Chromosome Condensation by a Human Condensin Complex in Xenopus Egg Extracts*

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13S condensin is a five-subunit protein complex that plays a central role in mitotic chromosome condensa-
tion. The condensin complex was originally identified and purified from Xenopus egg extracts and shown to have an ATP-dependent positive supercoiling activity in vitro. We report here the characterization of a human condensin complex purified from HeLa cell nuclear extracts. The human 13S complex has exactly the same composition as its Xenopus counterpart, being composed of two structural maintenance of chromosomes (human chromosome-associated polypeptide (hCAP)-C and hCAP-E) subunits and three non-structural maintenance of chromosomes (hCAP-D2/CNAP1, hCAP-G, and hCAP-H/BRRN) subunits. Human condensin purified from asynchronous HeLa cell cultures fails to reconfigure DNA structure in vitro. When phosphorylated by purified cdc2-cyclin B, however, it gains the ability to introduce positive supercoils into DNA in the presence of ATP and topoisomerase I. Strikingly, human condensin can induce chromosome condensation when added back into a Xenopus egg extract that has been immu-
nodepleted of endogenous condensin. Thus, the structure and function of the condensin complex are highly conserved between Xenopus and humans, underscoring its fundamental importance in mitotic chromosome dy-
namics in eukaryotic cells.

Chromosome condensation is an essential cellular process that ensures the faithful segregation of chromosomes in both mitosis and meiosis (1, 2). Recent studies in Xenopus egg cell-free extracts led to the identification of a five-subunit protein complex, termed 13S condensin, that plays a key role in this process (3, 4). The Xenopus 13S condensin complex is composed of two subcomplexes, an 8S core subcomplex (8SC) consisting of two structural maintenance of chromosomes (SMC) subunits (XCAP-C and -E) and an 11S regulatory subcomplex (11SR) containing three non-SMC subunits (XCAP-D2, -G, and -H) (3–6). Similar five-subunit complexes have also been identified from Schizosaccharomyces pombe (7) and Saccharomyces cerevisiae (8). Each of the condensin subunits is essential for cell viability in yeasts, and their mutations lead to defects in chromosome condensation and segregation in mitosis (7–12). Subunit composition of the putative condensin complex in human cells is not fully understood, although a complex containing hCAP-C, hCAP-E, and CNAP1 (homologous to XCAP-C, XCAP-E, and XCAP-D2, respectively) has been reported very recently (13).

13S condensin, when purified from Xenopus egg mitotic extracts, displays a DNA-stimulated ATPase activity and changes DNA structure in an ATP-dependent manner in vitro. It introduces positive supercoils into relaxed circular DNA in the presence of type I topoisomerases (14) and converts nicked circular DNA into positively knotted forms in the presence of a type II topoisomerase (15). The interphase form of 13S conden-
sin lacks these activities, although its subunit composition is the same as that of the mitotic form. It was found that mitosis-specific phosphorylation of the non-SMC subunits by purified cdc2-cyclin B can activate the ATP-dependent activities of 13S condensin in vitro (5, 15). Moreover, the ability of 13S conden-
sin to induce DNA supercoiling in the purified system is tightly coupled with its ability to promote chromosome condensation in the cell-free extracts (6). These results suggest strongly that the supercoiling and knotting activities are fundamental to condensin function and directly contribute to mitotic chromo-
some condensation. However, these in vitro activities have so far been detected only in the Xenopus condensin complex purified from egg extracts. It is therefore very important to deter-
mine whether the functional, as well as structural, properties of the condensin complex are conserved in different organisms and at different developmental stages.

In this paper, we report the purification of 13S condensin from HeLa cell nuclear extracts and describe its complete sub-
unit composition. We show that the human complex displays ATP-dependent supercoiling and knotting activities that are regulated by phosphorylation by cdc2-cyclin B in vitro. Finally, a functional complementation assay demonstrates that the human condensin complex can induce chromosome condensation in Xenopus egg extracts.

EXPERIMENTAL PROCEDURES

Cloning of cDNA for hCAP-G—By searching the human expressed sequence tag (EST) data base, we identified a set of partial cDNA sequences that potentially encode the human ortholog of XCAP-G (AW503468, AW194979, AW401913, BE278549, A628901, and AI761782). A nucleotide sequence assembled from these clones encoded a 768-amino acid polypeptide that is homologous to the C-terminus 3/4 of XCAP-G. The following two polymerase chain reaction primers were designed to amplify a human cDNA fragment using a 10 linker library as a template: 5hG1, 5'-CCCTCTAGAGCTATGCAGA-

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBankTM/EBI Data Bank with accession number(s) AF331796.
Figure 1. Sequence alignment of the CAP-G family. hCAP-G was aligned with its ortholog in *Xenopus laevis* (XCAP-G; AF111443), *S. pombe* (Cnd3; AB030214), and *S. cerevisiae* (Ycg1/Tcs5; YDR325W) using ClustalW. HEAT repeats are shown by rectangles. Amino acid residues that match the consensus of HEAT repeats (20) are shown by red, and other conserved residues are shown by blue.

![Figure 1](http://www.jbc.org/)

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AGCATCTTC-3' (XbaI tag sequence is underlined) and 3'GGATGACGGGATTTGATGGG-5' (BamHI tag sequence is underlined). A resulting 530-base pair fragment was used as a hybridization probe to screen a HeLa cell cDNA library (Stratagene). Eight positive clones were analyzed, and seven of them were found to contain the full coding sequence. One of the full-length clones (pHG104) was fully sequenced.

**Antibody Production**—Rabbit polyclonal antisera were raised against synthetic peptides corresponding to the C-terminal sequences of hCAP-G (GTVLPGQGATLHR; see Refs. 5 and 13), hCAP-G (EKSKLNLAQFLNEDLS; this study), and hCAP-G (GAHVEV; see Ref. 16), hCAP-D2/CNAP1 (TTPILRASARRHRR; see Ref. 17), hCAP-C (VAVNPKEIASKGLC; see Ref. 16), hCAP-E (KSKAKPPK-

**RESULTS AND DISCUSSION**

**Cloning of hCAP-G**—A search of the human EST data base identified a set of partial cDNA sequences that potentially encode the human ortholog of XCAP-G, a 130-kDa subunit of the *Xenopus* 13S condensin complex (4). On the basis of this information, we designed polymerase chain reaction primers, amplified the human cDNA fragment, and used it as a hybridization probe to screen a HeLa cell cDNA library. The longest open reading frame deduced from multiple clones encoded a 1,015-amino acid polypeptide with a calculated molecular mass of 114.1 kDa, which was highly homologous to XCAP-G along its entire length (62% identical; 74% conserved). We named this polypeptide human chromosome-associated polyptide-G (hCAP-G). Members of this class of condensin subunits had been reported from *S. pombe* (Cnd3; see Ref. 7) and *S. cerevisiae* (Cnd3; see Ref. 13), hCAP-G (EKSKLNLAQFLNEDLS; this study), and hCAP-G (GAHVEV; see Ref. 16), hCAP-D2/CNAP1 (TTPILRASARRHRR; see Ref. 17), hCAP-C (VAVNPKEIASKGLC; see Ref. 16), hCAP-E (KSKAKPPK-

**Immunodepletion and Rescue**—Immunodepletion of condensin from *Xenopus* egg extracts was performed as described previously (4, 6). For the rescue experiment, an amount of purified condensin was affinity-purified using anti-hCAP-G antibody, fractionated in a 5–20% sucrose gradient, and analyzed by immunoblotting.

**Other Assays**—Sucrose gradient centrifugation, supercoiling, and knotting assays were performed as described previously (4, 14, 15).

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subunits were very similar to those found in Xenopus egg extracts. In this experiment, we did not detect the presence of an 11S consisting of the non-SMC subunits only. This was not surprising, however, because even in the Xenopus egg extracts this subcomplex is present at a very low level (~1/10 of the 13S complex) and not detectable in the same assay (4). We then purified the human 13S complex by immunofluorescence column chromatography using the hCAP-G peptide antibody and fractionated it by sucrose gradient centrifugation (Fig. 2B, lower panel). Again, all the five subunits cofractionated at a single peak of 13S, confirming that they tightly associate with each other and form a complex. Taking these results together, we conclude that the 13S holocomplex of human condensin has exactly the same size and subunit composition as its Xenopus counterpart.

Phosphorylation-dependent Supercoiling and Knotting Activities—The Xenopus 13S condensin complex introduces positive supercoils into relaxed circular DNA in the presence of ATP and topoisomerase II in vitro (supercoiling assay; see Ref. 14). It also converts nicked circular DNA into positively knotted forms in the presence of ATP and topoisomerase II (knotting assay; see Ref. 15). The two activities are regulated by mitosis-specific phosphorylation of the non-SMC subunits (5, 15). We wished to test whether human condensin displays a similar set of activities. When the complex was affinity-purified from a nuclear extract of an asynchronously grown HeLa cell culture (Fig. 3A, lane 1), it exhibited little activity in the supercoiling and knotting assays (Fig. 3B, lanes 1–4). We reasoned that most of the purified complexes were in the interphase (unphosphorylated) form, thereby producing the negative result. To test this possibility, the purified condensin fraction was treated with cdc2-cyclin B (Fig. 3A, lane 2). This treatment phosphorylated the three non-SMC subunits, hCAP-D2, hCAP-G, and hCAP-H, as judged by [32P] labeling (Fig. 3A, lane 4). Interestingly, we found that the phosphorylated form of condensin was active in both of supercoiling and knotting assays (Fig. 3B, lanes 5–8). Neither of these activities was found in the purified cdc2-cyclin B fraction alone. The apparently less effective stimulation of the knotting activity compared with the supercoiling activity (Fig. 3B, lanes 4 and 8) is probably because of the less quantitative nature of the former assay; a similar observation was made with Xenopus condensin (15). As expected, two-dimensional gel electrophoresis demonstrated that the final products of the supercoiling assay were positively supercoiled (data not shown).
shown). These results show that the human condensin complex displays the same set of biochemical activities as its Xenopus counterpart. The cdc2-mediated stimulation of these activities also suggests that they contribute directly to mitosis-specific condensation of chromosomes in human somatic cells.

**Human Condensin Induces Chromosome Condensation in Xenopus Egg Extracts**—To further test for the functional similarity between the human and Xenopus condensin complexes, we set up a complementation assay in Xenopus egg extracts. When sperm chromatin was incubated in a control extract containing endogenous condensin (Fig. 4A, lane 1), it was converted into a mass of mitotic chromosomes (Fig. 4B, panel a). In a condensin-depleted extract (Fig. 4A, lane 2), however, no chromosome assembly occurred (Fig. 4B, panel b). When purified Xenopus condensin (Fig. 4A, lane 3) was added back into the extract, it restored the ability of the extract to condense chromosomes (Fig. 4B, panel c) as we reported previously (4, 6). Strikingly, we found that human condensin purified from a HeLa nuclear extract (Fig. 4A, lane 4) could also functionally complement the extract, inducing chromosome condensation very effectively (Fig. 4B, panel d). No pre-treatment with cdc2-cyclin B was required in this assay, suggesting that the human complex was phosphorylated by a protein kinase(s) present in the Xenopus egg extract and converted into an active complex.

In summary, the current work identifies, for the first time, all the five subunits of the 13S condensin complex purified from HeLa cells. Like Xenopus condensin, the human complex displays ATP- and phosphorylation-dependent supercoiling and knotting activities *in vitro*, providing strong lines of evidence that they are fundamental to condensin function (and thereby mitotic chromosome condensation), not only in early embryonic cells but also in somatic cells.

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