We have isolated SMAP (Smg GDS-associated protein; Smg GDS: small G protein GDP dissociation stimulator) as a novel Smg GDS-associated protein, which has Armadillo repeats and is phosphorylated by Src tyrosine kinase. SMAP is a human counterpart of mouse KAP3 (kinesin superfamily-associated protein) that is associated with mouse KIF3A/B (a kinesin superfamily protein), which functions as a microtubule-based ATPase motor for organelle transport. We isolated here a SMAP-interacting protein from a human brain cDNA library, identified it to be a homolog of Xenopus XCAP-E (Xenopus chromosome-associated polypeptide), a subunit of condensins that regulate the assembly and structural maintenance of mitotic chromosomes, and named it HCAP (Human chromosome-associated polypeptide). Tissue and subcellular distribution analyses indicated that HCAP was ubiquitously expressed and highly concentrated in the nuclear fraction, where SMAP and KIF3B were also present. SMAP was extracted as a ternary complex with HCAP and KIF3B from the nuclear fraction in the presence of Mg-ATP. The results suggest that SMAP/KAP3 serves as a linker between HCAP and KIF3B in the nucleus, and that SMAP/KAP3 plays a role in the interaction of chromosomes with an ATPase motor protein.

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank®/EBI Data Bank with accession number(s) AF200434.

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Complex Formation of SMAP with HCAP and KIF3A/B

region of HCAP (865–1217 aa) was isolated. A human brain cDNA library in azAPII (Stratagene) was screened to determine the full-length sequence using the positive clone as a probe. Multiple overlapping clones covering the entire coding region were sequenced to assemble the full-length sequence. The HCAP cDNA sequence reported in this work is based on the determination of cDNA sequences of both strands of the HCAP cDNA.

**Assay for Direct Interactions of SMAP with HCAP—GST-HCAP or GST (0.1 nmol) was mixed with MBP-SMAP or MBP (0.1 nmol) in 0.2 ml of Buffer A (25 mM Tris/HCl at pH 7.5, 5 mM MgCl2, 0.5 mM DTT, 3 mM/g liter cystealasin B, 0.2 mM PMSF, and 10 mg/liter leupeptin) and incubated for 30 min. They were homogenized in a Potter-Elvehjem Teflon-glass homogenizer and centrifuged at 1,000 g for 10 min. The supernatant was used as the cytoplasmic fraction. The pellet was resuspended in 10 volumes of Buffer B (10 mM triethanolamine/ HCl at pH 7.5, 5 mM MgCl2, 0.2 mM PMSF, and 10 mg/liter leupeptin) containing 1.62 M sucrose, layered on the top of a 2.3M sucrose cushion, and centrifuged at 160,000 × g for 1 h. The pellet was used as the nuclear fraction.**

**Immunostaining of COS-7 Cells—**The cells were fixed and stained with the anti-HCAP antibody (4 µg/ml protein) or preimmune rabbit IgG (4 µg/ml protein) as described (12). Fluorescein-conjugated donkey anti-rabbit IgG (Chemicon) was used as the second antibody. Chromosomes were stained with DAPI (Sigma). The stained cells were observed with a Zeiss Axiphot microscope (Carl Zeiss, Oberkochen, Germany) and photographed with a peltier cooling 3CCD color camera (C5810-01; Hamamatsu Photonics KK., Hamamatsu, Japan).

**Subcellular Fractionation of COS-7 Cells—**Subcellular fractionation of COS-7 cells was performed as described (11). Briefly, COS-7 cells grown in 10-cm dishes (1) were rinsed twice with cold phosphate-buffered saline and harvested by scraping. All manipulations were performed at 0–4 °C. Cells were pelleted in 7 volumes of homogenizing buffer (10 mM HEPES/KOH at pH 7.3, 10 mM KCl, 5 mM MgCl2, 0.5 mM DTT, 3 mM/g liter cystealasin B, 0.2 mM PMSF, and 10 mg/liter leupeptin) and incubated for 30 min. They were homogenized in a Potter-Elvehjem Teflon-glass homogenizer and centrifuged at 1,000 g for 10 min. The supernatant was used as the cytoplasmic fraction. The pellet was resuspended in 10 volumes of Buffer B (10 mM triethanolamine/ HCl at pH 7.5, 5 mM MgCl2, 0.2 mM PMSF, and 10 mg/liter leupeptin) containing 1.62 M sucrose, layered on the top of a 2.3M sucrose cushion, and centrifuged at 160,000 × g for 1 h. The pellet was used as the nuclear fraction.

**Immunostaining of COS-7 Cells—**The cells were fixed and stained with the anti-HCAP antibody (4 µg/ml protein) or preimmune rabbit IgG (4 µg/ml protein) as described (12). Fluorescein-conjugated donkey anti-rabbit IgG (Chemicon) was used as the second antibody. Chromosomes were stained with DAPI (Sigma). The stained cells were observed with a Zeiss Axiphot microscope (Carl Zeiss, Oberkochen, Germany) and photographed with a peltier cooling 3CCD color camera (C5810-01; Hamamatsu Photonics KK., Hamamatsu, Japan).

**Immunoprecipitation of SMAP and KIF3B with HCAP—**All manipulations were performed at 0–4 °C. The nuclear fraction of COS-7 cells (2.4 mg of protein) was resuspended with 0.8 ml of Buffer B containing 5 mM ATP, sonicated, and incubated for 1 h. After centrifugation at 60,000 × g for 20 min, the supernatant was used as the Mg-ATP extract. The pellet was resuspended with 0.8 ml of Buffer B. Twenty percent Nonidet P-40 was added to the Mg-ATP extract, giving a final concentration of 1%, and the mixture was immunoprecipitated with the anti-HCAP antibody, followed by Western blotting using the indicated antibodies as described (1).  

**Other Procedures—**SMAP, HCAP, and KIF3B transferred to nitrocellulose sheets after SDS-PAGE were detected using the ECL immunoblotting detection system (Amersham Pharmacia Biotech). The amount of each protein was determined by densitometric tracing at 420 nm with each purified protein as a standard in a linear range as described (13).

**RESULTS AND DISCUSSION**

We first attempted to isolate a SMAP-interacting protein from a human B-cell cDNA library by the yeast two-hybrid method with SMAP as bait. Among 4 × 10⁸ transformants, nine clones were positive for the screening. DNA sequencing of the insert DNAs of these clones revealed that three mants, nine clones were positive for the screening. DNA sequencing of the insert DNAs of these clones revealed that three 141,540 and was named HCAP (accession number AF020043) (Fig. 1A). The neighboring sequence of the first ATG was consistent with the translation initiation start site proposed by Kozak (14), and in-frame stop codons were present upstream of the first ATG. Moreover, the position of the in vitro translated product of full-length HCAP was similar to that of endogenous HCAP on SDS-PAGE (data not shown). We have concluded from these results that the isolated clone contains the open reading frame of HCAP. An initial data base search demonstrated that HCAP shared 53, 41, and 22% overall amino acid sequence identities with Drosophila DCAP (15), Aspergillus SUDA (16), and Xenopus XCAP-E, respectively. DCAP and SUDA are also SMC family members (15, 16). HCAP had a head-rod-tail structural organization, which the SMC family members commonly have (Fig. 1B) (7, 8). The head contained an NTP-binding motif and the tail contained a DBA-box. These results indicate that HCAP is an SMC family member. Recently, the full sequence of the rat basement membrane-chondroitin sulfate proteoglycan was directly submitted to GenBank™ (accession number U82626). The cDNA of this protein was isolated by screening the cDNA expression library with polyclonal antiseraum raised against basement membrane proteoglycans, and the properties of this protein were not reported (17), but this protein shared 98% amino acid sequence identity with HCAP.

We confirmed using the yeast two-hybrid method that the isolated HCAP indeed interacted with SMAP (Fig. 2A). In these experiments, the N-terminal two-thirds of SMAP (1–562 aa) and the C-terminal one-third of HCAP (865–1217 aa) were used. Bas(G12V) and Raf, known to interact in the yeast two-hybrid method (18), were used as positive controls. Moreover, we examined using the recombinant samples whether HCAP directly interacts with SMAP in a cell-free system. MBP-SMAP bound to GST-HCAP, but not to GST (Fig. 2B). MBP did not bind to GST-HCAP (data not shown). These results indicate that SMAP directly interacts with HCAP.

Western blot analysis using the anti-HCAP antibody detected only an ~140-kDa protein in all the rat tissues examined and the molecular mass was similar to a M, value calculated from the open reading frame (Fig. 3A). The subcellular distri-
mitotic chromosomes. Anaphase COS-7 cells were double stained with antibodies. Adaptin and lamin were used as cytoplasmic and nuclear markers, respectively (26).

Immunostaining of COS-7 cells with the anti-HCAP antibody were detected in both the nuclear and cytoplasmic fractions. Immunostaining of COS-7 cells with the anti-HCAP antibody showed that HCAP was highly concentrated in the nuclear fraction and hardly detected in the cytoplasmic fraction (Fig. 3). Chromosomes were not stained with preimmune rabbit IgG (data not shown). These results suggest that SMAP forms a complex with HCAP and/or KIF3A/B in the nucleus.

We, therefore, examined whether SMAP interacts with HCAP and/or KIF3A/B in the nuclear fraction. It has been shown that hydrolysis of ATP disrupts the interaction of an ATPase protein(s) with other protein(s); myosin I ATPase is extracted from an actin filament in the presence of Mg-ATP (19); and NSF ATPase is extracted from a complex of SNAPs and SNAP receptors in the presence of Mg-ATP (20). Moreover, it has recently been reported that 13 S condensin, a complex containing XCAP-C and -E, has a DNA-stimulated ATPase activity and that the XCAP-C and -E subunits are indispensable for this activity (21). These observations suggest that HCAP and/or KIF3A/B should be extracted with SMAP from the nuclear fraction in the presence of Mg-ATP. When the nuclear fraction of COS-7 cells was incubated in the presence of Mg-ATP, SMAP, HCAP, and KIF3B were extracted (Fig. 4A). None of these proteins was extracted in the absence of Mg-ATP. Most of SMAP and KIF3B, but only a small amount of HCAP, were extracted. Furthermore, SMAP and KIF3B were coimmunoprecipitated with HCAP with the anti-HCAP antibody from the Mg-ATP extract (Fig. 4B). These results suggest that SMAP forms a ternary complex with HCAP and KIF3A/B in the nucleus and that hydrolysis of ATP by HCAP and/or KIF3A/B disrupts their interactions with other proteins. The reason why only a small amount of HCAP was extracted is not known, but since it was estimated by Western blotting that in the nuclear fraction, the amount of HCAP was about 10-fold more than that of SMAP, only the SMAP-HPAC-KIF3A/B complex might be extracted.

Our present results indicate that SMAP forms a ternary complex with KIF3B and HCAP, a human SMC family member, and suggest that this ternary complex is associated with unknown proteins in the nucleus. XCAP-E forms at least two major complexes, 8 and 13 S condensins, and 8 S condensin contains two SMC family members, XCAP-C and -E, whereas 13 S condensin contains three subunits, XCAP-D2, -G, and -H, in addition to XCAP-C and -E (22). 13 S condensin is required for chromosome condensation, but the function of 8 S condensin remains unknown (22). Moreover, a recombination protein complex (RC-1) purified from calf thymus contains two SMC family members, the 160- and 130-kDa polypeptides, and two additional subunits, DNA polymerase ε and DNA ligase III (23). The 160- and 130-kDa polypeptides alone exhibit a DNA reannealing activity, whereas RC-1 has the activity to catalyze recombinational repair of double strand gaps and deletions in DNA in addition to the DNA reannealing activity (23). It is likely that an SMC family member interacts with different sets of subunits, thereby modifying and acquiring its unique functions. The SMAP-HPAC-KIF3A/B complex might have a novel function, different from those thus far reported in the SMC family members.

We have shown here that HCAP is associated with mitotic chromosomes as described for XCAP-E (6, 22). Sea urchin ki-
nesin II is associated with pericentriolar and intranuclear regions during prophase, with kinetochore-to-pole microtubules near the kinetochores during metaphase (24), suggesting that a mammalian counterpart, KIF3A/B, also binds to the spindle and plays a role in mitosis. Taken together, it is likely that HCAP forms a complex with SMAP and KIF3A/B in the interphase nucleus and that during mitosis the complex tethers chromosomes to the spindle and plays a role in chromosome movement, like the microtubule-dependent motor CENP-E (25). Further study is necessary to clarify the function of the SMAP-HCAP-KIF3A/B complex in the nucleus.

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