Identification of two distinct human SMC protein complexes involved in mitotic chromosome dynamics

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ABSTRACT The *s*tructural *m*aintenance of *c*hromosomes (SMC) family member proteins previously were shown to play a critical role in mitotic chromosome condensation and segregation in yeast and *Xenopus*. Other family members were demonstrated to be required for DNA repair in yeast and mammals. Although several different SMC proteins were identified in different organisms, little is known about the SMC proteins in humans. Here, we report the identification of four human SMC proteins that form two distinct heterodimeric complexes in the cell, the human chromosome-associated protein (hCAP)-C and hCAP-E protein complex (hCAP-C/hCAP-E), and the human SMC1 (hSMC1) and hSMC3 protein complex (hSMC1/hSMC3). The hCAP-C/hCAP-E complex is the human ortholog of the *Xenopus* chromosome-associated protein (XCAP)-C/XCAP-E complex required for mitotic chromosome condensation. We found that a second complex, hSMC1/hSMC3, is required for metaphase progression in mitotic cells. Punctate vs. diffuse distribution patterns of the hCAP-C/hCAP-E and hSMC1/hSMC3 complexes in the interphase nucleus indicate independent behaviors of the two complexes during the cell cycle. These results suggest that two distinct classes of SMC protein complexes are involved in different aspects of mitotic chromosome organization in human cells.

The eukaryotic genome undergoes dynamic structural changes during the cell cycle to carry out multiple functions. During interphase, genomic DNA wrapped around nucleosomes forms 10- to 30-nm chromatin fibers and serves as a template for replication and transcription. During mitosis, chromatin fibers condense to form 700-nm diameter chromosome structures to achieve proper segregation of genetic information during subsequent cell division. Although fundamental to the cell, the molecular mechanisms of such complex changes of higher order chromatin structure are not well characterized.

The *s*tructural *m*aintenance of *c*hromosome (SMC) genes, *SMC1* and *SMC2*, originally were identified in *Saccharomyces cerevisiae* as genes required for proper condensation and segregation of mitotic chromosomes (1, 2). *Xenopus* chromosome-associated protein-C (XCAP-C) and XCAP-E, the SMC homologs in *Xenopus* isolated from mitotic oocyte extracts, were shown to be required for the early stage of mitotic chromosome condensation *in vitro* and to be physically associated with condensed chromosomes as part of a multiprotein complex called "condensins" (3, 4). These studies revealed that the SMC family proteins are integral components of the machinery that modulates chromosome structure for mitosis. The predicted secondary structure of SMC proteins resembles

myosin and contains conserved head and tail domains with putative NTP-binding sites, as well as a coiled-coil region located in the middle (Fig. 1C) (for reviews, see refs. 5–9). The C-terminal conserved domain also is called the "DA" box (6). Based on this structure, it has been postulated that SMC family proteins may function as motor proteins analogous to myosin in promoting chromatin movement during its structural changes.

SMC family proteins can be divided into two subfamilies based on the sequence differences within the conserved C-terminal regions (2) (Fig. 1A). For example, XCAP-C in *Xenopus* and cut3 in *Schizosaccharomyces pombe*, which belong to the SMC1(XCAP-C) subfamily, appear to form complexes in an equimolar ratio with XCAP-E and cut14 belonging to the SMC2(XCAP-E) subfamily, respectively (3, 10). Gel filtration analyses suggest that they form a heterotetramer (5).

The SMC homologs have been found in a variety of organisms, including *Escherichia coli* and humans, underscoring their essential role in the cell. A *Caenorhabditis elegans* homolog DPY-27 was shown to be required for X chromosome dosage compensation (11), and a more distantly related family member was found in *S. pombe* to be involved in DNA repair (12), suggesting a potential common structure of SMC proteins required for multiple nuclear events during interphase. An avian SMC2 homolog was shown to be identical to chromosome scaffold protein II, a major component of the chromosome scaffold (13). In mammals, human SB1.8 was cloned based on homology to the SMC1(XCAP-C) subfamily proteins in lower eukaryotes, although no function has been described for it (14). Recently, the bovine homologs BSMC1 and BSMC2 were shown to be part of the DNA recombination repair complex RC-1 (15). Even though the sequences of only the first 20 amino acids of BSMC1 and BSMC2 are available, they share extensive homology to the corresponding regions of other SMC1(XCAP-C) and SMC2(XCAP-E) subfamily proteins, respectively. In particular, SB1.8 and BSMC1 are identical in this short stretch of N terminus residues, suggesting a similar recombination-related role for SB1.8 in humans.

Although these studies highlight the functional significance of the SMC protein family, it remains unclear how many family members are conserved in higher eukaryotes, and how their functional specificities are determined. The molecular mechanisms of SMC functions in chromosome condensation and other nuclear events remain elusive. Because little is known about the human SMC family proteins, we set out to system-

Abbreviations: SMC, structural maintenance of chromosomes; hSMC, human SMC; hCAP, human chromosome-associated protein; HCAP, human chromosome-associated polypeptide; XCAP, *Xenopus* chromosome-associated protein; Ab, antibody; DAPI, 4′,6-diamidino-2-phenylindole; P140, a protein of 140 kDa.
Data deposition: The sequences reported in this paper have been deposited in the GenBank database [accession nos. AF092563 (hCAP-E) and AF092564 (hCAP-C)].
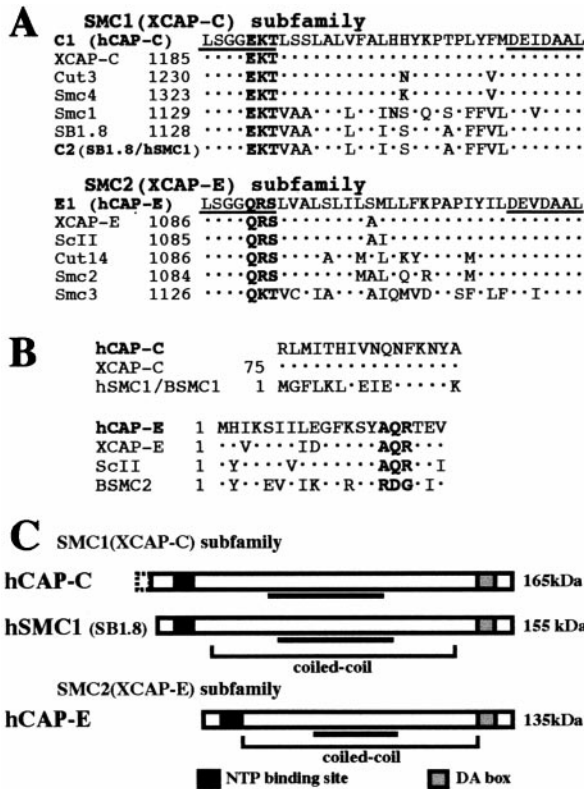§To whom reprint requests should be addressed. e-mail: kyokomor@uci.edu.

FIG. 1. Identification of three human SMC family proteins. *(A)* Sequence comparison of the conserved C-terminal domains of the SMC family proteins. Three PCR fragments C1, C2, and E1 obtained from human cDNAs are translated and compared with other family members [either SMC1(XCAP-C) or SMC2(XCAP-E) subfamily] in different species. Human SMC protein genes, to which the PCR fragments correspond, are indicated in parentheses. C1 (hCAP-C) and C2 (SB1.8/hSMC1) were aligned with XCAP-C (*Xenopus*) (3), cut3 (*S. pombe*) (10), Smc4 (*S. cerevisiae* data base), Smc1 (*S. cerevisiae*) (1), and hSMC1 (human SB1.8) (14). E1 (hCAP-E) was compared with XCAP-E (*Xenopus*) (3), scaffold protein II (chicken) (13), cut14 (*S. pombe*) (10), Smc2 (*S. cerevisiae*) (2), and Smc3 (*S. cerevisiae* data-base). Amino acids identical to C1 or E1 are shown as "·," and three amino acids that distinguish two subfamilies are shown in bold letters (EKT vs. QRS). The numbers represent the amino acid positions of each protein. The amino acid sequences used to design PCR primers are underlined. *(B)* Sequence comparison of the N-terminal sequences of SMC proteins in vertebrates. The N-terminal sequence of BSMC1 (identical to the corresponding region of SB1.8) is compared with XCAP-C and hCAP-C (see amino acid numbers). The N-terminal sequence of hCAP-E is compared with those of XCAP-E, Scaffold protein II, and BSMC2 (15). Three amino acids exhibiting nonconserved changes in BSMC2 are indicated in boldfaced letters. *(C)* A schematic diagram of three human SMC protein cDNA clones. hSMC1 and hCAP-E cDNA clones are full-length, whereas the hCAP-C cDNA clone lacks the region corresponding to the first 80 amino acids. The conserved NTP-binding motif and DA box in the N and C termini are shown by ■ and ▨, respectively. The diverged coiled-coil domain in the middle is indicated. The thick underlines represent the region of proteins against which antibodies used in this study were raised.

atically screen for SMC proteins in human cells and characterize their roles in the cell. Here we report the identification and cloning of multiple human SMC proteins and the presence of two biochemically and functionally distinct SMC complexes in human cells. One complex is the human ortholog of *Xenopus* XCAP-C/XCAP-E involved in chromosome condensation, whereas the other contains SB1.8, a homolog of BSMC1 linked to recombination (3, 14, 15). Our results suggest that the two complexes represent different classes of SMC complexes and are involved in distinct aspects of mitotic chromosome organization.

MATERIALS AND METHODS

cDNA Cloning of Human SMC Family Proteins. Based on conserved amino acid sequences within the C terminus of *Xenopus*, yeast and *C. elegans* SMC proteins (Fig. 1*A*), degenerate oligonucleotide primers were designed and "touch-down" PCRs were performed on various human cDNA libraries as described (16). PCR primers correspond to the first and last seven amino acid stretches of the sequences (Fig. 1*A*; underlined sequences). After cloning and sequencing, the specific PCR products were used as probes to screen a human teratocarcinoma cDNA library.

Antibody Production and Affinity Purification. Rabbit polyclonal antibodies were generated against bacterially expressed subdomains of the human SMC proteins identified above. Antibodies were subsequently affinity-purified by using antigen-affinity columns. Specificities of antibodies were confirmed by Western blot analysis of the endogenous proteins in crude HeLa nuclear extracts (Fig. 2*A*).

Immunofluorescent Staining and Confocal Microscopy Analyses. HeLa cells were fixed with acetone at −20°C for 5 min and air-dried for 15 min at room temperature. Alternatively, cells were fixed with paraformaldehyde followed by
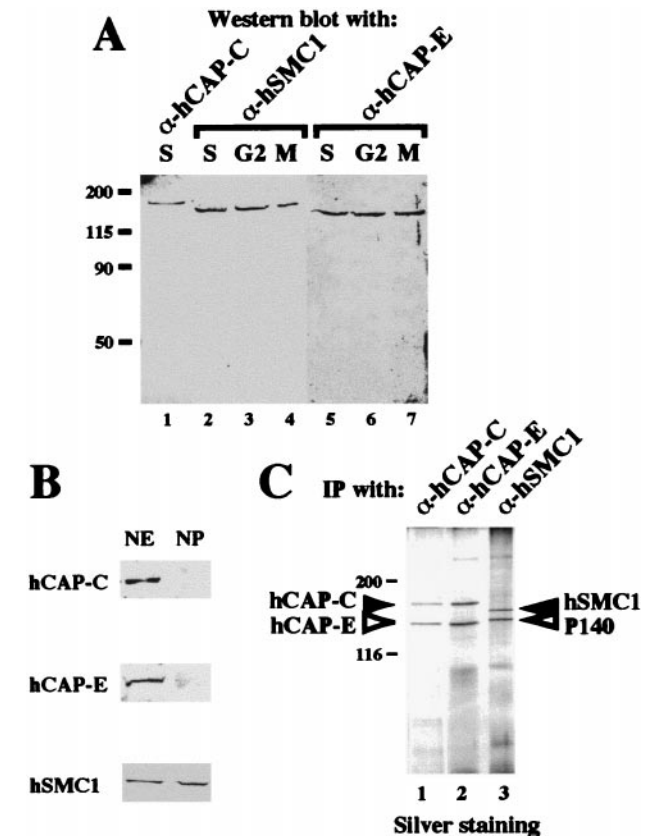

FIG. 2. Detection of the endogenous human SMC proteins. *(A)* Western blot analysis of the endogenous SMC proteins in a HeLa nuclear extract. HeLa crude nuclear extracts synchronized in S and M phases were applied to SDS/PAGE, transferred to a nitrocellulose membrane, and probed with the antibody indicated at the top. The sizes of the polypeptides detected are ≈165 kDa for hCAP-C (lane 1), 150 kDa for hSMC1 (lane 2), and 135 kDa for hCAP-E (lane 3). *(B)* Solubility of human SMC in the extract. Both nuclear extract (NE) and nuclear pellet (NP) (insoluble material left after the 0.4M salt extraction) were subjected to Western blot analysis, using the same antibodies as in *A*. *(C)* Silver staining of the heterodimeric human SMC complexes immunoprecipitated with antibodies specific for hCAP-C, hCAP-E or hSMC1 (lanes 1, 2, and 3, respectively). Immunoprecipitates were briefly washed with 1M guanidine-HCl. The polypeptide coprecipitated with hSMC1 is indicated as P140.

permeabilization with 2% Triton X-100. In addition, HT1080, an osteosarcoma cell line (kindly provided by Judith Campisi, Lawrence Berkeley Laboratories, Berkeley, CA) and IMR90, normal female human fibroblast cells (a generous gift from Eric Stanbridge, University of California, Irvine) were used. Cells were first blocked with PBS containing 0.02% saponin, 0.05% NaN$_3$, and 1% BSA with 0.1% fish gelatin and 4% normal goat serum at 37°C for 15 min and incubated with primary antibodies in the same buffer with 0.05% fish gelatin and 1% normal goat serum at 37°C for 30 min. Cy3-conjugated goat anti-rabbit IgG antibody (Ab) (The Jackson Laboratory) was used to detect the primary antibodies. DNA was detected by 4′,6-diamidino-2-phenylindole (DAPI) staining. Chromosome spreads were prepared either from HeLa or HT1080 cells according to the published protocol with minor modification (17). Briefly, the cells were resuspended in 0.5 ml of phosphate buffer and incubated for 10 min at 37°C. The cell suspension was placed on a glass coverslip in a tube and centrifuged until 3,000 rpm was reached in a Beckman GS-6R centrifuge. The cells were immediately fixed with 3% formaldehyde in PBS buffer for 5 min at 4°C. Cells on coverslips were then subjected to immunostaining as described above. The staining was analyzed by immunofluorescence microscopy or laser confocal microscopy.

Immunoprecipitation of the Endogenous SMC Protein Complexes. HeLa nuclear extract was prepared as described (16). Antibody was prebound to protein-A beads and incubated with the crude nuclear extract for 3 hr at 4°C. To detect protein species tightly bound to Ab beads, the beads were washed with a buffer containing 0.1 M and 1 M KCl in the presence of 0.1% Nonidet P-40, and then washed with 1 M guanidine-HCl. Samples were analyzed by SDS/PAGE and silver staining, or subjected to Western blot analysis.

Peptide Sequencing Analysis. Large-scale immunoprecipitation of the SMC complex (≈5 µg per protein species) was subjected to preparative SDS/PAGE and transferred to nictrocellulose membrane. Protein bands were excised and digested with trypsin. Microsequencing analysis was performed as described (16).

Antibody Microinjection. Affinity-purified antibody against human SMC1 (hSMC1) was concentrated by spin columns (Microcon, Millipore) to a final concentration of 3–4 mg/ml in a buffer containing 100 mM potassium aspartate and 20 mM Hepes (pH 7.2). Antibody was injected into mitotic HeLa cells. As controls, buffer alone or anti-glutathione *S*-transferase Ab prepared in the same manner were injected into mitotic HeLa cells on separate plates. Fura-2, pentapotassium salt (5.4 mM) (Molecular Probes) was coinjected as a marker for injection. Asynchronous HeLa cells were seeded on a glass coverslip that had been cemented with silicone sealant (Dow-Corning) over a machined hole in the bottom of a 35-mm tissue culture plate. Immediately before the injection, DMEM was replaced by a Ringer's solution (138 mM NaCl/2.7 mM KCl/1.06 mM MgCl$_2$/5.6 mM D-glucose/1.8 mM CaCl$_2$/2.4 mM Hepes, pH 7.25) without sodium bicarbonate to maintain a stable pH in the absence of CO$_2$. Grids were drawn on the coverslip to locate the positions of the injected cells. The culture plate then was placed on a 36°C heating stage on a Zeiss IM35 inverted microscope. The Nikon 40× glycerol immersion objective lens was wrapped in Tygon tubing connected to a water bath. Temperature above the lens was 36°C. Antibody was injected into mitotic HeLa cells at metaphase and anaphase. Mid/late metaphase and very late metaphase/early anaphase of HeLa cells can be distinguished by the morphology of the metaphase plate and cell shape under the microscope, as well as by the length of time necessary for the cell to reach cytokinesis. Under our conditions, it typically takes 45–60 min for the control cells at early metaphase to reach cytokinesis and cell division, and 15–25 min for very late metaphase/early anaphase cells. Cell cycle progression of the injected cells was monitored continuously under the microscope on the heating stage for 1 hr in comparison with that of uninjected cells on the same plate. Cells then were returned to the CO$_2$ incubator. The cells were fixed by acetone 2.5 hr postinjection. The injected antibodies subsequently were detected by immunostaining. Six to seven cells were injected in one experiment, and the experiments were repeated three times.

RESULTS

Identification of Multiple Human SMC Family Proteins. Degenerate oligonucleotide primers were designed to yield PCR products spanning the conserved C-terminal regions of the SMC1(XCAP-C) and SMC2(XCAP-E) subfamilies (see *Introduction*) from human cDNA library templates (Fig. 1*A*). Deduced amino acid sequences of two PCR fragments were characteristic of the SMC1(XCAP-C) subfamily and were designated C1 and C2. The third fragment belongs to the SMC2(XCAP-E) subfamily and was designated E1 (Fig. 1*A*). Using these PCR products as probes, we obtained three independent cDNA clones. The cDNA clone isolated by the C2 probe is identical to SB1.8 (14). The overall amino acid sequence identity of SB1.8 to Smc1 of *S. cerevisiae* is 25.4%, whereas SB1.8 shares only 17.5% identity to other SMC1(XCAP-C) subfamily proteins Smc4 (*S. cerevisiae*) and XCAP-C (*Xenopus*). Therefore, the protein product of SB1.8 is referred to as human SMC1 (hSMC1) (Fig. 1*C*). The cDNA clone obtained by the C1 probe is highly homologous to *Xenopus* XCAP-C, and therefore is termed human CAP-C (hCAP-C) (Fig. 1*C*). The hCAP-C clone was missing the first 80 amino acids found in XCAP-C. The homology of hCAP-C to XCAP-C includes the middle coiled-coil region, which is not conserved in hSMC1 (data not shown). Furthermore, hCAP-C and XCAP-C have additional N-terminal sequences compared with hSMC1, which share only a limited sequence homology (Fig. 1*B*). These data indicate that hCAP-C and XCAP-C belong to a subclass of the SMC1(XCAP-C) subfamily different from hSMC1. The full-length cDNA containing the E1 sequence belongs to the SMC2(XCAP-E) subfamily and is highly homologous to XCAP-E (79.8% identity), and it was termed hCAP-E (Fig. 1*C*). In addition, the hCAP-E protein shares 66.3% amino acid sequence identity with chicken scaffold protein II, and is more closely related to *S. cerevisiae* Smc2 (34.9% identity) than to Smc3 (17.0% identity) within the SMC2(XCAP-E) subfamily. Furthermore, hCAP-E and XCAP-E contain distinct amino acid differences in the N terminus compared with BSMC2, a partner of BSMC1 (Fig. 1*B*). Northern blot analysis revealed that hSMC1, hCAP-C, and hCAP-E proteins are encoded by mRNAs of ≈6.5, 5, and 4.5 kb, respectively (data not shown).

hCAP-C and hCAP-E Form a Heterodimeric Complex, Which Is Distinct from a hSMC1-Containing Complex. Affinity-purified antibodies directed against the diverged middle domain of hCAP-C, hSMC1, and hCAP-E (underlined in Fig. 1*C*) each recognized single distinct polypeptide species in crude HeLa nuclear extracts as determined by Western blot analysis (Fig. 2*A*). All three proteins are expressed throughout the cell cycle (Fig. 2*A*, hCAP-C, data not shown). Expression of the three proteins is observed in a variety of cell lines (HeLa, Daudi B cell line, Jurkat T cell line, SK2 neuronal cell line, and HepG2 liver cell line; data not shown). High salt extraction of nuclei revealed that hCAP-C and hCAP-E are highly soluble, whereas some of hSMC1 remains in the nuclear pellet (Fig. 2*B*), suggesting that hSMC1 may be associated with different components in the nucleus with tighter binding.

Immunoprecipitation from HeLa nuclear extracts revealed that hCAP-C and hCAP-E are associated with each other (Fig. 2*C*). Reciprocal immunoprecipitation confirmed that all the hCAP-C and hCAP-E molecules exist as a heterodimeric complex in the cell (Fig. 2*C*, compare lanes 1 and 2). This

notion is further supported by the copurification of hCAP-C and hCAP-E by using various column chromatography matrices (K.Y., data not shown). This interaction is highly stable and resistant to mild denaturation with 1 M guanidine hydrochloride. Similarly, the soluble fraction of hSMC1 is found in an equimolar interaction with a protein of 140 kDa (P140). P140 is distinct from hCAP-E in size (Fig. 2C). Antibodies against the N-terminal and middle regions of hCAP-E did not cross-react with P140 (data not shown). These results demonstrate that there are two distinct SMC complexes in human cells. One complex is composed of hCAP-C and hCAP-E, whereas a second complex consists of hSMC1 and P140. These heterodimeric complexes are referred to as hCAP-C/hCAP-E and hSMC1/P140 in the text. Although we call the complexes "heterodimeric" in the text to emphasize the equimolar association of the two SMC proteins, gel filtration analyses established that both endogenous complexes are larger than 600 kDa in size, indicating formation of a heterotetramer in solution similar to the XCAP-C/XCAP-E complex in *Xenopus* (data not shown) (3). The results indicate that a heterodimeric complex between SMC1(XCAP-C)- and SMC2(XCAP-E)-subfamily proteins is a highly stable molecular unit in human cells, and the partnering between the two subfamily members for complex formation is highly specific. This is in contrast to the SMC2(XCAP-E) protein MIX-1 in *C. elegans*, which appears to form two different complexes for X chromosome dosage compensation and mitosis (18). The hCAP-C/hCAP-E and hSMC1/P140 complexes are found throughout the cell cycle (data not shown). In addition, we failed to detect any obvious interaction between the two complexes.

hCAP-C/hCAP-E Is the Human Ortholog of *Xenopus* XCAP-C/XCAP-E, and Exhibits a Subcellular Localization Pattern Distinct from hSMC1/P140. To further analyze the specificity of the two SMC complexes, immunolocalization analysis was performed in HeLa cells (Fig. 3). The results demonstrated that the two human SMC complexes behave differently in the cell. Immunofluorescent staining with anti-hCAP-E antibody revealed that hCAP-E is associated with condensed chromosomes in mitotic cells and in mitotic cell chromosome spreads (Fig. 3A, panels 2 and 4), whereas anti-hSMC1 antibody staining showed that hSMC1 is excluded from mitotic chromosomes but present in the rest of the cell (Fig. 3B, panel 4). High sequence similarities (≈80%) between hCAP-C and hCAP-E with XCAP-C and XCAP-E, and the association of hCAP-C/hCAP-E with mitotic chromosomes strongly suggest that the hCAP-C/hCAP-E complex is the ortholog of the *Xenopus* XCAP-C/XCAP-E complex, and most likely is required for mitotic chromosome condensation (Fig. 1C) (3).

Distribution patterns of these complexes in the cell nucleus during interphase are also different (Fig. 3A, panel 2, and Fig. 3B, panel 2). Immunofluorescent staining of hCAP-E shows distinct dense speckles in the nucleus (Fig. 3A, panel 2), whereas hSMC1 exhibits a granular staining of the entire nucleus (Fig. 3B, panel 2). Similar localization patterns of SMC proteins also were observed in the normal human fibroblast IMR90 cells (data not shown). Together, these results indicate that the two SMC complexes are independently distributed in the cell throughout the cell cycle, and that the two complexes most likely function by distinct mechanisms.

The hSMC1/P140 Complex Is Involved in Mitosis. Immunofluorescent staining suggested that hSMC1/P140 may be excluded from mitotic chromosomes during mitosis, in contrast to the hCAP-C/hCAP-E complex. Furthermore, hSMC1 appears to be identical to BSMC1, which was shown to play a role in DNA recombination (15). These data suggest that hSMC1/P140 may have a role during interphase distinct from hCAP-C/hCAP-E. However, sequence comparison revealed that hSMC1 is related to Smc1 of *S. cerevisiae* involved in mitosis (1). Because hSMC1 is present in mitotic cells (Figs. 2A and
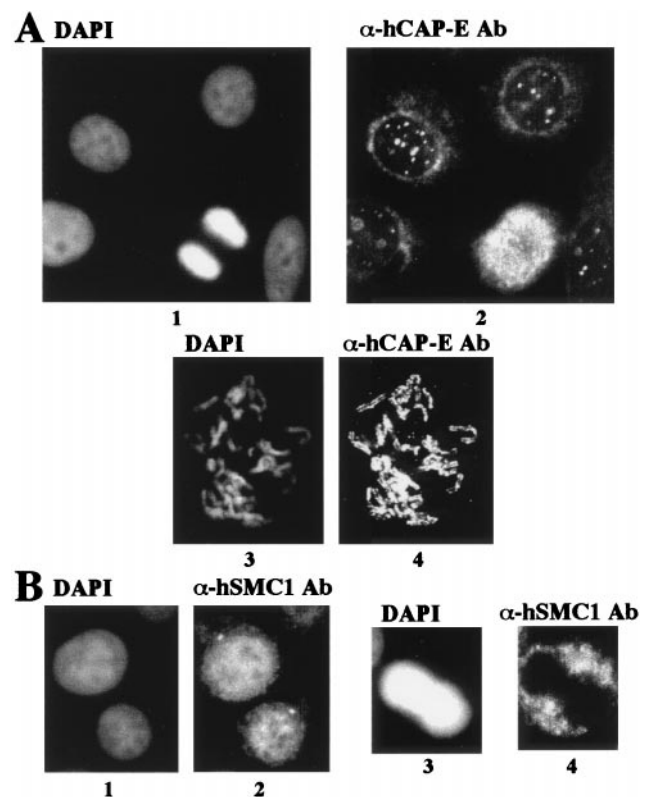

FIG. 3. Immunofluorescent antibody staining analysis of hCAP-C/hCAP-E and hSMC1/P140 complexes in cells. (A) Localization of hCAP-C/hCAP-E complexes. DNA was detected by DAPI staining (panels 1 and 3). Immunofluorescent staining with anti-hCAP-E Ab of interphase and mitotic HeLa cells (panel 2) and chromosome spread of HT1080 cells (panel 4). (B) Localization of hSMC1/P140 complexes. Panels 1 and 3 are DAPI. Interphase (panel 2) and mitotic (panel 4) cells were stained with anti-hSMC1 Ab.

3B, panel 4), we tested the possibility that hSMC1/P140 may play a role in mitosis by using antibody-microinjection assays. Antibody specific for the middle region of hSMC1 was used, which did not show any crossreactivity to other proteins in the nuclear extract (Fig. 2A). Antibody injected into metaphase cells efficiently blocked the progression of mitosis at metaphase (Fig. 4A). After injection, an irregular morphology of the metaphase plate was observed (Fig. 4C, panel 1). The same arresting effect also was observed by using antibodies against the C-terminal regions of hSMC1 (data not shown). A control injection with anti-glutathione S-transferase Ab had no effect (data not shown). Interestingly, injection of anti-hSMC1 antibody into the cells at the onset of anaphase failed to affect the progression of the mitotic process to cytokinesis and generation of two daughter cells (Fig. 4 B and C, panel 2), suggesting that hSMC1 is not required during anaphase. In contrast, microinjection of antibody specific for hCAP-C or hCAP-E failed to arrest metaphase cells (data not shown). This could be caused by a lack of neutralizing antibody populations in our polyclonal antibody preparations. A more likely possibility is that the antibody injection in the cells in metaphase may be too late to interfere with the function of hCAP-C/hCAP-E in chromosome condensation already initiated in interphase. Generation of antibodies against different epitopes of hCAP-C and hCAP-E and microinjection of antibodies at earlier time points in the course of chromosome condensation will be attempted to further analyze the function of hCAP-C/hCAP-E in the cell. Nonetheless these results indicate that hSMC1/P140 is involved in proper progression of metaphase in human cells.
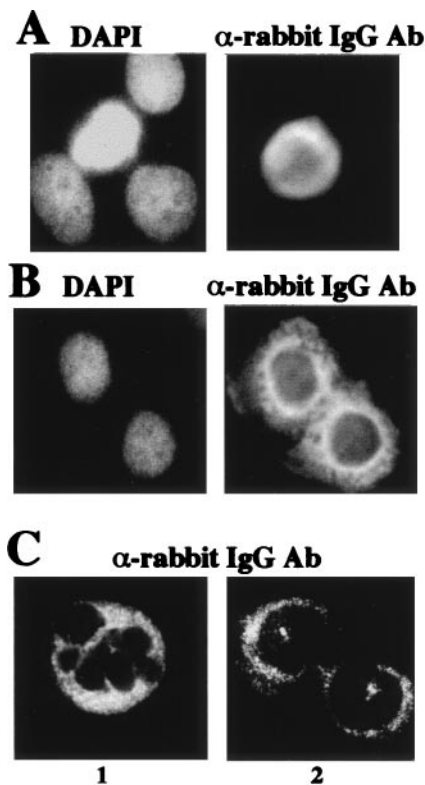
FIG. 4. The effect of microinjection of anti-hSMC1 Ab in mitotic HeLa cells. Cells were fixed after 2.5 hr and the injected antibodies were detected by immunofluorescent staining with anti-rabbit IgG Ab *(A)* A cell injected during mid/late metaphase is arrested in mitosis. *(Left)* DAPI staining of DNA. *(Right)* Anti-(α)-rabbit IgG Ab staining the injected anti-hSMC1-Ab as indicated at the top. *(B)* A cell injected at early anaphase subsequently went through cytokinesis and resulted in two daughter cells. Again, DAPI staining and antibody staining are in left and right panels, respectively, as indicated. *(C)* Laser confocal microscopic analysis of cells injected with anti-hSMC1. Cells injected with antibody during mid/late metaphase (panel 1) and at early anaphase that subsequently underwent cytokinesis (panel 2).

P140 Is the hSMC3 Protein. Because P140 tightly associates with hSMC1 in a manner similar to the hCAP-E and hCAP-C heterodimeric complex (Fig. 2*C*), we characterized P140 by obtaining peptide sequences from the purified endogenous complex. Six peptide sequences (KINQMATAPDSQR, RA-LEYTIYNQELN, KAKD, RSMEV, KTFMPK, and KATLVMK) obtained were found in the human chromosome-associated polypeptide (HCAP), a recently reported human SMC family protein gene product (19) (Fig. 5). Sequence analysis revealed that HCAP belongs to the SMC2(XCAP-E) subfamily, but is distinct from hCAP-E sharing only 19.5% amino acid identity. Consequently, HCAP is clearly not the ortholog of XCAP-E (19.3% identity), and is relatively closely related to Smc3 (29.9%) compared with Smc2 (18.3%) within
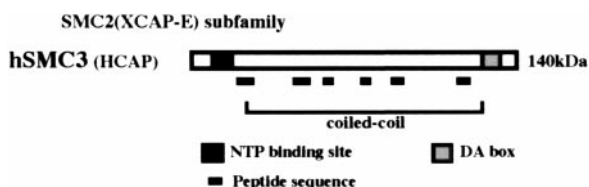

FIG. 5. A schematic diagram of the hSMC3 (HCAP) protein. The regions of protein corresponding to the peptide sequences obtained are indicated by the thick underlines. The conserved NTP-binding motif and DA box in the N and C termini are shown by ■ and ▨, respectively. The diverged coiled-coil domain in the middle is indicated.

the subfamily. Therefore, HCAP is the hSMC3 specifically complexed with hSMC1. Taken together, these results indicate that human cells express four distinct SMC proteins, hSMC1, hCAP-E, hSMC3, and hCAP-C, which are equivalent to Smc1, Smc2, Smc3, and Smc4 in *S. cerevisiae*, and form two separate complexes hSMC1/hSMC3 and hCAP-C/hCAP-E (Table 1).

DISCUSSION

Previous studies showed that several SMC family proteins play a role in mitosis in yeast (1, 2, 10, 20, 21). However, the molecular relationships of multiple SMC proteins were not completely understood. We report here the identification of four human SMC family proteins that form two distinct classes of heterodimeric SMC complexes in the cell, hCAP-C/ hCAP-E and hSMC1/hSMC3. We concluded that hCAP-C/ hCAP-E is the ortholog of *Xenopus* XCAP-C/XCAP-E required for mitotic chromosome condensation based on its specific association with condensed chromosomes in mitotic cells and high sequence similarity to XCAP-C/XCAP-E. In addition, we found that the second complex, hSMC1/hSMC3, is required for progression of metaphase during mitosis. These results suggest that two distinct SMC complexes are involved in mitotic chromosome organization in human cells.

A SMC1(XCAP-C) Subfamily Protein Specifically Interacts with a Member of the SMC2(XCAP-E) Subfamily. We identified four SMC family members that are components of two different heterodimeric complexes in human cells. hCAP-C and the previously cloned hSMC1 (SB1.8) (14), both of which belong to the SMC1(XCAP-C) subfamily, tightly interact with a SMC2(XCAP-E) subfamily protein hCAP-E and hSMC3, respectively. Despite the homology of the conserved head and tail regions, partnering of SMC family members is not interchangeable between the two SMC complexes. As is the case in human cells, four SMC genes are found in the *S. cerevisiae* genome (Smc1 and Smc4 are in the SMC1/XCAP-C subfamily, and Smc2 and Smc3 are the SMC2/XCAP-E subfamily members) (8, 22). The patterns of pairing appear to be evolutionarily conserved (Table 1) (8, 21, 22), even though overall amino acid sequence identity and similarity is not very high between the SMC proteins in yeast and humans (25–35% identity). The genetic interaction study (21) revealed that Smc1 and Smc3 are involved in sister chromatid cohesion in yeast, suggesting the formation of two separate heterodimeric complexes, Smc1/Smc3 and Smc4/Smc2, although these interactions have not been shown biochemically (Table 1) (23). Furthermore, the most recent report in *Xenopus* demonstrated that *Xenopus* embryos also express the second SMC complex containing XSMC1 and XSMC3 required for sister chromatid cohesion (24). Therefore, two classes of SMC heterodimeric complexes appear to be present in different organisms. This is different from *C. elegans*, in which three SMC1(XCAP-C) subfamily proteins and only one SMC2(XCAP-E) subfamily protein have been identified thus far (P. T. Chuang and B. J. Meyer, personal communication). *C. elegans* has one extra SMC1 subfamily protein, DPY-27, which is specialized for X chromosome dosage compensation (11). MIX-1, the only homolog of XCAP-E identified thus far in *C. elegans*, appears to play dual roles in mitosis and X chromosome dosage compensation (18). It is not known whether MIX-1 interacts with all three SMC1(XCAP-C) subfamily proteins or if there is an additional SMC2(XCAP-E) subfamily protein in *C. elegans*. In all cases, however, the interactions between the two subfamily proteins appear to be critical for their functional specificities.

It is not known whether there are more than two SMC heterodimeric complexes in human cells, analogous to the functionally specialized DPY-27/MIX-1 complex in *C. elegans* (11). Based on expression levels, the two complexes that we identified are the major populations in the cell. There are less

Table 1.   The two classes of heterodimeric SMC protein complexes containing SMC1(XCAP-C) and SMC2(XCAP-E) subfamily members

	Condensation	Cohesion	Ref.
S. cerevisiae	Smc4/Smc2	Smc1/Smc3	(1, 2, 22)
S. pombe	cut 3/cut14	?/?	(10)
C. elegans	?/MIX-1	?/?	
	DPY-27/MIX-1		(11, 18)
Xenopus	XCAP-C/XCAP-E	XSMC1/XSMC3	(3, 24)
Avian	?/ScII	?/?	(13)
Bovine	?/?	BSMC1/BSMC3 (formerly BSMC2)	(15, 23)
Human	hCAP-C/hCAP-E	hSMC1/hSMC3 (SB1.8/HCAP)	(this paper, 14, 19)

closely related family members found in yeast that play a role in DNA repair (12). Human homologs of these proteins have not been identified.

"Condensation-Related" and "Cohesion-Related" Classes of SMC Complexes. The role of various SMC proteins in mitosis has been described in different systems, including the requirement for XCAP-C/XCAP-E in mitotic chromosome condensation *in vitro* in *Xenopus* (3), as well as the critical roles of Smc1 and Smc2 in mitotic chromosome condensation and segregation *in vivo* in *S. cerevisiae* (1, 2). Sequence and localization analysis revealed that hCAP-C/hCAP-E is the human ortholog of XCAP-C/XCAP-E. In addition, we found that antibody specific for hSMC1 blocks progression of metaphase during mitosis. In light of the recent studies showing the role of Smc1 and Smc3 in sister chromatid cohesion in *S. cerevisiae* (20, 21) and the role of XSMC1 and XSMC3 in *Xenopus* (24), disruption of the metaphase plate caused by antibody against hSMC1 may be a reflection of the disruption of sister chromatid cohesion. More detailed phenotypic analysis of the antibody-injected human cells will be needed. Because HCAP (hSMC3) was found to localize on mitotic chromosomes (19), our failure to detect hSMC1, a partner of hSMC3, on mitotic chromosomes may be caused by steric hindrance. Further characterization of the hSMC1/hSMC3 complex will be necessary. Nonetheless, these results suggest that there are two classes of SMC heterodimeric complexes, "condensation-related" and "cohesion-related", both of which are involved in mitotic chromosome organization (Table 1). Thus far, it is not clear whether there is any functional cooperativity between the two SMC complexes during mitosis. In light of our data indicating distinct behaviors of the two different SMC complexes in the cell, the two complexes may act independently. It is important to understand the mechanisms of action of each complex and the relationship of the two complexes during mitotic chromosome organization.

Recombination and Metaphase Plate Organization. Previously, the bovine SMC1 homolog BSMC1 was shown to be part of the DNA recombination repair complex RC1 (15). Partial sequence comparison suggests that the hSMC1/hSMC3 complex may be the equivalent of BSMC1/BSMC2 and may have dual roles during interphase and mitosis, providing a potential link between recombination and mitotic chromosome organization. Because hSMC1/hSMC3 is present throughout the cell cycle, differential interactions with additional proteins may determine the cell cycle-dependent functions of hSMC1/hSMC3. Consistent with this notion, we found the interphase-specific association of three proteins of 120, 125, and 190 kDa in size with hSMC1/hSMC3 (K.Y., unpublished work), suggesting the possibility of the mitosis-specific interaction of other proteins with hSMC1/hSMC3. The functional specificities, mechanisms, and regulation of actions of the two human SMC complexes will be addressed further by identifying other

molecular partners that may differentially interact with the SMC proteins during the cell cycle.

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