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Identification of a Subunit of a Novel Kleisin-β/SMC Complex as a Potential Substrate of Protein Phosphatase 2A

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Summary

Protein phosphatase 2A (PP2A) holoenzymes consist of a catalytic C subunit, a scaffolding A subunit, and one of several regulatory B subunits that recruit the AC dimer to substrates [1, 2]. PP2A is required for chromosome segregation [3–6], but PP2A's substrates in this process remain unknown. To identify PP2A substrates, we carried out a two-hybrid screen with the regulatory B/PR55 subunit. We isolated a human homolog of *C. elegans* HCP6, a protein distantly related to the condensin subunit hCAP-D2, and we named this homolog hHCP-6. Both *C. elegans* HCP-6 and condensin are required for chromosome organization and segregation [7–11]. HCP-6 binding partners are unknown, whereas condensin is composed of the structural maintenance of chromosomes proteins SMC2 and SMC4 and of three non-SMC subunits [12]. Here we show that hHCP-6 becomes phosphorylated during mitosis and that its dephosphorylation by PP2A in vitro depends on B/PR55, suggesting that hHCP-6 is a B/PR55-specific substrate of PP2A. Unlike condensin, hHCP-6 is localized in the nucleus in interphase, but similar to condensin, hHCP-6 associates with chromosomes during mitosis. hHCP-6 is part of a complex that contains SMC2, SMC4, kleisin-β, and the previously uncharacterized HEAT repeat protein FLJ20311. hHCP-6 is therefore part of a condensin-related complex that associates with chromosomes in mitosis and may be regulated by PP2A.

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Results and Discussion

Identification of a B/PR55-Interacting Protein with Homologies to HCP-6 and hCAP-D2

To isolate targets of the B/PR55 regulatory subunit, we carried out a yeast two-hybrid screen by using the α isoform of B/PR55 as the bait and a HeLa cDNA library as the prey. We coexpressed the mammalian A subunit (α isoform) to provide the scaffold for interaction between the bait B/PR55 fusion protein and the yeast C subunits PPH21 and PPH22, which are each 76% identical to the mammalian C subunit. Apart from mimicking a B/PR55-containing PP2A complex, coexpression of the A subunit should lower the probability of obtaining A subunit as an interactor in the screen. In two screens performed, we isolated four cDNAs encoding different B/PR55 interacting proteins, none of which encoded the PP2A A subunit.

Here we describe the characterization of one of the B/PR55 interactors. A BLAST search (Figure 1A) revealed that its cDNA is identical to the 3′ coding region of a 5623 bp cDNA (GI:30156565) that encodes the hypothetical protein KIAA0056 consisting of 1498 amino acids with a predicted molecular weight of 169 kDa [13]. KIAA0056 cDNA's of various lengths were isolated in the two-hybrid screen, with the largest corresponding to the carboxy-terminal 288 amino acids and the smallest to the carboxy-terminal 190 amino acids (Figure 1A). This indicates that the C-terminal 190 amino acids of KIAA0056 can bind to B/PR55.

BLAST searches identified KIAA0056 as the mammalian ortholog of *C. elegans* holocentric protein 6 (HCP-6). HCP-6 is a kinetochore protein required for maintaining chromosomal rigidity and has partial sequence similarity with XCAP-D2/CNAP1/Cnd1, a non-SMC subunit of condensin [11]. The region between amino acid residues 920 and 1120 of KIAA0056/hHCP-6, which is homologous to CAP-D2, contains HEAT (huntingtin-elongation-A subunit-TOR) repeats, tandemly arranged bihelical structures that are thought to mediate protein-protein interactions in other proteins [14]. Various chromosome-associated proteins including condensin's non-SMC subunits hCAP-D2 and hCAP-G also contain HEAT repeats [15]. Using a modified search method based on a hidden Markov model (http://hmmer.wustl.edu), we identified several HEAT repeat-containing regions in KIAA0056 (Figure 1A).

hHCP-6 Is Phosphorylated during Mitosis and Dephosphorylated by PP2A in a B/PR55-Dependent Manner

Immunoprecipitation from lysates of NIH3T3 fibroblasts expressing HA-tagged hHCP-6 showed an interaction between hHCP-6 and endogenous B/PR55 (data not shown). Immunoprecipitation assays using polyclonal hHCP-6 antibodies (His1a) also revealed an interaction between endogenous hHCP-6 and B/PR55 in HeLa lysates (data not shown). However, this interaction was

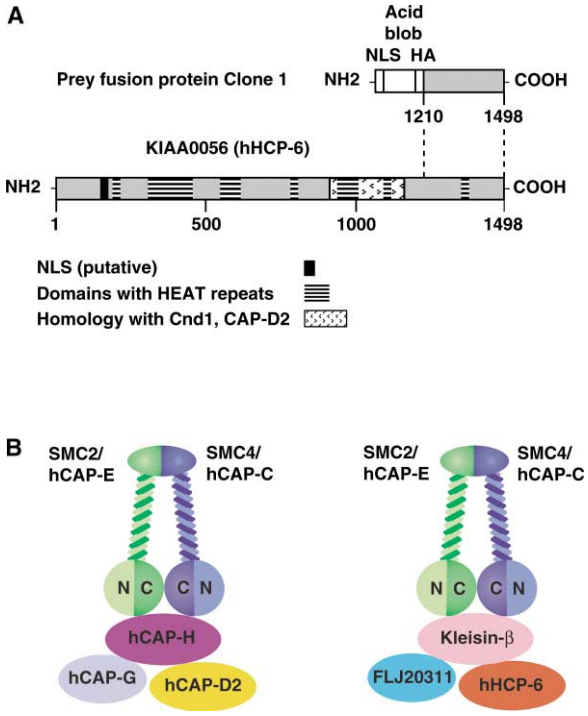

Figure 1. Schematic Presentation of KIAA0056/hHCP-6 and Model of Condensin and hHCP-6 Complex Composition

(A) The conserved domains of KIAA0056/hHCP-6. The B/PR55-interacting clone (prey fusion clone 1) is identical to the C-terminal 288 amino acids of KIAA0056. KIAA0056/hHCP-6 contains a putative nuclear localization signal (NLS) (black box), a region homologous to CAP-D2 (dotted box), and HEAT repeats indicated by horizontal lines.

(B) Condensin and the hHCP-6 complex possess a similar subunit composition. Both complexes share the heterodimeric SMC core and contain a kleisin (hCAP-H/kleisin-γ in condensin and kleisin-β in the hHCP-6 complex) and two HEAT repeat proteins (hCAP-D2 and hCAP-G in condensin and hHCP-6 and FLJ20311 in the hHCP-6 complex). The presentation of condensin and hHCP-6 complexes as V-shaped structures connected by a kleisin is hypothetical but is consistent with electron microscopy data [26] and with the subunit topology of cohesin, a related kleisin/SMC complex [27, 28].

very weak, raising the possibility that hHCP-6 and B/PR55 only associate transiently, possibly because hHCP-6 is a substrate of PP2A.

To test this possibility, we first asked if different post-translational modification states of hHCP-6 exist. When logarithmically proliferating HeLa cells were lysed directly in SDS sample buffer, hHCP-6 migrated predominantly as a band of 175–180 kDa, whereas hHCP-6 appeared as two bands with slower electrophoretic mobility in lysates from HeLa cells that had been arrested in prometaphase by nocodazole (Figure 2A, upper panel). In cells progressing through mitosis after synchronization and release from a double thymidine block, hHCP-6 underwent a similar change in electrophoretic mobility (Figure 2A, upper panel, lanes 2–4; for FACS analyses, see Figure S1A in the Supplemental Data available with this article online). Ten hours after the second thymidine release, the more slowly migrating forms of hHCP-6 had been partially converted back to the form that migrated more quickly, concomitant with the disappearance of cyclin B and the partial dephosphorylation of Cdc27, a subunit of the anaphase-promoting complex (APC) (Figure 2A, upper panel, lane 4). The more slowly migrating forms of hHCP-6 also disappeared when cells were released for 3 hr from a nocodazole arrest (Figure 2B, lane 3). We conclude that hHCP-6 is modified during mitosis.

To determine whether PP2A is required for the removal of hHCP-6's modification during mitotic exit, we treated nocodazole-arrested MCF-7 cells for 1 hr with DMSO, 0.1 μM okadaic acid (OA), or 1 μM OA, released the cells from the nocodazole block in the presence or absence of OA, and analyzed cell lysates 3 hr after the release by SDS-PAGE/immunoblotting (Figure 2B). We performed these experiments in MCF-7 cells whose treatment with 1 μM OA is known to inhibit PP2A specifically, whereas the activity of PP1 is unaffected [16]. As a control for the specific inhibition of PP2A, we analyzed the activation of the MAP kinases Erk1 and Erk2 by using a phospho-p44/42-specific antibody (Figure 2B bottom panel). It is known that inhibition of PP2A activates the MAP kinase pathway by inhibiting dephosphorylation of MEK and ERK [17, 18]. Only the OA-treated cells showed increased phospho-Erk levels, indicating PP2A inhibition. FACS analyses of DNA content revealed that cell division was inhibited by OA (Figure S1B), and immunoblotting showed that the electrophoretic mobility of Cdc27 remained retarded, indicating that Cdc27 had not been dephosphorylated and that cells had failed to exit mitosis (Figure 2B, middle panel). The electrophoretic mobility of hHCP-6 remained slightly reduced in the presence of 0.1 μM OA, whereas the mobility remained more strongly reduced in the presence of 1 μM OA (Figure 2B, upper panel, lanes 4 and 5). The incomplete block of hHCP-6 dephosphorylation at 0.1 μM OA correlates with partial inhibition of PP2A, which is indicated by the partial activation of ERK. This result implies that PP2A is required to reverse the mitotic modification of hHCP-6, either directly by dephosphorylating hHCP-6 or indirectly by blocking exit from mitosis.

Treatment of hHCP-6 with calf intestine alkaline phosphatase (CIP) removed its mitotic modification, indicating that hHCP-6 is modified by phosphorylation (Figure S2). To test if hHCP-6 is specifically dephosphorylated by PP2A and if this reaction depends on B/PR55, we immunopurified phosphorylated hHCP-6 from lysates of nocodazole-arrested MCF-7 cells and incubated aliquots of the immunoprecipitate with different immunopurified PP2A holoenzymes. The catalytic C subunit was isolated with the 7A6 antibody (Upstate), which recognizes a C-terminal 8 amino acid epitope. Because antibody binding to this epitope blocks the association of the B/PR55 subunit with the AC heterodimer [19], the 7A6 immunoprecipitates contain a mixture of the C subunit, AC heterodimers, and presumably trimers with B‴ subunits, but not trimers containing the B/PR55, B′/PR56 or B″/PR59 subunits (data not shown) [20]. PP2A consisting of AC associated with the B/PR55 subunit was immunoprecipitated with HA antibodies from lysates of NIH3T3 cells stably expressing HA-tagged B/PR55α. Only PP2A holoenzymes containing B/PR55α subunit but not tenfold excess of 7A6 immunoprecipitated holoenzymes had the ability to dephosphorylate

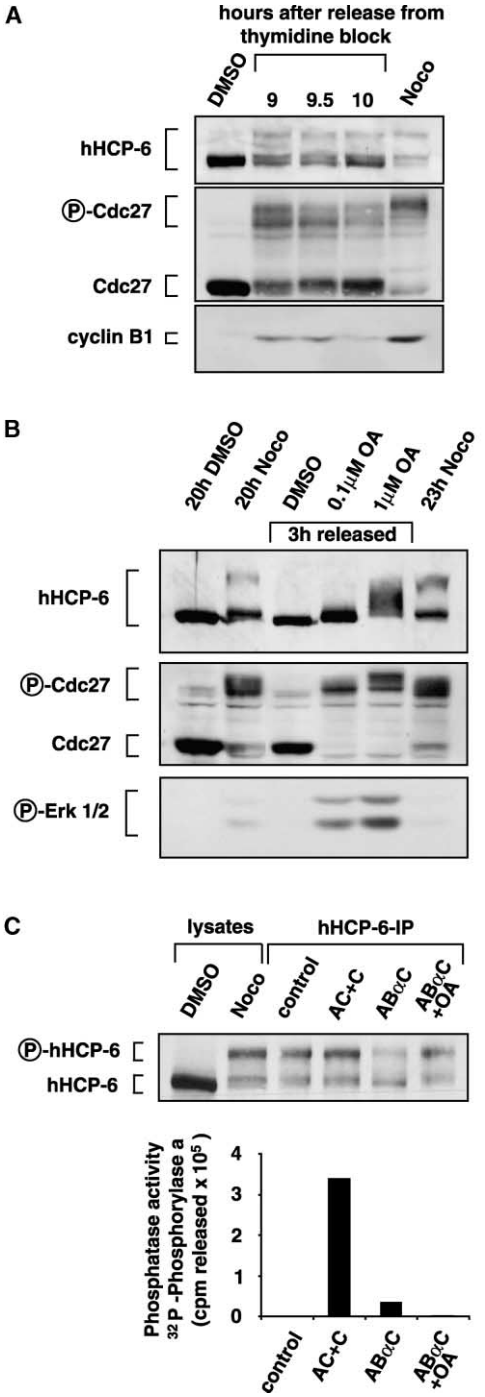

Figure 2. hHCP-6 Is Phosphorylated during Mitosis and Dephosphorylated by PP2A in a B/PR55-Dependent Manner

(A) hHCP-6 is posttranslationally modified in mitosis. Whole-cell lysates were prepared under denaturing conditions in SDS sample buffer from logarithmically proliferating HeLa cells (lane 1, DMSO control), from HeLa cells synchronously progressing through mitosis (9, 9.5, and 10 hr after a release from a double thymidine block), and from HeLa cells blocked in mitosis by nocodazole (lane 5). Lysates were analyzed by 7.5% SDS-PAGE and immunoblotting with antibodies against hHCP-6 (rabbit polyclonal antiserum His1a, raised against the C-terminal 143 residues of hHCP-6), Cdc27, and cyclin B1 (sc-245, Santa Cruz Biotechnology).

(B) PP2A is required to reverse the mitotic modification of hHCP-6. Whole-cell lysates were prepared under denaturing conditions in SDS sample buffer from logarithmically proliferating MCF-7 cells (lane 1), from nocodazole-arrested MCF-7 cells (lanes 2 and 6), and from MCF-7 cells that were released for 3 hr from a nocodazole block in the presence of DMSO (lane 3), 0.1 μM OA (lane 4), or 1 μM OA (lane 5). Lysates were analyzed by 7.5% SDS-PAGE and immunoblotting with antibodies against hHCP-6 (His1a), Cdc27, and phospho-p44/42 MAP kinase (Cell Signaling). Lanes 1–2 and 3–6 were not adjacent to each other on the original blot.

(C) Mitotically phosphorylated hHCP-6 is a substrate of a B/PR55-containing PP2A holoenzyme. Lysates were prepared from logarithmically proliferating (DMSO) or nocodazole-arrested MCF-7 cells as described in Figure S2 except that lysis buffer was supplemented with 50 mM sodium fluoride, 20 mM sodium pyrophosphate, 1 mM sodium orthovanadate, 10 mM β-glycerophosphate, and 4 mM EDTA. An aliquot of these lysates was analyzed by 7.5% SDS-PAGE and immunoblotting (lanes 1–2) with a monoclonal anti-hHCP-6 antibody (2B5) (raised against amino acids 706–917 of hHCP-6). Anti-hHCP-6 immunoprecipitates (with polyclonal antiserum #156 raised against amino acids 328–475 of hHCP-6) from lysates of nocodazole-arrested MCF-7 cells were incubated for 15 min at 30°C with buffer only (lane 3), with immunopurified PP2A holoenzymes (C subunit, AC heterodimer) lacking B/PR55 subunit (lane 4), or with a PP2A holoenzyme containing the B/PR55 subunit in the absence (lane 5) or presence (lane 6) of 1 μM OA. The PP2A-treated hHCP-6 immunoprecipitates were then analyzed by 7.5% SDS-PAGE and immunoblotting with the monoclonal anti-hHCP-6 antibody 2B5 (raised against amino acids 706–917 of hHCP-6). Phosphatase activities of immunopurified PP2A holoenzymes used in the hHCP-6 dephosphorylation assays are shown as a histogram.

hHCP-6 in vitro (Figure 2C, compare lanes 4 and 5). This activity was inhibited in the presence of OA (Figure 2C, lane 6). Taken together, these results suggest that the mitotically phosphorylated hHCP-6 is a substrate of a B/PR55-containing PP2A holoenzyme.

hHCP-6 Is a Nuclear Protein that Associates with Chromosomes in Mitosis

In *C. elegans*, HCP-6 displays a dotted staining in interphase nuclei and colocalizes with the kinetochore protein HCP-3 during mitosis [11]. To determine the subcellular localization of hHCP-6, we carried out cell fractionation and immunofluorescence experiments by using two different polyclonal hHCP-6 antibodies raised against different regions of hHCP-6. The specificity of anti-hHCP-6 (His1a) antibodies used in the immunofluorescence analyses (Figures 3A and 3B) was confirmed by immunoblot analysis (Figure S3). Cell fractionation of logarithmically proliferating HeLa and 293 cells revealed that the majority of hHCP-6 was found in the nucleoplasmic fraction, whereas only small amounts of hHCP-6 could be detected in the cytoplasmic fraction (Figure S4). In agreement with these results, hHCP-6 showed nuclear localization in interphase cells in immunofluorescence microscopy experiments (Figure 3A, panel b). The staining appeared as a small-dotted/granulated pattern overlapping with DNA but excluding the nucleoli throughout the nucleus. Nuclear localization was also seen when a GFP-tagged version of hHCP-6 was transiently expressed in HeLa cells (Figure S5). This localization is furthermore consistent with the presence of a putative NLS in the N terminus of the hHCP-6 coding sequence (Figure 1A). When transiently transfected into HeLa cells (Figure S5), a mutant hHCP-6 lacking the N-terminal 313 amino acids including the putative NLS

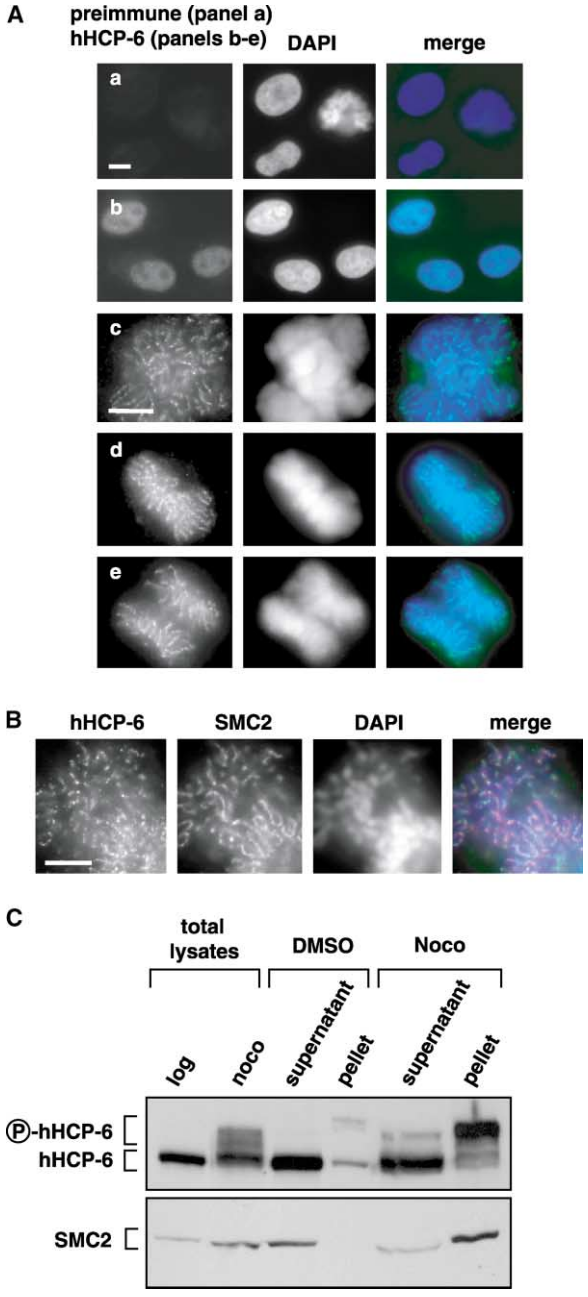

Figure 3. hHCP-6 Is a Nuclear Protein that Associates with Chromosomes in Mitosis

(A) Immunolocalization of hHCP-6 during the cell cycle. Logarithmically proliferating HeLa cells were immunostained either with preimmune serum (panel a) or anti-hHCP-6 antibodies (His1a) (panels b–e) and counterstained with DAPI, as indicated. Representative cells of interphase (a and b), prometaphase (c), metaphase (d), and anaphase (e) are shown. Similar results were obtained with anti-hHCP-6 antibodies (#156). Scale bars represent 10 μm.

(B) Colocalization of hHCP-6 and SMC2 on chromosomes. Cells were coimmunostained with anti-hHCP-6 (His1a) and SMC2 (polyclonal antibody raised against peptide CAKSKAKPPKGAHVEV) and probed with FITC and Texas Red, respectively. DNA was counterstained with DAPI, as indicated. Scale bars represent 10 μm.

(C) Chromosome-associated hHCP-6 is phosphorylated. Logarithmically proliferating (DMSO) or nocodazole-arrested HeLa cells were lysed under denaturing conditions in SDS sample buffer (lanes 1–2) or under native conditions (lanes 3–6) in a buffer consisting of 1% NP-40, 5 mM $MgCl_2$, 10 mM NaCl, and 20 mM Tris (pH 8.0) and supplemented with inhibitors (as described in Figure 2C), and the lysates were separated by centrifugation (14.000 rpm, 10 min) into a pellet and a supernatant fraction. Whole-cell lysates (lanes 1–2) and the supernatant and pellet fractions (lanes 3–6) were analyzed by 7.5% SDS-PAGE and immunoblotting with anti-hHCP-6 (His1a) and SMC2 antibodies.

was exclusively found in the cytoplasm, suggesting that the predicted NLS is functional.

In mitosis, hHCP-6 staining overlapped with the DAPI stain of condensed chromosomes (Figure 3A, panels c–e). In prometaphase, hHCP-6 decorated the entire lengths of the mitotic chromosomes in a dotted manner (Figure 3A, panel c). At the end of mitosis when chromosomes decondensed, the chromosomal staining of hHCP-6 disappeared and became more diffuse. In contrast to the holocentric kinetochore localization of *C. elegans* HCP6, no enrichment of hHCP-6 at kinetochores could be detected.

To determine if mitotic phosphorylation could have a role in the chromosome association of hHCP-6, we prepared lysates from logarithmically proliferating or nocodazole-arrested HeLa cells, separated chromatin/chromosomes from the soluble fraction by centrifugation, and analyzed the resulting supernatant and pellet fractions by immunoblotting (Figure 3C). In proliferating cells, hHCP-6 was mostly detected in the supernatant, with a minor fraction in the pellet, indicating that the bulk of hHCP-6 is not stably associated with chromatin in interphase despite its nuclear localization. Consistent with the small percentage of mitotic cells in a proliferating cell population, only a small portion of hHCP-6 was phosphorylated, but this form was clearly enriched in the pellet. When cells were arrested in mitosis, the fraction of hHCP-6 that was recovered in the pellet increased significantly. Also, under these conditions the pellet bound hHCP-6 consisted almost exclusively of the phosphorylated form. It is therefore possible that the chromosomal association of hHCP-6 is regulated by phosphorylation.

hHCP-6 Is Part of a Protein Complex that Contains SMC2, SMC4, Kleisin-β, and an Uncharacterized HEAT Repeat Protein

The partial homology between hHCP-6 and the condensin subunit hCAP-D2, the localization of hHCP-6 on mitotic chromosomes, and the previously described role of *C. elegans* HCP-6 in chromosome organization [11] raised the possibility that hHCP-6 is a previously unidentified subunit of condensin or part of a condensin-related complex. The existence of condensin-related complexes has recently been predicted by Schleiffer et al. based on bioinformatic analyses [21]. Condensin is composed of the subunits SMC2, SMC4, hCAP-D2, hCAP-G, and hCAP-H [22]. Schleiffer et al. noticed that hCAP-H is distantly related to the Scc1 subunit of cohesin, a complex that contains the SMC1 and SMC3 members of the SMC family, and they proposed naming Scc1 and hCAP-H kleisin-α and -γ, respectively. Schleiffer et al. further noticed that some eukaryotic genomes, including the human one, contain another gene distantly related

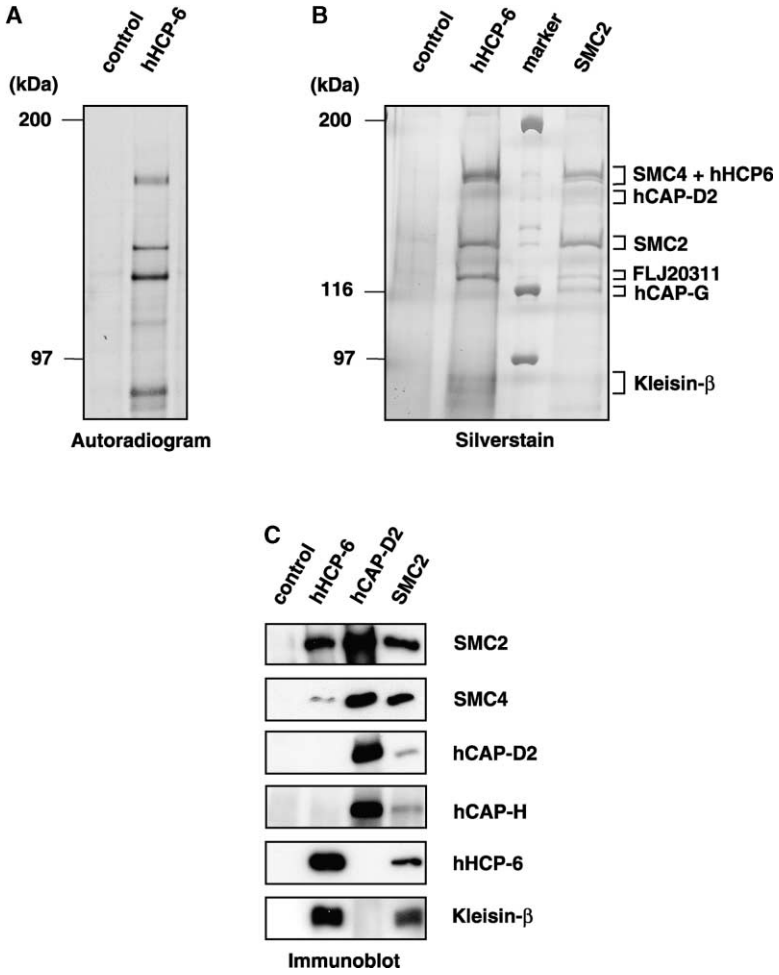

Figure 4. SMC2/hCAP-E and SMC4/hCAP-C Interact with Two Different Sets of Non-SMC Subunits

(A) Several proteins specifically coimmunoprecipitate with hHCP-6. Anti-hHCP-6 immunoprecipitates from lysates of ^{35}S-methionine-labeled HeLa cells were analyzed by SDS-PAGE and autoradiography. Lane 1: preimmune control; Lane 2: hHCP-6 (His1a).

(B) hHCP-6 is a subunit of a novel Kleisin-β/SMC complex. Preimmune serum, anti-hHCP-6 (His1a), and anti-SMC2 antibodies were used to obtain immunoprecipitates from HeLa cell extracts. Immunoprecipitates were analyzed by SDS-PAGE and silver staining. Excised bands (brackets) were analyzed by mass spectrometry as described [29, 30].

(C) SMC2 and SMC4 bind either to hHCP-6/Kleisin-β or to hCAP-D2 and hCAP-H. Preimmune serum, anti-hHCP-6 (His1a), anti-hCAP-D2, and anti-SMC2 antibodies were used to obtain immunoprecipitates from logarithmically proliferating HeLa cell lysates. Immunoprecipitates were analyzed by SDS-PAGE and immunoblotting. The immunoblot was sequentially incubated with SMC2, SMC4 (rabbit polyclonal antibody raised against synthetic peptide KSVAVNPKEIASKGLC), hCAP-D2, hHCP-6 (His1a), hCAP-H/kleisin-γ (rabbit polyclonal antibody raised against synthetic peptide CTEHYEEIEDYD YNNPNDTSN) and kleisin-β (rabbit polyclonal antibody raised against synthetic peptide CETPDPWQSLDPFDSLESK) antibodies.

to Scc1 and hCAP-H; they called this gene kleisin-β. To test if hHCP-6 is part of a complex that contains kleisin-β, we also raised antibodies against the hypothetical human kleisin-β protein.

First, we immunoprecipitated hHCP-6 from lysates of ^{35}S-methionine-labeled logarithmically proliferating HeLa cells and analyzed the immunoprecipitates by SDS-PAGE and autoradiography. The anti-hHCP-6 (His1a) antibodies specifically immunoprecipitated a set of high-molecular-weight proteins with apparent molecular masses of 175, 145, 135, and 85–90 kDa (Figure 4A). To determine the identity of these proteins, we analyzed hHCP-6 immunoprecipitates by SDS-PAGE, silver staining, and mass spectrometry (Figure 4B). The 175 kDa band contained both hHCP-6 and SMC4, the 145 kDa band contained SMC2, and the 135 kDa band contained a previously uncharacterized hypothetical protein called FLJ20311 (Figure 4B and data not shown). The silver stain analysis also revealed additional bands of weak intensity with apparent molecular masses around 90 kDa. Mass spectrometry identified these bands as kleisin-β (Figure 4B and data not shown). Immunoblotting with antibodies to SMC2, SMC4, hHCP-6, and kleisin-β confirmed the presence of these proteins in immunoprecipitates obtained with hHCP-6 antibodies, whereas none of them could be detected in control immunoprecipitates (Figure 4C). Antibodies to FLJ20311 are not available yet, and this protein could therefore not be analyzed in the same experiment.

The identification of SMC2 and SMC4 in hHCP-6 immunoprecipitates was unexpected because these two proteins were previously shown to be part of the condensin complex [23]. However, neither silver staining nor immunoblot analyses revealed the presence of the known non-SMC condensin subunits hCAP-D2, hCAP-H/kleisin-γ, and hCAP-G in hHCP-6 immunoprecipitates (Figures 4B and C). These observations raised the possibility that SMC2 and SMC4 are part of two different protein complexes, one containing the three known non-SMC subunits and the other one containing hHCP-6, FLJ20311, and kleisin-β. Double immunofluorescence microscopy with hHCP-6 and SMC2 antibodies were consistent with this possibility because SMC2 colocalized partly but not completely with hHCP-6 (Figure 3B). To test the possibility that SMC2 and SMC4 are part of two distinct complexes; we analyzed immunoprecipitates obtained with either hCAP-D2 antibodies or SMC2 antibodies by immunoblotting. SMC2, SMC4, hCAP-D2, and hCAP-H/kleisin-γ but not hHCP-6 and kleisin-β could be detected in the hCAP-D2 immunoprecipitates, whereas all proteins were found in the SMC2 precipitate (Figure 4C). We did not have hCAP-G antibodies, but

this protein could be specifically detected in SDS-PAGE/mass spectrometry analyses of SMC2 immunoprecipitates (Figure 4B).

These data reveal that hHCP-6 is part of a protein complex that contains SMC2, SMC4, kleisin-β, and the previously uncharacterized protein FLJ20311. Interestingly, FLJ20311 contains HEAT repeats (data not shown), as do hCAP-D2 and hCAP-G. The composition of the hHCP-6 complex is therefore remarkably similar to that of condensin in that both complexes share SMC2 and SMC4, both contain a member of the kleisin family (kleisin-β in the hHCP-6 complex and kleisin-γ in condensin), and both contain two additional HEAT repeat proteins (hHCP-6 and FLJ20311 in the hHCP-6 complex and hCAP-D2 and hCAP-G in condensin; Figure 1B). Because kleisin-β is related to kleisin-γ, and because hHCP-6 is related to hCAP-D2, it is possible that these proteins substitute for each other in the two complexes. Likewise, FLJ20311 may occupy the position of hCAP-G, although no clear homology beyond the presence of HEAT repeats can be detected between these two proteins.

Both condensin and *C. elegans* HCP-6 are required for the proper compaction of chromatin into mitotic chromosomes and for chromosome segregation [7–11]. These observations, the observed association of hHCP-6 with mitotic chromosomes, and the reported phenotype of kleisin-β RNA interference experiments in *C. elegans* [21] suggest that the hHCP-6 complex has a role in organizing mitotic chromosomes. After submission of this manuscript, Ono et al. reported the identification of the same hHCP-6/kleisin-β-containing complex, called condensin II by these authors, and provided direct evidence that this complex is required for chromosome condensation [24]. In this respect, it is interesting to note that we have detected hHCP-6 in interphase nuclei (Figure 3), whereas condensin has been found to associate with chromosomes only after nuclear envelope breakdown in prometaphase [25]. The finding that SMC2 is part of both condensin and the hHCP-6/condensin II complex could resolve the apparent contradiction between the observation that inactivation of SMC2 orthologs in both worms and chicken cells causes delays in chromosome condensation in prophase [7, 8, 10], despite the fact that condensin associates with chromosomes only in prometaphase [25]. It will therefore be interesting to analyze if the hHCP-6 complex has an earlier role in chromosome condensation than condensin. It will further be important to understand if the association of condensin and the newly identified hHCP-6 complex with chromosomes is controlled by phosphorylation and if the reverse reaction is under the control of PP2A. Interestingly, disruption of the B/PR55 subunit of PP2A in flies results in an *abnormal anaphase resolution* (*aar*) phenotype [3, 4] similar to the one seen in worm and fly condensin mutants [7–9]. It is conceivable that untimely or abnormally high phosphorylation of the hHCP-6 complex or condensin contributes to the phenotypes of *aar* mutants.

Supplemental Data

Additional data regarding the phosphorylation and intracellular localization of hHCP-6 can be found with this article online at http://www.current-biology.com/cgi/content/full/13/23/2058/DC1/.

Acknowledgments

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