18
The Mitotic Chromosome: Structure and Mechanics

John F. Marko

18.1
Introduction

Cell division requires replication of DNA followed by physical segregation of chromosomes and replicated chromatids. DNA replication is likely to leave duplicate sister chromatids wrapped around one another (catenated) at least once every few kilobases (Chapter 15). Then, as the cell enters mitosis, chromosomes are folded ("condensed") into neatly compacted and segregated mitotic chromosomes each composed of two separated sister chromatids (Figure 18.1). The mechanism of mitotic folding likely facilitates segregation of chromosomes and adjacent chromatids, but exactly how this occurs – both the folding and its coupling to topological and geometrical segregation – is largely a mystery [1].

Our incomplete understanding of chromosome organization follows in part from basic physical properties of chromatin. The dynamic nature of chromosomes resulting from active processes including gene positioning and cell cycle reorganization, plus continual thermal (Brownian) motions indicates that chromatin structure must be described statistically, rather than in terms of precise folds and structures. Furthermore, chromatin and chromosomes are soft materials, with rigidities far less than that of the molecules from which they are composed, leading to the pitfall that large-scale structure of chromosomes can be altered by preparations which leave protein and DNA molecular structures intact. Finally, the molecular mechanisms by which large-scale chromosome organization is controlled are simply not understood.

This chapter focuses on the large-scale architecture, mechanics, and molecular connectivity of mitotic chromosomes. Section 18.2 reviews protein components thought to define the structure of mitotic chromosomes, starting with histones and other key DNA-binding proteins, and then examining the "structural maintenance of chromosomes" (SMC) protein complexes known to be essential to formation and maintenance of mitotic chromosome structure [2–5]. The large-scale architecture of mitotic chromosomes is then discussed in Section 18.3.

Next, Section 18.4 reviews the mechanical properties of mitotic chromosomes: whole mitotic chromosomes are highly elastic, suggesting that the folded

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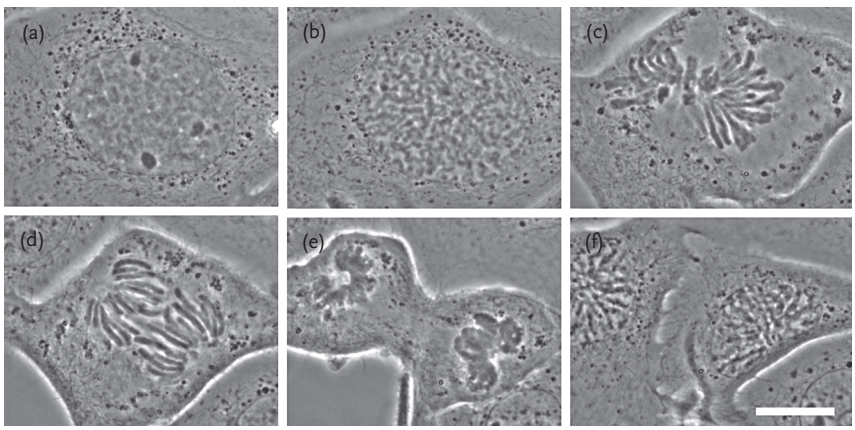

Figure 18.1 Cell division in newt epithelial cell, phase-contrast imaging. (a) Early prophase. (b) Late prophase showing long and thin prophase chromosomes. (c) Spindle-aligned metaphase chromosomes. (d) Separation of chromatids at anaphase. (e) Telophase chromosomes beginning to decondense. (f) Interphase nuclei in daughter cells. Bar, 20 μm. Images courtesy of M.G. Poirier.

chromatin in their interior may be appreciably unfolded without breakage of chemical bonds. Then, in Section 18.5, the results of experiments probing molecular connectivity of mitotic chromosomes are described. Those experiments suggest that non-DNA elements constraining mitotic chromatin are isolated from one another, that is, that mitotic chromosomes have a chromatin "network" or "gel" organization, with stretches of chromatin strung between "crosslinking" points. The as yet unresolved questions of the identities of the putative chromatin crosslinkers, their organization inside mitotic chromosomes, and the coupling of chromosome condensation to chromosome segregation are then discussed in the context of a model for the condensation–resolution process in Section 18.6 and the conclusion.

Some topics relevant to the mitotic chromosome are covered poorly or not at all. The most severe deficit is the lack of discussion of the function of the mitotic centromere and kinetochore, which could be the subject of an entire chapter [6]. Much of what we know about the large-scale structure of mitotic chromosomes comes from studies of large mammalian and amphibian chromosomes, and this chapter has a similar focus.

18.2
Structural Components of Mitotic Chromosomes

Proteomic experiments are moving towards providing a comprehensive catalog of mitotic chromosome proteins [7–10]. Here only a subset of the proteins in the mitotic chromosome selected for their DNA folding functions is discussed (Figure 18.2).

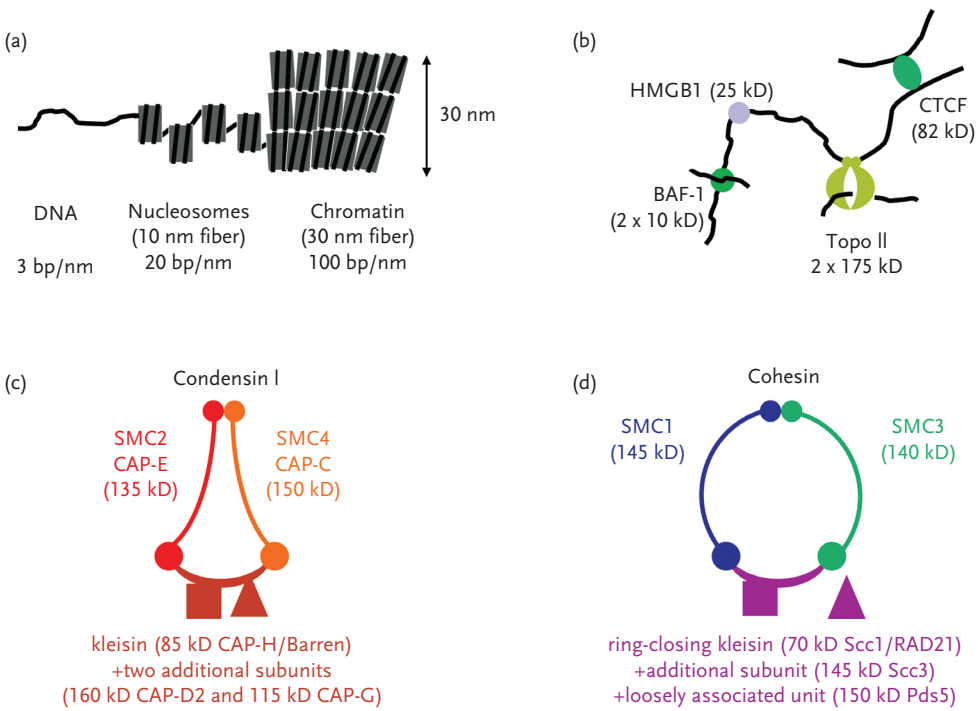

Figure 18.2 Cartoons of elements of eukaryote mitotic chromosomes, sketched roughly to the same scale. (a) DNA is complexed with histones to form nucleosomes, which then fold into chromatin fiber containing roughly 100 bp/nm; linker histones are not shown. (b) Small HMGB1 and BAF-1 proteins bend and crosslink DNA; a larger (10 nm) topo II is shown bound to one DNA, while passing a second DNA through it. A CTCF is shown linking two distant DNA loci. (c) Large (50 nm) condensin I complex composed of two long SMCs plus a bridging kleisin unit and two additional accessory proteins. Condensin II is not shown; its structure is similar to that of I (see text). (d) A cohesin complex composed of long SMCs plus kleisin and accessory units has a large open structure with a hole large enough to pass 30 nm chromatin fiber.

18.2.1 Chromatin Fiber

Chromosomes are composed of chromatin fiber, which consists of DNA complexed with histones into repeated nucleosome units as described in Chapter 3. Each $\approx$10-nm diameter nucleosome contains 147 bp of DNA wrapped around eight core histone proteins (two each of histones H2A, H2B, H3, and H4; total octamer mass $\approx$95 kDa); the structure of the nucleosome is known in atomic detail [11]. Given that there is one nucleosome for every $\approx$180 bp of DNA (of mass 110 kDa) the total mass of core histones is about the same as that of DNA. Formation of a nucleosome reduces the total 60 nm contour length of 180 bp of DNA to roughly 10 nm. Thus naked DNA, with 3 bp/nm, can be compacted into a string

of nucleosomes with roughly 20 bp/nm, a linear compaction of about sixfold (Figure 18.2a).

Chromatin fiber structure is sensitive to ionic conditions. When fibers are extracted into solution at sub-physiological 10 mM univalent salt concentration, they are observed in the electron microscope (EM) as 10-nm thick "beads on a string". Near the more physiological level of 100–150 mM univalent ions, nucleosomes stack into the 30 nm fiber (Chapter 8). An often used estimate is that when compacted into 30 nm form, there are about six nucleosomes per 10 nm of chromatin fiber length, or 100 bp/nm, about 30-fold shorter than the original DNA (Figure 18.2a), an estimate supported by X-ray studies of crystallized nucleosome arrays [12] and tetranucleosomes [13].

At physiological salt concentration (150 mM), lateral internucleosomal attractions tend to lead to aggregation of isolated fibers [14]. This sensitivity indicates that nucleosome–nucleosome interactions have a strong electrostatic component, and the variability of chromatin fiber structure with salt indicates that chromatin is soft and easily deformed. This softness and consequent variable structure of chromatin has made it difficult to arrive at consensus regarding 30 nm fiber folding. Perhaps the most extreme manifestation of this is recent cryo-EM studies, where rapid freezing is the only sample preparation, which indicate that *in vivo* chromatin is organized into a "liquid" of nucleosomes with no discernable 30 nm fiber organization [15].

Nucleosomes have associated with them linker histones (H1 or H5, $\approx$20 kDa). Linker histones have long been thought to be to be involved in compaction of chromatin fiber to a folded 30-nm thick form [16], but the details of how this occurs remain poorly understood. The questions of linker histone to nucleosome stoichiometry [17] and exactly how linker histone binds to chromatin are not settled [18]. However, experiments with *Xenopus* egg extracts have shown that varying the amount of linker histone dramatically affects large-scale structure of mitotic chromatids assembled *in vitro*. Comparison of experiments with native extracts, linker-histone-depleted extracts, and mock-depleted extracts revealed that the absence of linker histone resulted in an approximately twofold longer chromatid [19, 20]. When linker histone was added to the depleted extracts, a shorter chromatid was recovered. This important result shows that H1 strongly affects global mitotic chromatid folding, in an anisotropic way. This is made even more remarkable by single-molecule studies of *in vitro* assembled fibers which suggest that absence of H1 does not strongly impact local fiber compaction [21].

An important aspect of nucleosomes is their ability to be covalently modified via phosphorylation, acetylation, methylation, and ubiquitination, primarily along their N-terminal "tails" (see Chapter 4). Many of these modifications affect gene expression [22] and are maintained through cell division. Histone modifications provide mechanisms for "epigenetic" gene regulation (expression patterns that persist through cell division that are not strictly based on DNA base sequence [23]; see also Chapter 2). To survive the cell cycle, epigenetic marks must be robust against the displacement of transcription factors and inhibition of transcription which occurs during mitosis, and histone modifications satisfy this constraint [24, 25].

Other histone modifications are modulated synchronously with the cell cycle, with some modifications thought to play a role in controlling mitotic chromatin folding [26]. An important example of this latter type of modification is phosphorylation of serine 10 of histone H3 (H3S10), which occurs first in pericentric heterochromatin and then spreads across chromosomes at the beginning of mitosis in a wide range of organisms [26]. H3S10 is then dephosphorylated at anaphase. Although there is no evidence that this modification directly changes nucleosome packing or tail–tail interactions, it is known that H3S10 phosphorylation leads to the release of heterochromatin protein 1 (HP1), suggesting that this modification is involved with chromosome condensation via the release of chromatin from heterochromatic constraints [27].

18.2.2
Condensin Structural Maintenance of Chromosome (SMC) Complexes

SMC proteins are large ($\approx$150 kDa) ATPases folded into long (50 nm) coiled coils terminated by globular domains (Figure 18.2c–d). Together with additional "kleisin" and accessory proteins, SMCs form large complexes that play major roles in chromosome condensation and segregation, as well as in other aspects of chromosome dynamics. The SMC–kleisin complexes of interest here are the condensin and cohesin SMC complexes [2, 28].

Condensins consist of two SMCs (a heterodimer of a 135 kDa SMC2 and a 150 kDa SMC4) complexed with a smaller kleisin (85 kDa) and at least two additional accessory units (Figure 18.2c). Condensins were first characterized in yeast [4, 5] and *Xenopus* [3]. It was soon realized that one of the SMCs had been identified as non-histone "scaffold protein II" from mitotic chromosomes [29]. EM visualization indicates that the two SMCs bind together to form a hinged structure nearly 100 nm in length if extended, suggesting a function as a chromatin–chromatin linker. Experiments with *Xenopus* egg extracts established that the SMC units were essential for the establishment and maintenance of mitotic chromatid structure [3].

Further experiments with *Xenopus* egg extracts and human cells revealed that two distinct condensins (I and II) are involved in mitotic chromosome condensation [30]. These two complexes are built on the same SMC2–SMC4 heterodimer, but have different kleisin and accessory units (condensin II, not shown in Figure 18.2c, contains distinct CAP-H2, CAP-D3, and CAP-G2 units). Condensin I and II appear to have distinct architectural functions; in human cells, depletion of the condensin I-specific G subunit led to poorly condensed, fat, and fuzzy metaphase chromosomes, while depletion of the condensin II-specific G2 subunit led to "curly" chromosomes [30].

The dynamics of the two condensin units are quite different. Vertebrate condensin II loads onto chromosomes in the nucleus, participating in prophase chromosome condensation, while condensin I is cytoplasmic and loads onto chromosomes only after nuclear envelope breakdown (NEB) [31–33]. Condensin II appears immobile on human chromosomes even during prophase, while

condensin I is highly mobile, exchanging on a roughly 4-min timescale throughout mitosis [34].

The roles and dynamics of condensin I and II complexes vary in different organisms. Yeast contains only condensin I, and in *Drosophila*, condensin I has been reported to dominate mitotic chromosome condensation and to be necessary for stability of chromosomes during mitosis [35]. It is loaded onto chromosomes in early prophase, and remains highly dynamic throughout mitosis, undergoing binding–unbinding turnover on a timescale of a few minutes [36].

Estimates of the number of condensin complexes on mitotic chromosomes are in the range of one per 10–30 kb of DNA [9, 37]. In chromosomes assembled using *Xenopus* extracts, it has been estimated that there is one condensin per 5–10 kb [38]; 10 kb contains about 60 nucleosomes, or about 100 nm of 30 nm chromatin fiber.

Condensin activity on individual DNA molecules has been observed. Single-DNA experiments revealed that purified *Xenopus* condensin I is able to condense single DNAs by roughly 75 nm steps, in an ATP-dependent reaction [39]. This result establishes that condensin has an ATP-dependent DNA-condensing function in a biochemically defined system.

A second and important single-DNA experiment of [39] started by introducing condensin without ATP; no condensation occurred. Then, all condensin in solution was washed away. Finally ATP alone was introduced, triggering stepwise condensation of DNA. Thus, condensin is able to associate with DNA in the absence of ATP, and then after ATP becomes available, to reorganize *along* DNA so as to condense it. A second *in cis* capability of condensin is generation of chiral knots and supercoiling along DNA [38, 40, 41], suggesting a chiral DNA bending function, regulated by phosphorylation [42].

In vivo experiments suggest that condensin subunits may be to some extent dispensable for chromosome condensation. Using a conditional knockout system it has been observed that, in the absence of one of the condensin SMCs, mitotic chromosome condensation was delayed but eventually proceeded [43]. When isolated, the mitotic chromosomes appeared more easily damaged and less mechanically robust; *in vivo*, vertebrate chromosomes lacking condensin become disorganized during anaphase [44]. In another study, depletion of non-SMC condensin I or II subunits led to defective chromosome segregation and made it more likely that chromosomes would be damaged by spindle forces during mitosis; however chromosome condensation did occur [34]. Condensin appears essential for imparting the mechanical robustness and "structural memory" to mitotic chromosomes necessary for them to survive mitosis [45].

18.2.3 Cohesin SMC Complexes

Eukaryote cells also contain cohesin complexes, which like condensins are based on a heterodimer of ≈ 50-nm long coiled-coil SMC proteins and a kleisin unit, plus additional subunits (Figure 18.2d) [28]. Cohesins have a more open, ring-like form,

appearing as asymmetric polygons in EM studies large enough to encircle chromatin fibers [46, 47].

Cohesins associate with DNA before S phase [48, 49]. After DNA replication, cohesins link the sister DNAs together, holding them together until anaphase, when a regulated protease cuts the cohesin allowing sister separation [46, 50]. In metazoan cells, much of the cohesin initially loaded is removed after S-phase, during prophase and prometaphase. However, an appreciable amount of cohesin remains near centromeres [51], and at least some cohesin stays bound along arms of vertebrate mitotic chromatids up to the point when anaphase segregation occurs [52]. Interestingly, recent studies indicate that partial cohesion depletion suppresses chromosome condensation more strongly than cohesion [53], and also that suppression of cohesion removal has deleterious effects on sister chromatid resolution [54], both studies emphasizing the interplay between chromatid condensation and cohesion [55].

Cohesin associates with DNA at sequence-defined locations. In budding yeast it has been established the spatial distribution of cohesion units changes after their initial loading, eventually becoming concentrated at regions of convergent transcription spaced by roughly 10–15 kb [56, 57]. Experiments of [46–48] support a model whereby cohesins topologically link sister chromatids together, and are able to slide during their redistribution, while other authors have presented evidence suggesting that cohesin binds to individual chromatids [58] and that cohesins are reorganized by transcription-driven dissociation [59]. The situation is markedly different in mammalian cells, where cohesin has been observed to bind DNA in a manner highly correlated with binding of the transcriptional insulator CTCF [60, 61].

It should be noted that a third SMC complexes is found in all eukaryotes, based on an SMC5–SMC6 heterodimer. The function of this third unnamed complex is as yet not completely understood, but it does appear to be involved in both higher-order chromatin organization and DNA repair [62]. SMC-containing complexes are also found in prokaryotes, the prime example being the MukBEF complex in *Escherichia coli*, which is based on a homodimer of the MukB SMC. MukB was identified genetically via a chromosome segregation defect [63]. Overexpression of MukB has been observed to cause chromosome overcondensation *in vivo* [64], to condense DNA in single-molecule experiments *in vitro* [65], and in similar experiments, to be able to bridge pairs of DNAs [66]. Estimates of 1000 bsSMC condensins in *Bacillus subtilis* [67] suggest that there is roughly one bacterial condensin per 10 kb of (replicated) DNA, not terribly different from the eukaryote ratio. Finally, SMCs are found in archaeal species [68], making them a chromosomal protein that can be found in all three domains of life.

18.2.4
Topoisomerase II

Topoisomerase (Topo) II is a large dimeric protein (each polypeptide chain is $\approx$175 kDa) responsible for passing DNA through DNA in an ATP-dependent

manner, so as to resolve DNA entanglements such as those between sister chromatids resulting from DNA replication. Estimates for amounts on mitotic chromosomes vary over the range of one topo IIα for every 20–50 kb of DNA [9, 69]. Remarkably, topo II has been demonstrated to be able to use energy liberated during ATP hydrolysis to selectively (non-randomly) remove DNA entanglements [70]. This topological simplification activity is essential to condensation and segregation of mitotic chromosomes [71]. Furthermore, topo II has been shown to be more effective than topoisomerase I for relaxation of DNA superhelical stress (supercoiling) in chromatin [72]. Metazoan cells contain topoIIα and β isoforms; during mitosis topo IIα is mainly resident on chromosomes while topo IIβ is mainly cytoplasmic [73]. Observations of GFP–topo IIα fusion proteins *in vivo* show it to rapidly exchange on and off chromosomes [74, 75].

Topo II has been suggested to not only efficiently disentangle DNA, but also to play a structural role in mitotic chromosomes. Analysis of non-histone proteins in mitotic chromosomes found "scaffold protein I" [29], later identified as topo II [69, 76]. EM studies have indicated that topo II can bind a crossover of two DNAs [77], and topo II has been observed to be able to recondense protease-decondensed chromosomes [78]. Immunofluorescence experiments have observed topo II localized in chromatid–axial patterns in mitotic chromosomes [33, 79–82] (Figure 18.3). However, although topo II is required for assembly of mitotic chromatids using *Xenopus* egg extracts, 500 mM univalent salt treatment extracts topo II after assembly, without causing noticeable changes in chromatid structure [71]. While essential for the chromatin condensation process, topo II does not appear to be an essential structural element of mitotic chromosomes.

18.2.5 Other Chromosomal Proteins

A number of other chromosomal proteins are present in mitotic chromosomes in numbers comparable to histones. Examples of proteins likely to be important to chromatin folding are high mobility group (HMG) proteins and the barrier to integration factor (BAF-1) protein [7, 83]. HMG proteins have a range of functions [84]: HMGA proteins bind AT-rich DNA, HMGB proteins bend DNA [85], and HMGN proteins reorganize nucleosomes. Intriguingly, BAF-1 molecules (10 kDa) organize into dimers, with two DNA-binding domains capable of interacting with and linking two different DNA helices [86–88].

Interphase chromatin contains a vast number of transcription factors, which in addition to controlling gene expression, play a role in constraining nucleosome positioning and therefore in higher-order folding of interphase chromatin [89]. However, since most transcription factors are removed from chromatin during mitosis, they probably do not play a major role in defining mitotic chromosome structure. A very important exception to this is CTCF, which remains bound to mitotic chromosomes [90]. Since CTCF can bind chromatin loops, and is known to have binding positions correlated with cohesins in mammalian cells [60, 61], it may well play an organizational role in mitotic chromosome folding.

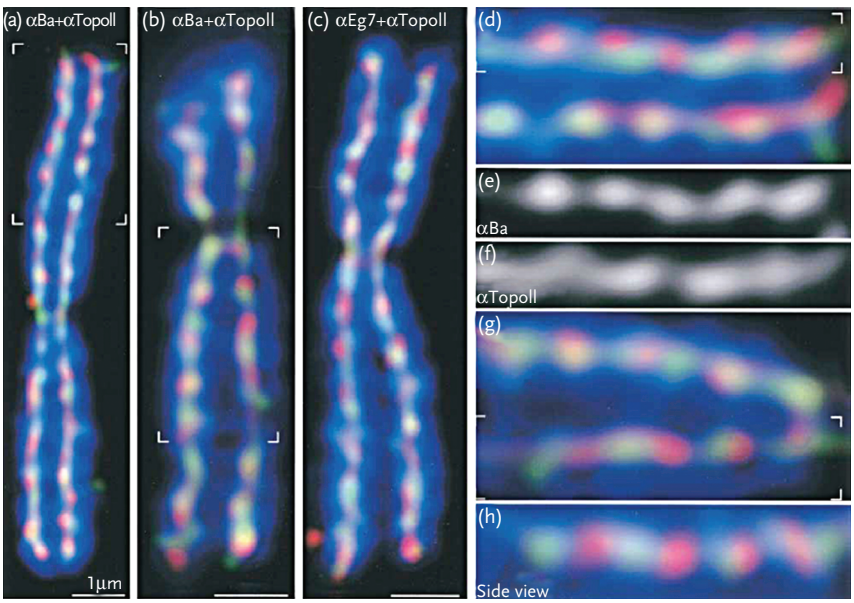

Figure 18.3 Condensin and topo II distributions on HeLa metaphase chromosomes. Chromosomes were stained with DAPI (blue), anti-topo IIα (green), anti-condensin I [αBa, red in (a, b)], and anti-condensin II [Eg7, red in (c)]. Antibody signals occur along the chromatid axis, with condensin and topo II in alternating or coiled regions. (d) Higher magnification images of the box in (a). (e, f) Individual antibody signals of (d). (g) Higher magnification image of the boxed region in (b). (h) A side of the boxed region of (b) obtained from a series of images taken along the focusing axis. Bars, 1 μm. Reprinted from *Developmental Cell* **4**, Maeshima K. and Laemmli U.K., A two-step scaffolding model for mitotic chromosome assembly, Pages 467–480, Copyright (2003), with permission from Elsevier and Cell Press.

18.3
Large-Scale Organization of Mitotic Chromosomes

Mitotic plant and animal chromosomes have a "noodle" shape at metaphase (Figure 18.1), with two parallel chromatids (Figures 18.3 and 18.4) held together by cohesins. The longest human metaphase chromosome is roughly 10 μm long, and slightly less than 2 μm in width, with 247 Mb of DNA folded into each linear chromatid. The longest metaphase newt (*Notophthalmus viridescens*) chromosome is about 20 μm long [91] and slightly more than 2 μm in width. Here the focus is on chromatin packing along the linear arms of the chromosome without discussion of the specialized chromatin folding at centromeres [92].

Our understanding of chromatin folding in mitotic chromosomes at sub-optical scales (<200 nm) is largely based on EM studies. EM visualization of DNA loops extending from a protein-rich chromosome body after histone depletion [93], plus visualization of structures consistent with a loop organization in serially sectioned fixed cells suggest a model for mitotic chromosome structure based on chromatin

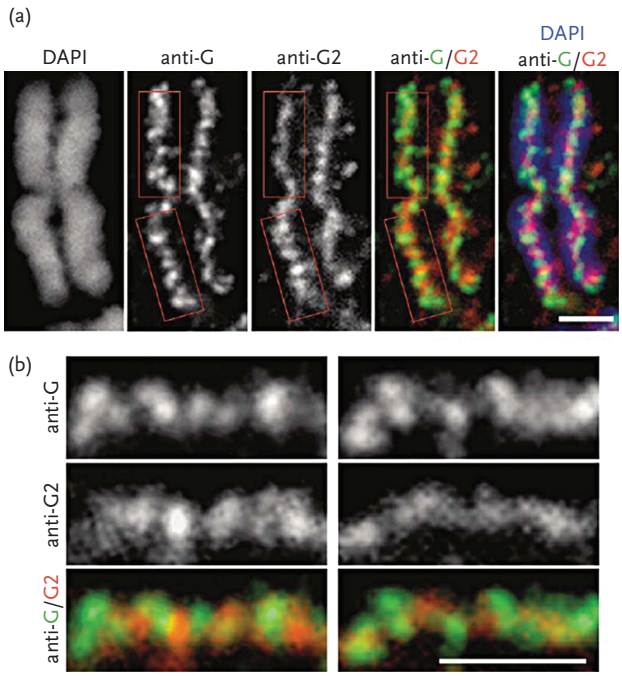

Figure 18.4 Condensin I and condensin II distribution on HeLa chromosomes. (a) Metaphase HeLa chromosome stained with DAPI (blue) and biotinylated anti-hCAP-G (condensin I) and anti-hCAP-G2 (condensin II). Condensins occupy chromatid-axial distributions, with condensin I and II in separate alternating or coiled regions. Right panels show merged images. Bar, 2 μm. (b) Higher magnification images of boxed regions of (a) show alternating condensin I and II domains, with condensin I (green in merge) possibly exterior to condensin II (red). Bar, 2 μm. Reprinted from *Cell* **115**, Ono T., Fang Y., Spector D.L., and Hirano T., Spatial and temporal regulation of Condensins I and II in mitotic chromosome assembly in human cells, Pages 109–121, Copyright (2003), with permission from Elsevier and Cell Press.

loops connected to a non-histone–protein-rich chromaid–axial "scaffold" [81, 94–98]. In human cells, mitotic loops observed in EM experiments are 50–100 kb in size.

Other EM studies suggest a hierarchical folding formed from a succession of coils or folds at progressively larger length scales [99–101]. Proposals have also been made for mitotic chromosome structure which combine loop and helix folding motifs [80, 102–104], and which include an axial "glue" acting on a hierarchically folded chromosome [82].

The general idea that folded domains of chromatin are attached to a chromatid–axial structure is further supported by many studies which have observed axial distribution of nonhistone chromosome structural proteins (Figures 18.3 and 18.4). Topo II has been observed to be axially or helically organized in mitotic chromosomes [33, 75, 79, 81, 82, 105, 106], although the degree to which an axial distribution

is observed appears sensitive to experimental details [71]. Immunofluorescence studies of expanded chromosomes revealed a punctuate, discontinuous distribution of topo II [33, 107, 108]. Live-cell experiments with dyed topo II [109] and GFP fusion proteins [74, 75] disagree as to the degree of its axial localization. It has been suggested that topo II is found where DNA interlocks occur [105, 109], which in conjunction with topo II's dynamic exchange on and off chromosomes might be responsible for the variability in axial localization observed experimentally. The data might be unified if a portion of topo IIα is rather stably bound along chromatid axes, with less stably bound topo IIα populating the chromatid exteriors.

Condensin units have also been observed to be axially organized in mitotic chromatids (Figures 18.3 and 18.4) [3, 30, 32, 33, 81, 82]. Immunofluorescence studies indicate that in animal cells, condensin II may be localized nearer to the chromatid axis than condensin I [30], reflecting the loading of condensin II before condensin I. The same study suggests that condensin I and II may have alternating or helically interwound axial distributions (Figure 18.4). A similar alternating distribution along chromatid axes was observed for condensins and axial topo II (Figure 18.3) [33].

18.4 Mechanics of Mitotic Chromosomes

During mitosis, mitotic chromosomes in animal cells are subjected to spindle forces in the nanonewton (nN, or 10^{-9} N) range [110], sufficient to cause twofold stretching and sharp bending. These large forces involve the coordinated action of many molecular force-generating proteins (i.e., kinesins and polymerizing/depolymerizing microtubules); individual motor proteins typically generate forces of a few piconewtons (pN, or 10^{-12} N), corresponding to work done on the order of 1 kcal/mol per nanometer of motion directed by irreversible chemical reactions (e.g., ATP hydrolysis coupled to protein conformational change). Spindle-generated forces in chromosomes are known to play a role in regulation of the mitotic apparatus [110–114]. More broadly, chromosome mechanics is thought to be central to a variety of aspects of chromosome dynamics [115–120].

Mechanical studies of chromosomes are also useful for studying chromosome structure, for example, through observation of how modification or removal of specific molecules impacts chromosome mechanics. Several of the experiments discussed in the previous section used observations of qualitative changes in chromosome mechanical stability following interference with or depletion of condensins [3, 30, 34, 43] to infer their chromosome-folding functions.

18.4.1 Chromosome-Stretching Experiments

Several groups have carried out mechanics experiments on individual mitotic chromosomes, using glass micropipettes as manipulation and force measuring

tools [104, 121–126]. Similar experiments have been carried out on unreplicated *Xenopus* chromatids assembled using egg extracts [127, 128]. The methods used for these experiments are broadly similar to microneedle-based manipulation of meiotic metaphase chromosomes inside grasshopper spermatocytes [110, 129] and to classic studies of lampbrush chromosome structure [130, 131]. Other manipulation experiments have used microneedles to remove whole genomes from cells to allow experiments with various biochemical reagents [78, 132, 133].

18.4.2 Mitotic Chromosome Stretching Elasticity

Mitotic chromosomes have robust elasticity ("elasticity" indicates reversible deformability), returning to native length after up to fivefold extensions (Figure 18.5) [110, 122, 126]. This extensibility has been used to increase the resolution of chromosome banding [135]. Nicklas [110, 129] was the first to quantify the elasticity of mitotic-like chromosomes (actually meiotic metaphase I and II chromosomes), using microneedles to push and hook chromosomes *inside* grasshopper cells, by pushing on the cell membrane. Bending of the microneedle provided a way to measure forces, and Nicklas found that roughly nN forces caused chromosomes to be stretched to double their native length *in vivo*.

Recent experiments on mitotic newt chromosomes removed from cells and manipulated with micropipettes showed that they could be doubled in length by roughly 1 nN forces [122, 123, 126], in good accord with Ref. [129]. The stretching force increases nearly linearly with extension for elongations of up to four times the native length, allowing one to summarize the elastic response with a single number, the "force constant", or the slope of the force versus elongation curve. Similar results were obtained for chromatids reconstituted using *Xenopus* egg extracts [127]. Interestingly, a broad distribution of chromosome force constants was obtained from single-chromosome stretching experiments [110, 123]; it is not clear whether this variation is due to mitotic stage or is chromosome-specific.

Spindle-scale forces (for animal chromosomes, about 1 nN) on a whole newt chromosome are insufficient to remove histones from DNA. Stretching experiments on assembled chromatin fibers in buffer (typically 10–100 mM NaCl, pH 7.5) Nucleosome removal by force alone (without the aid of histone chaperones) occurs only for forces in excess of about 10 pN [136–138]. However, across a whole animal chromosome of ~ 1.5 μm cross-sectional diameter and therefore ~ 2 μm^2 cross-sectional area, several thousand 30-nm chromatin fibers pass through each chromosome cross-section. Therefore, nN forces on a whole chromosome reduce to roughly pN forces per chromatin fiber, insufficient force to dislodge histones. However, this level of force is sufficient to stretch out a chromatin fiber from a folded (30 nm) to extended (10 nm, or "beads on a string") conformation; in this force range chromatin fibers have been observed to display a nearly linear force versus extension response [136, 137]. The linear reversible elastic range of stretching of whole chromosomes can be attributed to the unfolding elasticity of chromatin fiber without disturbing histone binding [139].

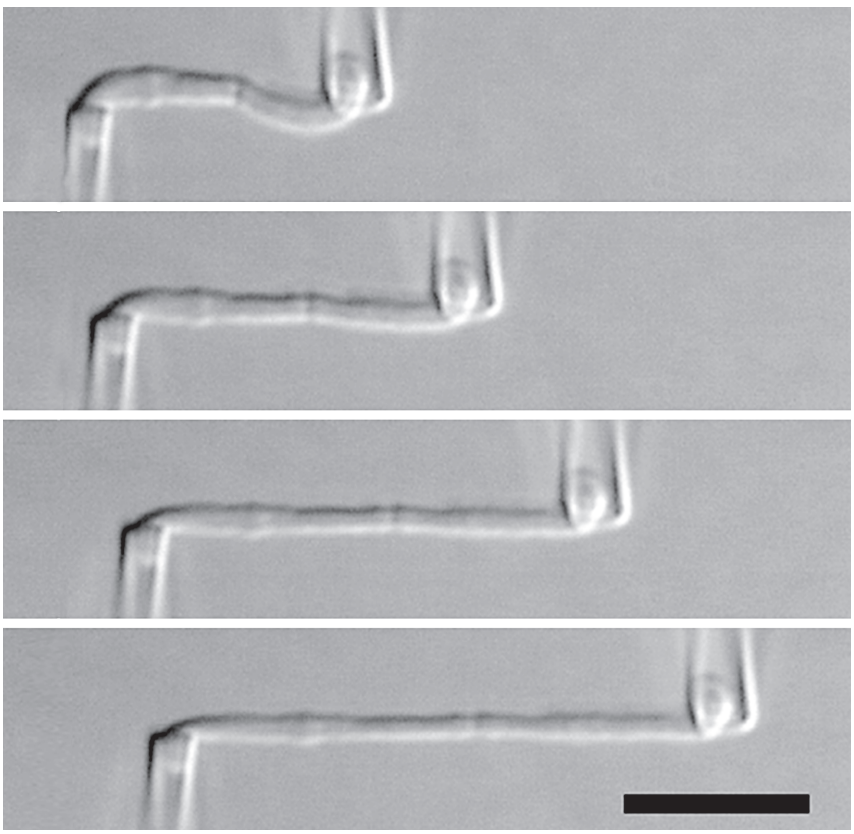

Figure 18.5 Chromosome stretching experiment. Pipettes are used to hold a mitotic chromosome, with left pipette fabricated with a deflection force constant ~1 nN/μm to allow chromosome tension to be measured. Top image shows relaxed chromosome. As the right pipette is moved, the left pipette is observed to deflect from its zero-force position (thin white line). Digital image analysis allows pipette deflections to be measured to about 10 nm accuracy, translating to about 10 pN force resolution. Bar, 10 μm. Adapted from [134].

To describe the elastic properties of a material, one often quotes its elastic modulus. This expresses what stress (force per area) would be required to double an object's length, if the initial linear elasticity were extrapolated. For a mitotic chromosome, this stress is about 500 Pa [121, 122, 140] (1 Pa = Pascal = 1 N/m^2 is the SI unit of pressure and stress). A 500 Pa modulus is low, even for a very loose high-polymer gel. 1% agarose gels have a modulus of about 10 kPa (10 000 Pa), plexiglass and folded biomolecules (B-DNA and globular protein domains) have moduli near 1 GPa (10^9 Pa), and covalently-bonded materials (metals, glasses) have moduli in excess of 10 GPa. The modulus is useful since it expresses the strength of the interactions holding a material together, in a way which is independent of size or shape. Table 18.1 lists moduli of mitotic chromosomes studied to date.

Table 18.1 Physical properties of mitotic chromosomes. Ranges for values indicate the width of distribution of measured values, not measurement errors.

Chromosome type	Experiment conditions	Stretching (Young) modulus (Pa)	Bending rigidity (J m)	References
Drosophila metaphase chromosome	*In vivo*	ND	$\sim 6.0 \times 10^{-24}$	[141]
Grasshopper metaphase I and anaphase I chromosome	*In vivo*	200–1000 (ave. 430)	ND	[110, 142]
S. cerevisae pachytene chromosome	Cell culture medium	ND	$\sim 5.0 \times 10^{-26}$	[119]
Newt (*N. viridescens*) prometaphase chromosome	Cell culture medium	100–1000	$1.0–3.0 \times 10^{-22}$	[122, 125, 126, 134, 143]
Newt prometaphase chromosome	*In vivo*	ND	$2.0–5.0 \times 10^{-23}$	[134]
Xenopus prometaphase chromosome	Cell culture medium	200–800	$0.5–2.0 \times 10^{-23}$	[134]
Xenopus prometaphase chromatid	Cell culture medium	~ 300	$\sim 5.0 \times 10^{-24}$	[134]
Xenopus reconstituted chromatid	*Xenopus* Egg extract	1000	1.2×10^{-26}	[127, 128]

ND: quantity not directly measured.

Mitotic chromosomes have a modulus roughly one-millionth of the modulus of the molecules from which they are composed, indicating that they are loosely internally linked. Their extensibility of up to five times without apparent damage indicates that the internal structure must involve loosely compacted domains of chromatin that can readily unfold under force. Further evidence for unfolding of polymer-like folded domains is given by dynamic experiments that show a slow, viscous response to applied forces consistent with the elastic response of a flexible polymer network [122, 144, 145]. Chromosome experiments require very slow (100 s) extension–relaxation cycles to stay in mechanical equilibrium; rapid stretching can cause a buildup of large viscous forces and irreversible changes to chromosomes [126].

By contrast, following extension to fivefold or greater extensions and forces in the 10–20 nN range, mitotic chromosomes are permanently lengthened, suggesting that internal "links" holding chromatin in its compacted form are being broken [122]. Similar irreversible elasticity is seen for unreplicated mitotic chromatids following sufficient extension [127]. After slow extensions beyond about 30 times native length followed by relaxation, mitotic chromosomes end up not only longer than native, but also thicker, without appreciable loss of histones [122]. This suggests that if sufficient numbers of chromatin interconnects are broken up, the

then less constrained chromatin swells up. Experiments in the irreversible stretching regime involved up to 20 nN forces, corresponding to several pN forces per chromatin fiber by the cross-sectional argument mentioned above. This is insufficient force to quickly break chemical bonds [146], but is sufficient to break protein–DNA and protein–protein interactions. Irreversible stretching behavior of chromosomes is most likely due to disruption of chromatin-crosslinking elements.

18.4.3 Mitotic Chromosome Bending Stiffness

The bending stiffness of a chromosome can be measured without applying external stresses. Any small flexible rod undergoes random bending fluctuations at room temperature by thermal forces; the stiffer the rod, the smaller the fluctuations. The approach of measuring thermal bending fluctuations has been widely used to study mechanical properties of biopolymers and biopolymer complexes (e.g., [147]). One usually measures the length over which thermally excited bends occur, or the "persistence length" [127, 147]. The bending stiffness is just the persistence length times a thermal energy factor ($k_BT = 4 \times 10^{-21}$ J where T is absolute temperature, essentially the same for all biologically relevant temperatures). Thus, the bending constant is measured in Joule-meters (Table 18.1).

When prometaphase chromosomes are isolated from either newt or *Xenopus* cells, very small bending fluctuations are observed: the "persistence length" is found to be many times the length of the chromosome [134]. By contrast, when unreplicated *Xenopus* chromatids assembled using egg extracts are observed (after dilution into suitable buffer to avoid non-thermal fluctuations generated by condensins and other ATPases), one sees drastic thermal bending fluctuations by large angles, and one measures a persistence length of roughly 2 μm, much shorter than the 20-μm long chromatids [127]. Reconstituted *Xenopus* chromatids have a bending stiffness about 500 times less than *Xenopus* chromosomes [134], indicating a profound difference in internal structure between unreplicated egg-extract chromatids and prometaphase chromosomes from differentiated cells.

A rod made of a material with a well defined elastic stretching modulus has a bending stiffness which is proportional to that modulus. Given stretching moduli and bending stiffness for chromosomes, one can ask whether they are consistent with this uniform-elastic-medium result. For both newt and *Xenopus* chromosomes from tissue culture cells, the bending stiffnesses are consistent with their being made of a uniform elastic medium with stretching modulus of 500 Pa [134].

In contrast, the *Xenopus* egg-extract chromatids are thus about 500 times easier to bend than one would expect for a uniform elastic medium, suggesting that egg-extract chromatids have the organization of a halo of chromatin attached to a very thin internal elastic structure, that is, with no crosslinking in the exterior halo region [127]. If two such chromatids were linked together by cohesins as in the prometaphase chromosomes, the resulting structure would be much more difficult to bend, possibly explaining the large difference in bending modulus between egg-extract chromatids and somatic-cell chromosomes.

Bending fluctuations have also been used to estimate stretching modulus in systems where stretching experiments would be very difficult or impossible due to the small size of the chromosomes involved. Bending fluctuations of *Drosophila* embryo mitotic chromosomes in colchicine-poisoned cells led to an estimate of 10 Pa, significantly smaller than the 500 Pa measured for amphibian tissue culture cell chromosomes [141]. It would be quite interesting to know the corresponding stretching modulus; recall that *Drosophila* chromosomes are thought to be dominated by highly dynamic condensin I [36]. Finally, a recent experiment [119] has observed bending fluctuations of isolated yeast (*Saccharomyces cerevisae*) meiotic chromosomes, measuring a persistence length ~ 12 μm or a bending modulus $\sim 5 \times 10^{-26}$ J m.

18.4.4
Reversible Folding and Unfolding of Mitotic Chromosomes by Salt

As mentioned above, chromatin fibers can be unfolded from 30 to 10 nm fiber form by shifting a univalent salt concentration to low (10 mM) values where electrostatic repulsion overwhelms nucleosome stacking interactions [14]. Similar experiments for whole chromosomes lead to dramatic results: mitotic chromosomes can abruptly decondense and recondense in response to cycles in univalent salt concentration [132, 148–151].

Chromosome elastic response after inducing unfolding with salt followed by return to native buffer treatments matched the pre-treatment response [143], suggesting refolding to a near-native state with little or no loss of protein. For univalent salt (NaCl) both low salt ($<$100 mM) and high salt (>100 mM) conditions led to chromosome unfolding. Thus, maximum chromosome compaction as a function of NaCl concentration occurred for essentially physiological (100 mM) levels. At low salt, decondensation is due to electrostatic repulsion driving adjacent nucleosomes apart, essentially unfolding 30 nm chromatin to the 10 nm form. At high salt, attractive electrostatic interactions favoring chromosome compaction become screened by high ion density, leading to expansion of the chromosome. This unfolding is dramatic; for 400 mM NaCl a newt chromosome reaches a volume roughly five times larger (longer and thicker) than its native state.

For divalent salt ($MgCl_2$) different results were found [143]. Low divalent concentrations (10 mM) led to compaction of the chromosome (the opposite effect of the univalent salt), possibly due to Mg^{2+}-mediated attractions between single negative charges along chromatin fibers. At high divalent concentrations (100 mM), chromosome expansion was observed, again likely due to screening out of charge interactions. In all cases where univalent and divalent salts were used, the chromosomes rapidly recovered their native elasticity when the flow of ions was stopped.

These experiments indicate that far from being tightly bound together, chromatin in mitotic chromosomes is greatly compacted by relatively weak electrostatic interactions which can be easily disrupted. The native state is easily recovered following its disruption. Interestingly, by use of trivalent cations, the volume of a chromosome can be reduced by about one-third. Thus, the native state is well

below its maximum density; much of the mitotic chromatid volume is mobile small molecule species, presumably mostly water. Notably both the unfolding (expansion) and hypercondensation (contraction) driven by salt was always observed to be isotropic, with length changed by the same factor as width [143].

18.5
Molecular Connectivity of Mitotic Chromosomes

18.5.1
Nucleases Disintegrate Mitotic Chromosomes

Elasticity experiments indicate that mitotic chromosomes are highly extensible. A main question one is led to ask is whether this extensibility and elasticity is due to DNA (chromatin) extensibility, or whether chromosome elasticity comes from extensibility of protein structures, for example, SMCs. A closely related question is whether the chromatin in a mitotic chromosome is folded by being looped or attached to a protein scaffold which is stably connected by protein–protein interactions, or alternately whether non-histone proteins which stabilize mitotic chromatin are essentially disconnected from one another so as to act as chromatin "crosslinks."

One way to attack these questions is to use enzyme digestion to determine how the mechanical properties of chromosomes are modified by cleavage of different molecular components. Classic experiments of this type [152, 153] showed that DNAase fragmented amphibian lampbrush chromosomes (meiotic prophase), and that this was not done by RNAase and proteases. Quantitative DNAase cleavage experiments determined that lampbrush chromosomes contained four parallel DNA molecules (i.e., the four chromatids present at meiotic pachytene) [131]. Later experiments studied the access of restriction enzymes to loop domains in lampbrush chromosomes [154].

This approach has been used to examine the effect of cutting nucleic acid on mechanical properties of individual mitotic chromosomes. Digestion of DNA has long been known to disrupt mitotic chromatin [132, 148]. Experiments with micrococcal nuclease and frequently blunt-cutting restriction enzymes (Figure 18.6 shows an experiment with the four-base cutter AluI) revealed that even isolated DNA cuts first rapidly eliminates newt mitotic chromosome elasticity, then causes cleavage of the whole chromosome, collapse of the remaining chromatin into a spherical droplet indicating all loss of elasticity and memory of its original shape, and finally dissolution of the chromosome [155]. Experiments on reconstituted *Xenopus* chromatids obtained similar results [128]. Since cutting of DNA alone leads to complete disruption of the mitotic chromosome, non-histone proteins are not connected together [155]. Instead proteins such as topo II and SMC complexes are disconnected from one another, and must act as crosslinkers to form a "gel" or "network" of chromatin. Experiments with a series of specificities of blunt-cutting restriction enzymes allow a rough estimate of the inter-crosslink distance of approximately 15 kb [155].

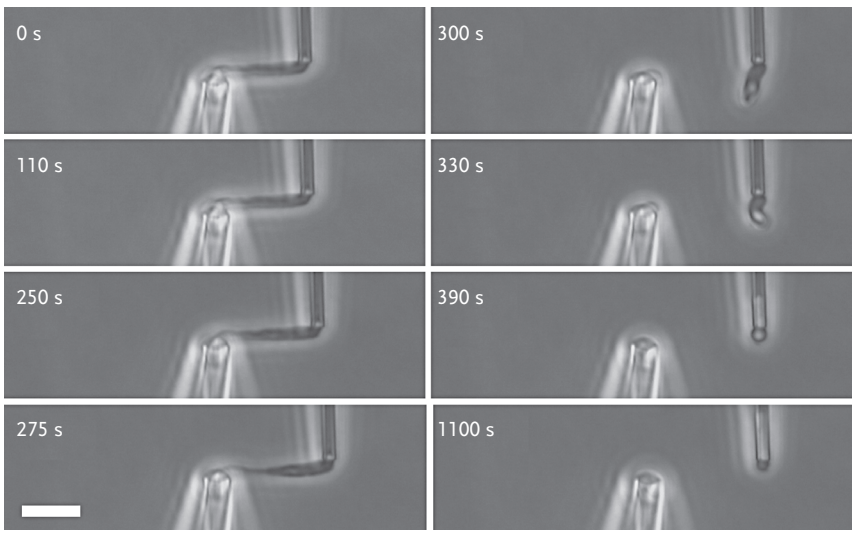

Figure 18.6 Digestion of newt mitotic chromosome by four-base specificity blunt-cutting restriction enzyme AluI. Initial (0 s) image shows native chromosome under low tension (100 pN). As digestion proceeds, force measuring pipette (right) relaxes, indicating that chromosome has lost elastic modulus (250 s). Additional digestion thins (275 s) and cleaves (300 s) chromosome; additional digestion converts chromosome to "droplet" of chromatin fragments (390 s) and finally eliminates most of the chromosome outside the right pipette (1100 s). Bar, 10 μm. Figures courtesy of M.G. Poirier.

18.5.2
Proteases Gradually Expand but Do Not Cleave Chromosomes

Trypsin and proteinase K treatments of whole genomes cause a volume expansion of human mitotic chromosomes [132]. Force measurement experiments on *Xenopus* reconstituted chromatids [128] showed that the elastic stiffness was gradually reduced by protein digestion. Protease experiments on newt mitotic chromosomes obtained similar results: exposure to either trypsin or proteinase K gradually decondensed and softened chromosomes but without ever entirely eliminating their elastic response or cleaving them (Figure 18.7), and with more length increase than width increase [125]. It was also found that partial digestion of mitotic chromosome protein induced sensitivity of the elastic modulus to six-base-specificity blunt-cutting restriction enzymes. All of these effects are consistent with a network organization of the mitotic chromosome, with a strong degree of anisotropy of folding to allow strong lengthening in response to mild protein digestion [82, 125].

18.5.3
DNA Topology: Entanglement and Supercoiling of Chromatin in Mitotic Chromosomes

Another potential contribution to connections between different chromatin segments in a mitotic chromosome are topological constraints on DNA. Given that

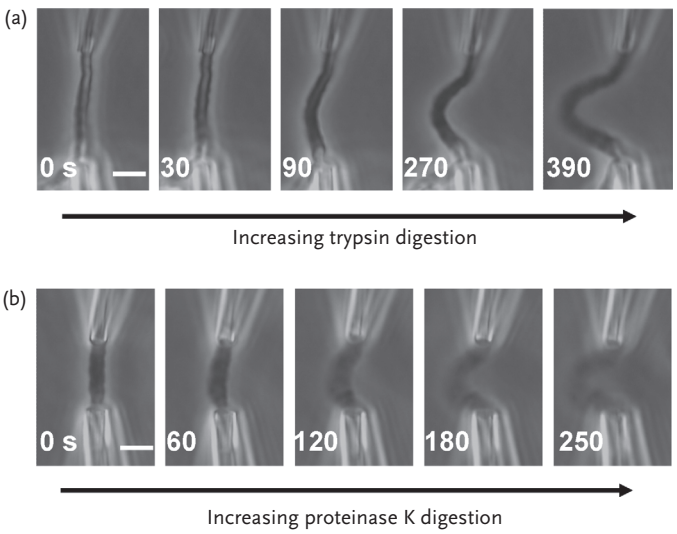

Figure 18.7 Decondensation driven by digestion of protein in newt mitotic chromosome. (a) Progressive lengthening and widening of chromosome resulting from increasing trypsin digestion; digestion time shown in seconds. Expansion is anisotropic, with length increasing more than width. Chromosomes remain elastic during these digestion experiments. (b) Similar effects of proteinase K. Bars, 5 μm. Adapted from [125].

mitotic chromosomes contains a tremendous length of chromatin packed into a small volume, entanglements are likely to be present. A recent experiment has shown that exposure of isolated chromosomes to topo IIα relaxes their elastic modulus in an ATP-dependent manner [156]. Given that parallel experiments showed a much smaller effect of topo IB, a DNA supercoiling–relaxing enzyme, the simplest interpretation of this result is that DNA in the mitotic chromosome is self-entangled. Notably, condensin SMCs have been shown to have a propensity to generate positive-writhe knotting of DNA *in vitro* [38, 41], and topo II is known to be required for chromosome *condensation* [157–160]. However, topo IB is less effective at relaxing supercoiling in chromatin than topo II [72], so it is possible that chromatin supercoiling also plays some role in mitotic chromosome compaction.

18.5.4 Interchromosome Linkers

A feature of chromosome structure evident whenever mitotic chromosomes are microdissected from animal cells is that different chromosomes (replicated chromatid pairs) are connected together by thin, highly extensible filaments. These have been observed in chromosome isolation experiments for many years [132, 161, 162], but have always been controversial since they contradict the common wisdom that different chromosomes are separate gene linkage units. Definitive observation of such filaments inside a live cell has not been reported, and observing these filaments outside the cell always invites the criticism that they are an artifact of chromosome isolation [163].

A number of authors have reported that mitotic interchromosome linkers are cut by nucleases [123, 132], and therefore that they are based on DNA. Linkers between mitotic newt chromosomes are encountered during most chromosome isolations; once in every few dozen experiments one observes a loose chromosome free of such linkers. Although their mechanical effects are obvious, interchromosome linkers can barely be observed by phase contrast or DIC, indicating that their thickness is in the range of 100–200 nm.

A study of chaffinch (bird) chromosomes revealed filaments containing a centromeric satellite DNA extending between nonhomologous metaphase chromosomes [164, 165]. Interchromosome filaments containing centromeric satellite DNA and CENP protein have also been observed in mouse tissue culture cells by [166]. The function of interchromosome filaments remains an enigma.

18.6
A Model for Mitotic Chromosome Structure and Function

18.6.1
Mitotic Chromosomes are Chromatin Networks

Biochemical and biophysical results put constraints onto models of how the mitotic chromosome is folded. DNA digestion experiments indicate that the basic organization of the mitotic chromosome is that of a chromatin network or gel with non-DNA crosslinking elements which are not bound to one another [155]. Note that "crosslinking" does not necessarily imply covalent binding; the chromatin crosslinkers of interest here may act via non-covalent protein–DNA, protein–protein, or even topological interactions [28]. It must also be noted that digestion experiments do not rule out an inhomogeneous spatial distribution of crosslinks inside chromatids. However, recent EM studies observed a surprisingly regular network of chromatin in the interior of egg-extract-assembled chromosomes [167].

Chromosome elasticity experiments combined with single-chromatin fiber-stretching experiments are consistent with isolated scaffold elements. If the crosslinks were bonded together into a contiguous protein scaffold, one would not expect such a large range of elastic force response, since folded proteins are known to be relatively rigid: condensin-folded structures along single DNAs [39] require 10 pN forces to be broken, and coiled-coils require even higher 20 pN forces to be uncoiled [168]. The known high degree of extensibility of chromatin fiber [21, 135, 136] can simply explain the large extensibility of mitotic chromosomes at relatively low forces, but only if chromatin crosslinking elements are not bound to one another.

18.6.2
What Are the Crosslinking Elements?

Current data suggests SMC complexes as prime candidates for crosslinkers [169]. Animal condensin units can by themselves condense DNA [39] and are essential

to chromatid condensation in the egg-extract system [3]. Depletion of condensins in cells impairs chromosome condensation and causes chromosomes to be mechanically weak [30, 31, 34, 43]. Finally, estimates for the numbers of condensins on animal chromosomes are consistent with inter-crosslink distances inferred from digestion experiments [155].

Cohesins have a chromatin-crosslinking function in mitotic chromosomes, given that they hold sister chromatids together, possibly by a topological mechanism [28]. They appear to be mobile and affected by transcription in yeast [56]. Cohesins provide crosslinks between sister chromatids which persist until anaphase.

It is possible that there are other as yet uncharacterized mitotic crosslinking elements, given that condensin depletion experiments suggest that the cell may have alternatives to condensins to drive chromosome condensation [31, 34, 43–45, 108].

18.6.3
SMC-Crosslinked Chromatin Network Model of Mitotic Chromosome Condensation

The results discussed above, combined with the conclusions of [33, 82, 98, 101, 104, 169–174] suggest the following scenario for vertebrate chromosome condensation (Figure 18.8). Numbers are approximate and apply to the human case.

Cohesins are bound before DNA replication. As DNA replication proceeds, it may drive the condensation and segregation of sister chromatids, for example, through the extrusion of replicated DNA domains between cohesion zones [175, 176]. Cohesins become organized into intermittent clusters along replicated sister chromatids, at positions programmed by DNA sequence. Mechanisms for cohesion positioning appear to vary by species: DNA transcription plays a major role in yeast [49, 56, 57], while interactions with other proteins (including CTCF) position the cohesins in mammalian cells [60, 61]. The common outcome in all organisms is establishment of well separated, sequence-programmed points of cohesion, preceding mitotic condensin activity.

Next, during prophase, condensin II binds to chromosomes, and acts to condense the parallel sisters (Figure 18.8a–c). If condensin II acts *in cis* along DNA (as observed in single molecule experiments [39]) then crosslinking and potential topological relinking of sisters does not occur. Instead, remnant sister catenation is pushed out of the condensin-rich regions, to form tight DNA crossings favored by topo II [105], and generating alternating condensin- and topo II-rich regions [33].

A plausible mechanism for condensin II to accomplish chromatin condensation *in cis* is for it to initially bind short, contiguous segments of chromatin of length similar to its ≈ 50 nm size (also comparable to the ≈ 30 nm persistence length of chromatin fiber [137, 177]) and then to gradually reorganize *in cis*, or alternately to stimulate binding of additional condensin units at neighboring chromatin sites, so as to progressively condense chromatin between cohesin "boundaries" [171, 172]. The outcome would be a series of segregated loop-like chromatin domains, separated by cohesin clusters along the chromatid axis, and a highly contracted

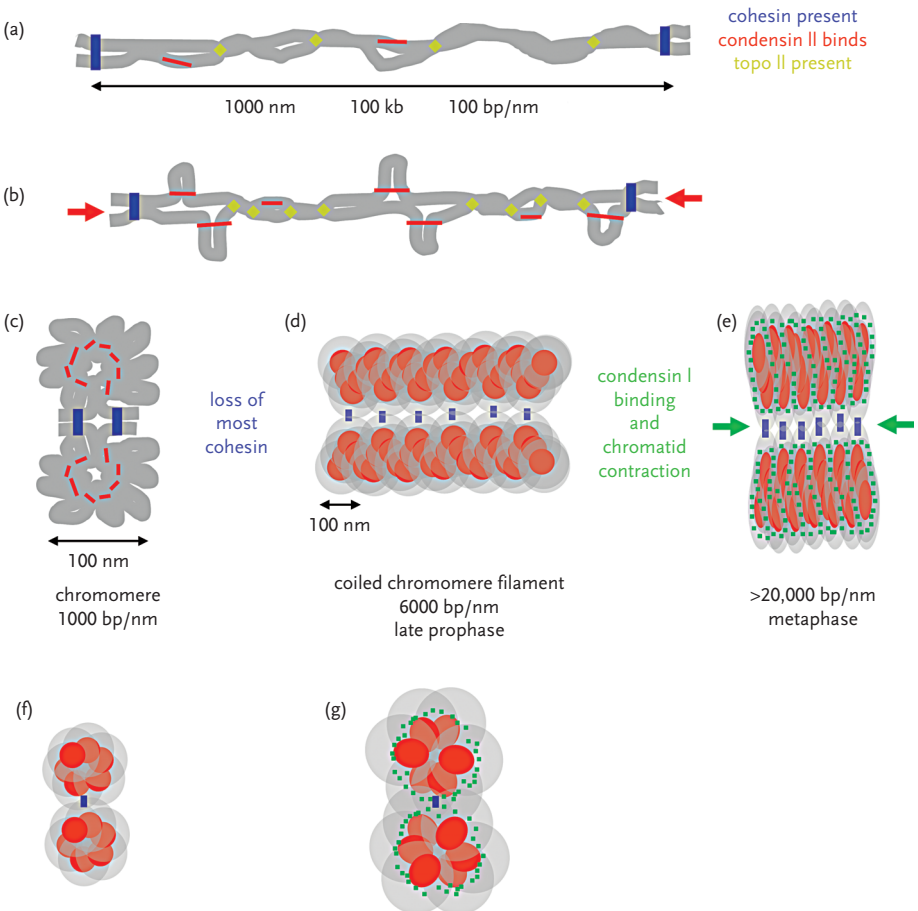

Figure 18.8 Model of human mitotic chromosome folding. (a) Part of the replicated sister chromatids (gray lines represent 30 nm fiber containing 100 bp/nm). Roughly 1000 nm of 30 nm fiber (100 kb of DNA) is between between successive cohesin domains (blue bars). (b) Binding of condensin II (red bars) during early prophase begins to organize loops of chromatin, gradually shortening chromosome; topo II (yellow diamonds) binds to and resolves chromatid crossings trapped between condensin-rich regions. (c) At end of prophase, the chromatin length between cohesion blocks has been absorbed into condensin-looped regions; the result is a chromatin "rosette" or "chromomere" of roughly 100 nm diameter with a condensin II core. Topo II and adjacent looped regions of chromosome are not shown for clarity. (d) Parallel chromatids corresponding to a series of rosette chromomeres organized as in (c). Gray balls (100-nm diameter) correspond to rosette/chromomere structures of (c) [note change of scale relative to (c)]. Red balls indicate their condensin II-rich cores. Successive chromomeres are stacked, folded or coiled to achieve a packing density of 6000 bp/nm, about 2000-fold higher than linear DNA. (e) Binding of additional bulk chromatid-condensing factors including condensin I (green dots) compresses chromosome along length, increasing its width. Length compaction at this stage is roughly 20 000 bp/nm, approaching 10 000-fold relative to DNA. (f) End-on view of prophase chromosome of (d); chromatids contain chromomeres (gray circles) with condensin II-rich cores (red balls). (g) End-on view of metaphase chromosomes of (e); addition of condensin I (green) compresses chromosome lengthwise, increasing chromatid diameters.

chromosome. These loop-like chromatin domains might be folded or interwound by topological effects of condensin [38, 41] or by binding of metal ions [169, 178]. Condensin locations may be sequence-programmed: evidence exists supporting defined yeast condensin binding sites spaced by roughly 10 kb [179]. Furthermore, a study in yeast revealed condensin to be colocalized with the cohesin loading factor Scc2/4, at sites for the RNApol III transcription factor TFIIIC [180].

Local reorganization *in cis* over distances of tens of kilobases would make the condensin II distribution appear stationary at optical scales [34], while still generating a large amount of compaction and without adding links between sister chromatids. Quite to the contrary, tension built up between adjacent chromatids would drive topo II to gradually segregate them [33, 116].

This scheme organizes prophase chromatids into a string of rosette-like "chromomere" structures [181] of a size similar to the observed folding intermediates [1, 82, 99, 100, 182–184]. For human chromosomes, these proposed structures contain about 1000 nm of 30-nm fiber (100 kb of DNA), with a condensed volume of roughly 10^6 nm^3 and therefore with a diameter of roughly 100 nm (Figure 18.8c).

The chromomeres can be folded or coiled (e.g., like nucleosomes in 30-nm fiber) only if there is a gradual loss of cohesin along chromatid arms [54, 55, 104]: the cohesins of Figure 18.8c oppose longitudinal condensation beyond roughly 1000 bp/nm, with higher compaction factors requiring cohesin removal. Removal of cohesin and further folding gives a further sixfold compaction, generating at a 6000 bp/nm mid-prophase chromatid (Figure 18.8d; note that the gray balls represent chromomere units of roughly 100 nm diameter).

Volume conservation indicates that the chromosome becomes thicker by an amount approximately equal to the square root of the length compaction. For the 60-fold length compaction of chromatin fiber into the human prophase chromatid described above this is a factor of eight (times the 30-nm fiber thickness), resulting in segregated prophase chromatids that are 250 nm thick with a condensin II-enriched core region [82].

Then, at NEB, condensin I binds, acting as a highly mobile [34], reversible chromatin crosslinker. Condensin I acting as a reversible crosslinker in the chromosome interior drives chromatids to adopt a configuration with lower surface area, driving longitudinal compaction and transverse thickening after NEB (Figure 18.8e) [82]. This effect is analogous to surface tension driving the shape of a liquid droplet to be spherical; but for a chromosome, the underlying chromatin network opposes the formation of a sphere and maintains an anisotropic shape. The result is a metaphase chromatid which is shortened and thickened relative to prometaphase, with a condensin II-rich core, covered by a layer of condensin I (Figure 18.8e).

The final metaphase length to width ratio is determined by mechanical balance of condensin I condensation versus chromatin network elasticity. Condensin I-driven longitudinal compaction may force buckling or folding of the central nonhistone protein-rich chromatid axis [80, 102]. Sufficient crosslinking by condensin I to drive longitudinal compaction also provides mechanical stabilization: consistent with this, depletion of condensin I has been observed to significantly weaken metaphase chromosomes [34].

In experiments where some condensin subunits are depleted or mutated, condensation might still be driven by partial condensin activity, by other DNA-condensing proteins, or by direct nucleosome–nucleosome attraction, leading to eventual segregation of adjacent chromatids [34]. Note that *trans*-fiber crosslinking factors must bind and unbind, either by being ATP-cycled, or by simply binding rather weakly, in order to ensure chromatid segregation [116].

This model predicts trends across species: the larger the distance between cohesin domains, the greater is the length compaction. Metaphase cohesin interdomain distances in vertebrates must be much greater than the 15 kb observed in yeast; for *Xenopus* the cohesin density has been estimated to be one per 400 kb [170]. Inter-cohesin domain distances should correlate with mitotic loop size and possibly with convergent transcription domain size [56] and replicon size [185]. Notably, condensin binding sites in yeast have been found to be correlated with DNA replication landmarks [179].

18.6.4
Lengthwise Condensation and Chromosome Segregation

The condensation process described above is "lengthwise condensation," where the long chromosomal fiber is progressively condensed along its length by *in cis* folding. This type of folding is highly distinct from that of classical polymer collapse (e.g., formation of a compact globule in a bad solvent), whereby a polymer is indiscriminately stuck to itself and its neighboring polymers. The gradual increase of stiffness and decrease of overall length generated by lengthwise condensation strongly drives the disentanglement of nearby chromosomes from one another. Topoisomerases are biased to progressively disentangle different chromosomes from one another as lengthwise condensation proceeds [186]. In this way a locally controlled structural transformation of the chromosome to its mitotic form can drive chromatid and chromosome segregation.

Lengthwise condensation suppresses *trans* contacts since chromatin–chromatin contacts are made between the surfaces of adjacent folding intermediates as condensation proceeds. In this case one can expect the average number of contacts between nearby chromosome segments which are N bases apart to vary as $1/N$, the same behavior encountered in the "random crumpled globule" model of interphase chromosomes [187]. At the sequence scale where one reaches the chromosome width (roughly 20 Mb for human metaphase), the contact probability should dramatically decrease, since at larger scales the chromosome is linear. The contact distribution could be measured by the use of "chromosome conformation capture" [187] applied to purified metaphase chromosomes.

18.6.5
Stretching and Bending Elasticity

In this model cohesin and condensin I and II are not bonded together, but act as isolated chromatin crosslinkers, so that a whole chromosome can be stretched

simply by the stretching of chromatin fibers between the network nodes. As mentioned above, extension of individual chromatin fibers accounts for the roughly nN force needed to double the length of a vertebrate chromosome. The fivefold reversible elastic response of whole chromosomes correlates well with the sixfold extension obtained when chromatin is converted from 10 to 30 nm form. The action of condensin I and possibly other crosslinking mechanisms through the body of the chromatid generates the mechanical coupling needed to generate bending elasticity.

Beyond a fivefold extension, one can expect to start to break crosslinking elements at roughly 20 nN forces (given 2000 fibers in parallel, this corresponds to 10 pN per condensin, the force required for condensin disruption [39]). The result is permanent chromosome lengthening and widening, with lengthening predominating due to the prometaphase condensin I-driven contraction [122].

The small bending stiffness of egg-extract-reconstituted chromatids [126] may be a result of the unreplicated chromatids having regions of individual chromatin fibers between chromomeric domains which can act as "hinges." Consistent with this, under large extension egg-extract chromatids extend by the formation of thin, extended fibers between thicker chromosome domains [127], not seen for comparable extensions of chromosomes from animal cells [122]. Furthermore, and also supporting this hypothesis, are observations of [188] that the bending stiffness varies along the egg-extract chromatids.

The difference between the egg-extract and somatic cell chromosomes may also be a consequence of the embryonic developmental state of the egg extracts. The condensin I to condensin II ratio in egg extracts is about 5 : 1, while in somatic HeLa cells it is closer to 1 : 1 [30]. Metaphase chromosomes in *Xenopus* embryos are twice as long and substantially narrower than those in swimming larvae [189], the difference in condensation perhaps being due to different ratios of condensin I and II, cohesin domain or replicon size [190], or developmental variation in linker histone usage [19].

18.6.6
Effects of Cutting and Removing Molecules

For this model cutting DNA sufficiently frequently results in a loss of elasticity (due to disconnection of chromatin), with cleavage of the chromosome only if the crosslink elements are not bound together. Insufficient cutting (less than one cut per crosslink) does not change chromosome elasticity.

If protein is cut instead, histone tails and other exposed protein structures along the chromatin fibers are cleaved, causing chromatin fiber unfolding and lengthening. This drives gradual isotropic expansion, similar to that observed for shifts in univalent salt concentration [143]. However, crosslinkers are also cleaved, possibly causing a less symmetric effect: the hypothesis of a condensin I-driven longitudinal compaction discussed above can explain the anisotropic unfolding of prometaphase newt chromosomes observed to result from protease treatment [125]. Under the assumption that condensin I is the last major crosslinker added to

chromosomes, it is the crosslinker most exposed to general proteolysis. Cutting condensin I releases constraints that longitudinally compacted the chromosome, leading to longitudinal expansion. Of course general proteolysis also cuts histone tails and other proteins along chromatin which contribute to the transverse swelling observed during the proteolysis of whole chromosomes.

Specific cleavage of condensin (e.g., using engineered kleisins with suitable specific protease sites) on assembled metaphase chromosomes could be instructive. There may be large differences between cleavage of condensin I and condensin II units, given their differing functions in chromosome compaction [30]. Based on the above model, condensin I cleavage should produce lengthening and narrowing and a reduction in elasticity of metaphase chromosomes, while condensin II cleavage should produce little effect (since condensin I acts as a more general stabilizer and crosslinker "on top" of condensin II). However, simultaneous condensin I plus condensin II cleavage should produce lengthening and widening similar to that seen by forced chromosome unfolding [122]. Of course, the possibility remains that additional cross-linking elements may be present in sufficient numbers to maintain some chromosome integrity when condensin is disrupted. An interesting question is how having condensin I or II entirely absent could affect condensin cleavage experiments.

Network, hierarchical folding, and radial loop models are unified by the model described above. If histones are suddenly removed, there is a release of DNA length and a large degree of swelling of the chromosome, as a more severe and irreversible version of the result of swelling of chromosomes by high or low univalent salt [143]. In the case of histone removal, loop-like domains of DNA are observed [93], a result of the domain structure of early prophase condensation.

18.7 Open Questions

Despite the large amount of progress made analyzing mitotic chromosome structure and dynamics, we are only starting to understand exactly how chromosomes are folded up during cell division and how that folding is coupled to chromosome and chromatid segregation. A pressing question regarding mitotic chromosome organization is what molecular interactions are responsible for converting the chromosomes from their relatively decondensed interphase form to their highly condensed and geometrically regular mitotic form. As discussed above, nucleosome modifications coincident with this conversion have been discovered, but the questions of exactly how (or if) mitotic marks like H3S10 phosphorylation are interpreted by chromosome condensation machinery remain open.

The next level of mitotic chromatin compaction appears likely to be mediated by condensin SMC complexes. However, exactly how condensins are targeted and bind to DNA (or to chromatin) and reorganize it, how ATP hydrolysis is coupled to this process, and how condensation activities of condensins are regulated, are all

poorly understood. Furthermore, the way that condensins organize together into their chromatid–axial pattern observed in microscopy experiments is obscure. Condensins appear to be chromatin-condensing machines, yet we have limited knowledge of their mechanistic function.

At the largest scales, mitotic chromatids become self-organized into remarkably regular linear structures which are fully segregated from other chromosomes and their adjacent duplicate sisters. How chromatin folding (mediated by molecules tens of nanometers in size) is able to precisely select the formation of micron-wide chromosome arms while driving out entanglements between sister chromatids and separate chromosomes remains an intriguing puzzle. Perhaps the use of emerging technologies, including high-throughput chromosome conformation analysis [187], single-molecule analysis of chromatin assembly and condensation machinery [39], and nanometer-precision tracking of molecular interactions inside live cells using ultra-sensitive fluorescence microscopy [191], will provide some answers to these many questions.

Acknowledgments

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