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Condensin and cohesin display different arm conformations with characteristic hinge angles

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Structural maintenance of chromosomes (SMC) proteins play central roles in higher-order chromosome dynamics from bacteria to humans. In eukaryotes, two different SMC protein complexes, condensin and cohesin, regulate chromosome condensation and sister chromatid cohesion, respectively. Each of the complexes consists of a heterodimeric pair of SMC subunits and two or three non-SMC subunits. Previous studies have shown that a bacterial SMC homodimer has a symmetrical structure in which two long coiled-coil arms are connected by a flexible hinge. A catalytic domain with DNA- and ATP-binding activities is located at the distal end of each arm. We report here the visualization of vertebrate condensin and cohesin by electron microscopy. Both complexes display the two-armed structure characteristic of SMC proteins, but their conformations are remarkably different. The hinge of condensin is closed and the coiled-coil arms are placed close together. In contrast, the hinge of cohesin is wide open and the coiled-coils are spread apart from each other. The non-SMC subunits of both condensin and cohesin form a globular complex bound to the catalytic domains of the SMC heterodimers. We propose that the "closed" conformation of condensin and the "open" conformation of cohesin are important structural properties that contribute to their specialized biochemical and physiological functions.

Introduction

In eukaryotic cells, the assembly of metaphase chromosomes is achieved by a coordinated balance of two mechanistically distinct processes, chromosome condensation and sister chromatid cohesion (for review see Koshland and Strunnikov, 1996; Dej and Orr-Weaver, 2000; Hirano, 2000; Nasmyth et al., 2000). Recent genetic and biochemical studies have shown that condensation and cohesion are regulated by two structurally related protein complexes, condensin and cohesin, respectively. Both complexes contain structural maintenance of chromosomes (SMC)* ATPases as their core subunits. Condensin is a five-subunit complex that is composed of two SMC subunits (SMC2/CAP-E and SMC4/CAP-C) and three non-SMC subunits (CAP-D2, -G and -H) (Hirano et al., 1997; Sutani et al., 1999; Freeman et al., 2000; Kimura et al., 2001). The cohesin complex consists of SMC1, SMC3 and at least two non-SMC subunits (Scc1/Mcd1/RAD21 and Scc3/SAs) (Losada et al., 1998; Toth et al., 1999; Losada et al., 2000; Sumara et al., 2000; Tomonaga et al., 2000).

The condensin complex purified from *Xenopus* egg extracts binds directly to double-stranded DNA and displays a DNA-stimulated ATPase activity. Condensin has the ability to reconfigure DNA structure in an ATP-hydrolysis–dependent manner. It introduces positive supercoils into relaxed circular DNA in the presence of type I topoisomerases (Kimura and Hirano, 1997), and converts nicked circular DNA into positively knotted forms in the presence of a type II topoisomerase (Kimura et al., 1999). The same set of activities has been found in the human condensin complex purified from a HeLa cell nuclear extract (Kimura et al., 2001). On the other hand, the cohesin complex displays DNA-binding properties that are remarkably different from those of condensin (Losada and Hirano, 2001). It induces the formation of large protein–DNA aggregates and stimulates intermolecular catenation (rather than knotting) of circular DNA molecules in the presence of topoisomerase II. These results are consistent with our proposal that condensin acts as an intramolecular DNA cross-linker to compact DNA, whereas cohesin acts as an intermolecular DNA cross-linker to hold two sister chromatids together (Hirano, 1999). It remains

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*Abbreviations used in this paper: ABC, ATP-binding cassette; SMC, structural maintenance of chromosomes.

Key words: ABC ATPases; chromosome condensation; coiled-coil; sister chromatid cohesion; structural maintenance of chromosomes

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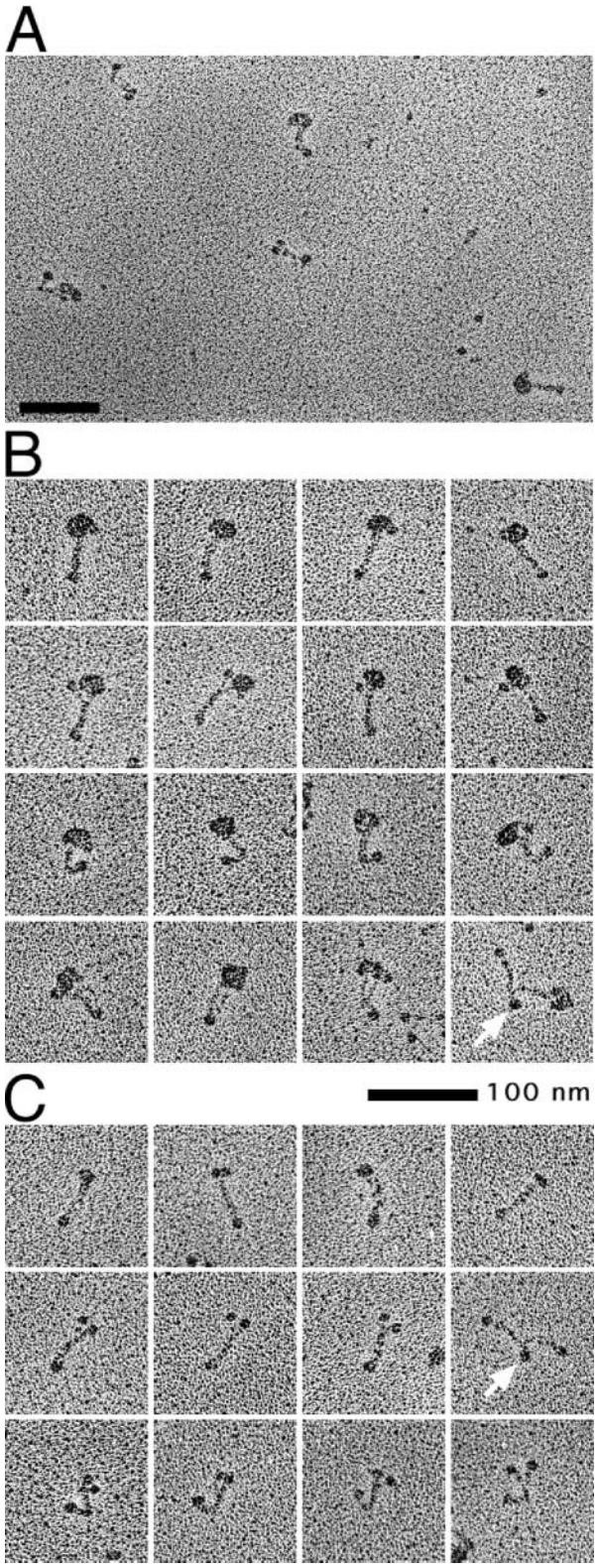

Figure 1. **Electron micrographs of the human condensin complexes.** (A) An example field of molecules. (B) The structure of holocomplexes can be classified into three major groups on the basis of the configuration of their coiled-coil arms: 'folded-rod' (first row) and 'ends-split' (second row)—a subset of which have a bend in the coils (third row)—and 'coils-spread' (fourth row). The last panel in the fourth row is an example of a rare 'open-V'. (C) The two major forms of SMC heterodimers (hSMC2-hSMC4) are again folded-rod (first row) and ends-split (second row), some with bent coils (third row). Open-V configurations are rare (last panel, second row). The hinge domain is indicated by an arrow on the open-V molecules. B and C are the same magnification. Bars, 100 nm.

unknown, however, how the two SMC protein complexes might be able to distinguish between the two different modes of DNA interactions.

SMC proteins are conserved not only in Eukarya but also in Bacteria and Archaea (for review see Cobbe and Heck, 2000). As judged by EM, the SMC homodimer from *Bacillus subtilis* (BsSMC) is composed of two antiparallel coiled-coil arms connected by a flexible hinge (Melby et al., 1998). A recent biochemical analysis of BsSMC has shown that hinge-mediated opening and closing of SMC dimers may be mechanistically coupled with their dynamic interactions with DNA (Hirano et al., 2001). It is a reasonable speculation that eukaryotic SMC complexes share these structural features, but the structures have not been directly visualized until now.

In the present study, we have visualized vertebrate condensin and cohesin complexes by EM. We find that both SMC protein complexes share the two-armed structure, but display different arm conformations with characteristic hinge angles. The hinge of condensin is largely closed, whereas that of cohesin is wide open. In both complexes, the non-SMC subunits associate with the catalytic end domain(s) of the SMC dimer and not with the hinge domain. Our results suggest that condensin and cohesin have evolved to acquire differentiated structures so that they can efficiently support their specialized functions in condensation and cohesion, respectively.

Results and discussion

EM of human condensin

The condensin complex was purified from a HeLa cell nuclear extract by immunoaffinity column chromatography using an antibody specific to one of the non-SMC subunits, hCAP-G (Kimura et al., 2001). The resulting protein fraction was rotary shadowed and visualized by electron microscopy. An example field of the molecules is shown in Fig. 1 A. We examined a total of 146 molecules, and found that most of them had rod-shaped structures with variable conformations. Approximately half of the molecules showed an obvious enlargement in one of the terminal domains, which we interpret to be the non-SMC subcomplex consisting of hCAP-D2, -G and -H. Therefore, this population corresponds to the five-subunit holocomplex of condensin. The other half of the molecules lacked the large globular domain, and were very similar to the bacterial SMC dimers (Melby et al., 1998). These molecules are most likely heterodimers of hCAP-E/SMC2 and hCAP-C/SMC4. We speculate that the condensin complex may be unstable in the buffer used for rotary shadowing, or that the globular complex of the non-SMC subunits may dissociate from the SMC dimer upon contact with the mica (see Schürmann et al., 2001, for discussion of this mechanism).

The conformations observed among the holocomplexes could be classified into four groups. The first group, which accounted for 49% of the complexes, had a rod-shaped

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Table I. **Measured dimensions of human condensin and cohesin complexes**

Molecule	Total length of molecule[a]	Diameter of hinge	Diameter of terminal complex[b]	Diameter of catalytic domain
	nm	*nm*	*nm*	*nm*
Condensin holocomplex	67 ± 6	7.6 ± 0.9	21 ± 4	–
SMC2-SMC4 heterodimer	58 ± 4	8.0 ± 0.9	–	6.6 ± 0.8
Cohesin holocomplex	64 ± 7	8 ± 1	17 ± 4	–
SMC1-SMC3 heterodimer	59 ± 4	8 ± 1	–	7.2 ± 0.9

All measurements listed have been corrected for a presumed 1 nm shell of metal.
[a]Measured from the outer edge of the hinge domain to the outer edge of the globular termini/terminal complex, along one of the coiled-coil arms.
[b]Measured when the catalytic domains and non-SMC subunits were shadowed as a single globular particle.

structure with a large globular domain at one end and a smaller one at the other (Fig. 1 B, first row). The total length of the molecules was 67 nm, and the width of the rods was ~4.5 nm (Table I), consistent with the interpretation that a single rod is composed of a fully folded SMC dimer (Melby et al., 1998; Hirano et al., 2001). In accordance with the original classification of SMC structures proposed by Melby et al. (1998), we call this conformation "folded-rod". We interpret that the small globular domain corresponds to the hinge region, whereas the larger domain at the opposite end contains the two SMC catalytic heads and the non-SMC subcomplex. The second group (27%) had an 'ends-split' conformation in which a small globular domain (one head of the SMC dimer) was split off from the large one (Fig. 1 B, second row). Around one third of the molecules in each of these two groups had a sharp bend in the folded coiled-coils (Fig. 1 B, third row). The third group (21%), 'coils-spread', had the two coiled-coil arms separated from each other, but the two SMC catalytic heads and the non-SMC subcomplex were closely attached at one end of the molecules (Fig. 1 B, fourth row, first three panels). The fourth and extremely rare (3%) conformation was "open-V" in which the arms of the SMC dimer were completely splayed apart and the non-SMC subcomplex attached to only one of terminal domains (Fig. 1 B, fourth row, last panel).

Interestingly, the coiled-coil and hinge conformations of the heterodimers were similar to those of the holocomplexes. Approximately 32% were folded-rod (Fig. 1 C, first row), 44% were ends-split (Fig. 1 C, second row, first three panels), and 12% were coils-spread (unpublished data). Again, some of the folded-rod and ends-split molecules exhibited a sharp bend (Fig. 1 C, third row). The remaining 12% of the heterodimers were open-V molecules. Even in this group, however, the hinge was not completely open: the two coiled-coil arms were often associated with each other near the hinge and splayed apart toward the ends (Fig. 1 C, second row, last panel). This structural feature was also found in a holocomplex with the open-V conformation (Fig. 1 B, fourth row, last panel). These observations suggest that the hinge of the SMC2-SMC4 heterodimer is largely closed.

EM of human cohesin

An antibody specific to the SMC3 subunit was used to purify the human cohesin complex from a HeLa cell nuclear extract by immunoaffinity column chromatography (Losada and Hirano, 2001). This procedure yields a large population of holocomplexes and a much smaller population of SMC1-

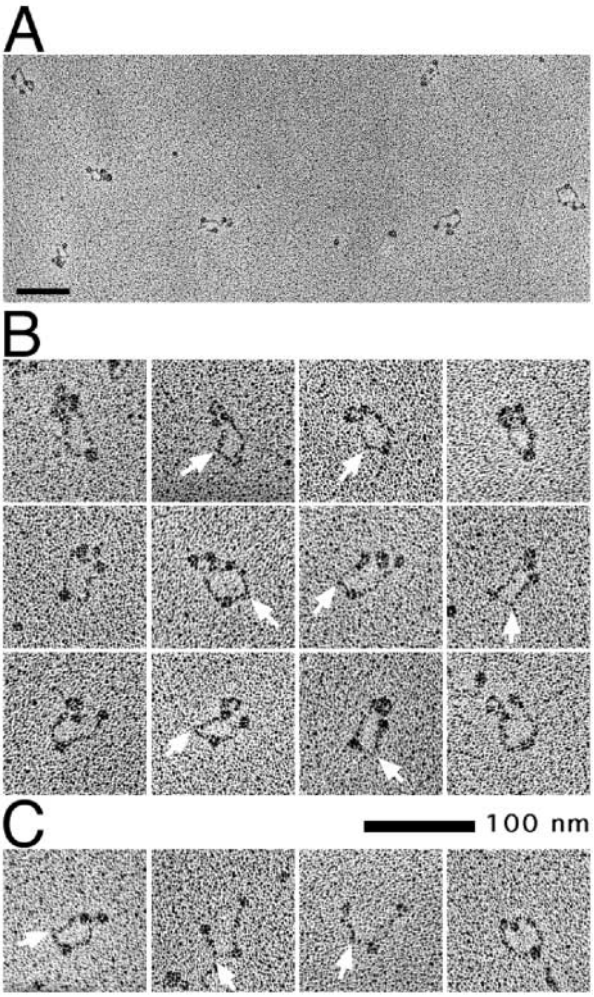

Figure 2. **Electron micrographs of the human cohesin complexes.** (A) An example field of molecules. (B) The structure of holocomplexes can be classified into two major groups on the basis of the configuration of their coiled-coil arms. In the first row the molecules are in a 'coils-spread' conformation, with their catalytic domains and the non-SMC proteins mostly superimposed. In the second and third rows the molecules are in an open-V conformation with the heads somewhat separated. The non-SMC complex appears either as a separate globule between the catalytic domains (second row), or bound to one of them (third row). A kink is often observed at a fixed position in one of the two coiled-coil arms (indicated by arrows). (C) SMC heterodimers (hSMC1-hSMC3) show mostly open-V configurations. Again, one of the coiled-coil arms often displays a kink (indicated by arrows). B and C are the same magnification. Bars, 100 nm.

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SMC3 heterodimers, as described previously (Losada et al., 2000). An example field of the corresponding rotary shadowed molecules is shown in Fig. 2 A. Similar to condensin, cohesin had the characteristic two-armed structure of the SMC dimer. A globular complex of non-SMC subunits was associated with the SMC catalytic domains in 59 of the 66 molecules (89%) examined (Fig. 2 B). The remaining 7 molecules were interpreted to be SMC1-SMC3 heterodimers (Fig. 2 C). In striking contrast to condensin, the hinge of the cohesin complex was almost always open, and the coiled-coil arms were splayed apart. The hinge angle of the cohesin holocomplexes was measured to be 88 ± 36 (n = 52) degrees, whereas that of the condensin complexes was 6 ± 12 (n = 57) degrees. The larger standard deviation for the hinge angle of cohesin may indicate its greater flexibility compared with the hinge of condensin.

The structure of cohesin holocomplexes was somewhat variable at the head region. Some molecules (47%) showed a large globular particle with a complex structure, which is most likely the result of a close association or superposition of the two catalytic domains and the non-SMC subcomplex (Fig. 2 B, first row). In other molecules (33%), the large particle was resolved into three separate globular domains, with the non-SMC subcomplex laying between the two catalytic SMC domains (Fig. 2 B, second row). A third group (20% of the molecules) showed the two SMC catalytic domains separated from each other, with a globular complex associated with one of them (Fig. 2 B, third row). Despite the apparent separation, the three domains stayed very close to each other in these images, suggesting that this configuration might have been created by partial disruption of the complex upon contact with the mica (Schürmann et al., 2001). In the population of heterodimers, two different conformations were observed with almost equal frequency. The first one was open-V with the catalytic domains of the molecule well separated (Fig. 2 C, two middle panels). The second one was also open at the hinge, but the two end domains were in close proximity (Fig. 2 C, left and right).

There were two other structural differences between condensin and cohesin. First, the globular complex of non-SMC subunits was noticeably smaller for cohesin. This is consistent with the difference in the total molecular mass of the non-SMC subunits between the two complexes (~350 kD for condensin and ~210 kD for cohesin). Second, one arm of cohesin frequently showed a sharp bend or kink ~1/3 of the way from the hinge to the catalytic domain (Fig. 2 B, arrows). The angle at the kink was measured to be 102 ± 26 (n = 52) degrees. Since a similar kink was also observed in one of the arms of the SMC1-SMC3 heterodimers (Fig. 2 C, arrows), this structural feature appears to be inherent to the SMC coiled coil.

EM of *Xenopus* condensin and cohesin

To confirm that the differences observed between condensin and cohesin are indeed intrinsic to the two SMC complexes, they were purified from a different source, *Xenopus* egg interphase extracts, and visualized by rotary shadowing. We found that the basic characters of the two *Xenopus* complexes were the same as those of their human counterparts. The coiled-coil arms were largely closed in the condensin holocomplexes (Fig. 3 A, first row) as well as in the SMC2-SMC4 heterodimers

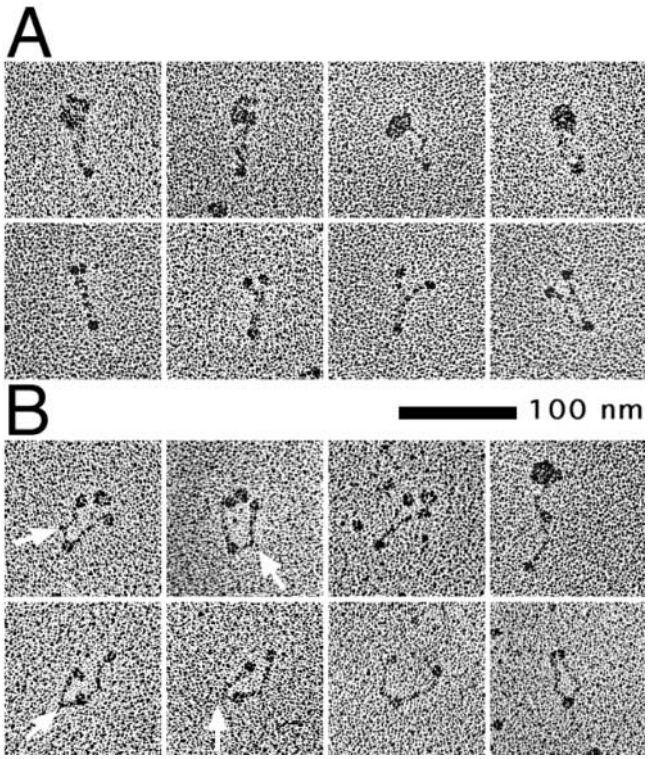

Figure 3. **Electron micrographs of the *Xenopus* condensin and cohesin complexes.** (A) Holocomplexes of *Xenopus* condensin (first row) and heterodimers of XCAP-E/XSMC2 and XCAP-C/XSMC4 (second row). (B) Holocomplexes of *Xenopus* cohesin (first row) and heterodimers of XSMC1 and XSMC3 (second row). Arrows in B indicate the position of the kink in the coiled-coil arm. Bar, 100 nm.

(Fig. 3 A, second row). In contrast, the SMC arms adopted wide-open conformations in the cohesin complexes (Fig. 3 B, first row) and in the SMC1-SMC3 heterodimers (Fig. 3 B, second row). A kink was again observed in one of the coiled-coil arms of cohesin (Fig. 3 B, arrows). Dimensions of the *Xenopus* holocomplexes and heterodimers were the same as their human counterparts, within 1–2 nm (unpublished data).

It has been shown that the DNA supercoiling and knotting activities of *Xenopus* condensin are activated by mitosis-specific phosphorylation of the non-SMC subunits (Kimura et al., 1998, 1999). To test whether this cell cycle–specific modification might induce a conformational change of condensin, the complex was purified from *Xenopus* egg mitotic extracts and visualized in the same way. Little, if any, difference was found, however, between the mitotic and interphase forms of condensin in either general appearance or measured dimensions (unpublished data).

Comparison of two-armed ATP-binding cassette (ABC) ATPases from bacteria to humans

The central hinge of the BsSMC is highly flexible, displaying a wide range of conformations from folded-rod to open-V (Melby et al., 1998; Hirano et al., 2001). In contrast, we show here that each of the two eukaryotic SMC protein complexes has its unique hinge angle: the coiled-coil arms of condensin are largely closed, whereas those of cohesin are open with an average angle of ~90 degrees (Fig. 4 A). Similar, if not identi-

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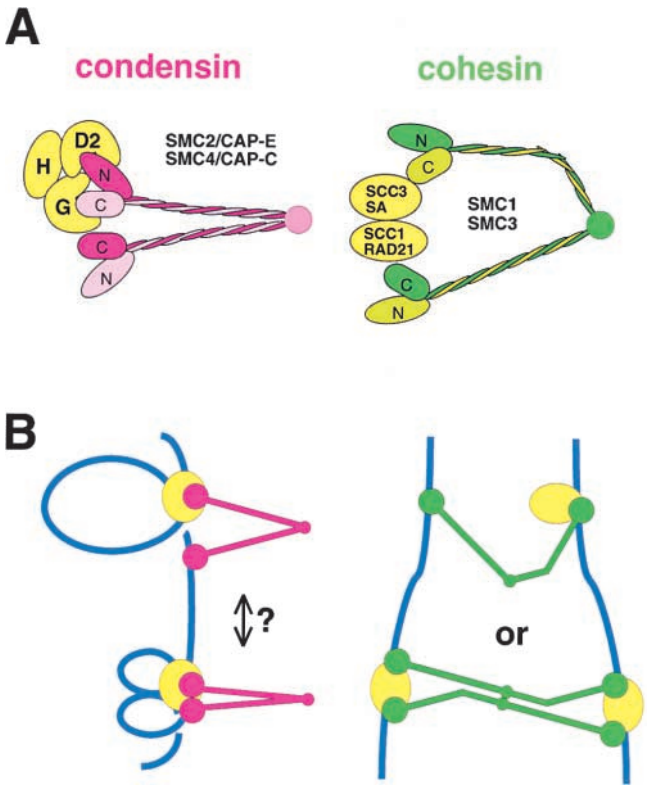

Figure 4. **Models.** (A) Molecular architecture of condensin and cohesin. N and C indicate NH_2-terminal and COOH-terminal non-helical domains, respectively, of the SMC subunits. By analogy to BsSMC, we assume here that the coiled-coil arms of the eukaryotic SMC heterodimers are arranged into an antiparallel fashion. The relative positions of the non-SMC subunits (shown in yellow) are arbitrary. (B) Hypothetical models of the interactions of condensin (left) and cohesin (right) with DNA.

cal, hinge angles are observed in the corresponding SMC heterodimers, suggesting that the conformational difference between condensin and cohesin is, at least in part, intrinsic to their SMC subunits. The closed and open conformations may be determined by either the hinge structure itself, the interactions between the two coiled-coil arms, or both. In the case of condensin, for example, the closed hinge angle could be further stabilized by association of the coiled-coil arms. A sharp kink observed in one of the cohesin arms is likely to be another intrinsic property of its SMC subunits.

The EM clearly shows that the non-SMC subunits bind the catalytic domains of the SMC heterodimer in both condensin and cohesin complexes. The non-SMC subunits of condensin appear to associate with one of the catalytic ends of the SMC2-SMC4 heterodimer, since one free domain is frequently seen (Fig. 1 B, second row). Our previous biochemical study has shown that these non-SMC subunits are essential to activate the SMC ATPase in vitro (Kimura and Hirano, 2000). The current results strongly suggest that this activation is achieved by a direct contact between the non-SMC subunits and the SMC catalytic domain(s). In the cohesin complex, the non-SMC subunits bind both ends of the SMC dimer and appear to keep them in close proximity. In fact, some of the SMC1-SMC3 heterodimers show a more open configuration with the catalytic domains spread farther apart. It should be noted that the position of the non-SMC subunits of cohesin, as revealed by the current work, is not consistent with the model in which the Scc1 subunit bridges two SMC dimers at the hinge and thereby holds sister chromatids together (Uhlmann et al., 1999).

The structure of the SMC protein complexes shares a number of common features with that of Rad50–Mre11, a protein complex involved in double-strand DNA break repair (for review see Haber, 1998). Like SMCs, Rad50 forms a dimer in which two coiled-coil arms are connected by a central hinge, and its catalytic termini are composed of ABC ATPase domains (Hopfner et al., 2000; Lowe et al., 2001). The overall conformation of the Rad50–Mre11 complex is more similar to that of cohesin than to that of condensin (Anderson et al., 2001; Hopfner et al., 2001). The hinge of the Rad50 homodimer tends to be open and the Mre11 subunits bridge the two catalytic end domains of Rad50. It is tempting to speculate that the structural similarities between Rad50–Mre11 and cohesin could reflect a common mechanism of action, as both complexes have been implicated in double-strand break repair (Birkenbihl and Subramani, 1992; Sjogren and Nasmyth, 2001).

Functional implications for the actions of condensin and cohesin

On the basis of their cellular functions, we previously hypothesized that condensin and cohesin might function as intra- and intermolecular DNA cross-linkers, respectively (Hirano, 1999). DNA-binding properties of purified condensin and cohesin in vitro support this model (Kimura and Hirano, 1997; Kimura et al., 1999, 2001; Losada and Hirano, 2001). A recent mechanistic study using a bacterial SMC homodimer has shown that the two different modes of SMC–DNA interactions may be coupled with global conformational changes of the SMC dimers (Hirano et al., 2001). The current EM images of "closed" condensin and "open" cohesin are consistent with this idea, and further suggest that the conformations of the two eukaryotic SMC complexes are structurally differentiated to support their specialized functions. In the case of condensin, the two catalytic domains of the closed SMC dimer may bind to contiguous DNA segments, and thereby the complex would preferentially act as an intra-molecular DNA cross-linker (Fig. 4 B, left). In the case of cohesin, an open hinge would favor the interaction of the two catalytic ends of the SMC dimer with noncontiguous DNA segments, allowing the complex to act as an intermolecular DNA cross-linker. However, almost all the images of cohesin holocomplexes show that both catalytic domains bind the non-SMC subunits, placing them in close proximity. One possibility is that, upon binding to DNA (or with a certain signal), one catalytic domain of cohesin is released from the non-SMC subunits and the wide hinge angle allows the complex to adapt a more open configuration. This conformational change would in turn facilitate the freed end to bind a DNA segment from the sister chromatid (Fig. 4 B, right, top). Moreover, as implied from the analysis of the bacterial SMC protein (Hirano et al., 2001), two SMC dimers may associate with each other, and thereby establish and strengthen the linkage between the sister chromatids (Fig. 4 B, right, bottom). In this scenario,

cleavage of one of the non-SMC subunits in anaphase might destabilize the DNA binding of one or both end(s) of the cohesin complex, allowing the sister chromatids to be pulled apart by the action of the spindle microtubules. Clearly, future studies are required to determine the conformations of condensin and cohesin bound to DNA, and to understand how the DNA-binding properties are regulated by the ATP-binding and hydrolysis cycle.

Materials and methods

Purification of condensin and cohesin

The human condensin and cohesin complexes were purified by affinity chromatography from HeLa cell nuclear extracts as described previously (Losada et al., 2000; Kimura et al., 2001; Losada and Hirano, 2001) with minor modifications. After the binding and washing steps, columns were equilibrated with 20 vol of a buffer containing 0.2 M ammonium bicarbonate, 10 mM Hepes, pH 8.0, and 5% glycerol. The peptides used for elution were dissolved in the same buffer at 0.5 mg/ml. The peak fraction, without further concentration, was supplemented with 1 mM DTT and kept on ice until used. The *Xenopus* condensin and cohesin complexes were purified in a similar way from high-speed supernatants of *Xenopus* egg extracts (Kimura and Hirano, 1997; Losada et al., 2000), using the same elution buffer described above. We noticed that *Xenopus* complexes were less stable than their human counterparts in this buffer at 4°C, and higher populations of heterodimers were apparent in the corresponding EM images. The populations of holocomplexes increased when the purified protein samples were frozen and kept at –70°C before use. In such case, the glycerol concentration of the elution buffer was raised to 10%.

Rotary shadowing and EM

Rotary shadowing and EM were performed as described previously (Melby et al., 1998; Anderson et al., 2001) with minor modifications. Glycerol gradient centrifugation was omitted, and fractions containing condensin or cohesin in the elution buffer were directly sprayed onto mica after adding glycerol to 40%. Specimens were rotary shadowed (Fowler and Erickson, 1979), viewed under a transmission electron microscope (Model 301; Philips Electron Optics) and photographed at 50,000×. Negatives were scanned and imported into the programs Adobe Photoshop 5.5 and NIH image for analysis and measurements of the molecules.

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