13S Condensin Actively Reconfigures DNA by Introducing Global Positive Writhe: Implications for Chromosome Condensation

Keiji Kimura,[†] Valentin V. Rybenkov,^{*} Nancy J. Crisona,^{*} Tatsuya Hirano,^{†‡} and Nicholas R. Cozzarelli^{*‡} ^{*}Department of Molecular and Cell Biology University of California Berkeley, California 94720-3204 [†]Cold Spring Harbor Laboratory Cold Spring Harbor, New York 11724

Summary

Xenopus 13S condensin converts interphase chromatin into mitotic-like chromosomes, and, in the presence of ATP and a type I topoisomerase, introduces (+) supercoils into DNA. The specific production of (+) trefoil knots in the presence of condensin and a type II topoisomerase shows that condensin reconfigures DNA by introducing an ordered, global, (+) writhe. Knotting required ATP hydrolysis and cell cycle-specific phosphorylation of condensin. Condensin bound preferentially to (+) supercoiled DNA in the presence of ATP but not in its absence. Our results suggest a mechanism for the compaction of chromatin by condensin during mitosis.

Introduction

The DNA of eukaryotic chromosomes must be elaborately folded to fit within the confines of the nucleus. The degree of folding changes locally through chromatin remodeling to allow specific transcription of individual genes (Workman and Kingston, 1998; Pollard and Peterson, 1998) and globally to allow chromosome segregation during the cell cycle (Koshland and Strunnikov, 1996). The latter is the subject of our report. DNA is compacted by successive folding about 10⁴-fold within a mitotic chromosome. The first level of compaction is the left-handed wrapping of DNA around the core histones, yielding about a 5-fold shortening (Luger et al., 1997). The nature of the subsequent steps in compaction has remained elusive, but recent studies indicate that the SMC (structural maintenance of chromosomes) proteins are major contributors.

SMC proteins were originally identified in yeast as key elements of chromosome segregation and have since been recognized in a wide range of organisms (reviewed by Koshland and Strunnikov, 1996; Jessberger et al., 1998; Hirano, 1999). They are also related to the wellstudied *Escherichia coli* MukB protein (Hiraga, 1992). SMC proteins are among the most abundant nonhistone chromosomal proteins (Hirano and Mitchison, 1994) and are major components of the mitotic chromosome scaffold (Saitoh et al., 1994). The structural features shared by SMC proteins include an amino-terminal ATP-binding

[‡]To whom correspondence should be addressed (e-mail: hirano@ cshl.org [T. H.], ncozzare@socrates.berkleley.edu [N. R. C.]).

region (containing the Walker A motif), two long coiledcoil regions connected by a nonhelical hinge, and a carboxy-terminal conserved sequence termed the DA box (perhaps related to the Walker B motif). Recent biochemical studies have shown that SMC proteins associate with non-SMC proteins, forming complexes with diverse roles in chromosome structure and function. The best studied SMC protein complex is 13S condensin from Xenopus (Hirano and Mitchison, 1994; Hirano et al., 1997). 13S condensin purified from Xenopus egg extracts consists of two SMC subunits (XCAP-C and -E) and three non-SMC subunits (XCAP-D2, -G, and -H) (Hirano et al., 1997). The non-SMC subunits are phosphorylated in a mitosis-specific manner and play regulatory roles in condensin functions (Hirano et al., 1997; Kimura et al., 1998). Blocking experiments with antibodies demonstrated that 13S condensin is required for both the assembly and structural maintenance of mitotic chromosomes in a Xenopus cell-free system. Genetic studies in bacteria, yeast, worms, and Drosophila have proved that condensin homologs are essential for chromosome condensation and segregation in vivo (Saka et al., 1994; Strunnikov et al., 1995; Bhat et al., 1996; Britton et al., 1998; Lieb et al., 1998).

How can the addition of 13S condensin lead to the dramatic reconfiguration of interphase chromatin into mitotic-like chromosomes? Important clues are that the process requires ATP hydrolysis and that the SMC proteins have a structure reminiscent of motor proteins that convert chemical energy into movement. The Bacillus subtilis SMC protein and the analogous MukB of E. coli are antiparallel dimers, in contrast with the parallel protomer arrangement in other motor proteins (Melby et al., 1998). The antiparallel configuration provides an opportunity for the amino- and carboxy-terminal domains of condensin to interact so that each end of the protein could bind ATP and DNA. The antiparallel orientation of the SMC protomers also suggests that motion fueled by ATP hydrolysis would change the relative position of the terminal domains within a single protein. Thus, the widely separated terminal domains may bind DNA and then bend or twist the DNA in between (Melby et al., 1998). The interphase chromatin fibers may thereby be actively pulled into the tighter mitotic structure.

The first indication of how an SMC protein changes DNA conformation is the recent finding that 13S condensin introduces (+) supercoils into a closed circular DNA in the presence of a type I topoisomerase (Kimura and Hirano, 1997). Supercoiling required 13S condensin purified from mitotic extracts and ATP hydrolysis. Condensin isolated from interphase extracts showed almost no supercoiling activity, suggesting that supercoiling may play a role in mitotic chromosome condensation.

We envision three possible mechanisms by which 13S condensin plus topoisomerase I (topo I) could (+) supercoil DNA (Figure 1A). In model I, 13S condensin overwinds the DNA at its binding site and thereby increases double helical twist. Compensating (-) supercoiling in the condensin-free DNA is then relaxed by topo I, and



Wrap III. (+) Global writhe



Figure 1. Models for (+) Supercoiling by 13S Condensin

(A) Three models are shown for how 13S condensin could deform DNA so that (+) supercoils result after treatment with a type I topoisomerase. DNA is represented by a double line, and condensin is schematized as a bidentate ligand for DNA as described in Melby et al. (1998). Model I. (+) Twist. Condensin binding overwinds DNA, causing an increase in twist and a compensating (-) supercoil in condensin-free DNA. Relaxation of the (-) supercoil by the topoisomerase causes the DNA to become (+) supercoiled. Model II. (+) Wrap. A (+) node results from a right-handed helical wrapping of DNA around 13S condensin. The compensatory (-) supercoil is then relaxed by the topoisomerase. Model III. (+) Global writhe. Condensin binding stabilizes a large (+) supercoil, which remains after relaxation of the compensatory (-) supercoil.

(B) The same three models as in (A) but with a nicked DNA substrate. (C) Node sign and local geometry. The sign of a node is determined by which of the crossing DNA segments is on top and the path of the DNA connecting the segments. The (-) and (+) nodes shown (solid lines) have the same relationship of underpassing and overpassing DNA segments and thus identical local geometry. Because the path of the rest of the DNA indicated by the arrows and the dashed lines is different, the sign of the nodes is different.

the DNA becomes (+) supercoiled. In model II, constrained (+) supercoiling arises from the right-handed wrapping of DNA around condensin. As in model I, compensatory (-) supercoiling is generated that, upon relaxation, yields (+) supercoiled DNA. In these two models, 13S condensin acts locally, changing the structure of DNA at its binding site. In contrast, in model III, 13S condensin is postulated to introduce global (+) writhe by forming a (+) supercoiled loop in the DNA. As above, the compensatory (-) supercoils are relaxed by topo I and the DNA becomes (+) supercoiled. Many proteins locally change DNA twist or writhe, as required by models I and II. Perhaps the best known examples are the untwisting of DNA during open complex formation by bacterial RNA polymerase (Sasse-Dwight and Gralla, 1989) and the left-handed coiling of DNA around core nucleosomes (Luger et al., 1997). In most cases, twist or writhe decreases but there are a few examples of a local increase. The binding of the drug netropsin in the minor groove of DNA tightens up the double helix and results in (+) supercoiling of the DNA in the presence of a topoisomerase (Snounou and Malcom, 1983). The right-handed wrapping of DNA around archael histones is the probable cause of the (+) supercoiling that results when a topoisomerase is added (Musgrave et al., 1991).

Model III is the most intriguing as it involves a direct reconfiguration of DNA by 13S condensin. In the first two models, the major compaction is indirect and derives from the induction of compensatory (-) supercoiling (e.g., see Kimura and Hirano, 1997). Compaction by these compensatory supercoils would be vulnerable, however, to the ubiquitous topoisomerases in the cell, unlike the global (+) writhe in model III that is braced by condensin. Although we know of no examples of global supercoiling caused by protein binding, as required in model III, we suggest two ways in which such supercoiling could be introduced. First, 13S condensin could be bound to the crossing segments at the base of a (+) supercoil. Bidentate DNA-binding proteins that stabilize loops are well known (Mukherjee et al., 1988; Rippe et al., 1995), and scaffold proteins have long been suggested to be chromosomal loop fasteners (Laemmli et al., 1978). 13S condensin would have to do more than close a DNA loop, however, because protein binding to crossing DNA segments cannot distinguish a (+) from a (-) crossing. As illustrated in Figure 1C, this distinction requires that the path of the DNA between the crossing segments must also be recognized. Second, a global (+) supercoil could result from phased nonplanar bends in DNA stabilized by a framework of condensin molecules.

Model III can be readily tested. Supercoiling of DNA greatly increases the probability of knotting by a type II topoisomerase (Liu et al., 1979; Wasserman and Cozza-relli, 1991). With a nicked DNA substrate, no compensatory (–) supercoils will be generated in any of the three models for condensin action (Figure 1B). If model III is correct, however, knotting can still result from the linking by the topoisomerase of two condensin-braced (+) supercoils within a single DNA molecule. No knotting is expected with either of the other two models. The path of a nicked DNA is hardly changed in model I. In model II wrapping of the DNA is maintained by direct binding to condensin, leaving no accessible DNA loops as substrate for strand passage by the topoisomerase.

We found that the addition of 13S condensin to nicked DNA resulted in chiral knotting by a type II topoisomerase. We conclude, therefore, that model III is correct. The topology of the knots was highly informative. The knots were almost exclusively (+) trefoils (three crossing knots), which implies that condensin produces an ordered array of (+) solenoidal supercoils. Efficient knotting required the mitotic form of 13S condensin and ATP hydrolysis. We suggest that 13S condensin actively



Figure 2. Topoisomerase II-Dependent Knotting of DNA by 13S Condensin

(A) Knotting assay. 2.5 fmol of nicked JHX-1 DNA (3 kb) was incubated with 180 fmol of affinity-purified 13S condensin (lanes 5 and 6) or with buffer alone (lanes 3 and 4) in the presence (lanes 4 and 6) or absence (lanes 3 and 5) of 25 fmol of phage T2 topo II. The DNAs were purified, subjected to electrophoresis through a 0.7% agarose gel, and visualized by Southern blotting. Lane 1, knotted JHX-1 DNA marker (M); lane 2, nicked circular DNA used as substrate (S). The positions of nicked circular (OC), linear (L), and knotted DNA (Kn) are indicated.

(B) Antibody blocking of knotting. Knotting reactions in the presence of topo II were set up with 13S condensin (lanes 2–7) or with buffer alone (lane 1). Before adding the nicked circular substrate, the mixtures were preincubated with 1 μ g of control IgG (lane 3), 1 μ g of anti-XCAP-E peptide antibody (lanes 4 and 5), or 1 μ g of anti-XCAP-G antibody (lane 7). For lanes 5 and 6, the XCAP-E peptide (0.1 mg) was added as a binding competitor. The fraction of DNA that was knotted is 0% (lane 1), 6.1% (lane 2), 5.7% (lane 3), 0.2% (lane 4), 4.3% (lane 5), 5.3% (lane 6), and 1.0% (lane 7).

compacts DNA via promotion of an ordered (+) writhe during the formation of mitotic chromosomes.

Results

Production of Knots by 13S Condensin

Of the three models for introduction of (+) supercoils into DNA by 13S condensin diagrammed in Figures 1A and 1B, only in model III, introduction of a global (+) writhe, will there be substantial compaction and coiling of nicked DNA leading to knotting by a type II topoisomerase. We used phage T2 topoisomerase to test this because the knotting promoted by supercoiling has been well characterized for the T-even phage topoisomerases (Wasserman and Cozzarelli, 1991).

The substrate for knotting was a singly nicked 3.0 kb plasmid. The DNA was incubated for 60 min at 22°C with immunoaffinity-purified 13S condensin, phage T2 topo II, and ATP. After deproteinization, the DNA was analyzed for knots by gel electrophoresis along with a reference knot ladder. We found that 13S condensin did indeed induce formation of knots, overwhelmingly 3-noded knots (trefoils) (Figure 2A). Knotting required both 13S condensin and topo II. The knotted structure was confirmed by 2D gel electrophoresis (data not shown).

Because these experiments used a large molar excess



Figure 3. Kinetics of Knotting and Requirement for ATP Hydrolysis by 13S Condensin

(A) Time course and 13S condensin dependence of the knotting reaction. Nicked circular JHX-1 DNA was mixed with increasing amounts of 13S condensin at the ratios shown (lanes 4–12) or with buffer alone (lanes 1–3). The reactions were incubated for 5, 15, or 60 min as indicated, and then topo II was added and the incubation continued for another 5 min. DNA was purified and analyzed by Southern blotting. The fraction of DNA that was knotted is 0% (lanes 1–3), 1.0% (lane 4), 1.2% (lane 5), 0.7% (lane 6), 4.3% (lane 7), 2.9% (lane 8), 1.6% (lane 9), 2.7% (lane 10), 2.5% (lane 11), and 1.6% (lane 12). The positions of nicked circular (OC), linear (L), and knotted DNA (Kn) are indicated.

(B) Requirement for ATP hydrolysis for knotting. In the first incubation, nicked circular JHX-1 DNA was incubated with 13S condensin in the presence of 1 mM ATP (A, lanes 2 and 5), 1 mM AMP-PNP (P, lanes 3 and 6), or no nucleotide (-, lanes 1 and 4) for 60 min. Then a mixture containing topo II and the following nucleotides (lane 2, 1 mM AMP-PNP (P); lane 3, 1 mM ATP [A]; lane 4, 1 mM ATP and 1 mM AMP-PNP (AP); lanes 1, 5, and 6, no nucleotide [-]) were added and incubation continued for another 5 min (2nd inc.). DNA was purified and analyzed by Southern blotting. The fraction of knotted DNA was 0.2% (lane 1), 3.6% (lane 2), 0.2% (lane 3), 1.8% (lane 4), 4.2% (lane 5), and 0.3% (lane 6). The positions of nicked circular (OC), linear (L), and knotted DNA (Kn) are indicated.

of 13S condensin to DNA, it was important to show that knotting was not the result of a contaminant in the condensin preparation. We found strong inhibition of knotting by antibodies against a peptide of XCAP-E (Figure 2B, lane 4), which was blocked by the eliciting peptide (Figure 2B, lane 5). Antibodies to XCAP-G also inhibited knotting (Figure 2B, lane 7). Therefore, 13S condensin is required for knotting.

Kinetics and Stoichiometry of Knotting

We next investigated the effects of 13S condensin concentration and time of incubation on the restructuring of DNA that leads to knotting (Figure 3A). In this experiment, condensin was added at time zero. Topo II and ATP were added at various times thereafter, and the reactions were stopped 5 min later. Knots were formed quickly, reaching a maximum level after only 5 min, and did not increase over 2 hr, even when the topoisomerase was present from time zero (Figure 3A and unpublished data). As before, the knots were primarily 3-noded. Knotting was optimal at a ratio of 36 condensins for each 3.0 kb plasmid. In parallel, we measured (+) supercoiling by 13S condensin in the presence of topo I and found that it had a similar kinetics and enzyme dependence as knotting (data not shown). Based on these results and the chirality of the knots presented below, we conclude that (+) supercoiling and knotting are manifestations of the same condensin-provoked conformation of DNA.

Requirement for ATP Hydrolysis

We next tested whether condensin-caused knotting required ATP hydrolysis and not simply ATP binding, as was found for (+) supercoiling by 13S condensin (Kimura and Hirano, 1997). A complication in this experiment is that ATP hydrolysis is needed for turnover of topo II and therefore for efficient knotting. To circumvent this problem, we used a two-step protocol. The DNA was first incubated for 60 min with 13S condensin and either buffer alone, ATP, or the nonhydrolyzable ATP analog, AMP-PNP (Figure 3B, 1st incubation). We assume that the action of AMP-PNP is a model for the consequences of ATP binding as opposed to its hydrolysis. Then, topo II and either ATP or AMP-PNP were added as indicated and the reactions incubated for an additional 5 min (Figure 3B, 2nd incubation). Knotting cannot occur during the first incubation with condensin because there is no topoisomerase. However, if condensin is able to restructure the DNA during the first incubation, knotting will take place during the second incubation. The controls showed that ATP (Figure 3B, Jane 5) but not AMP-PNP (Figure 3B, Jane 6) supported knotting when present throughout both incubations. We compare next the reactions displayed in lanes 2, 3, and 4 that contained both ATP and AMP-PNP during the incubation with topoisomerase, when knotting can occur. When ATP was present in the first incubation with 13S condensin, DNA was efficiently knotted during the second incubation (lane 2) even though AMP-PNP was added along with the topoisomerase. When the order of nucleotide addition was reversed and the first incubation contained AMP-PNP, negligible knotting was found (lane 3). When no nucleotide was present in the first incubation, but ATP and AMP-PNP were in the second incubation, knot formation was reduced (lane 4). Because the conditions in these three reactions (lanes 2, 3, and 4) were the same in the second incubation, the difference in knotting must be a consequence of which nucleotide was present in the first incubation with condensin. We conclude that 13S condensin requires ATP hydrolysis to establish the DNA conformation that is knotted by topo II.

Cell Cycle Regulation of Knotting Activity by 13S Condensin

Condensin activity is tightly regulated during the cell cycle. Interphase condensin is deficient in inducing (+) supercoiling compared to the mitotic form (Kimura et al., 1998). The (+) supercoiling activity of the mitotic form of 13S condensin is due to specific phosphorylation, because the interphase form is activated by phosphorylation by Cdc2–cyclin B. We found that knotting has a similar cell cycle dependence (Figure 4). The mitotic condensin is far more active in the knotting reaction than the interphase form. With 32 molecules of 13S condensins per 3.0 kb plasmid, 10.2% of the DNA was



Figure 4. Cell Cycle–Dependent and Phosphorylation-Stimulated Knotting and Supercoiling Activity of 13S Condensin

(A) Knotting assay. 13S condensin was purified from a mitotic (M-condensin) or an interphase (I-condensin) extract and then treated with Cdc2-cyclin B (+cdc2) or with buffer alone (-cdc2). Increasing amounts of the treated fractions (M-condensin -cdc2, lanes 2-4: M-condensin +cdc2; lanes 6-8: I-condensin, lanes 10-12; I-condensin +cdc2, lanes 14-16) were used for a standard knotting assay. A reaction containing no 13S condensin was treated with Cdc2-cyclin B (lanes 5 and 13) or with buffer alone (lanes 1 and 9), and used as a negative control. DNAs were purified, resolved by electrophoresis through a 0.7% agarose gel, and visualized by Southern blotting. The fraction of knotted DNA was 0% (lane 1), 1.8% (lane 2), 10.2% (lane 3), 10.8% (lane 4), 0% (lane 5), 1.8% (lane 6), 11.3% (lane 7), 7.1% (lane 8), 0% (lane 9), 0.4% (lane 10), 1.6% (lane 11), 4.8% (lane 12), 0% (lane 13), 0.7% (lane 14), 3.7% (lane 15), and 7.5% (lane 16). The positions of nicked circular (OC), linear (L), and knotted DNA (Kn) are indicated.

(B) (+) Supercoiling assay. A standard supercoiling assay was performed using the same fractions as those in (A). The positions of relaxed circular DNA (RC) and a ladder of (+) supercoiled DNAs (Sc) are indicated.

The approximate molar ratios of 13S condensin to DNA in both (A) and (B) were 16:1 (lanes 2, 6, 10, and 14), 32:1 (lanes 3, 7, 11, and 15) or 64:1 (lanes 4, 8, 12, and 16).

knotted with the mitotic form but only 1.6% with the interphase form (Figure 4A, lanes 4 and 12). We detected only marginal (+) supercoiling with interphase condensin but robust supercoiling with the mitotic counterpart (Figure 4B, lanes 4 and 12). For both knotting and (+) supercoiling, the activity of the interphase form was greatly increased by phosphorylation with Cdc2–cyclin B (Figures 4A and 4B, lanes 12 and 16).

Determination of the Topology of Knots

We determined the topology of the knots induced by 13S condensin to gain further insight into the DNA architecture stabilized by 13S condensin. If model III is correct (Figure 1), then the knots should be chiral. These experiments employed a longer circular DNA than above, 7.0 kb, to make it easier to follow the knotted DNA path by electron microscopy and to facilitate the formation of more complex knots. We first used a 60 min incubation with mitotic 13S condensin and topo II.



Figure 5. Topology of Knots Made by 13S Condensin and Phage T2 Topoisomerase

Electron micrographs of knots formed by reaction of the 7 kb plasmid pAB3 with 13S condensin. The number and sign of the knot crossings and the type of knot are indicated below each micrograph.

As measured by gel electrophoresis, 12% of the DNA was knotted, with trefoils 20-fold more abundant than 4-noded knots (data not shown). The DNA was coated with RecA protein so that the overpassing and underpassing DNA strands could be followed by microscopy (Crisona et al., 1994). Examples of the knots found are shown in Figure 5.

The results were striking. Forty-four of the 48 trefoils scored were (+) (Table 1, Exp. 1). Thus, knotting has the same chirality as supercoiling. Moreover, only a scattering of knots with more than 3 nodes was obtained (Table 1). Formation of two of these more complex knots, the granny (which is a composite of two trefoils) and the 5-noded torus knot, requires at least two strand passages by a topoisomerase (Dazey Darcy and Sumners, 1997). To lessen the complications of multiple strand passages, we reduced the time of incubation with topo II from 60 min to 5 min. The EM results were again definitive: 52 of 53 trefoils were (+) (Table 1, Exp. 2). Some 5-noded torus knots were still found and these were exclusively (+), as in the first experiment. We show in the discussion how these knots and the granny knots can be produced by a simple iteration of the mechanism that yields the (+) trefoil.

Although mitotic condensin was much more effective than interphase condensin in restructuring DNA, some knots were formed with interphase condensin. These knots have the same topology as those induced by mitotic condensin (Table 1). We suggest that specific deformation of DNA is already programmed into interphase 13S condensin but that phosphorylation increases its efficiency in bringing about the restructuring of DNA.

Effect of (+) and (-) Supercoiling on Binding of 13S Condensin to DNA

To understand better the role of ATP in condensin action, we investigated the effects of ATP on the binding of 13S condensin to (+) and (-) supercoiled DNA using a filter binding assay. DNA is retained by the filter only

Knot Type	Number Found			
	Mitotic Condensin			Internhase
	Exp. 1	Exp. 2	Total	Condensin
(+) 3-noded	44	52	96	34
(-) 3-noded	4	1	5	1
4-noded*	3	1	4	0
(+) 5-noded torus	3	4	7	2
(+) 5-noded twist	1	0	1	0
(-) 5-noded twist	2	0	2	1
(+) 6-noded granny	2	0	2	3

pAB3 DNA was reacted with 13S condensin and topo II and prepared for electron microscopy after coating with RecA protein to ease identification of knot topology. The topology of the knots (Wasserman and Cozzarelli, 1986) identified are given. With mitotic condensin, incubation with topo II was for 1 hr in experiment 1 (Exp. 1) and for 5 min in experiment 2. With interphase condensin, incubation with the topoisomerase was for 5 min. (*) There is only a single 4-noded knot.

if it is bound to condensin. A mixture of (+) supercoiled DNA, (-) supercoiled DNA, and nicked DNA was incubated with various amounts of 13S condensin in the standard reaction buffer (low salt buffer) with or without ATP. The mixture was passed through the filter, which was then washed successively with three solutions: low salt buffer, buffer plus 1 M NaCl (high salt buffer), and buffer plus 0.5% SDS (SDS buffer). The low salt buffer elutes DNA not bound to condensin, whereas the other two solutions elute bound DNA.

Figure 6A displays the electrophoresis patterns in the presence of chloroquine for DNA in the low salt, high salt, and SDS buffer eluates. We calculated the fraction of DNA bound to 13S condensin as the ratio of DNA in the high salt and SDS eluates to the total DNA in all three washes. The fraction of each DNA bound is plotted in Figure 6B as a function of the ratio of condensin to DNA. We draw two conclusions. First, in the absence of ATP, 13S condensin binds equally well to (+) supercoiled DNA, (-) supercoiled DNA, and nicked DNA (Figure 6B, left panel). Second, ATP destabilizes 13S condensin binding to all three DNAs as shown by the lower recovery of DNA from the high salt and SDS washes (Figure 6B, right panel). The effect of ATP on condensin binding, however, was greatest with (-) supercoiled DNA, minimal with (+) supercoiled DNA, and intermediate with nicked DNA.

To confirm these results, we analyzed the DNA shown in lanes 10–12 of Figure 6A by 2D gel electrophoresis (Figure 6C). The preferential reduction of 13S condensin binding to (–) supercoiled DNA by the addition of ATP is apparent from the elution of most of the (–) supercoiled DNA in the low salt wash versus elution of most of the (+) supercoiled DNA in the SDS wash. The simplest interpretation of these results is that 13S condensin binds well to DNA irrespective of topology in the absence of ATP, but that the addition of ATP causes a conformational change in condensin that introduces (+) writhe and compensatory (–) supercoils in the DNA. The energy for this deformation of DNA weakens condensin binding overall, but the effect on binding is minimized



Figure 6. Effect of ATP and Supercoiling Sign on the Binding of 13S Condensin to DNA

(A) Filter binding assay. Mixtures of (+) and (-) supercoiled JHX-1 DNAs were incubated with increasing amounts of 13S condensin (lanes 4-15) or with buffer alone (lanes 1-3) for 60 min in the absence (upper panel) or in the presence (lower panel) of 1 mM ATP. Each reaction mixture was passed through a glass fiber filter and the filter was washed successively with reaction buffer (low salt wash, L); reaction buffer plus 1 M NaCl (high salt wash, H); and buffer containing 0.5% SDS (SDS wash, S). The DNAs present in the low salt (lanes 1, 4, 7, 10, and 13), high salt (lanes 2, 5, 8, 11, and 14), and SDS washes (lanes 3, 6, 9, 12, and 15) were purified and electrophoresed through a 0.7% agarose gel containing 100 mg/ml chloroquine and visualized by Southern blotting. The positions of nicked circular (OC), negatively supercoiled (NS), and positively supercoiled DNAs (PS) are indicated. The approximate molar ratios of 13S condensin to DNA present in the reaction mixtures are indicated at the top.

(B) Quantification of the binding assays. The amounts of (+) supercoiled (open circles), (−) supercoiled (closed circles), and nicked (closed squares) JHX-1 DNAs recovered in each wash were quantified using an image analyzer. The ratios of bound DNA (H+S) to total DNA (L+H+S) are shown in the absence (left) or presence (right) of 1 mM ATP.

(C) 2D gel analysis. DNA from lanes 10–12 in (A) were analyzed by 2D gel electrophoresis. Low-salt, high-salt, and SDS washes are shown in the absence of ATP (left) and in the presence of ATP (right). The positions of nicked circular (OC), (–) supercoiled (NC), and (+) supercoiled DNAs (PS) are indicated.

by (+) supercoiling, because it cancels the compensatory (-) supercoiling, and is exacerbated by (-) supercoiling.

Discussion

13S Condensin Produces Organized (+) Solenoidal Supercoils in DNA

We conclude that (+) supercoiling in the presence of a type I topoisomerase and (+) knotting in the presence of a type II topoisomerase are manifestations of the same DNA conformation induced by 13S condensin. The chief evidence is that both topological changes are chiral and have the same sign, (+). In addition, both topology changes require ATP hydrolysis (Figure 3B; Kimura and Hirano, 1997) and are regulated by mitosis-specific phosphorylation of condensin (Figure 4; Kimura et al., 1998). The time courses for supercoiling and knotting are similar, and both are inhibited by antibodies to XCAP-E (Figure 2B; Figure 3A; Kimura and Hirano, 1997).

The two topological changes give complementary information. Positive supercoiling is the simpler reaction, because any net stabilization of (+) writhe or an increase in twist would lead to (+) supercoils in the presence of a topoisomerase. As a result, (+) supercoiling is a better quantitative measure of DNA conformational changes by 13S condensin. The production of (+) trefoils, however, requires a more organized, regular, and global change in DNA conformation and thus gives a deeper insight into the 13S condensin–stabilized conformation (see below).

The chirality of the knots produced by 13S condensins is striking. Of the 136 trefoils analyzed, 130 were (+) (Table 1). This is a minimal estimate of 13S condensinimposed chirality because the small fraction of knots in the substrate and the knots produced by topo II action on DNA free of condensin could be either (-) or (+). We presume that condensin action leads exclusively to (+) trefoils.

Because 13S condensin causes the production of a large amount of (+) trefoils in nicked DNA we further conclude that it introduces a global (+) writhe as proposed in model III (Figure 1B). Condensin cannot merely introduce a local writhe, such as a right-handed wrap of DNA around condensin (Figure 1B, model II), because this would have scant effect on the overall conformation of nicked DNA. For the same reason we eliminate an increase in double helical twist upon 13S condensin binding (Figure 1B, model I). From the introduction of (+) supercoiling with topo I we could only infer a net effect of condensin on DNA. Condensin could also have induced substantial, but less, (-) supercoiling. Unlike supercoils, (+) knots do not cancel (-) knots, and thus we conclude that the supercoiling by 13S condensin is exclusively (+).

It is highly informative that few knots with more than three nodes were formed by a single strand passage by topo II in the presence of condensin. With a short incubation (5 min) to limit strand passage by topo II to largely a single round, only two of the 88 single-passage knots analyzed were not a trefoil (Table 1). Gel electrophoresis confirmed that over 90% of the knots formed were trefoils. We draw two conclusions from the highly preferential production of trefoils. First, the (+) supercoils stabilized by condensin are solenoidal rather than plectonemic (braided). If the condensin-stabilized supercoils were plectonemic, a ladder of (+) knots called twist knots would instead be produced (Wasserman and



Figure 7. Models for Supercoiling and Knotting Directed by 13S Condensin

DNA is shown as a single solid line with variable thickness for depth cueing. The substrate has several condensin-stabilized (+) supercoils, but only two adjacent supercoils are shown in (A) and (B). (A) Production of (+) trefoils. The supercoil at the top is in the plane of the page and the other supercoil is positioned at an angle to the first, as shown. A (+) trefoil results from the linking of the supercoils by a type II topoisomerase.

(B) Lack of formation of a 4-noded knot. The supercoil out of the plane of the page has its opposite edge adjacent to the supercoil in the plane of the page. Strand passage by a topoisomerase would yield a 4-noded knot.

(C) Formation of a (+) pentafoil. The substrate has a (+) trefoil formed as in (A) and an additional condensin stabilized (+) supercoil positioned as in (A) but below the plane of the page. Linking of the

Cozzarelli, 1991). Given the (+) chirality of the condensin-stabilized supercoils, the solenoids must be right-handed.

Our second conclusion from the knotting data is that the (+) supercoils formed by condensin are themselves organized into a higher order structure. Figure 7A illustrates how topo II could link two (+) supercoils stabilized by 13S condensin and specifically give rise to (+) trefoils. The two (+) solenoidal supercoils provide two of the three (+) nodes of the trefoil. We conclude that topo II-mediated strand passage is directed to result in the third (+) node of the trefoil by the higher-order architecture of the condensin-bound DNA. If two (+) supercoils could link equally well in any orientation, then 4-noded knots would be formed, as shown in Figure 7B, in amounts equal to trefoils.

The topology of the rare, more complex knots induced by condensin provide further support for our model. A second round of strand passage by topo II that links a (+) supercoil to a (+) trefoil formed in the first round in the same way that the trefoil was formed would produce a (+) 5 torus knot (Figure 7C). If a second round by a topoisomerase links two (+) supercoils not involved in making a trefoil in the first round, then a granny knot made of two (+) trefoils will result. The total of 14 granny and 5-noded torus knots found had exclusively the predicted topologies (Table 1).

How could 13S condensin stabilize an organized (+) writhe? The simplest model is that 13S condensin introduces a nonplanar, right-handed bend in DNA (Figure 7D). Condensins bind preferentially to four-way junctions (Kimura and Hirano, 1997), a common feature of proteins that bend DNA (Bhattacharyya et al., 1991; Zlatanova and van Holde, 1998). Planar bending of the DNA would form achiral knots and therefore cannot explain the results. The nonplanar bends must also be phased to give the organized supercoils found. This could be achieved if the condensins touch each other to form a protein infrastructure. If the condensins form a framework for DNA, then cooperativity is expected. The available data are consistent with cooperativity, but do not prove it. With sub-saturating amounts of condensin, less (+) supercoiling and knotting was obtained but not a different topology. Also, the superior binding of condensin to longer DNA (Kimura and Hirano, 1997) can be explained by cooperativity.

An alternative model for the introduction of (+) writhe by 13S condensin is analogous to the loop expansion model proposed for bacterial MutS action (Allen et al., 1997; Figure 7E). If, at the expense of ATP, DNA initially wraps around 13S condensin in a right-handed fashion but then loops out from the condensin, a growing (+)

⁽⁺⁾ supercoil and the (+) trefoil produces a (+) 5 torus knot.

⁽D and E) Possible mechanisms for 13S condensin stabilization of a (+) supercoil. A segment of DNA is shown as two parallel lines and condensin as a bidentate ligand.

⁽D) Nonplanar bending of DNA by 13S condensin. The condensins touch to maintain phasing and to maintain the spatial relationship between successive supercoils.

⁽E) Chiral loop expansion. The DNA wraps around condensin, which dictates the local crossing geometry and topology that are maintained as the DNA slides past condensin to enlarge the (+) supercoil.

supercoil would result. This model, by itself, fails to explain the paucity of 4-noded knots, because it does not organize the (+) supercoils into a higher-order structure.

The addition of 36 molecules of 13S condensin to a 3 kb DNA leads to an increase in linking number of 3.3 (Kimura et al., 1998). Our estimate of the number of condensins bound to 3 kb of DNA ranged from 10–25 or a mean of five condensins per supercoil (K. K. and T. H., unpublished data). The relatively high stoichiometry of protein to DNA required is more consistent with the nonplanar bending model. Each condensin-stabilized supercoil would be about 900 bp in size if they occupied the whole length of the plasmid. Given that the distance between the DNA-binding sites in a single 13S condensin could be as large as 1000 Å (Melby et al., 1998), five condensins could easily stabilize a supercoil of this size.

An important feature of 13S condensin is that it provides an active reconfiguration of DNA. We conclude that ATP hydrolysis is needed to maintain the global (+) writhe because AMP-PNP, a nonhydrolyzable analog of ATP, did not suffice. What could be the role of ATP hydrolysis? In the loop expansion model, ATP hydrolysis could promote specific wrapping and unidirectional movement of the DNA to generate a growing (+) supercoil (Figure 7E). In the nonplanar bend model (Figure 7D), the binding of 13S condensin could introduce (+) writhe only when ATP is hydrolyzed. Such a model is consistent with the filter binding data (Figure 6). The addition of ATP caused a destabilization of 13S condensin binding to DNA that is greatest with (-) supercoiled DNA, intermediate with nicked DNA, and minimal for (+) supercoiled DNA. These data are easily explained if condensin binds well to DNA in the absence of ATP but the induction of (+) writhe requires ATP. The compensatory (-) supercoils would be dissipated by a nick, exacerbated by (-) supercoiling, and absorbed by (+) supercoiling.

Implications for Mitotic Chromatin Folding

How might the activity of 13S condensin contribute to the conversion of interphase chromatin to mitotic chromosomes? A prior model for condensin-mediated compaction depended on compensatory (-) supercoiling (Kimura and Hirano, 1997), which would easily be removed by the abundant topoisomerases in the cell. The formation of a global (+) writhe by 13S condensin, reported in this study, compacts DNA more directly, suggesting more plausible mechanisms underlying mitotic chromosome condensation. The proposed action of 13S condensin is also fundamentally different from a loop fastener in the radial loop model of chromosome organization (Laemmli et al., 1978; Earnshaw, 1991). A simple bidentate protein binding to crossing segments of DNA cannot distinguish between (+) and (-) nodes (see Figure 1C). Nor can it support a preferential formation of (+) trefoils over 4-noded knots. The model we propose also provides a natural explanation for the requirement for a type II topoisomerase in chromosome condensation (Uemura et al., 1987) to accommodate the organized supercoiling of DNA by condensin.

Because the action of 13S condensin on chromatin

is mitosis specific (Hirano et al., 1997; Kimura et al., 1998; Figure 4), we suggest that (+) solenoidal supercoiling is a mitosis-specific strategy for chromatin organization. Given our limited knowledge of chromatin structure, however, it is not straightforward to extrapolate our current results obtained with naked DNA to the action of 13S condensin on protein-laden chromosomes.

In mitotic chromosomes assembled in vitro, we estimate that there is one 13S condensin per 5-10 kb of DNA. A similar value has been obtained in S. pombe chromosomes (one complex per 8 kb of DNA; Sutani and Yanagida, 1997). Given the gigantic size of condensin and its unique shape, and if cooperative action is assumed, the density of 13S condensin on chromosomes might be sufficient to initiate a (+) solenoidal organization of nucleosome fibers or a higher order structure (Belmont and Bruce, 1994). Future ultrastructural analyses of native chromosomes, combined with full reconstitution of chromosomes in vitro, should provide more insights into how the condensin-mediated solenoidal formation might compact mitotic chromatin. Whatever the underlying mechanism might be, our current study provides a fundamental implication that a specific chirality of chromatin organization is determined by an energy-dependent action of a protein complex.

Experimental Procedures

Enzymes

The 13S condensin complex was purified from mitotic or interphase *Xenopus* egg extracts by adsorption to and elution from an antibody column (Kimura and Hirano, 1997; Kimura et al., 1998).

Knotting Assay

Negatively supercoiled plasmid DNA (JHX-1: Kimura et al., 1998), singly nicked by treatment with pancreatic DNase I in the presence of ethidium bromide, was the substrate for knotting. Five nanograms of the nicked circular DNA were incubated with 13S condensin in the presence of 6.5 ng of T2 topoisomerase II at 22°C for 60 min. The reaction mixtures (4 µl) also contained 10 mM potassium-HEPES (pH 7.7), 50 mM KCl, 2 mM MgCl₂, 0.1 mM CaCl₂, 1 mM MgATP, 5 mM EGTA, 1 mM dithiothreitol (DTT), and 0.5 mg/ml bovine serum albumin (BSA). In some experiments, nicked DNA was first incubated with condensin at 22°C for 60 min, and then 6.5 ng of topo II was added and incubation continued for another 5 min. Reactions were terminated by the addition of 60 μ l of 20 mM Tris-HCl (pH 8.0), 20 mM EDTA, 0.5% SDS, 10 µg/ml yeast tRNA, and 0.5 mg/ml proteinase K and incubated at 37°C for 40 min. The DNA was extracted with phenol, precipitated with ethanol, subjected to electrophoresis through a 0.7% agarose gel with TBE buffer, and detected by Southern blotting. To generate knot markers, we treated JHX-1 DNA with an excess of phage T2 topoisomerase in the absence of ATP (Wasserman and Cozzarelli, 1991) and then nicked the DNA with DNase I. For the antibody inhibition experiment, an affinity-purified antibody raised against the C-terminal sequence of XCAP-E or XCAP-G was used (Hirano et al., 1997).

Supercoiling Assay

The supercoiling assay was done as described previously (Kimura and Hirano, 1997). In some experiments, a relaxed circular DNA was first incubated with 13S condensin and then calf thymus topo I was added.

Filter Binding Assay

The filter binding assay was done as described (Roca and Wang, 1992) with minor modifications. Five nanograms of (+) supercoiled JHX-1 DNA was incubated with 13S condensin. The final reaction mixture (5 μ l) contained 10 mM potassium-HEPES (pH 7.7), 50 mM

KCI, 2 mM MgCl₂, 0.1 mM CaCl₂, 5 mM EGTA, 1 mM DTT, and 0 mM or 1 mM MgATP. After 60 min at 22°C, the mixture was filtered through a 7 mm Millipore glass fiber filter (APFC02500). Each filter was preincubated in 200 μl of 0.1 mg/ml salmon sperm DNA in 10 mM potassium-HEPES (pH 7.7), 50 mM KCI, 2 mM MgCl₂, 0.1 mM CaCl₂, 5 mM EGTA, 1 mM DTT, and 0.1 mg/ml BSA. The preincubated filter was placed on the cap of a 1.5 ml Eppendorf tube that had a small drainage hole. A reaction mixture was placed on the filter, and the filtrate was recovered by centrifugation. The filter was washed 3 times with 50 µl of reaction buffer containing 0.1 mg/ml BSA: 3 times with 50 μl of reaction buffer plus 1 M NaCl; and 3 times with 50 µl of buffer containing 10 mM Tris-HCl (pH 8.0), 1 mM EDTA, and 0.5% SDS. DNA in the washes was phenol extracted, ethanol precipitated, subjected to electrophoresis through a 0.7% agarose gel containing 100 µg/ml chloroquine, and visualized by Southern blotting. Two-dimensional gel electrophoresis was done as described (Kimura and Hirano, 1997).

Electron Microscopy

Seven-kilobase plasmid DNA was pretreated with E. coli topo IV to remove most of the preexisting knots (Rybenkov et al., 1997). In the first experiment with mitotic condensin (Table 1), 60 ng of singly nicked DNA was incubated with 1.5 nmol of 13S condensin and 50 ng of T2 topoisomerase in a 40 ml reaction containing 10 mM potassium-HEPES (pH 7.7), 50 mM KCI, 2 mM MgCl₂, 0.1 mM CaCl₂, 1 mM MgATP, 5 mM EGTA, 1 mM DTT, and 0.5 mg/ml BSA at 22°C for 60 min. In the second experiment with mitotic condensin and the experiment with interphase condensin, the DNA was incubated with condensin for 60 min and then with topo II for 5 min. The reactions were terminated by the addition of 20 mM Tris-HCI (pH 8.0), 20 mM EDTA, 0.5% SDS, 10 μg/ml yeast tRNA, and 0.5 mg/ ml proteinase K and incubated at 37°C for 40 min. Purified DNA was denatured in the presence of glyoxal, coated with RecA protein to visualize DNA crossings, and examined by electron microscopy (Crisona et al., 1994).

Acknowledgments

This work was supported by NIH grant GM 31655 (to N. R. C.) and GM 53926 (to T. H.). T. H. was supported by the Pew Scholars Program in the Biomedical Sciences and K. K. was supported by fellowships from the Leukemia Society of America and the Robertson Research Fund. We thank Mark Krasnow for valuable discussions.

Received April 19, 1999; revised June 14, 1999.

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