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# 10

# Transcription factories and chromosome structure

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## 10.1 INTRODUCTION

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? What are the basic principles that determine the cylindrical shape?

Many models assume that chromosomes are constructed from a hierarchy of helices: the double helix first being coiled into a nucleosome (~11 nm dia.), nucleosomes into solenoids (~30 nm dia.), solenoids into chromatid fibres (225—250 nm dia.), and so on to give a cylinder. However, despite what our textbooks say, and despite the irrefutable evidence that solenoids are found *in vitro* in buffers containing <100 mM monovalent ion, there remains no evidence that solenoids exist *in vivo* (van Holde and Zlatanova, 1995). For example, solenoids cannot be seen in vitrified sections of metaphase CHO or HeLa cells that have been neither fixed nor stained, and it is unlikely that solenoids did exist but were missed because microtubules with the same mass/unit-length can be seen in the cytoplasm (McDowall *et al.*, 1986). There is also no agreement about higher-order coiling (Manton, 1950): gyres 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 biological diversity and/or differences in the conditions at the time of analysis instead of some general structural principle.

We will summarize an alternative model that explains why chromosomes are cylindrical (Cook, 1995). It takes as its starting point the observations of the early microscopists that chromosomes are formed from strings of chromomeres that condense at the end of interphase, to split and segregate during mitosis, and then decondense in the daughter cells (Wilson, 1928). It is to be contrasted with most current models that involve some form of helical coiling or ill-defined interactions between DNA and/or proteins (see, for example, DuPraw 1966; Bak *et al.*, 1977; Paulson and Laemmli, 1977; Rattner and Lin, 1985; Zatsepina *et al.*, 1989; Manuelidis, 1990; Rattner, 1992).

## 10.2 STUDIES USING 'PHYSIOLOGICAL' CONDITIONS

The study of nuclear and chromosome structure is bedevilled by the problem of artefacts. Chromatin is densely packed and poised in a metastable state so that even small changes in tonicity cause it to aggregate into an unworkable mess. As a result, unphysiological conditions are almost invariably used to isolate chromosomes, nuclei and nuclear substructures like matrices and scaffolds; then argument often rages as to what extent such isolates reflect structures found *in vivo* (reviewed by Cook, 1988). To sidestep these problems, we have developed conditions that allow us to analyse structure under conditions close to the physiological. The practical problems caused by chromatin aggregation at an isotonic salt concentration are overcome by encapsulating cells in agarose microbeads (50—150 $\mu$m dia.) before permeabilizing them in a 'physiological' buffer (Jackson *et al.*, 1988). The chromatin — now protected by agarose — 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 have been analysed using 'physiological' conditions and the approaches illustrated in figure 10.1.

The results can be summarized as follows. 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;



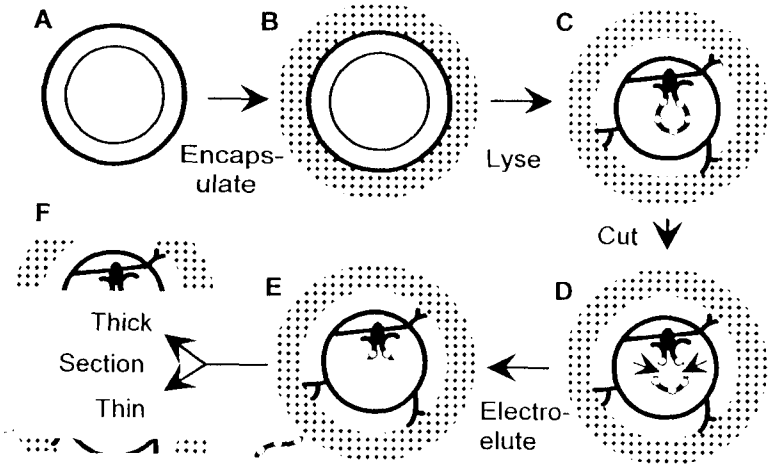



**Fig. 10.1** A procedure for analysing chromatin structure using 'physiological' conditions. (A) HeLa cells are (B) encapsulated in an agarose bead (dotted surroundings). After permeabilization, the cytoskeleton, lamina, internal nucleoskeleton, associated transcription factory (oval) and DNA loop covered with nucleosomes (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. [From Cook (1995) with the permission of The Company of Biologists Ltd.] 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 $8.6 \times 1/(10/100) = 86$ 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 (ie factories) containing the incorporated analogue are immunolabelled using antibodies against Br-RNA (Jackson *et al.*, 1993; see also Wansink *et al.*, 1993).

importantly, this length does not change during mitosis, implying that the molecular ties must persist (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 which makes counting difficult, but we nevertheless estimate that there are ~2000 in a HeLa cell, or roughly the number of mitotic bands or chromomeres (unpublished results). The bright ones contain >50 active polymerases, plus associated templates and components of the splicing apparatus. We have christened them 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).

## 10.3 NUCLEOLAR ORGANIZING REGIONS AND TRANSCRIPTION FACTORIES

We will assume that the remnants of transcription factories containing RNA polymerases II and III are equivalent to most of the mitotic chromomeres seen during mitosis. (We are currently collecting evidence for this assumption.) However, excellent evidence already exists that the transcription factories found in nucleoli (which contain RNA polymerase I) are equivalent to the mitotic 'chromomeres' of the nucleolar organizing region.

Nucleoli contain several 'fibrillar centres' surrounded by a 'dense fibrillar component'; in turn, these are embedded in a 'granular component' (Spector, 1993). Fibrillar centres — which are 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).



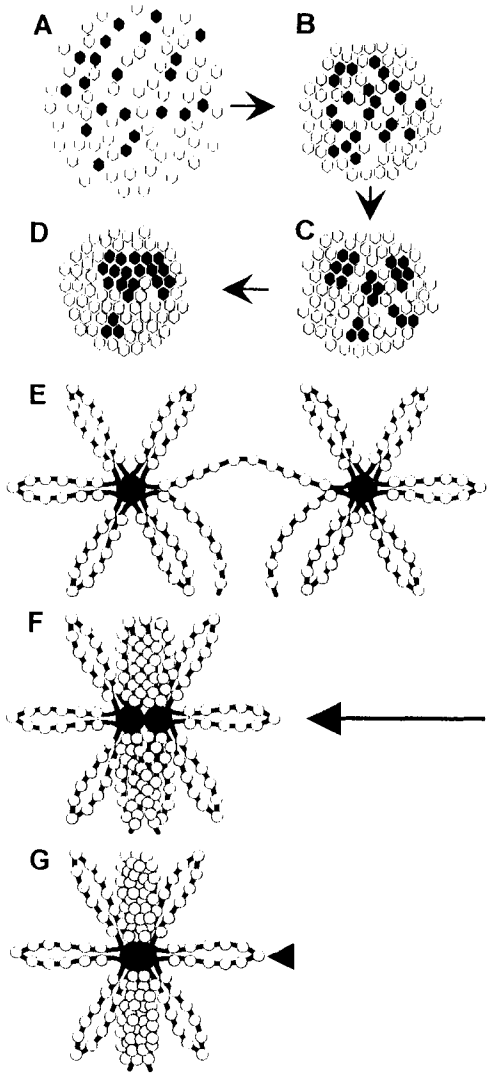
Each fibrillar centre is often associated with one, or a few, active ribosomal cistrons and the total number of centres is directly related to the transcription rate. For example, the ~234 in a fibroblast fall to ~156 on serum-starvation (Jordan and McGovern, 1981) and the ~9 in a peripheral blood lymphocyte 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 fibrillar centres aggregate into the nucleolar organizing regions on the chromosomes (Scheer and Rose, 1984; Roussel *et al.*, 1993).

It is 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 of mitosis. Groups of small factories rich in genes would condense into Giemsa-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.

## 10.4 A DIGRESSION ON CELLULAR ADHESION

But why do chromomeres condense into a cylinder and not a sphere? The principles involved can be exemplified by reference to the development of tissue layers during embryogenesis.

When disaggregated neural cells from frog embryos are randomly intermixed, they aggregate into a sphere and then sort into a semblance of the original tissue, with ectoderm surrounding the endoderm (Fig. 10.2A—D; Townes and Holtfreter, 1955). This process is simply explained if cells differ only in their adhesiveness for one another (Steinberg, 1964; Armstrong, 1989). First, the cells will aggregate; then, as random movement creates contacts, contacts between the more-adhesive cells will tend to persist, until eventually a sphere of less-cohesive cells forms around a core of the more-adhesive ones.






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 do sort into low-expressers surrounding a core of high-expressers.

We can model interphase chromosomes as strings of less-adhesive nucleosomes running between, and looping from, more-adhesive factories (Fig. 10.2E—G). In mitosis, increased adhesiveness will drive 'sticky-end' aggregation to the most compact and stable structure — a cylinder of nucleosomes around a chromomeric core in which inter-nucleosomal and inter-chromomere interactions are maximized.

**Fig. 10.2** (A-D) Cell sorting through differential adhesiveness and (E-G) an analogous 'sticky-end' aggregation and sorting during prophase. [From Cook (1995) with the permission of The Company of Biologists Ltd.] A-D: When two cell types (light and dark) are (A) mixed, (B) they aggregate into the most compact form, a sphere. As contacts created by random movement between the more-adhesive (dark) 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. E-G: (E) A string of less-adhesive nucleosomes (small circles on loops) is attached to two more-adhesive factories (large circles). (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.

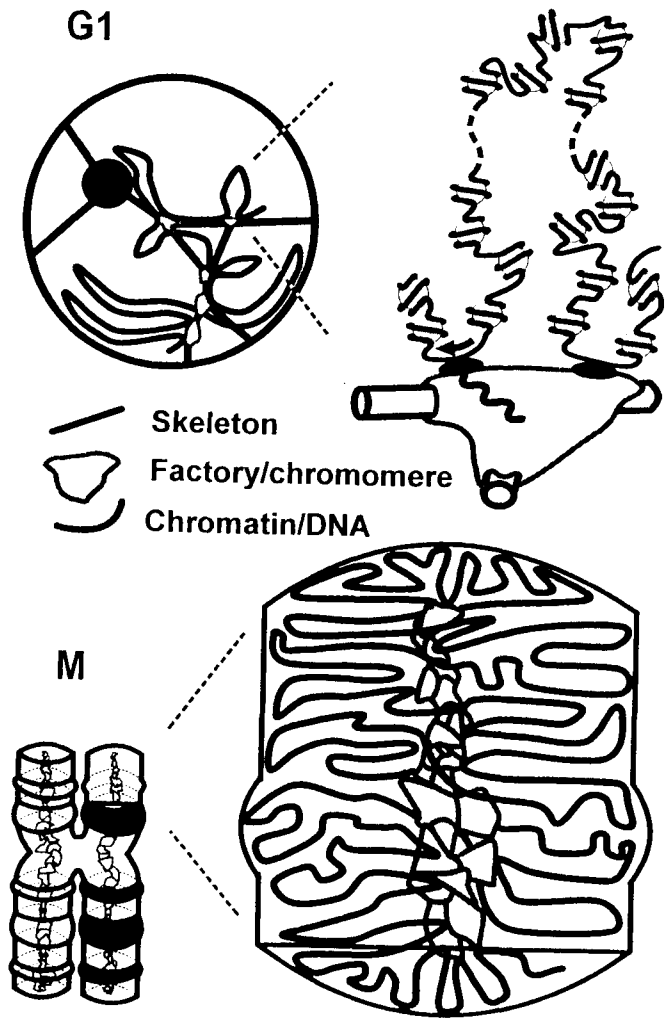





**Fig. 10.3** A model for the structure of a human chromosome. [From Cook (1995) with the permission of The Company of Biologists Ltd.] Upper: Structure during G1 phase. Transcription factories are located at nodes on a lamin-containing endoskeleton and their number and size depend upon transcriptional activity. If there are 1250, 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 (ie 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 (ovals). (In transcriptionally-inactive regions, transcription factors are the sole molecular ties.) A transcript (wavy line) is extruded as the template slides through the left-hand complex; this template movement 'opens' adjacent chromatin. The DNA duplex winds around nucleosomes (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 (enlargement, right). Factories associated with more shorter loops (and active genes) give narrower cylinders; examples include (I) Giemsa-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).

## 10.5 A MODEL

These considerations lead to a model involving three fundamental levels of organization: nucleosomes, loops and transcription factories/chromomeres (Fig. 10.3). During interphase, loops are tied through RNA polymerases or transcription factors to factories; increased transcription counteracts the tendency of factories to fuse and generates more, smaller, factories to which relatively more genes are attached in shorter loops. During mitosis, the skeleton depolymerizes, transcription ceases and proteins are phosphorylated, increasing adhesiveness between factories and between nucleosomes. Chromosome condensation would begin in heterochromatin and proceed bidirectionally to give cylindrical strings of chromomeres that first fuse into one large cylinder per (entangled) chromatid pair, which then splits into two 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. 10.3B). In different metaphases, regions rich in transcribed genes (in short loops) associated with (small) factories will condense to form Giemsa-light bands in roughly the same places, but individual genes do not occupy absolutely fixed positions. On entry into G1, 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.

## 10.6 CONCLUSIONS

This model is deliberately designed to be a minimalist one, and will obviously have to be embroidered as new details appear. It requires 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. Its major assumption is that 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 nonspecific (i.e. between nucleosomes and between chromomeres) or fewer but more specific (ie between polymerases/transcription factors and transcription units). 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 (Krawiec and Riley, 1990). The persistence of 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.



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# 11

# The replication of ribosomal RNA genes in eukaryotes

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## 11.1 INTRODUCTION

Replication of chromosomal DNA is a crucial process in the cell cycle of eukaryotes that is still poorly understood. Initiation of replication occurs when a cell is going to divide. However, entry into mitosis depends upon the successful termination of DNA replication. Initiation and termination of DNA replication are two relevant events for dividing cells.

Initiation of DNA replication in eukaryotic chromosomes occurs at numerous discrete sites along the parental DNA (Huberman and Riggs, 1968). In yeast, there is evidence that these sites are specific DNA sequences known as replication origins. Some DNA fragments of the budding yeast *Saccharomyces cerevisiae* confer to the plasmids containing them the ability to replicate autonomously once per S phase. Recently, it was demonstrated that many of these DNA fragments, named ARSs (for *a*utonomously *r*eplicating *s*equences), function as true replication origins not only when inserted into a plasmid but also in their chromosomal context. Sequence comparison and point mutation analysis led to the identification of an 11—bp ARS consensus sequence (ACS) (A/TTTTATA/GTTTA/T) that is essential for ARS function. This ACS is not sufficient, however, for efficient ARS activity. A less conserved AT-rich sequence located 3' to the T-rich strand of the ACS is also required. The ease of unwinding of this 3' region, named DUE (for *DNA unwinding element*), seems to be important for ARS function (Natale *et al.*, 1993). In a detailed functional analysis of the ARS1, Marahrens and Stillman (1992) found