

Packaging the Genome: the Structure of Mitotic Chromosomes

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Mitotic chromosomes are essential structures for the faithful transmission of duplicated genomic DNA into two daughter cells during cell division. Although more than 100 years have passed since chromosomes were first observed, it remains unclear how a long string of genomic DNA is packaged into compact mitotic chromosomes. Although the classical view is that human chromosomes consist of radial 30 nm chromatin loops that are somehow tethered centrally by scaffold proteins, called condensins, cryo-electron microscopy observation of frozen hydrated native chromosomes reveals a homogeneous, grainy texture and neither higher-order nor periodic structures including 30 nm chromatin fibres were observed. As a compromise to fill this huge gap, we propose a model in which the radial chromatin loop structures in the classic view are folded irregularly toward the chromosome centre with the increase in intracellular cations during mitosis. Consequently, compact native chromosomes are made up primarily of irregular chromatin networks cross-linked by self-assembled condensins forming the chromosome scaffold.

Key words: genome DNA, chromosome, condensins, scaffold.

INTRODUCTION

The human body is made up of 60 trillion cells that originate from a single fertilized egg. This suggests that the cells in the body go through roughly 46 rounds of cell division or mitosis. During mitosis, chromosomes, bundles of DNA, form condensed structures to ensure the faithful transmission of the duplicated genomic DNA. The term 'chromosomes' was derived from the Greek for 'coloured body', reflecting the fact that condensed chromosomes were clearly visible with dyes under primitive light microscopes. Chromosomes have fascinated biologists for over 100 years, since long before DNA was known to carry genetic information. This review article focuses on the structure of mitotic chromosomes.

How is the 2 m of genomic DNA that is present in each human cell packaged into compact mitotic chromosomes that are 10,000 times shorter? As shown in Fig. 1A, a long DNA molecule with a diameter of 2 nm is wrapped around the core histone octamer, which consists of the histone H2A, H2B, H3 and H4 proteins, and forms a 'nucleosome', which is the most basic package of DNA (1). The structure of a nucleosome core particle has been resolved at the atomic level (2). The nucleosome appears to be folded into 30 nm chromatin fibres. Several models of this folding have been suggested (3), including the ordered helical solenoid (4), twisted helices, and zigzag ribbon (5, 6). Note that as we describe later, the existence of such continuous 30 nm chromatin fibres in native chromosomes is still controversial. Furthermore, how the

nucleosome or 30 nm fibre is compacted into a mitotic chromosome with a diameter of about 0.7 μ m remains even less clear and is a basic challenge in cell biology (Fig. 1A).

CLASSICAL VIEWS

Chromosomes consist of roughly equal amounts of DNA, histones and non-histone proteins. In the 1970s, people believed that mitotic chromosome condensation is achieved by histone modification, especially the phosphorylation of histone H1 that happens specifically at the G2-M transition (7). Histone H1 is a linker histone that can stabilize 30 nm chromatin fibres structurally by linking nucleosomes (8). The prevailing model at that time suggested that the mitotic phosphorylation of histone H1 created a mitotic chromatin fibre that somehow self-assembled into higher-order structures.

In the late 1970s, Laemmli and colleagues proposed a novel hypothesis that chromosome structure arises from a set of non-histone proteins that fold the chromatin fibres into loops. To prove this, they isolated histone-depleted chromosomes (Fig. 1B) (9) by selectively removing the histones from isolated mitotic chromosomes gently by competition with an excess of the polyanions dextran sulfate and heparin. This approach was effectively able to separate the structural contributions of the histones from those of certain non-histone proteins. After removing the histones, they found that the DNA remains highly organized by a residue of non-histone proteins whose structure retains the size and shape of the original chromosomes (Fig. 1B) (9). Therefore, Laemmli called the central axial structure made from these non-histone proteins the 'chromosome scaffold' (Fig. 1B). The scaffold consists of a subset of non-histone proteins that includes two major high-molecular-weight proteins, Sc1 (170 kDa)

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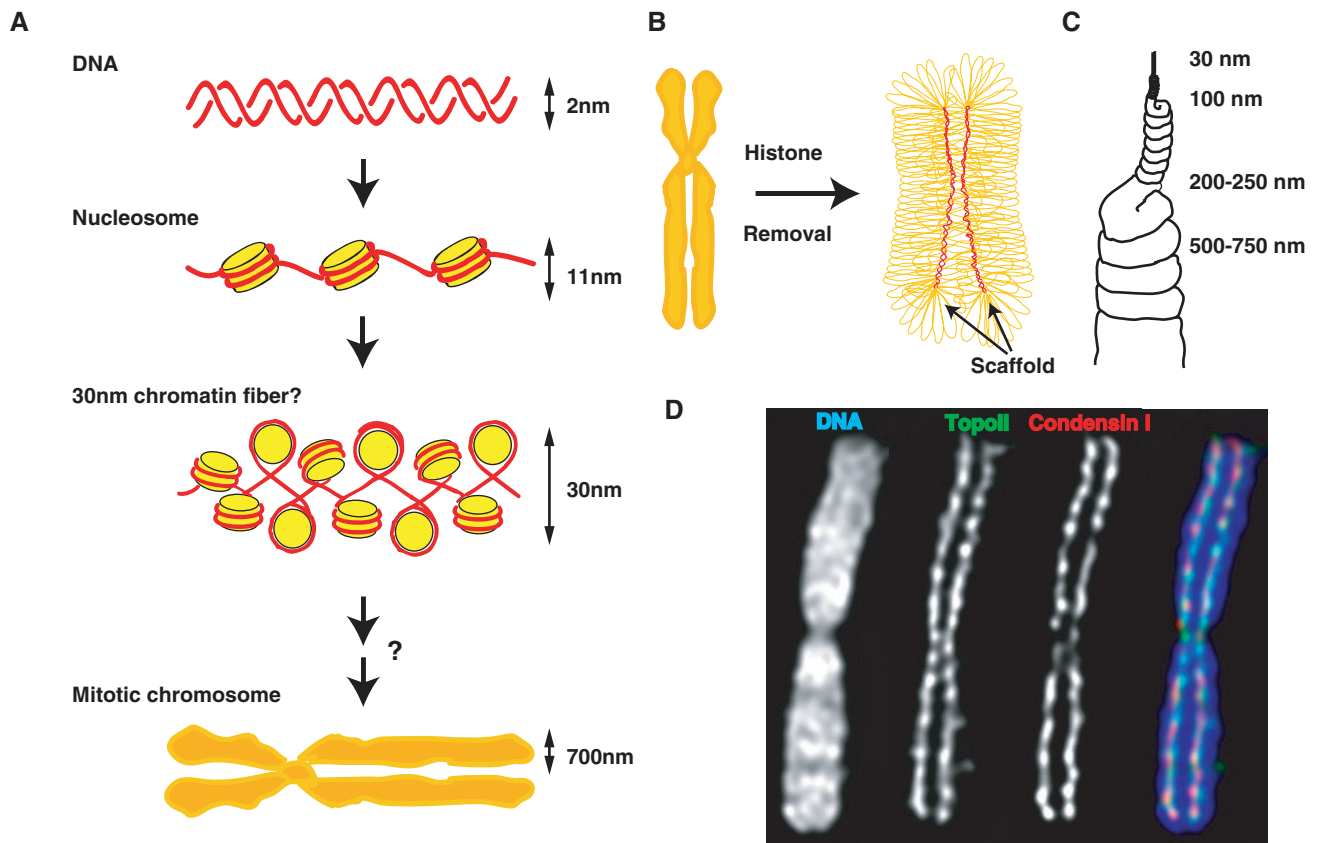


Fig. 1. (A) A long DNA molecule with a diameter of 2 nm is wrapped around a core histone octamer that consists of H2A, H2B, H3 and H4 histone proteins, and forms a 'nucleosome' with a diameter of 11 nm. The nucleosome is assumed to be folded into 30 nm chromatin fibres (1), although the existence of such continuous 30 nm chromatin fibres in native chromosomes remains controversial. Furthermore, it remains unclear how this 30 nm fibre is compacted into the chromatid with a diameter of about 0.7 μm . (B) Schematic representation of a histone-depleted chromosome. The histones were removed from the isolated mitotic chromosome gently by competition with an excess of the polyanions dextran sulfate and heparin. During this process, the positively charged histones in the chromatins are exchanged for the highly negatively charged polyanions. After removing the histones, the DNA remains highly organized by the

'chromosome scaffold' (drawn in red), keeping the size and shape of the original chromosomes (9). (C) 'Hierarchical helical folding model'. This model assumes that the 30 nm chromatin fibres are folded into 100 nm fibres and then progressively into 200–250 nm fibres that coil to form the final mitotic chromosomes. (D) Major chromosome scaffold components have axial distributions at the center of each chromatid in the compact chromosomes. An isolated human chromosome was stained using antibodies for topoisomerase II α and the condensin I component hCAP-H. The antibody labelling shows axial structures with a diameter of ~ 200 nm within the chromosome body stained with DAPI. Note that the staining for topoisomerase II α and condensin I is localized in an alternating manner, forming a 'barber pole' structure (26). The images are adapted from (26) with the permission of Elsevier.

and Sc2 (135 kDa), and a number of minor proteins (10). Sc1 was later identified as topoisomerase II (11, 12). Topoisomerase II is an evolutionary conserved protein, and can untangle DNA and relax the interwound supercoils in a DNA molecule by passing one DNA molecule through a transient double-stranded break in another [for a review, see (13)]. The requirement of topoisomerase II for chromosome condensation was clearly demonstrated by using elegant fission yeast genetics (14) and a useful *in vitro* system in which chromosomes are assembled by adding nuclei to extracts prepared from *Xenopus laevis* eggs (15, 16).

Strong evidence for chromatin loops was subsequently obtained from several electron microscopy studies (17–19). As we describe later, isolated chromosomes become swollen in a low-salt buffer, which helps in dissecting the chromosome architecture. Studies of such

swollen chromosomes suggested that the chromatin fibre generally folds in a radial fashion (away from the centre) rather than with a longitudinal orientation (Fig. 2A). Cross-sectional images of the swollen chromosomes appear star-like; the fibres converge on the central axis, suggesting tethering of the chromatin fibre to the axial element, forming loops (Figs 2A and B) (17–19). This loop structure of mitotic chromosomes may be analogous to that of the lampbrush chromosomes in amphibian oocytes (20) or the meiotic prophase chromosomes in various organisms (21), which are organized into an enormous number of large chromatin loops emanating from a linear chromosome axis. Evidence clearly implies that chromatin loops are a fundamental organizing unit of chromosomes.

In 1978, another classical model, the so-called 'hierarchical helical folding model', was proposed from

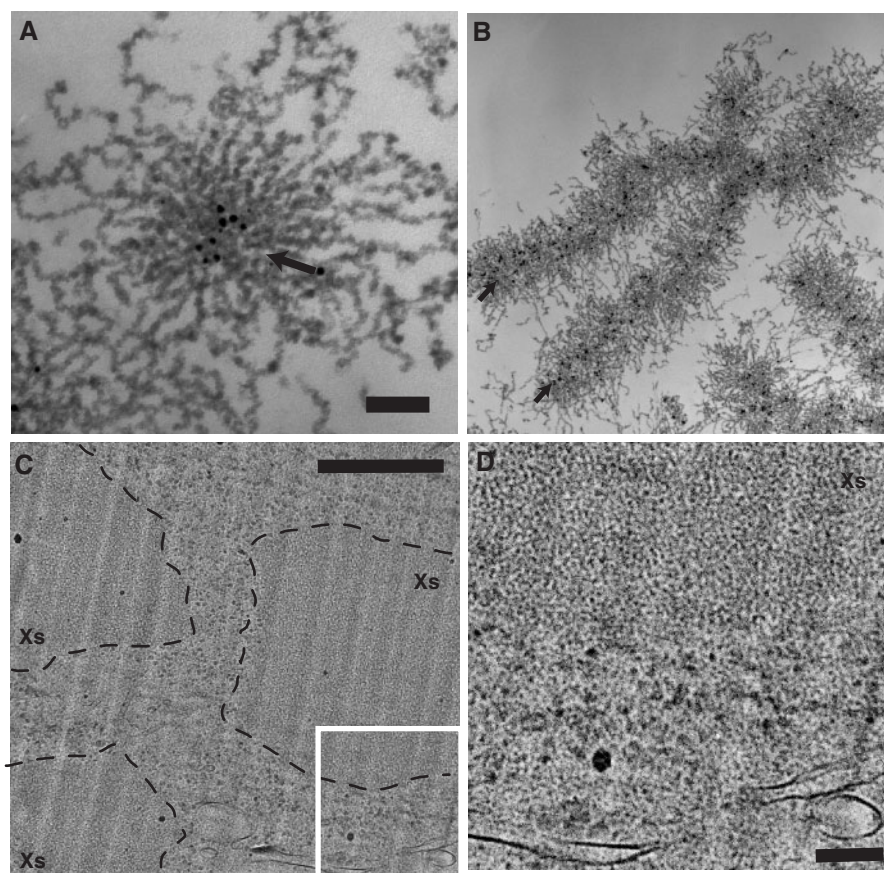


Fig. 2. (A) and (B) Conventional electron microscopy views of isolated chromosomes, swollen in a low-salt buffer containing 1 mM Mg^{2+} . Uniform 30 nm chromatin fibre loops that diverge radially from the centre are clearly visualized in cross- (A) and longitudinal- (B) sections of the chromosomes (19). The chromosomes seem to consist of radial chromatin loops that are somehow tethered centrally by the scaffolding protein condensin I, mapped using immuno-gold (shown by arrows). The images are reproduced from (19) with the permission of

Springer. The bar indicates 200 nm. **(C) Cryo-EM images of a frozen-hydrated section of mitotic HeLa cells.** The compact areas, outlined by the dashed line, are cross-sections of mitotic chromosomes (Xs), which are surrounded by cytoplasm full of electron-dense ribosomes and other particles. The selected area is magnified in (D). Note the grainy, homogeneous texture of the chromosome (Xs). No higher-order or periodic structures are recognized, such as 30 nm chromatin fibres. The scale bar indicates 500 nm in (C) and 100 nm in (D).

extensive observations using high-voltage electron microscopes (Fig. 1C) (22). This model postulates that the 30 nm chromatin fibres are folded into 100 nm fibres and then progressively into 200–250 nm fibres that coil to form the final mitotic chromosomes (Fig. 1C), *i.e.* a hierarchy in the chromosomes based on regular helical structures. Indeed, electron microscopy observation showed that chromosomes appeared to be assembled from chromatin fibres of various diameters (23). More recently, analysis using engineered chromosomes with large copy numbers of *lac* operator repeats revealed 250 nm-diameter coiling domains in the chromosome, supporting this model (24).

Although these two models appear incompatible, both structures may coexist in chromosomes. Chromosomes often become X-shaped with helically folded chromatids after prolonged treatment with a microtubule-depolymerizing drug, like nocodazole or colcemid (25, 26). Such chromosomes show helical coiling of a fibre that is in turn composed of radial loops (27).

DISCOVERY OF CONDENSIN

In 1994, three groups independently made a landmark discovery in chromosome study. Hirano and Mitchison identified a series of chromosome-associated polypeptides (CAPs) in *Xenopus* egg extracts (28). Of these, the two abundant proteins, CAP-C and CAP-E, were shown to have sequence similarity with a family of proteins in budding yeast, which was later called the 'structural maintenance of chromosomes' (SMC) family (29). Indeed, antibody-blocking experiments revealed that CAP-E is essential for maintaining the structure of condensed chromosomes (28). Almost simultaneously, Saitoh and Earnshaw showed that a major chromosome scaffold component, Sc2, is a chicken homologue of an SMC protein (30). Yanagida's group also demonstrated that Cut3 and Cut 14 identified as fission yeast mutants showing 'cell untimely torn' phenotype, are homologues of the SMCs, and both involved in the chromosome condensation (31).

Subsequent characterization of the CAPs led Hirano and his colleagues to the discovery of a protein complex called condensin, which consists of five different subunits, including a heterodimer of CAP-C (SMC4) and CAP-E (SMC2) (Fig. 3A) (32). When condensin is depleted from *Xenopus* extracts using a specific antibody, mitotic chromosome condensation is defective, and chromatin forms swollen puffs, not a compact structure. When purified condensin complex is added back to the depleted extracts, chromosome condensation recovers, implying that the complex has a key role in the chromosome condensation process.

It turned out that the condensin family is conserved from bacteria to mammals [for a review, see (33, 34)], suggesting a universal role of this family in compacting the genome. Although it is not known exactly how the complex functions in the condensation process, interesting clues came from the discovery that condensin can introduce positive supercoils into closed circular DNA (Fig. 3B) (35). This activity requires ATP-hydrolysis and is dependent on the mitosis-specific phosphorylation of the complex (36, 37).

Further biochemical and cytological analyses of the chromosome scaffold demonstrated that it is composed predominantly of condensin complex and topoisomerase II α (26). Both components have an axial distribution with a diameter of ~200 nm at the centre of each chromatid in the swollen and compact chromosomes (Fig. 1D) (26, 30, 38, 39), and even in the chromosomes in living cells (40, 41). Although there is an on-going argument that the scaffold structure is an artifact resulting from the non-specific aggregation of non-histone proteins in the histone-depleted chromosomes (42), these findings support the physiological relevance of the chromosome scaffold structure (26, 43).

Recently, in vertebrates, condensin II was isolated as a second condensin complex that is composed of the common heterodimer of SMC2 and SMC4 and three non-SMC subunits related to, but distinct from those in condensin I (Fig. 3A) (44, 45). Condensins I and II showed an axial localization in chromosomes in a rather complementary manner (44). Experiments knocking down condensin I or II separately or together have revealed their distinct functions in part (41, 46). The condensin I is localized in the cytoplasm until the nuclear envelope breaks down, whereas condensin II is located in the nuclei during interphase. Consistent with this observation, the depletion of condensin I did not affect prophase chromosome condensation (41). In contrast, the knockdown of condensin II significantly delayed the initiation of prophase chromosome condensation (41).

A PARADOX INVOLVING CONDENSIN AND TOPOISOMERASE II

There would be no doubt of the universal roles of the condensin family and topoisomerase II in genome compaction. However, available genetic or RNAi data from various organisms, including the fly (47), nematode (48), and chicken (43) indicate that condensin mutants mainly have a segregation defect, but do not cause

dramatic abnormalities in chromosome morphology. Earnshaw and colleagues showed that metaphase chromosomes in condensin-knockout chicken DT40 cells can still condense normally, as in wild-type cells, although they had frequent chromosomal bridges during anaphase (49). Surprisingly, this defect recovered with the expression of either cyclin B3 or dominant negative Repo-Man, which is unable to recruit the protein phosphatase PP1 γ to anaphase chromosomes. Therefore, they inferred a novel chromosome condensation activity, regulator of chromosome architecture (RCA), which enables metaphase chromosome condensation without condensins (49). As we discussed later, these results clearly indicate that condensins are required for the structural integrity of chromosomes, but not for chromosome condensation. As well as condensins, knockdown of the topoisomerase II α in fly or human cells also resulted in chromosome segregation, but no prominent condensation defect (50–52), while it was essential for chromosome condensation in the fission yeast (14) and *Xenopus* egg extract system (15, 16). It is again much less clear whether topoisomerase II is required for chromosome condensation in such cells. In the higher eukaryotic organisms, which have more complex systems, proteins might acquire more specialized function or functional redundancy to ensure their long-term survival.

ROLE OF CATIONS IN CHROMOSOME CONDENSATION

Besides protein factors, like condensins or topoisomerase II, cations are essential participants in chromosome condensation. As we mentioned above, mitotic chromosomes become very swollen following the depletion of Ca²⁺ or Mg²⁺. This process is completely reversible. Almost 40 years ago, Cole demonstrated that the repeated removal and addition of Mg²⁺ resulted in cycles of chromosome swelling and compaction (53). Deoxyribonucleic acid (DNA) has a negatively charged phosphate backbone that produces electrostatic repulsion. In the presence of cations, DNA condensation results from charge neutralization, since the binding of cations specifically to the DNA phosphates decreases the overall electrostatic repulsion between adjacent DNA regions. In the case of chromatin, it was reported that the negative charges of DNA are ~60% neutralized by core histones that have tails with positively charged lysine and arginine residues (54). Therefore, the remaining ~40% of the DNA charge must be neutralized by other factors, like histone H1, non-histone proteins, and cations. In fact, using secondary ion mass spectrometry (SIMS), Strick *et al.* (54) reported that the cations Ca²⁺, Mg²⁺, Na⁺ and K⁺ are highly enriched in mitotic chromosomes, as compared to interphase nuclei, suggesting a potentially important role in chromosome condensation.

Chromatin 'opening' and 'closing' with DNA charge neutralization may also be involved in global gene regulation as a result of histone tail modification, such as phosphorylation, acetylation, and methylation (1). Although there are many divalent cations available in cells, not all the divalent cations seem to function in

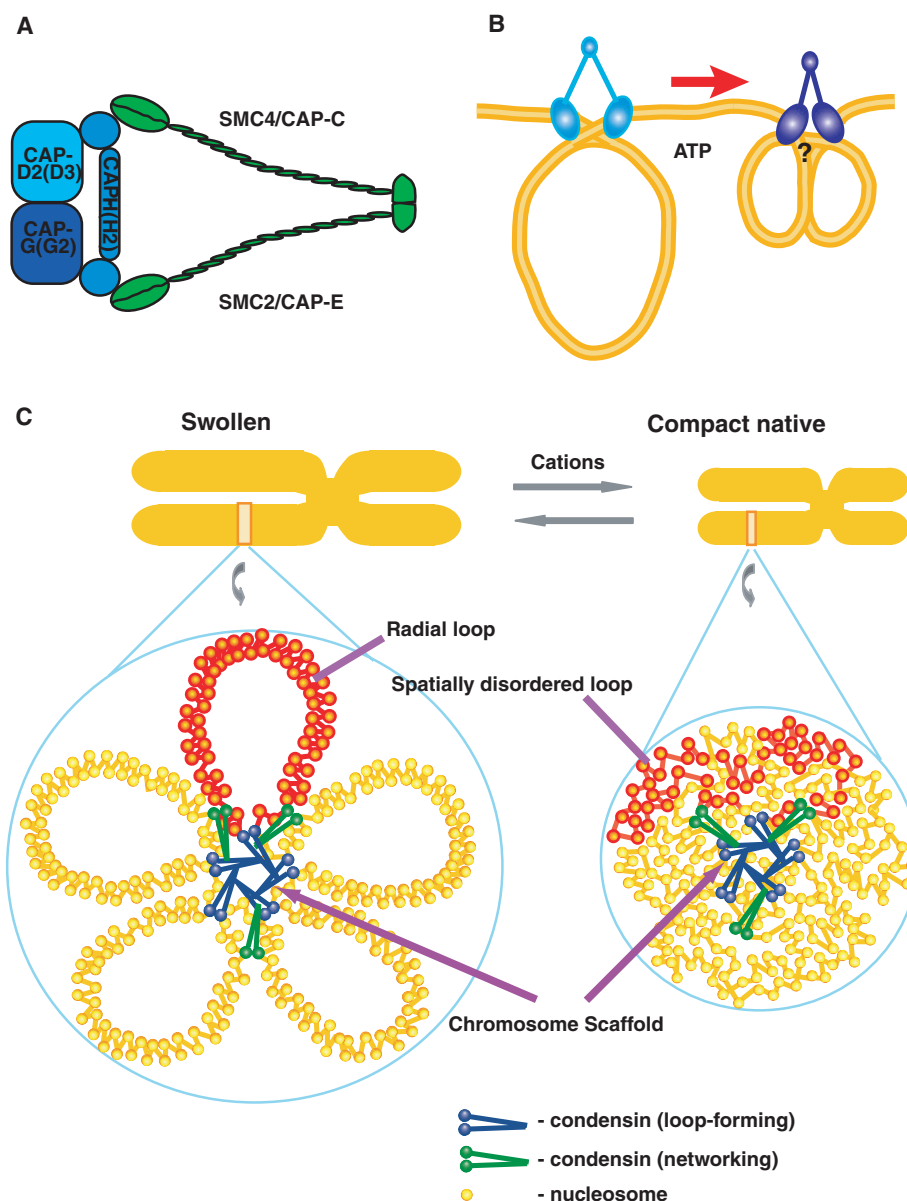


Fig. 3. (A) Schematic representation of the structure of condensin. In vertebrates, there are two types of condensin, condensin I and condensin II. Condensin I consists of five different subunits, a heterodimer of SMC4 (CAP-C) and SMC2 (CAP-E) and three non-SMC subunits: CAP-D2 (Eg-7), and CAP-G, and CAP-H (Kleisin γ , Barren). In vertebrates, condensin II has the SMC2 and SMC4 heterodimer in common and three distinct non-SMC subunits: CAP-D3, CAP-G2, and CAP-H2 (Kleisin γ). **(B) Condensins can introduce positive supercoils into closed circular DNA via ATP hydrolysis.** However, it is not known how this condensin activity functions in the condensation process. **(C) A proposed model of the mitotic chromosome structure.** A cross-section of an isolated chromosome swollen in a low-salt buffer shows radial chromatin loops that are somehow tethered centrally by condensin (Figs 2A and B). Our model supposes two

events for building a robust chromosomal architecture. First, condensins bind to certain specific sites in the genome chromatin to make loops (loop-forming activity). Second, condensins further cross-link neighbouring chromatin loops (networking activity) and form anisotropic self-assembly structures in a cooperative manner, like a scaffold. With the increase in intracellular cations during mitosis, the loop structures fold irregularly toward the chromosome scaffold containing abundant condensins. Due to this collapsing-loop process, compact native chromosomes are made up primarily of irregular chromatin networks cross-linked by self-assembled condensins, forming the chromosome scaffold. Note that no continuous 30 nm chromatin fibres are visible in the compact native chromosomes. In the absence of condensins (49), chromosomes are still condensed by RCA, but with loss of their structural integrity.

this process. Using scanning x-ray fluorescence microscopy (SXFM), we recently found that Zn^{2+} , which is a major divalent cation with a larger ion radius, is virtually excluded from the mitotic chromosomes,

whereas it is enriched in the nucleoplasm in interphase (Maeshima and Shimura *et al.*, unpublished). Therefore, there might be strict structural preferences of DNA for cations, even for the purpose of charge neutralization.

UNIFORM TEXTURE OF CHROMOSOMES: IRREGULAR FOLDING?

Isolated chromosomes become swollen in a low-salt buffer containing 1 mM Mg^{2+} . When such chromosomes were observed using conventional electron microscopy after being embedded in plastic sections, uniform 30 nm chromatin fibre loops that diverged radially from the centre were clearly visualized (Figs 2A and B) (19). As shown in Figs 2A and B, chromosomes seem to consist of radial 30 nm chromatin loops that are somehow tethered by scaffolding proteins such as condensin or topoisomerase II α centrally (19). Although this is a rather classical view of chromosome organization, it must be kept in mind that the samples were fixed chemically, dehydrated and embedded in plastic. What do chromosomes look like in living cells?

Using cryo-electron microscopy (cryo-EM), Dubochet and his colleagues observed frozen hydrated sections of mammalian mitotic cells that had not been fixed, stained, or embedded in plastic (55). Surprisingly, the chromosomes showed a homogeneous and grainy texture with ~11 nm spacing [(55), also see Figs 2C and D]. Neither higher-order nor periodic structures including 30-nm chromatin fibres were recognized [(55), also see Fig. 2D]. It is unlikely that 30 nm chromatin fibres existed, but were missed, because microtubules of comparable mass/unit-length could be seen in the cytoplasm (56). Dubochet *et al.* proposed that the basic structure of the chromosomes is a liquid-like compact aggregation of 11 nm nucleosome fibres and not 30 nm chromatin fibres. We continued with efforts to observe frozen hydrated mitotic chromosomes using cryo-EM, and obtained similar results (Figs 2C and D) (Eltsov *et al.*, unpublished).

Very recently, Kong *et al.* (57) reported the 3D structure of *Xenopus* chromosomes assembled *in vitro* using electron microscopy tomography. They used cryo-substitution, which is based on rapid freezing of a sample that is then embedded in plastic at low temperature. In the cryo-substituted chromosomes, they detected organized 30–40 nm domains, but no continuous fibre-like structure, such as 30-nm fibres. No other regular ultrastructural organization was observed. Such 30–40 nm domains may be aligned nucleosomal clusters that are spaced regularly and highly interconnected. They concluded that the *in vitro* assembled chromosomes consist of a complex network of closely spaced small chromatin domains (57).

The absence of a continuous 30 nm fibre-like structure in the assembled chromosomes and in cryo-EM observations suggests that such fibres are not a prerequisite for chromosome condensation. This conclusion is supported by the finding that chromosome assembly in *Xenopus* extracts can proceed without the linker histone H1, which supposedly stabilizes the 30-nm fibres (58). There are also mouse cell lines with very low amounts of H1 (59). In addition, histone H1 is highly mobile in the chromosome in live cells (60). These data again support the absence of 'static' continuous 30 nm chromatin fibres in native chromosomes.

As we mentioned and Belmont pointed out (61), the images of 30 nm chromatin fibres within mitotic

chromosomes came from isolated chromosomes partially swollen in low-salt buffers like 1–2 mM Mg^{2+} . In conventional electron microscopic observations, the formation of 30 nm chromatin fibres might be stabilized through intra-chromatin chemical cross-links (e.g. glutaraldehyde fixation) and shrinkage with alcohol dehydration during sample preparation.

FUNCTIONS OF REPETITIVE SEQUENCES IN THE GENOME

Once the almost entire human genome had been sequenced, we learnt that it would not be an exaggeration to say that the human genome consists mainly of repetitive DNA sequences. Among the repetitive sequences, satellite DNAs are clustered in discrete areas, such as the centromere, whereas other groups such as short interspersed nuclear elements (SINEs) and long interspersed nuclear elements (LINEs) are dispersed throughout the genome. Are these really 'junk'? Do they have any functions? Although there are many proposed functions, we would like to consider the possibility that repetitive sequences could be involved in genome compaction. Indeed, the genomic regions, which are abundant with satellite DNAs, are condensed throughout the cell-cycle (e.g. centromere).

Double-stranded DNA has the property of self-assembly in aqueous solutions containing physiological concentrations of divalent cations. Ohyama and his colleagues found that DNA molecules preferentially interact with molecules with an identical sequence and length, even in a solution containing heterogeneous DNA species (62). Therefore, it is tempting to postulate that this attractive force due to repetitive sequences functions in the process of chromosome condensation. In addition, there is evidence implicating LINEs (L1s) in X-chromosome condensation (63). It is well known that one of the female X-chromosomes is highly condensed as a Barr body (63). Bailey *et al.* (64) showed that the level of LINEs in X-chromosomal DNA was roughly double that in autosomes. They also found that the level was highest in a region near the X-inactivation centre and lowest in a region of the short arm that contains numerous genes that escape inactivation (64). These data are consistent with the postulate that the density of LINEs favours the extent of inactivation or condensation. In addition, LINEs are concentrated mainly in the dark G-bands in autosomes, where chromatin is condensed as heterochromatin. Although chromosomes cannot be condensed without the help of protein factors like condensins, it is highly possible that chromosomes acquired huge repetitive sequences to benefit genome compaction in the course of evolution.

A MODEL OF CHROMOSOME STRUCTURE

By its nature, chromatin forms aggregates in the presence of the proper concentrations of divalent or multivalent cations (65). However, mitotic chromosome condensation is not just a process of chromatin aggregation. Chromatin fibres must be organized in a manner that keeps the epigenetic marks of transcriptionally

active and inactive genes in the genome, which are somehow retained during chromosome condensation and de-condensation (66, 67). For instance, it is known that a number of gene promoters do not tightly condense in mitotic chromosomes *via* the so-called 'bookmarking' mechanism during mitosis [for a review, see (68)].

Again, how is the 2 m of genomic DNA packaged into compact mitotic chromosomes while maintaining the organization of genomic information? When isolated chromosomes become swollen under low-salt conditions, they seem to consist of radial chromatin loops that are somehow tethered centrally by scaffolding proteins (Figs 2A and B). Considering a structural analogy with meiotic and lampbrush chromosomes, we postulate that chromatin loops are the fundamental organizing unit of chromosomes (Fig. 3C). Such swollen chromosomes again become compacted in the presence of increased levels of divalent cations, as if chromosomes have a 'memory' for structural maintenance (53). Using Cole's technique, Hudson *et al.* (43) elegantly demonstrated that the morphology of isolated chromosomes from condensin-knockout cells is disrupted after the first round of swelling and compaction. This interesting finding implies that condensins are required for maintaining the structural integrity of chromosomes, and suggests that condensins promote the cross-linking of chromatin fibres, thereby forming loops.

With cryo-microscopic observations, chromosomes show a homogenous, grainy texture, and no higher-order or periodic structures (Figs 2C and D) (55). As the intracellular cations increase during mitosis, we postulate that the loop structures fold irregularly toward the chromosome scaffold, which contains abundant condensins (Fig. 3C). This loop collapse process might be enhanced by the attractive force of repetitive sequences dispersed throughout the genome. Therefore, the compact native chromosome would be made up primarily of an irregular chromatin network further cross-linked by condensins (Fig. 3C). This is consistent with a model based on micropipette analyses of mitotic chromosomes in which a uniform chromatin network is folded isotropically by protein components (69).

To make long rod-shaped (but not spherical) chromosomes, the formation of an anisotropic (with polarity) scaffold structure is essential. Whatever we call this scaffold, *i.e.* 'axis', 'core', or 'glue' (39), it is very likely that condensins play a major role in its formation. An immuno-electron microscopy study of condensins showed a traceable condensin array near the centre of the chromosome cross-sections (19), suggesting the existence of an anisotropic self-assembly structure of condensins that captures chromatin fibres. In fact, it has been reported that condensin can aggregate in the presence of DNA (70). Our model supposes two events for building a robust chromosomal architecture (Fig. 3C). First, condensins bind to certain specific sites in the genome chromatin to make loops (loop-forming activity). Epigenetic marks like histone modifications can affect the specific binding of condensins, which suggests a mechanism for modulating the loop size and chromosome compaction. Second, condensins further cross-link neighbouring chromatin loops (networking activity) and form anisotropic self-assembly structures in a co-operative

manner, like a scaffold (Fig. 3C). Further biochemical and structural investigations of condensins would provide an important clue to the self-assembly structure. Finally, it should be emphasized that, in our model, the orientations of chromatin fibres in native chromosomes are isotropic and not radial (Fig. 3C). Accordingly, our proposed model is compatible with the available data concerning the scaffold/radial loop model (24).

In conclusion, we have discussed the structural aspects of mitotic chromosomes and proposed a model. The chromosome structure and its condensation process are directly linked to the global regulation of DNA replication (71) and gene expression in the genome. Now that we know the almost entire genome sequences of many organisms, including *Homo sapiens*, an understanding of chromosome structure would provide a novel insight into genome science and would allow us to decipher the 'hidden codes' to determine higher-order chromosome architecture.

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