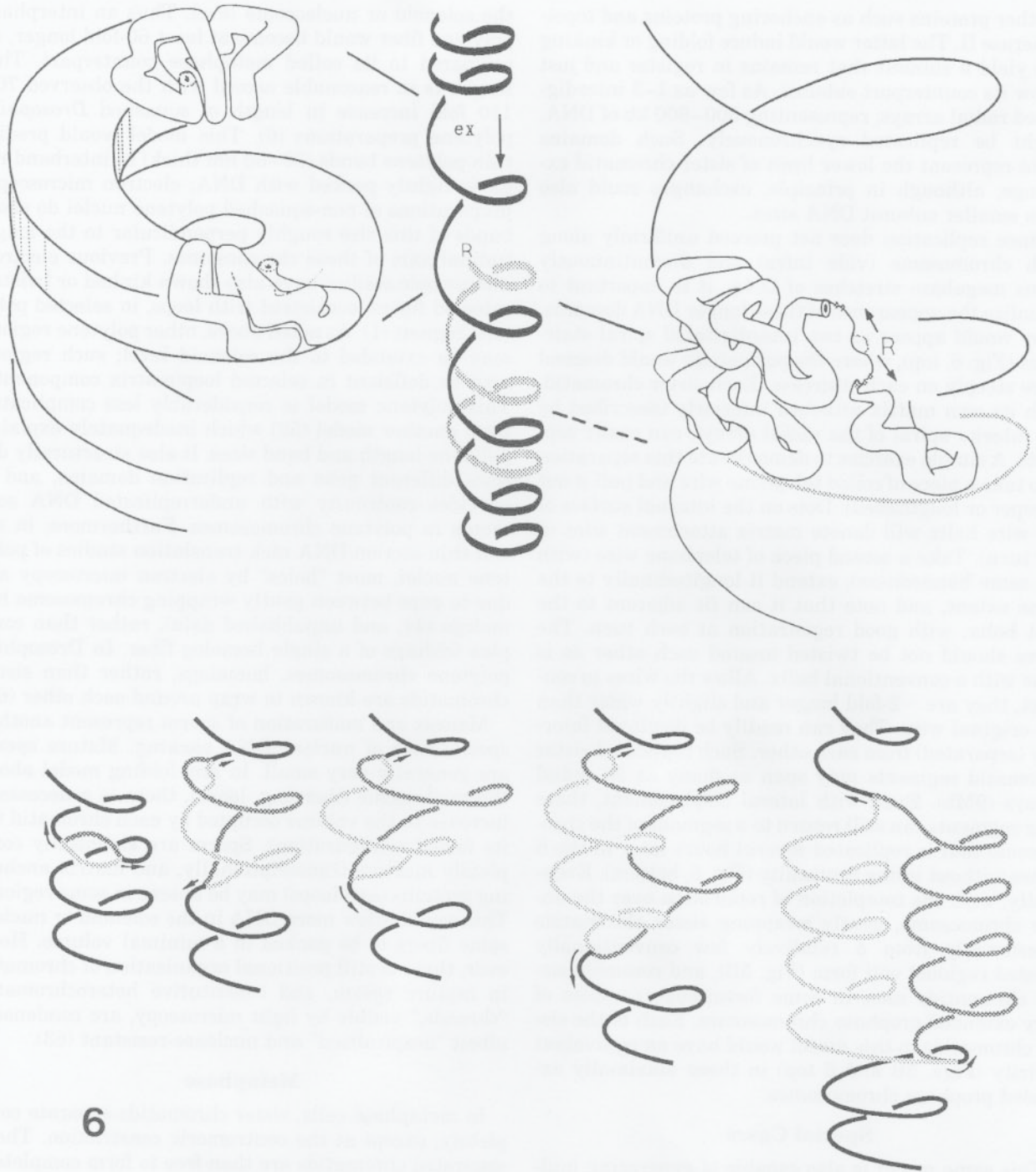
(see Figure 6 for replication details). Sister chromatids eventually wrap around each other to form a prophase chromosome. **C)** With metaphase contraction, sister chromatids separate to form coils. One sister chromatid is modeled in both a schematic and wire frame model, and one incompletely contracted region is depicted to show this process. A single radial array (gray) represents the orientation of this unit with respect to the 240×700 nm coil.

Replication

In dividing cells prior to metaphase, each homolog must be replicated. We propose that replication may proceed first at the level of each loop in the 300kb radial array. A schematic detail of this is shown in Figure 6, top. In this model, it should be noted that DNA in each 60 kb sector can easily slide into and out of a centrally positioned replication complex (55). Replication origins in mammalian DNA are located at 50–300 kb apart (26), and these sizes are represented between the subunits and span of each radial array. A sliding mechanism would facilitate the rapid completion of replication in multiple 300 kb segments. Since replication is bidirectional and semiconservative, synchronously replicating loops, or longer radial arrays, would

rapidly join each other (Fig. 6, bottom). Additionally, each radial array can incorporate replication complexes at its center, in close apposition to matrix anchoring sites. Replication complexes are thought to be relatively immobile, and attached to the matrix during DNA synthesis (11,16,54–56).

During replication, sister chromatids would first be interdigitated with each other in almost perfect register (at each subunit and radial array, Figure 6, top), to yield an ~ 2 -fold longer and slightly thicker chromosome segment. A slight relaxation of the matrix proteins, which may have contractile properties, would allow each replicated segment to closely parallel its sister. In the model, as DNA is replicated at each fork (Fig. 6, top) it would rapidly bind to histones, and then



6

FIG. 6. Replication and longitudinal extension of radial arrays. **Top:** At left, two representative loops (4 subunits) are shown forming the general curve of radial arrays; this path corresponds to a portion of a euchromatic fiber that is longitudinally extended (ex, center). With replication (R), two spirals formed by the arrays nest (interdigitate) in extended regions. At right, is a detail of replicating sister chromatids shown on the black and dotted lines. The interdigitated arrays and their subunits are in register. Arrows denote bidirectional replication from a replication fork at R; (the corresponding upper subunit is omitted for clarity). Newly replicated DNA may rapidly bind histones and selected high affinity matrix components (such as topoisomerase II) which will facilitate folding of nascent subunits.

Bottom: A diagram of two sister chromatid segments as they arise from a haploid DNA segment (black). Semiconservative DNA replication is denoted by dotted lines of different density in each sister. Arrows indicate direction of replication, and each turn of the helix represents one 60×240 nm radial array. Nested replicated regions can stack in a single column (without significantly increasing the diameter of the 240 nm fiber), and can also be displaced laterally from each other without losing continuity from nonreplicated regions. Replication beginning at only one origin is demonstrated here; adjacent large replicating domains (from several origins) can join to form enormous chromosome band structures that have synchronous replication characteristics. The separation process may be extremely rapid.

to other proteins such as anchoring proteins and topoisomerase II. The latter would induce folding or kinking and yield a subunit that remains in register and just below its counterpart subunit. As few as 1–3 interdigitated radial arrays, representing 300–900 kb of DNA, might be replicated synchronously. Such domains could represent the lower limit of sister chromatid exchange, although in principle, exchanges could also span smaller subunit DNA sizes.

Since replication does not proceed uniformly along each chromosome (vide infra) and discontinuously spans megabase stretches of DNA, it is important to visualize the appearance of these longer DNA domains. They would appear as two interdigitated spiral staircases (Fig. 6, top), where the peripatetic would descend more steeply on each staircase. Each sister chromatid, with its own matrix attached interiorly (described by the interior spiral of the radial array), can easily separate. A simple exercise to demonstrate this separation is to take a piece of coiled telephone wire and pull it out (steeper or lengthened). Dots on the internal surface of the wire helix will denote matrix attachment sites (5 per turn). Take a second piece of telephone wire (with the same handedness), extend it longitudinally to the same extent, and note that it can fit adjacent to the first helix, with good registration at each turn. The wires should not be twisted around each other as is done with a conventional helix. Allow the wires to contract; they are ~2-fold longer and slightly wider than the original wire. They can readily be displaced laterally (separated) from each other. Such replicated sister chromatid segments may span as many as 30 radial arrays (9Mb). Even with lateral displacement, these long segments can still return to a segment of the chromosome that is replicated several hours later in the S phase without losing continuity (Fig. 6, bottom). Eventually, with the completion of replication over the entire chromosome, gently wrapping sister chromatids (presumably from a relatively few conventionally twisted regions) will form (Fig. 5B), and resemble sister chromatids seen in some fortuitous examples of very extended prophase chromosomes. Each of the sister chromatids in this model would have an equivalent helicity (Figs. 5B and 6 top) in these maximally extended prophase chromosomes.

Special Cases

This same model is also capable of generating multiply-replicated polytene chromosomes with ease. With each round of homolog DNA duplication, chromosomes would appear as increasingly longer and somewhat wider fibers, consistent with interdigitated extended fibers described above. At some point in the repeated replication, radial arrays would be forced to unwind completely to form linear arrays of radial subunits that are 60 nm in width. Each replicated strand would be laterally offset in register. In some regions, such as in some transcriptionally active puffs and interbands, this unwinding might be even more extensive, i.e. to

the solenoid or nucleosome level. Thus an interphase polytene fiber would become at least 60-fold longer, as compared to its coiled metaphase counterpart. This length is in reasonable accord with the observed 70–110 fold increase in length of squashed *Drosophila* polytene preparations (6). This model would predict thin polytene bands (30–60 nm thick) in interband regions tightly packed with DNA; electron microscopic preparations of non-squashed polytene nuclei do show bands of this size roughly perpendicular to the longitudinal axis of these chromosomes. Previous electron microscopic studies have also shown kinked or twisted solenoid fibers, consistent with loops, in selected polytene regions (1). As noted above, other polytene regions may be extended to the solenoid level; such regions may be deficient in selected loop/matrix components. This polytene model is considerably less complicated than another model (52) which inadequately explains polytene length and band sizes. It also structurally defines different gene and replication domains, and it provides continuity with underreplicated DNA segments in polytene chromosomes. Furthermore, in serial thin section/DNA nick translation studies of polytene nuclei, most "holes" by electron microscopy are due to gaps between gently wrapping chromosome homologs (44, and unpublished data), rather than complex foldings of a single homolog fiber. In *Drosophila* polytene chromosomes, homologs, rather than sister chromatids are known to wrap around each other (6).

Meiosis and maturation of sperm represent another special case of nuclear DNA packing. Mature sperm are generally very small. In any folding model above the nucleosome fiber (e.g. loops), there is a necessary increase in the volume occupied by each chromatid (in its folded configuration). Sperm are essentially completely inactive transcriptionally, and matrix anchoring proteins (and loops) may be absent in some regions. This would allow more DNA in the solenoid or nucleosome fibers to be packed in a minimal volume. However, there is still positional organization of chromatin in mature sperm, and constitutive heterochromatin "threads," visible by light microscopy, are condensed, albeit "despiralized" and nuclease-resistant (63).

Metaphase

In metaphase cells, sister chromatids separate completely, except at the centromeric constriction. These separated chromatids are then free to form completely contracted coils. In our model (Fig. 5C), these coils are formed by a simple winding of the 240-nm-wide interphase fiber. Each radial array would appear as a disk-like 60-nm feature, roughly perpendicular to the axis of the coil in perfectly fixed or ideal three-dimensional preparations. This model of contracted metaphase coils is a modification of the one we previously presented (64), but accommodates larger amounts of DNA at this level of folding since the twist of each coil descends through the center and effectively utilizes the central helical space. (In less contracted transitions a central

Table 1
DNA Compaction at Each Level of Folding (in base pairs), With Corresponding Dimensions

Level	Base pairs	Dimensions (d = diameter, h = height)	Comments
DNA	0.01kb	3nm d.	DNA double helix
Nucleosome	0.2kb	10nm d.	2 turns DNA per histone complex (Fig. 1)
Solenoid	1.2kb	10nm × 30nm d.	per turn (Fig. 1)
Radial subunit	30kb	60h × 90 × 120 nm sector	25 solenoids (see text & Fig. 4)
Typical radial loop	60kb	60h × 90 × 190 nm sector	2 radial subunits (one matrix/replication site)
Radial Array	300kb	60h × 240nm d.	10 radial subunits (5 loops) per array multiple radial arrays of 240nm d. form the extended interphase fiber (Fig. 5)
Condensed Coil	9Mb	240h × 700nm d.	29.1 radial arrays, contracted metaphase
a. chromosome 1 (haploid)	270–300Mb	700nm d. × 6.4 to 8 μ m	29–33 condensed coils (400 Giemsa band level)
b. human genome (diploid)	6,000Mb	49–61.5 μ m ³	5 μ m spheroid = 65.5 μ m ³

space can exist). In its most contracted state, each sister chromatid can contain ~29 radial assays in each coil level, with a minimum of 4 radial arrays descending through the center to form one coil turn of ~9Mb. This structural model is consistent with the lack of large holes in thin section studies, as well as with the helical or zigzag appearance of separated metaphase coils known for many years (31,50). As described above, matrix or scaffold proteins in this model are at the center of each 240 nm fiber, and thus would not require additional space at the center of the coil. The appearance of matrix (scaffold) proteins at the center of disrupted metaphase chromosomes may be more apparent (preparation dependent) than real (49). Recent studies with antibodies to topoisomerase II have shown that this matrix bound enzyme does in fact follow the metaphase coil (5); it is possible that the observed opposite handedness of helical coils in each sister chromatid materializes after separation of the sister chromatids. Opposite handed sister chromatid cells may also be influenced by special isolation conditions.

Summary of Calculations

Table 1 summarizes (in base pairs) each level of the model from the DNA double helix through the volume occupied by diploid human DNA (6×10^9 bp) in a highly contracted state. The DNA lengths at the levels above the radial array are representative rather than absolute, since, as pointed out above, DNA lengths in each radial array may vary somewhat. In extended interphase and prometaphase domains, large stretches of DNA will be represented by one or multiple radial arrays of 240 nm, whereas in highly contracted metaphase chromosomes or in maximally coiled interphase domains that are constitutively heterochromatic, the structural motif will be 700 nm in diameter and contain considerably more DNA. As shown in this table, a maximally contracted human chromosome 1 in metaphase, is from 6.4–8 μ m long. This metaphase length can easily incorporate 27–33 coils (~9Mb per coil), equivalent to 250–300Mb of DNA in each haploid fiber. This value is in good accord with the estimated value of 270Mb for this chromosome. Furthermore, ob-

served coiled arrays are consistent with the modeled dimensions; coils are visible in aldehyde fixed metaphase preparations by scanning electron microscopy (40,60,64), and Giemsa-banded chromosomes also display regular surface invaginations consistent with the coils proposed (25). Each coil modeled is roughly equivalent to an average Giemsa band (at the 400-band level). Indeed, in more condensed examples of chromosome 1 at this level, standard maps show 27 distinct Giemsa bands, and three of these (at 1p31,1q12, and 1q31) are longitudinally thicker than the average band by ~2–3-fold. Thus there would be the equivalent of 27 + 4 typical Giemsa bands (31 average Giemsa bands) in terms of modeled coils. This number is in good accord with the model which calculates overall averages.

Table 1 also shows that in a small largely heterochromatic nucleus of 5 μ m in diameter, there would be adequate space for 3 nucleoli of 1 μ m diameter (total volume = 1.6 μ m³), with additional residual room (>4 μ m³) for extension and transcription of relevant interphase chromosome segments that are necessary for biological maintenance. Although the molecular mechanisms involved in coiling of diploid interphase chromosomes, or of replicated metaphase chromosomes is unclear at the present time, it is possible that loss of selected matrix components may facilitate condensation. In this context, it is of interest that when most hnRNA is removed, interphase matrices collapse into many tightly-coiled structures [20]. Moreover, metaphase chromosomes are known to lose selected proteins that decorate interphase chromosomes; transcriptional activity is decreased in metaphase, and probably several transcriptional-associated components are also depleted during this coiling transition.

Bands and Subbands

At this point it is useful to discuss more rigorously the molecular sizes and definition of mammalian chromosomal "bands." Giemsa banding conventionally shows light and dark regions of variable size, with a total of ~400 bands in more contracted metaphase spreads. In maximally extended prometaphase preparations there are ~2,000 Giemsa bands (68) per 3 ×

10^9 bp. Thus an average high resolution Giemsa band contains 1.2–1.5Mb, and the smallest such band is likely to contain 0.3–0.6Mb. Giemsa staining is complex, and likely to depend both on DNA sequence, local chromosome folding and protein–RNA interactions with chromatin (cf. Introduction). However, the smallest high resolution Giemsa band corresponds to 1–2 radial arrays in our model, and an average high resolution Giemsa band in prometaphase containing 5 radial arrays (1.5Mb) would be 300×240 nm. This is in reasonable accord with the observed dimensions of stained Giemsa bands in such preparations.

Pulse-field gel electrophoresis (PFGE) can be used to measure independently and accurately linear DNA lengths in selected regions of known morphology, and can complement structural studies. Double minutes of 3Mb by PFGE in uncleaved chromosomal DNA appear as a pair of ~ 300 nm diameter microscopic structures (18). Although double minutes may not be folded in exactly the same manner as integrated chromosomal sequences, these DNA measurements are in accord with the structural dimensions modeled above (equivalent to 5 radial arrays in each of the two minutes). PFGE can also be used to assess potential sequence motifs that may define discrete chromosomal bands. After cleavage of chromosomal DNA with restriction enzymes that cut very infrequently in the genome, specific DNA sequences can be detected in discrete linear pieces of DNA that range from 200–>700kb, depending on the enzyme used. For example, after endonuclease cleavage, PFGE bands in this size range can hybridize to repeated DNA sequences of the “minisatellite” class that are restricted to a single chromosomal locus; one or more copies of such long linear DNAs can define a specific Giemsa-dark domain at 1p36.3 (7,47). This minisatellite band also replicates later in S (see Fig. 9), and is equivalent in size to several 300kb radial arrays. Thus a high proportion of specific DNA sequences may delineate selected arrays of Giemsa-band size. Minisatellite-rich arrays may possess discrete structural tendencies (such as a tendency for increased coiling), and possibly signify functional characteristics, such as later replication. Giemsa-light bands may be defined, in part, by different sequence motifs. Unmethylated stretches of CpG rich DNA, associated with transcribed regions of DNA (2,38) also display PFGE Not I fragments in this same size range. These latter fragments may reasonably define more euchromatic or extended interphase domains that tend to be early-replicating.

Are there other DNA sequence motifs that molecularly define chromosomal bands in this size range, and do they have any functional significance? We have previously shown by in-situ hybridization that relatively AT rich, long interspersed repeated sequences (LINES) are morphologically segregated in arrays that roughly correspond to Giemsa-dark bands. In contrast, Giemsa-light regions on the chromosome have a relatively high proportion of short interspersed GC rich Alu repeats, or



FIG. 7. Pulse-field gel Southern blot of human DNA digested with *Nru* I and hybridized with a cloned human LINE repeat (39). Electrophoresis was done at 175 volts with one-minute pulses for 22 hours. Autoradiograph demonstrates the presence of large fragments of DNA in the 300–600 kb range that are enriched in these sequences. (The DNA on these PFGE blots detected by ethidium bromide staining showed only an intense fluorescent smear in this size range.) These long LINE-rich DNA fragments are likely to represent one to several contiguous radial arrays as described in Figs 4A & 5A. Different patterns are seen with other rare cutting restriction endonucleases, and LINE-rich PFGE bands may span several megabases (9). Such DNA motifs may in part contribute to folding or contraction characteristics of selected arrays. Lane 2 is a darker autoradiograph of lane 1. The molecular weight size markers on the left are yeast chromosomes from *S. cerevisiae*. The markers on the right are concatamers of lambda DNA from 50–>300 kb.

SINES (40,46). This molecular segregation of LINE and SINE motifs into structurally distinct compartments has recently been confirmed (30). In extended interphase chromosomes, LINE repeats are detected as ~ 250 nm wide spots of varying length (46,47). From the structural model above it can be predicted that linear DNAs of 300 kb to >1.5 Mb should contain clusters of LINE sequences. If the above in-situ observations and calculations are correct, it should be possible to confirm independently the presence of discrete PFGE bands that are rich in LINE motifs, visible above a background smear, in the size range of a very high to high resolution chromosome band. The presence of highlighted PFGE bands will also corroborate the non-random distribution of these sequences on chromosomes. We have found that this is indeed the case, and Figure 7 shows that discrete LINE rich bands are clearly visible in this size range. Other data has shown LINE motifs may be selectively concentrated in ~ 3 Mb

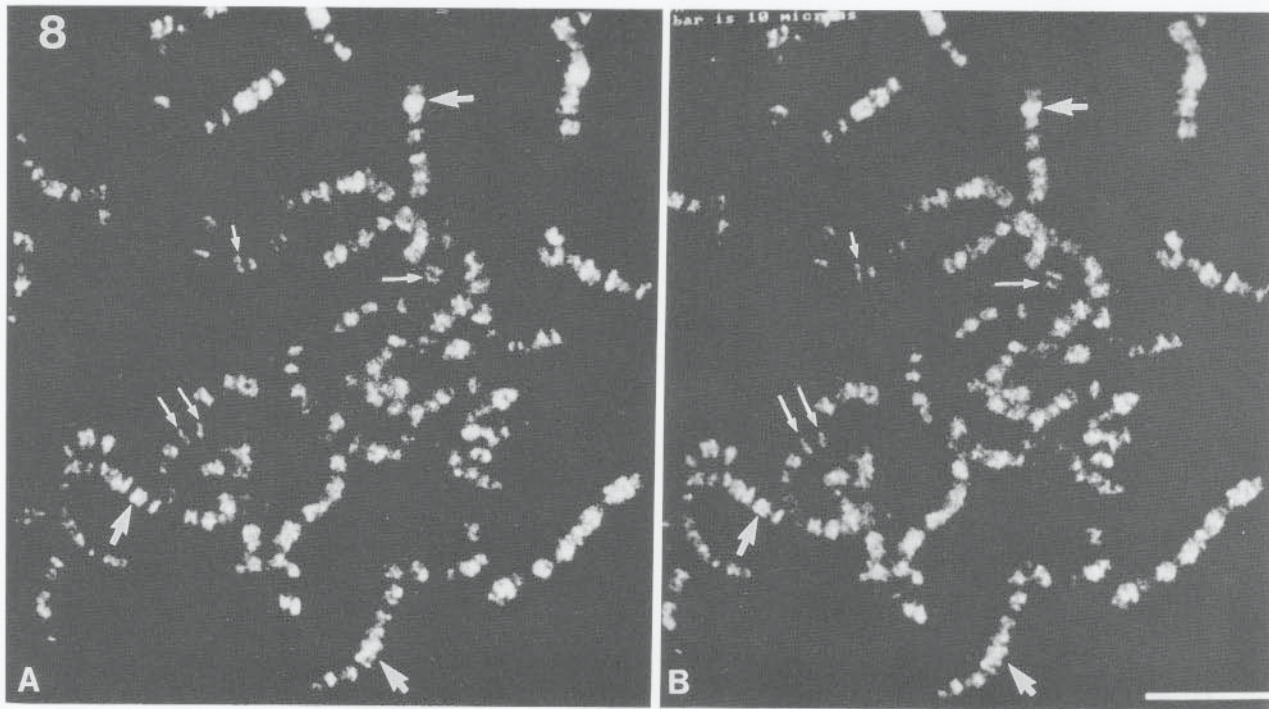


FIG. 8. Simultaneous detection of BrdUrd later replication domains detected with rhodamine secondary antibodies (A) and a biotinylated 1.9kb LINE repeat (39) decorated with streptavidin-FITC (B). In this, as in most other prometaphase spreads there is a general coincidence of later replicating and LINE rich domains. However, LINE rich domains are generally not as well demarcated as replication bands, and

some LINE and replication bands on C group chromosomes are not coincident. Note fine bands (small arrows) as well as thicker bands (larger arrows) that are the same in each panel. Methods for in-situ hybridization and detection essentially as described (37) and BrdUrd pulses were for 6 hr.

linear pieces of DNA that are SINE-poor (9). Each of these repeat motifs might confer distinctive coiling or recognition properties on selected chromosomal regions as originally proposed (40). Such properties and motifs are compatible with a general, although not absolute, "compartmental strategy" adopted by chromosomal bands (9,27).

Two functional aspects of Giemsa bands have been suggested by Holmquist and his colleagues (23,28). First, GC rich (Giemsa-light) regions are replicated early during the S phase, whereas Giemsa-dark, LINE rich regions are replicated later. Second, housekeeping genes, actively transcribed in all cells, tend to segregate in Giemsa-light regions, whereas tissue specific genes, that are largely inactivated, predominate in Giemsa-dark regions. Do the data support the prediction that LINE rich domains will be late replicating? In Figure 8 we demonstrate that LINE sequences can closely correspond to later replicating regions. Simultaneous detection of LINE and later replication domains reveal extremely fine bands, as well as thicker bands, that are coincident for both motifs.

Electron microscopy further confirms the variable sizes of replication domains. In prometaphase chromosomes pulsed with bromodeoxyuridine (BrdUrd) for the detection of later replicating regions (Fig. 9), the small late replicating 1p36.3 domain is labeled, and is com-

patible in dimension with a few radial arrays. Other labeled bands are larger and appear more condensed. These regions are consistent with organized sets of bands that are in the order of 9Mb (1 coil). This replication banding pattern is consistent with our model (Fig. 6) in that it indicates synchronous replication over enormous linear stretches of DNA that appear to be compartmentalized. Figure 9 also shows that later replication bands generally, but not absolutely, correspond to Giemsa dark bands.

Thus chromosomal bands, with characteristic replication, DNA sequence and genetic features can be assigned to structural motifs described in the current model. Sizes from ~300 kb, equivalent to one radial array of ~9Mb (1 coil) can be detected by PFGE methods, by Giemsa banding and by replication banding. Other specific recognition factors, such as specific proteins which have yet to be characterized, may further increase our understanding of compartmental mechanisms. However, as a first approximation, we suggest that LINE rich, later replicating domains may in general maintain a relatively more contracted (coiled) configuration in interphase and prometaphase as compared to GC rich, early replicating and transcriptionally active domains. In fact, later replicating regions of interphase nuclei display many more or less loosely coiled arrays (Fig. 10). In contrast, early repli-

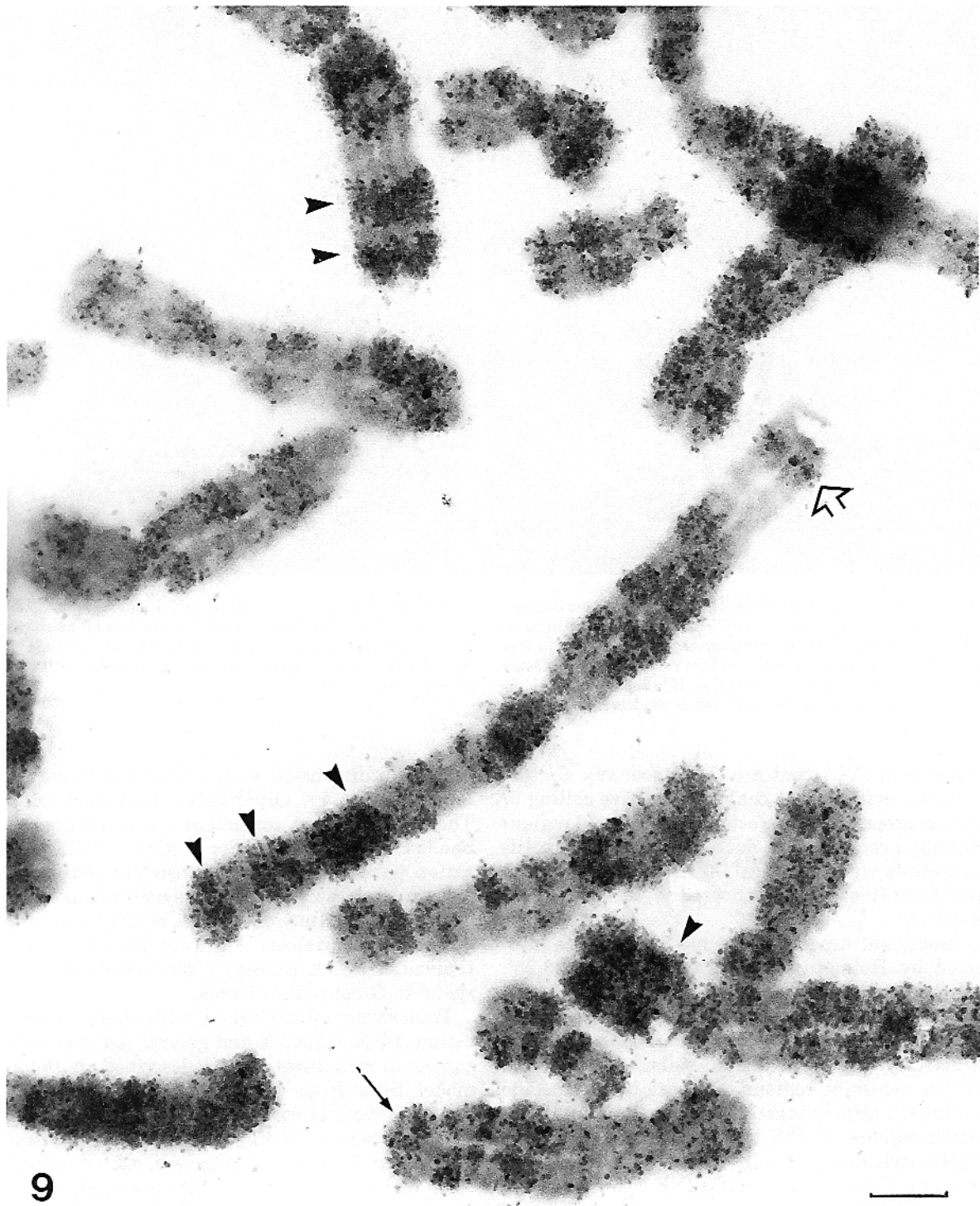


FIG. 9. Electron micrograph showing late replicating domains (BrdUrd-peroxidase labeled) in prometaphase spread. Open arrow points to labeled fine domain at 1p36.3, likely to be the site of a minisatellite (47); the surrounding extended early replicating regions that are unlabeled have previously been shown to contain a high concentration of GC rich SINES (46). The length of chromosome 1 in this prometaphase preparation (that is not maximally extended) is 10 μ m.

Arrowheads show larger labeled domains that are likely to contain \approx 9Mb of DNA (one or more coils). Small arrow shows label details that are consistent with radial arrays in a coil. BrdUrd was incorporated for 6 hr. prior to collection of cells, and preparation was denatured at 50°C for 40 min. Preparations were infiltrated with paraloid and attached to grid as described (48). Bar is 1 μ m.

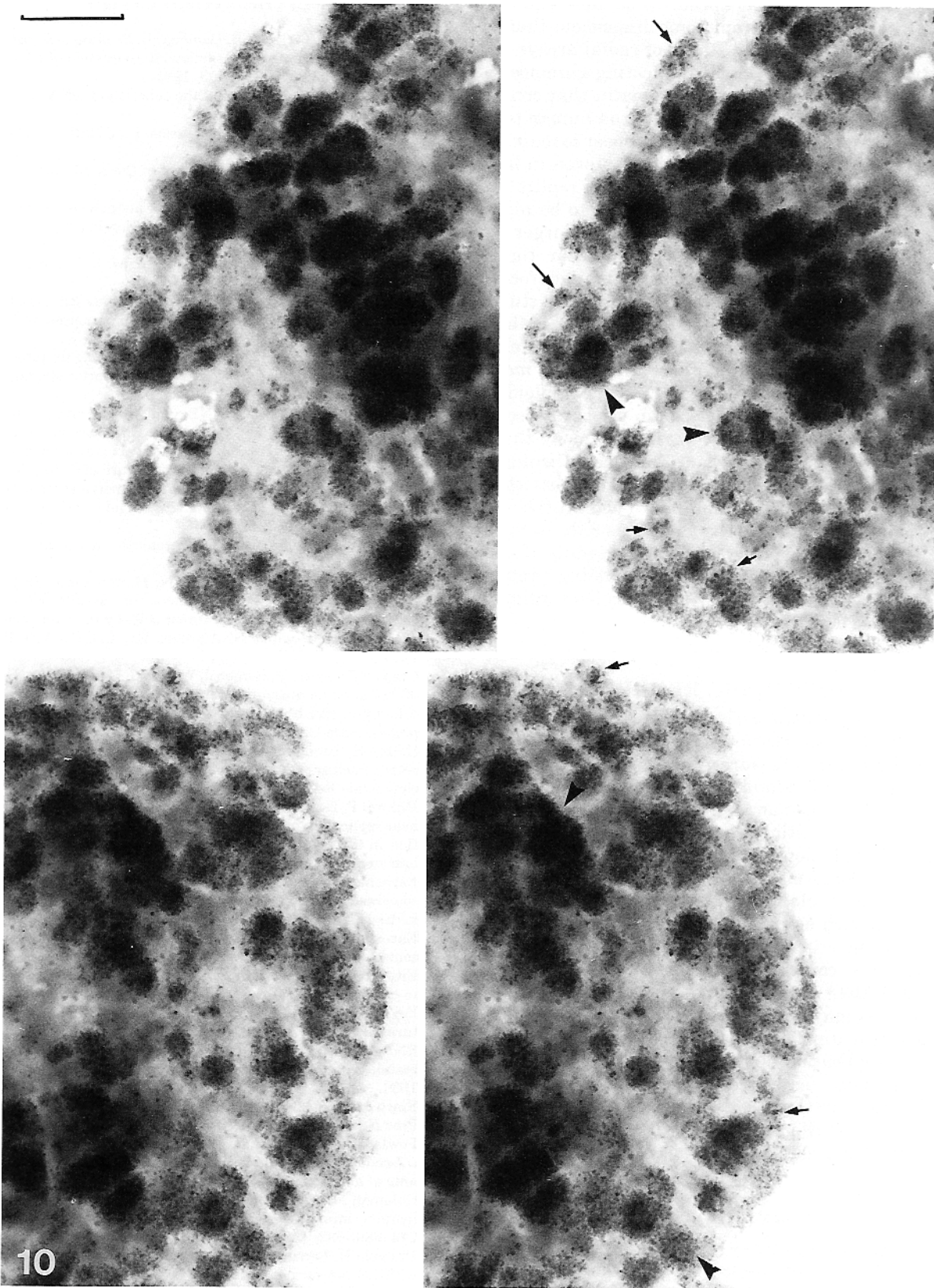


FIG. 10. Stereo pairs of a nucleus with a later replication pattern showing many heterochromatic, large coiled regions. Arrowheads point to a few coiled regions, small arrows point to fine bands consistent with radial arrays in more extended interphase fibers. Methods as in Figure 9. Mounted for cross-eyed viewing. Bar is 2 μ m.

cating (GC rich) domains appear to be more extended (53,54), and delineate chromosome dimensions that are consistent with less coiled stacks of radial arrays.

At the extreme, the latest replicating chromosome domains of constitutive heterochromatin that contain long stretches of tandem repeats (e.g., on human paracentromeric regions 1q12 and 9q12) appear to maintain a highly coiled metaphase configuration even in large interphase neurons (cf. section 1). During replication, very late replicating regions also appear to be highly and characteristically condensed (54); these larger replicated domains in the nucleus can appear tightly coiled and do indeed contain constitutive heterochromatic DNA sequences in double-label detection studies (unpublished data). Finally, the estimate of 9Mb for one coil is in fair agreement with the length of satellite DNAs that are paracentromeric. For example, mouse satellite DNA ($\sim 3 \times 10^8$ bp or $\sim 10\%$ of the haploid genome) contains an average of 7.5Mb per chromosome, and there may be an ~ 2 -fold variation among the 40 mouse chromosomes as well as some lengthening of this structure as it enters the centromeric constriction. In human cells, PFGE studies show constitutive heterochromatic domains can be as long as 9Mb in linear DNA length (9). The preferential tight binding of some satellite DNAs to a subset of tight-binding proteins (66) may also confer a highly stable coiled configuration on these domains.

CONCLUDING REMARKS

We have presented a flexible model of interphase and metaphase structure that is based on both molecular and structural features. We believe this model is useful for the understanding of selected DNA domains, such as euchromatin and heterochromatin, and can accommodate sequential transcription, replication and structural transitions with ease. It can also accommodate unique conformations, such as polyteny. Although the exact role of selected DNA and protein sequences in this model, such as those involved in modified folding patterns or matrix attachment, are not completely understood, the coincidence of banding patterns with structural, DNA sequence, and replication motifs is remarkable. It may be that some of the structural forms themselves (extended to coiled) are informational. For example, replication machinery may more easily bind to euchromatic (extended) arrays. Thus replication would proceed in temporally defined waves from 1) Giemsa-light to 2) Giemsa-dark to 3) constitutive heterochromatic domains.

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