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COMMENTARY

A chromomeric model for nuclear and chromosome structure

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SUMMARY

The basic structural elements of chromatin and chromosomes are reviewed. Then a model involving only three architectural motifs, nucleosomes, chromatin loops and transcription factories/chromomeres, is presented. Loops are tied through transcription factors and RNA polymerases to factories during interphase and to the remnants of those factories, chromomeres, during mitosis. On entry into mitosis, increased adhesiveness between nucleosomes and between factories drives a 'sticky-end' aggregation to the most compact and stable structure, a cylinder of nucleosomes around an axial chromomeric core.

Key words: chromomere, nucleoskeleton, RNA polymerase, factory

INTRODUCTION

'We are thus brought, finally, to one of the most fundamental conceptions of cytology and genetics, namely, that the spireme-threads are linear aggregates of much smaller self-perpetuating bodies, aligned in single series, and in definite order.' As chromosomes condense, these bodies, or chromomeres, may 'aggregate or fuse to form larger ones' and 'be compound bodies having perhaps a definite internal architecture.'

These quotations are taken from the concluding section on chromosome structure in E. B. Wilson's classic review of cell biology published in 1928 (Wilson, 1928); they summarize a half-century of careful microscopic analyses of chromosomes of many different organisms. Strings of chromomeres were traced from interphase as they condensed, split and segregated during mitosis, to decondense in the daughter cells. Whilst the compound term spireme-thread (spirema: a thing wound or coiled, a skein) expresses an ambivalence as to whether the string condensed spirally, the reader is left in no doubt that chromosomes are built around chromomeres.

Despite the wealth of evidence available to Wilson, chromomeric models fell out of favour, largely because they did not lead to a plausible explanation of why chromosomes have the shape that they have. Furthermore, Wilson presumed that some vestige of the chromomere persisted during interphase, but no suitable structure could be found. As a result, most current models involve some form of helical coiling or ill-defined interactions between DNA and/or proteins to account for chromosome structure (Fig. 1). However, a good candidate for the interphase counterpart of the mitotic chromomere has now been uncovered. Here, the basic structural elements of chromatin and chromosomes that any model must take into account will first be reviewed and then an up-dated chromomeric model will be presented; it helps explain how the interphase fibre is converted into a mitotic chromosome and why a chromosome has its characteristic shape.

A number of inter-related factors complicate analysis of chromosome structure. First, their tight packing and high density makes it difficult to visualize their interior and limits access of probes like antibodies. Second, chromatin is poised in a metastable state so that even small changes in tonicity cause it to aggregate into an unworkable mess. Therefore unphysiological conditions are used in almost all isolation procedures; for example, conventional cytological preparations are made by hypotonically swelling cells, followed by fixation/extraction in methanol-acetic acid and an explosive spreading at an air-liquid interface. Third, chromosomal substructures have sizes below the resolution of the light microscope, necessitating electron microscopy which brings problems associated with preserving structure in vacuo. Therefore, studies on isolates made using more physiological conditions or on rapidly-frozen specimens will be stressed.

BASIC FEATURES OF CHROMOSOME ORGANIZATION

Condensation is the hallmark of mitosis. Why, then, does the chromatin fibre not condense into the most compact form, a sphere? *Why are chromatids cylindrical, and not spherical?*

Helical hierarchies

It is a truism that extended biological structures are helical at the molecular level, but not at the macro-molecular level; actin filaments and DNA are helical, but cells and trees, which are nevertheless made of those helical molecular assemblies, are

not. This truism arises because assembly of molecular sub-units into a non-helical structure requires that each sub-unit is positioned next to its neighbours with no axial rotation; even the slightest adds up over many sub-units to give a helix. Therefore we might expect a hierarchy of helices in a chromosome: the double helix first being coiled into a nucleosome (~11 nm diam.), nucleosomes into solenoids (~30 nm diam.), solenoids into chromatid fibres (225-250 nm diam.), and so on to give a cylinder (Fig. 1E). At each level in the hierarchy, the sense of coiling and pitch between monomers would be precisely defined. According to this view, one might solve (in a crystallographic sense) the structure of a mitotic chromosome and expect some remnants of the hierarchy to be retained into interphase. However, when biological structures become as long as cells, they also become sufficiently flexible that other forces, like those involved in growth, can overcome the weaker forces maintaining helicity. A long disordered structure may well relax into a helix when the other forces are removed in vitro, so the crucial question is whether any coiling then seen actually pre-existed in vivo.

There is no reason to believe that the DNA coils seen in nucleosomal crystals do not exist in vivo, but the evidence for coiling at the next level of organization is controversial. Chromatin released from nuclei by digestion with a nuclease has, in 1 mM monovalent ion, the canonical 'beads-on-a-string' structure visible by electron microscopy (Thoma et al., 1979). As the ionic strength is raised progressively, the string folds into a series of helices with a fairly constant pitch but increasing numbers of nucleosomes per turn; at ~60 mM, the resulting 'solenoid' is ~25 nm wide, with 6-8 nucleosomes/turn and an 11 nm pitch. But both right- and left-handed solenoids are seen in the original micrographs, implying some disorder. Moreover, others interpret rather similar structures in terms of strings of 'superbeads' (Hozier et al., 1977; Strätling et al., 1978). Increasing the salt concentration further has little effect until chromatin precipitates at ~100 mM, so these experiments provide no direct evidence that solenoids exist at a physiologically-relevant salt concentration (i.e. ~150 mM monovalent ion).

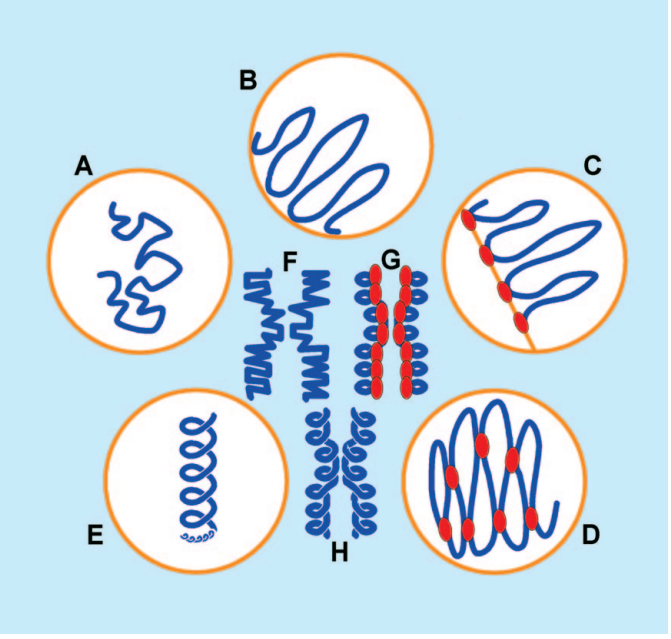

Fig. 1. Some models for chromosome structure during G1 phase and mitosis. Possible G1 structures include (A) a randomly-folded fibre, loops attached (B) to the lamina (e.g. Gerace and Burke, 1988), (C) to chromomeres (e.g. Zatsepina et al., 1989) or scaffolds (e.g. Paulson and Laemmli, 1977), (D) to proteins bound at specific sites on the fibre, or (E) a coiled-coil (e.g. Rattner, 1992). After duplicating DNA and dissolving skeletons, mitotic structure might be dictated by interactions (F) within randomly-folded chromatids (e.g. DuPraw 1966), (G) between chromomeres or scaffolding elements (e.g. Manuelidis, 1990), or (H) between gyres that tighten a coiled-coil (e.g. Bak et al., 1977). Hybrid models (not shown) might involve coiled-coils and loops attached to a skeleton or combinations of radial loops and helical folding (e.g. Rattner and Lin, 1985). Chromatids could be arranged symmetrically, as in G and H.

There is also no evidence for solenoids in vitrified sections of metaphase CHO or HeLa cells that have been neither fixed nor stained (McDowall et al., 1986). Chromosomes have a homogeneous 'grainy' texture and optical diffractograms are best explained by a compact association of 11 nm particles interacting in a manner akin to molecules in a liquid. It is unlikely that solenoids did exist but were missed, because microtubules with the same mass/unit-length can be seen in the cytoplasm. Electron tomography of interphase nuclei embedded at low temperatures also reveals ribbons of zig-zagging nucleosomes and not solenoids; variable lengths of linker DNA seem to enter and exit individual nucleosomes at various angles in the disordered structure, instead of the more precise arrangement expected of a solenoid (Horowitz et al., 1994).

There is also controversy over the coiling of the chromonemal fibre (Manton, 1950): gyres can vary with growth temperature in *Tradescantia* (Swanson, 1942), they may appear constant in number but variable in sense in hypotonically-swollen human chromosomes (Ohnuki, 1968), of opposite sense in a minority of the sister chromatids of isolated HeLa 'scaffolds' (Boy de la Tour and Laemmli, 1988), or right-handed in polytene *Drosophila* chromosomes (Hochstrasser and Sedat, 1987). Coupled with the gap in the hierarchy at the level of the solenoid, such variability smacks more of variability in growth rate or in the conditions at the time of analysis than of some general structural principle. Moreover, an attractive feature of coiled-coil models is that progressive rotation can progressively condense the coil, but chromatids condense without rotation; human vimentin genes tend to retain the same external position on sister chromatids, irrespective of chromatid length (Baumgartner et al., 1991).

30 nm fibres

A ~30 nm fibre is widely believed to be another architectural motif but, again, there is little agreement on its precise dimensions, the amount of DNA/unit-length and its existence in vivo (e.g. van Holde, 1988). Recent careful work suggests that a true biological variation underlies this controversy (Woodcock, 1994). It seems that 30 nm fibres are found in transcriptionally-inactive cells like chicken erythrocytes with condensed chromatin and 'long' (i.e. >210 bp) nucleosomal repeats, but not in active cells with 'typical' repeats of 160-200 bp. For example, 30 nm fibres are clearly seen in frozen-hydrated sections of nuclei of starfish sperm that were swimming in sea water up to the moment of cryo-immobilization (Woodcock,

1994). In contrast, most active nuclei contain no such fibres visible in vitrified sections (Dubochet et al., 1988).

Most chromatin is transcriptionally inert, even in 'active' nuclei, so we might expect most to form 30 nm fibres, but it does not. Why? Presumably, transcription of a few sequences somehow unfolds fibres throughout the nucleus.

Chromatin loops and underlying sub-structures

Another recurrent structural motif involves attaching the chromatin fibre in loops to a sub-structure. Apparently decisive evidence is provided by phase-contrast microscopy of unfixed 'lampbrush' chromosomes of amphibians (Callan, 1977). Such structures are prepared by puncturing an oocyte, extruding the nucleus and then removing its envelope in 0.1 M KCl/NaCl; the jelly-like clump of chromatin slowly disperses to reveal loops attached to a chromomeric axis (Macgregor and Varley, 1988). But there is no hint of individual chromomeres or loops in electron micrographs of intact nuclei; instead the chromatin appears as one granular aggregate. Therefore, it is quite possible that transcription units/loops are stripped off the granules during dispersal. Indeed, possible intermediates in such a process, small granules, are often seen scattered around loops (e.g. Mott and Callan, 1975).

Supercoiling provides additional evidence for looping. Supercoils are lost spontaneously from linear DNA as the molecule can spin about its axis; therefore, the existence of supercoiling implies that DNA is tied down (i.e. looped) to prevent rotation. 'Nucleoids' isolated by lysing interphase cells in >1 M NaCl sediment in gradients containing ethidium like superhelical DNA (Cook and Brazell, 1975); electron microscopy also reveals naked superhelical loops attached to a residual 'cage' (Jackson et al., 1984). The contour length of such loops remains unchanged during mitosis, so their basic structure must persist (Jackson et al., 1984). Significantly, supercoiling and the cage are both lost as transcriptionally-active chick erythroblasts mature into inert erythrocytes, pointing to a connection between looping and transcription (Cook and Brazell, 1976). Whilst such loops could be created artifactually on isolation, the total number actually falls slightly, and the remaining ones are attached through the same points as those seen in isolates made using isotonic buffers (Jackson et al., 1990). (Note that unrestrained supercoils are found only locally in eukaryotic chromatin; Jupe et al., 1993.)

Nuclear 'matrices' are superficially similar to nucleoids but are isolated using both hypo- and hyper-tonic steps (reviewed by Getzenberg et al., 1991). It is often assumed that they are associated with superhelical loops, but the DNase treatment generally used during isolation ensures that they are not. ('In situ matrices' with superhelical DNA are prepared more like nucleoids; Vogelstein et al., 1981.) Moreover, during the initial stages of isolation, the number of loops (and so attachments) first increases and then decreases (Jackson et al., 1990), making it difficult to be certain which, if any, of those seen finally actually existed in vivo.

An axial 'scaffold' associated with (unsupercoiled) loops was originally observed by electron microscopy of histone-depleted chromosomes (Fig. 1G; Paulson and Laemmli, 1977). Specific scaffold-associated regions (SARs) are attached to the major protein in the isolate, topoisomerase II (Mirkovitch et al., 1984; Earnshaw and Heck, 1985). Scaffolding models are attractive because isolated scaffolds look like chromosomes, their molecular ties are defined and the topoisomerase is strategically placed to decatenate and/or condense loops by altering supercoiling (Saitoh and Laemmli, 1994). But scaffolds retain their morphology when topoisomerase II is removed (Hirano and Mitchison, 1993; Swedlow et al., 1993) and five out of six of their loops are created during isolation (Jackson et al., 1990).

Looping has also been inferred from the rate at which nucleases solubilize the chromatin of isolated nuclei (Igó-Kemenes and Zachau, 1977). Cutting an unlooped fibre should first release two long fragments that are then shortened, but the expected long fragments are not seen; rather, the kinetics are consistent with two cuts releasing one short fragment from a 75 kbp loop. But again, long unlooped fibres do aggregate as nuclei are isolated (Jackson et al., 1990). In principle, tightly-attached sequences can be identified by detaching most DNA with a nuclease and pelleting the sub-structure, but pellets of cages, matrices and scaffolds contain different sequences and it is not clear which reflects a structure found in vivo (Cook, 1988).

If any consensus can be drawn from these conflicting results obtained using hypo- and hyper-tonic conditions, it is probably that the chromatin fibre is looped, but how, and to what, is not clear. Indeed, one model even involves attaching loops to the one skeleton that we are confident does exist, the peripheral lamina (Fig. 1B; Gerace and Burke, 1988); chromatin's high concentration close to lamin proteins and its affinity for them are consistent with this (Traub and Shoemann, 1994).

Chromosome bands

After various treatments, certain reagents stain some chromosomal regions more intensely to give chromosome-specific bands: G/Q bands are AT-rich, contain facultative heterochromatin and are late-replicating; R bands are GC-rich, contain ~80% known genes plus many *alu* sequences, and are early-replicating; C bands generally contain tandem repeats of centromeric DNA (Craig and Bickmore, 1993). The total number of bands depends on the degree of resolution but, to a broad approximation, up to 1,250 can be seen in prematurely-condensed chromosomes, by 'replication' banding and in prophase (Hameister and Sperling, 1984; Drouin et al., 1990). As cells progress into mitosis, individual R bands fuse more quickly and in greater numbers than G bands to reduce the total (Drouin et al., 1991). Trypsin treatment is usually used to generate G bands, but a similar pattern of slightly thicker regions can be seen in untreated human metaphase spreads by atomic force microscopy (Musio et al., 1994), in prematurely-condensed chromosomes (Gollin et al., 1984) and in nucleoids (Mullinger and Johnson, 1980). Although homologous chromosomes are similarly shaped, they rarely have exactly the same banding patterns or lengths, and this is why progress on the automation of karyotyping is so slow. Even so, any model for chromosome structure should be able to account for these bands.

Chromosomal proteins

Despite considerable effort, the list of proteins implicated in chromosome structure is limited. It includes histone H1 (which is hyper-phosphorylated during mitosis; Bradbury et al., 1974), RNA polymerases I and II (Matsui et al., 1979), topoisomerase II (see above), and XCAP-C and E (Hirano and Mitchison, 1994). The latter were identified after mixing sperm chromatin in a mitotic extract from *Xenopus* eggs and then spinning the

assembled chromosomes through sucrose; the two proteins, which are homologous to a scaffold protein (Saitoh et al., 1994), remained bound. They are recruited to the chromomeric core as chromosomes assemble, whilst antibodies against XCAP-C inhibit this process.

It is often assumed that the residual proteins (e.g. topoisomerases, XCAPs) bound to an extracted chromosome (e.g. a scaffold) necessarily determine its shape, but this is not so: shape survives extraction with a strong detergent like dodecyl sulphate which removes all the proteins discussed above (Cook, 1984). Partial deproteinization of DNA packed as tightly as a mitotic chromosome will inevitably generate a tangle that roughly reflects the original shape, and then the mere association of any residual proteins with the tangle tells us little about their structural role. Moreover, disrupting the function of any proteins involved in condensation, whether they be topoisomerases, XCAPs, histones or kinases, using antibodies (as in the case of XCAP-C) or inhibitors would be expected to affect the condensed shape. Therefore there remains little good evidence for the (currently-fashionable) role invoked for topoisomerase II and the XCAPs in maintaining shape, although both are clearly required to generate shape.

STUDIES USING 'PHYSIOLOGICAL' CONDITIONS

Arguments whether isolated structures are generated artifactually by the hyper- or hypo-tonic conditions used are best countered by the use of more physiological conditions. Fortunately, the practical problems caused by chromatin aggregation at an isotonic salt concentration can be overcome by encapsulating cells in agarose microbeads (50-150 μm diam.) before permeabilizing them in a 'physiological' buffer (Fig. 2; Jackson et al., 1988). The chromatin, now protected by agarose, does not aggregate and can be pipetted freely; it is accessible to probes like antibodies and enzymes, its DNA is intact and polymerases retain their activity. The basic structural features of HeLa nuclei, including a strong candidate for the chromomere of interphase, have been analyzed using 'physiological' conditions and the approaches illustrated in Fig. 2.

The results can be summarized as follows. The lamins, which are members of the intermediate-filament family of proteins and which form an exo-skeleton that underpins the nuclear membrane (Gerace and Burke, 1988), are also part of an internal nucleoskeleton (Jackson and Cook, 1988; Hozák et al., 1995). Chromatin loops with an average contour length of 86 kbp are attached to this skeleton; this length does not change during mitosis (Jackson et al., 1990). Surprisingly, loops, whether part of the natural chromosome or a transfected 'minichromosome', are attached through promoters/enhancers and transcribed sequences to polymerizing complexes on the skeleton (Jackson and Cook, 1985, 1993). The active polymerases that mediate attachment are concentrated into discrete 'foci' (Jackson et al., 1993; Wansink et al., 1993). (The term 'polymerase' is used to describe the large cluster of polypeptides in the active complex.) The foci have variable sizes and shapes, with the largest apparently formed by fusion; this makes counting difficult, but in a HeLa cell there are 300-500 bright ones and several thousand in all, or roughly the number of mitotic bands (F. Iborra, A. Pombo, D. A. Jackson and P. R. Cook, unpublished results). The bright ones contain >50 active polymerases, plus associated templates and components

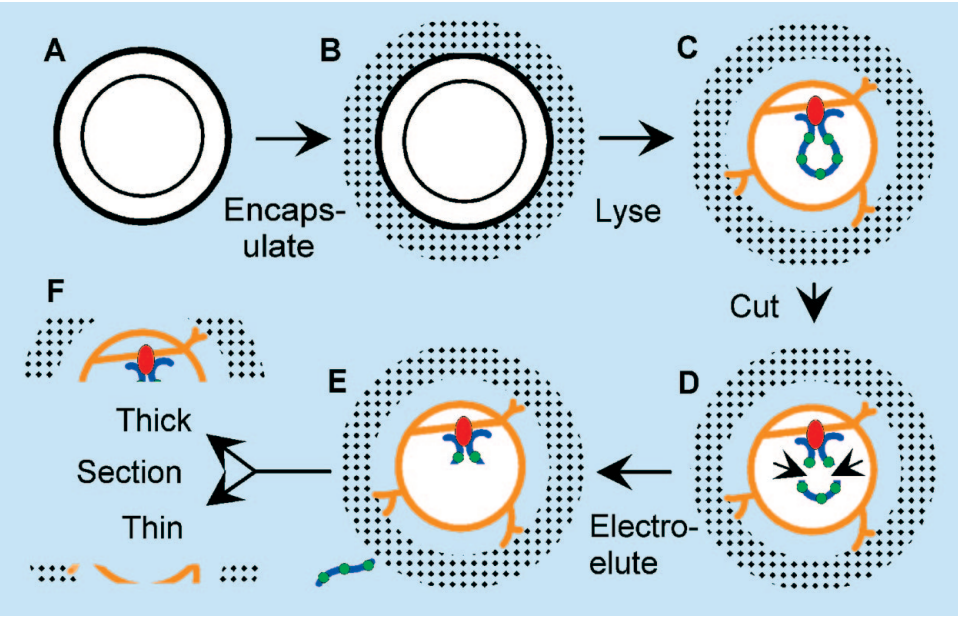

Fig. 2. A procedure for analyzing chromatin structure using 'physiological' conditions. (A) HeLa cells are (B) encapsulated in an agarose bead (dotted surroundings). (C) After permeabilization, the cytoskeleton, lamina, internal nucleoskeleton (all in brown), associated transcription factory (red oval) and DNA loop (blue line) covered with nucleosomes (green circles) all become accessible to molecular probes. (D) Added endonucleases can now diffuse through the agarose and cut chromatin loops (arrows) so that (E) most chromatin can be removed electrophoretically. (F) Skeletons, whether in the nucleus or cytoplasm, are best visualized by electron microscopy of thick sections. (Redrawn from Jackson and Cook, 1985.) This procedure has been used to characterize: (i) An internal lamin-containing nucleoskeleton, once obscuring chromatin is removed (Jackson and Cook, 1988; Hozák et al., 1995). (ii) The contour length of loops, from the average length and percentage of remaining DNA fragments (if fragment length is 8.6 kbp and 10% remains, contour length is 8.6×1/(10/100) = 86 kbp). It does not change during mitosis, so the molecular ties holding loops persist (Jackson et al., 1990). (iii) Sequences remaining after elution; they are mainly promoters, enhancers and transcribed sequences, implying that engaged polymerases, which can still 'run-on' along residual fragments, mediate attachment to the skeleton (Jackson and Cook, 1985, 1993). (iv) Sites of transcription. Permeabilized cells (either before or after cutting and elution) are allowed to make RNA in the presence of Br-UTP, and then sites (i.e. factories) containing the incorporated analogue are immunolabelled using antibodies against Br-RNA (Jackson et al., 1993; Wansink et al., 1993).

of the splicing apparatus, so they are called transcription 'factories' by analogy with the replication factories that are also fixed to the skeleton and contain all the machinery necessary to duplicate >20 replicons simultaneously (Nakamura et al., 1986; Hozák et al., 1993, 1994b).

NUCLEOLAR TRANSCRIPTION FACTORIES

Extra-nucleolar transcription factories (containing RNA polymerases II and III) have not yet been well-characterized by electron microscopy, but their nucleolar counterparts containing RNA polymerase I have (Spector, 1993). Nucleoli contain several 'fibrillar centres' surrounded by a 'dense fibrillar component'; in turn, these are embedded in a 'granular component'. Fibrillar centres, which are located at nodes on the skeleton, store the required enzymes and transcription seems to occur as rDNA slides through polymerases on their surface. Nascent rRNA is then extruded into the dense fibrillar component and, after termination, it moves to the granular component to complete its maturation. Therefore these factories, which are several hundred nanometers in diameter, contain the active machinery on the surface of storage cores, the fibrillar centres (Hozák et al., 1994a).

There is detailed information on the development of these factories. Each fibrillar centre is often associated with one, or a few, active ribosomal cistrons and the total number of centres is directly related to the rate of rRNA synthesis. For example, the ~234 in a fibroblast fall to ~156 on serum-starvation (Jordan and McGovern, 1981) and the ~9 in a peripheral blood lymphocyte rise to ~80 as it is stimulated to divide (Haaf et al., 1991). In other words, increasing transcription increases surface area and so the number of polymerases accessible to promoters. When nucleoli disassemble during mitosis, most nucleolar components disperse (Hernandez-Verdun and Gautier, 1994) but, remarkably, all polymerase I and most of the transcription factor, UBF, remain bound as the remnants of the centres aggregate into the nucleolar organizing regions on the chromosomes (Scheer and Rose, 1984; Roussel et al., 1993). Therefore, the polymerase I factories of interphase are directly related to mitotic chromomeres.

It is then attractive to suppose that factories containing polymerases II and III are built similarly. Increased transcription would disaggregate cores, increasing surface area and the number of accessible polymerases. When transcription ceases during mitosis, most core material would disperse and the rest would aggregate with other remnants to give the chromomeres

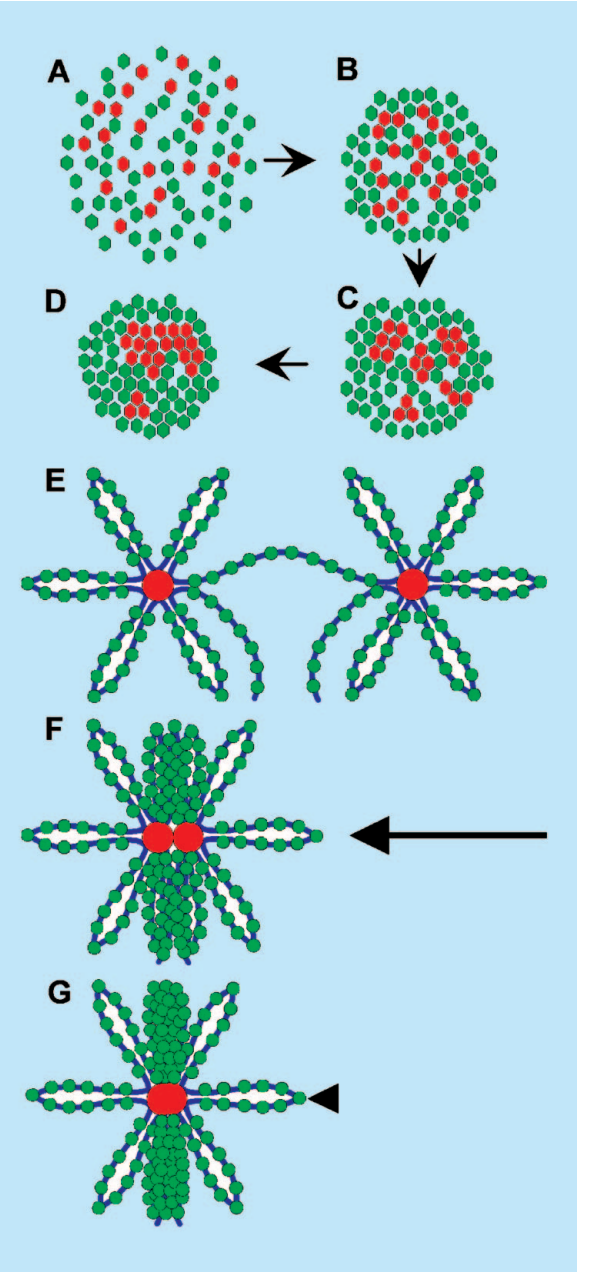

Fig. 3. (A-D) Cell sorting through differential adhesiveness and (E-G) an analogous 'sticky-end' aggregation and sorting during prophase. (A-D) When two cell types (green and red) are (A) mixed, (B) they aggregate into the most compact form, a sphere. (C) As contacts created by random movement between the more-adhesive (red) cells persist for longer, they clump internally. (D) Clumps of more-adhesive cells continue to aggregate until the less-adhesive cells surround a few large clumps, although sorting is rarely perfect. (Redrawn from Steinberg, 1964.) (E-G): (E) A string of less-adhesive nucleosomes (green) is attached to two more-adhesive factories (red). (F) As factories touch (arrow) during condensation, they stick together; large factories are probably the first to fuse. Displaced loops create a higher density in the plane of contact; for geometrical reasons this density is ~5× and ~10× higher if 86-kbp loops stretch 350 nm from factories of 50 and 25 nm diameter, respectively. (G) As the touching halves of the two factories fuse into a cylinder (arrowhead), this central density increases further by one-third. The nucleosomal concentration is now much higher around the middle of the cylinder than at an end; this enhances nucleosomal aggregation and ensures that the next factory to fuse will do so at a more-accessible end. Moreover, the next factory is generally close by and tethered through a short inter-factory loop, as it split from its neighbour earlier during interphase when a larger loop attached to generate two smaller loops, one of which became the inter-factory loop. Additional factories now bind at the ends, elongating the cylinder. These geometrical considerations, and the principles illustrated in A-D, ensure that chromatids are cylindrical (and not spherical) and that loops initially incorporated into the 'wrong' cylinder (usually a sister chromatid) will sort into the 'right' one.

of mitosis. Groups of small factories rich in genes would condense into Geimsa-light bands. And as the RNA polymerases and transcription factors that tie the chromatin into loops remain bound, those loops would retain their contour length.

A DIGRESSION ON CELLULAR ADHESION

How randomly-arranged chromatin condenses into a mitotic cylinder is one of the central issues that must be addressed by any model. The cylinder even survives translocation into a foreign environment, for example when several Mbp of *S. pombe* DNA are integrated into a mouse cell (McManus et al., 1994). Clearly, species-specific interactions cannot be involved. If such cylinders are not built using helices or highly specific interactions, we must look to other means exploited by nature to generate simple higher-order structures.

When disaggregated neural cells from frog embryos are randomly intermixed, they aggregate into the most compact form, a sphere, and then sort into a semblance of the original tissue, with ectoderm surrounding the endoderm (Fig. 3; Townes and Holtfreter, 1955). Many different cells will sort in this way and the process can be modelled if cells differ only in their adhesiveness for one another. Then random movement creates contacts and those between the more-adhesive cells tend to persist; eventually a sphere of less-cohesive cells forms around a core of the more-adhesive ones (Steinberg, 1964; Armstrong, 1989). This model has been tested directly (Steinberg and Takeichi, 1994). L cells are not normally very adherent, but become more so if they express (after transfection of the appropriate cDNA) the adhesion molecule, cadherin, on their surface; mixtures expressing different amounts of cadherin sort into low-expressers surrounding a core of high-expressers.

We can model interphase chromosomes, which are confined to discrete nuclear regions (Engh et al., 1992), as strings of less-adhesive nucleosomes running between, and looping from, more-adhesive factories (Fig. 3E-G). In mitosis, increased adhesiveness drives ‘sticky-end’ aggregation to the most compact and stable structure, a cylinder of

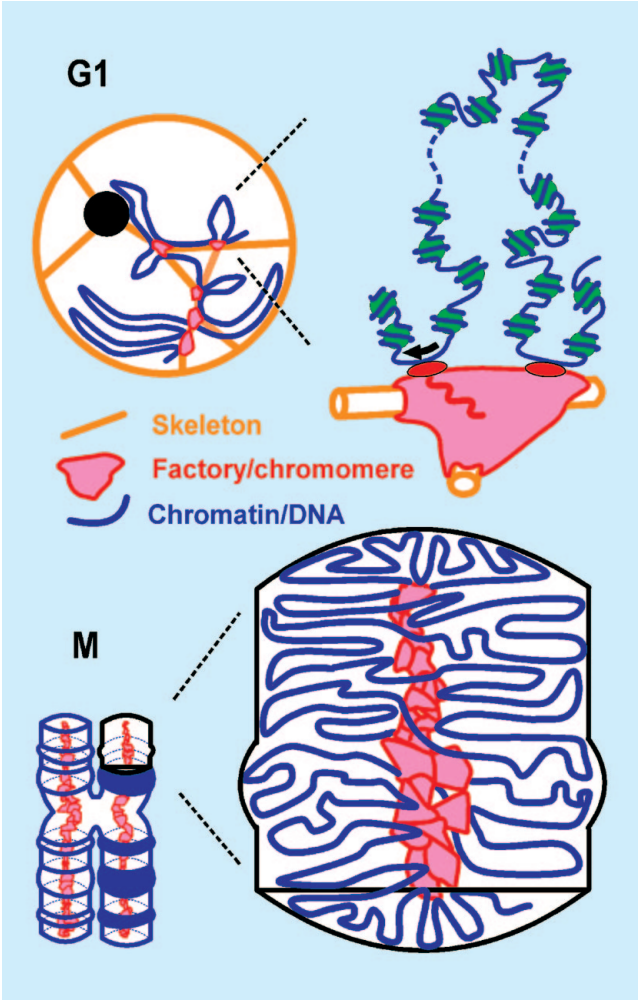

Fig. 4. A model for the structure of a human chromosome. Upper: structure during G1 phase. Transcription factories are located at nodes on a lamin-containing endo-skeleton and their number and size depends upon transcriptional activity. If there are 1,250, each might be ~50 nm diameter, contain ~25 active RNA polymerases and be associated with ~56 chromatin loops (usually, but not invariably, derived from one chromatid) with a range of contour lengths (i.e. 5 sets of 11 loops centred around ~7.5, 50, 75, 100 and 175 kbp, respectively; average 86 kbp). ~10% chromatin then lies within ~8.6 kbp of a factory, so genes within it are close enough to polymerases to attach and be transcribed; the remainder is too remote and condenses on to the lamina or nucleolus as heterochromatin. Increased transcription generates more, smaller, factories each associated with fewer, shorter, loops. The enlargement (right) shows a loop attached through a transcription unit and a promoter/enhancer to a polymerizing complex and transcription factor (red ovals). (In transcriptionally-inactive regions, transcription factors are the sole molecular ties.) A transcript (wavy red line) is extruded as the template slides through the left-hand complex; this template movement ‘opens’ adjacent chromatin. The DNA duplex winds around nucleosomes (green circles) in the loop; variations in linker length (±2 bp) and entry-exit angle (±15°) generate an irregular zig-zagging fibre (Horowitz et al., 1994) that extends, on average, ~350 nm from a factory. Lower: mitotic structure. Human chromosome 16, which contains ~100 Mbp DNA (3% of genome), is modelled, assuming each chromatid is 3,400×800 nm. (The dimensions of ‘native’ chromosomes vary significantly, depending on condensation.) On entry into mitosis, skeletons depolymerize, transcription ceases and nucleosomes plus ~38 factory remnants aggregate and then sort into a cylindrical nucleosomal ‘cloud’ surrounding a chromomeric axis, as described in Fig. 3E-G. Despite these rearrangements, loops retain their attachments and contour lengths. A typical factory/chromomere, plus an average loop extended on each side, fit within the width; longer loops fold back on themselves and are the major determinants of width (enlargement, right). Factories associated with proportionally more shorter loops (and active genes) give slightly narrower cylinders; examples include (i) Geimsa-light bands (5% thinner), (ii) several Mbp of yeast DNA translocated into a mouse genome (the closely-spaced yeast genes are transcribed during interphase and so will be in short loops; McManus et al., 1994) and (iii) active rDNA loops which give the ‘secondary constriction’ of the nucleolar organizing region (Robert-Fortel et al., 1993). The design principles are, almost certainly, further modified in other specialized chromosomal regions; for example, the several Mbp of non-transcribed α1-satellite probably condense into a spherical centromeric ‘chromomere’ through interactions involving CENP-B, rather than polymerases or transcription factors (Yoda et al., 1992).

nucleosomes around a chromomeric core in which inter-nucleosomal and inter-chromomere interactions are maximized. Then, many different loop-chromomere, nucleosome-nucleosome and chromomere-chromomere interactions maintain chromosomal shape, rather than the few typical of scaffolding models.

A MODEL

These considerations lead to a model involving three fundamental levels of organization: nucleosomes, loops and transcription factories/chromomeres. Loops are tied through transcription factors or RNA polymerases to factories during interphase and to chromomeres, which are the remnants of those factories, during mitosis. Each level is characterized by flexibility without having a fixed structure.

During interphase, a zig-zagging nucleosomal ribbon is looped to factories strung along an internal lamin-containing nucleoskeleton (Fig. 4, top); the ribbon slides past attached polymerases, changing its contour length from one moment to the next so it cannot revert to the heterochromatic 30 nm fibre found in inert cells. Increased transcription counteracts the tendency of factories to fuse and generates more smaller factories to which more genes are attached in shorter loops.

During mitosis, the skeleton depolymerizes, transcription ceases (Shermoen and O'Farrell, 1991) and proteins (including histone H1) are phosphorylated, increasing adhesiveness between factories and between nucleosomes. Chromosome condensation would begin in heterochromatin and proceed bidirectionally (Hiraoka et al., 1989) to give cylindrical strings of chromomeres that first fuse into one large cylinder per (entangled) chromatid pair, which then splits into two, from an end toward a centromere (Sumner, 1991), as individual chromatids sort out. Longer strings would give longer cylinders of similar width. Each cylinder would contain nucleosomes arranged around a disordered chromomeric axis (Fig. 4B) which might be helical locally where a run of uniformly-sized factories/chromomeres condensed at a constant rate, or where large ones were so positioned that they forced the appropriate bending (Sorsa, 1986). The hypotonic treatment used by Ohnuki (1968) could relax the structure into a more complete helix. In different metaphases, regions rich in transcribed genes (in short loops) associated with (small) factories will condense to form Geimsa-light bands in roughly the same places, but individual genes do not occupy fixed positions; rather, certain factories (and associated genes) might tend to end up on the outsides of a pair of chromatids, perhaps due to their size relative to their neighbours, whilst others end up more internally, as is the case (Baumgartner et al., 1991). Whilst cells in different tissues have transcription patterns which are generally similar, differences in detail will probably be reflected by small, but nevertheless detectable, differences in the fine structure of the banding pattern in mitosis. On entry into G_1, the decondensing chromatids would be confined to discrete nuclear domains by their neighbours. Simultaneously, the appropriate intermediate filaments would repolymerize between decondensing chromomeres, around the nuclear periphery and throughout the cytoplasm to form the internal nucleoskeleton, lamina and cytoskeleton that together integrate cellular space.

CONCLUSIONS

This model lies centrally within the historical tradition summarized by Wilson's statement that heads this piece. It is also a minimalist one, requiring only three basic structural motifs in the organizational hierarchy, the nucleosome, chromatin loop and factory/chromomere, to explain why nuclei and chromosomes have the shapes they have. It involves one major assumption: interphase transcription foci/factories are the precursors of mitotic chromomeres, which is supported by the established relationship of nucleolar foci/factories with the mitotic nucleolar organizing regions. The interactions involved are either numerous and non-specific (i.e. between nucleosomes and between chromomeres) or fewer but more specific (i.e. between polymerases/transcription factors and transcription units). They will inevitably generate both helical and disordered structures depending on how smoothly condensation occurs. The structural role suggested for RNA polymerase as one loop tie is perhaps surprising, but the enzyme plays just such a role in looping bacterial DNA; indeed, the cluster of polymerases at the core of the bacterial nucleoid provides the prototype for a chromomere (Krawiec and Riley, 1990). The persistence of all ties through mitosis inevitably means that transcriptional patterns will be inherited by daughter cells. Importantly, the best evidence for the model is derived from studies on intact or cryo-fixed cells, or those permeabilized in 'physiological' buffers.

Wilson's 'self-perpetuating bodies' are then the organizers of nuclear structure. But they also organize function; their RNA polymerases ensure that they are transcription centres, and, by nucleating the formation of processing and replication sites (Spector, 1993; Hassan et al., 1994), they are also intimately involved in other, vital, nuclear functions.

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