A Potential Role for Human Cohesin in Mitotic Spindle Aster Assembly*

Received for publication, April 16, 2001, and in revised form, August 30, 2001
Published, JBC Papers in Press, October 4, 2001, DOI 10.1074/jbc.M103364200

Heather C. Gregsone‡, John A. Schmiesing‡, Jong-Soo Kim‡, Toshiki Kobayashi‡‡, Sharleen Zhou‡, and Kyoko Yokomori‡‡

From the ‡Department of Biological Chemistry, College of Medicine, University of California, Irvine, Irvine, California 92697-1700 and the ‡Department of Molecular and Cell Biology, Howard Hughes Medical Institute, University of California, Berkeley, California 94720-3202

The cohesin multiprotein complex containing SMC1, SMC3, Scc3 (SA), and Scc1 (Rad21) is required for sister chromatid cohesion in eukaryotes. Although metazoan cohesin associates with chromosomes and was shown to function in the establishment of sister chromatid cohesion during interphase, the majority of cohesin was found to be off chromosomes and reside in the cytoplasm in metaphase. Despite its dissociation from chromosomes, however, microinjection of an antibody against human SMC1 led to disorganization of the metaphase plate and cell cycle arrest, indicating that human cohesin still plays an important role in metaphase. To address the mitotic function of human cohesin, the subcellular localization of cohesin components was re-examined in human cells. Interestingly, we found that cohesin localizes to the spindle poles during mitosis and interacts with NuMA, a spindle pole-associated factor required for mitotic spindle organization. The interaction with NuMA persists during interphase. Similar to NuMA, a significant amount of cohesin was found to associate with the nuclear matrix. Furthermore, in the absence of cohesin, mitotic spindle asters failed to form in vitro. Our results raise the intriguing possibility that in addition to its well demonstrated function in sister chromatid cohesion, cohesin may be involved in spindle assembly during mitosis.

Proper chromosome segregation in mitosis is essential for the maintenance of chromosome integrity. Sister chromatid cohesion, which ensures the pairing of the two sister chromatids, is a prerequisite for proper chromosome segregation at anaphase. Cohesin is a multiprotein complex required for sister chromatid cohesion in eukaryotes. Cohesin was first characterized in Saccharomyces cerevisiae, which is composed of two structural maintenance of chromosome (SMC) family proteins, Smc1p and Smc3p, as well as the non-SMC components Scc1 and Scc3p (Refs. 1–3 and see review in Ref. 4). A similar complex was identified in Schizosaccharomyces pombe, Xenopus, and humans (5–7). In S. cerevisiae, cohesin functions in both the establishment and maintenance of sister chromatid cohesion from S phase to metaphase (1, 2). Consistently, cohesin in S. cerevisiae stays associated with chromosomes from the late G′ phase until the onset of anaphase (3). Scc1p is a homolog of Rad21 identified earlier in S. pombe (8). Proteolytic cleavage of Scc1p by the cysteine protease separin, which is indirectly promoted by the anaphase-promoting complex at the end of metaphase, is responsible for the destruction of cohesin and the initiation of sister chromatid segregation in anaphase (9). A similar observation was made in S. pombe, although S. pombe cohesin stays associated with chromosomes throughout the cell cycle (7). Studies of S. cerevisiae identified the in vivo binding sites of cohesin on the chromosome arms at estimated intervals of 13 kb with the highest concentration at the centromeric regions (10–12). Similar centromeric clustering of cohesin was also observed in S. pombe (7).

In metazoans, the protein composition of cohesin is conserved, although there are two Scc3p homologs, stromal antigen 1 (SA1) and 2 (SA2), forming separate cohesin complexes in the cell (5, 6). Interestingly, chromosomal association of cohesin in metazoans differs from that of yeast cohesin. In a Xenopus oocyte cell-free system, depletion of cohesin during interphase prior to entering mitosis inhibited subsequent mitotic cohesion of sister chromatids, suggesting that the complex is required for establishing cohesion following DNA replication (13). In contrast to S. cerevisiae, however, metazoan cohesin was found to dissociate from chromosomes prior to entering mitosis (6, 13, 14). Therefore, it is unclear how sister chromatid cohesion is maintained during metaphase. A recent study using Myc-tagged human RAD21 (hRAD21/Scc1) suggested that at least a small amount of hRAD21 is in the centromeric regions during metaphase and is subject to a similar proteolytic cleavage as in yeast (15). Similar localization was reported with the endogenous hRAD21 protein (16). In Xenopus, SA1 was shown to localize between two sister chromatids in vitro, suggesting that a low amount of cohesin may continue to associate with metaphase chromosomes in higher eukaryotes, reminiscent of buttons on a jacket securing a few critical regions of sister chromatids together (5). It was reasoned that the chromosomes of higher eukaryotes must condense to a higher level and that...
the presence of too many cohesin molecules may interfere with efficient chromosome condensation. However, the observed localization represents a very minor population of cohesin (estimated to be ~5%) (5), and the role of metazoan cohesin in metaphase cohesion has not been clearly demonstrated. Furthermore, it is not known whether cytoplasmic cohesin plays any role in mitosis.

Despite the fact that the majority of cohesin is in the cytoplasm in mitotic cells, we previously showed that the injection of an antibody specific for hSMC1 into human mitotic cells blocked the progression of metaphase and led to disorganization of the metaphase plate, suggesting a role for cohesin in mitosis (14). However, its mechanism was unknown. The same antibody failed to detect any hSMC1 at the interface between the two sister chromatids, which would be the predicted site for cohesin localization. To further address the mitotic role of human cohesin, we characterized the human cohesin complex containing hSMC1, hSMC3, hRAD21, and SA1/SA2 and performed detailed immunostaining and biochemical analyses of cohesin subcellular localization in human cells. Here we report that cohesin localizes at the spindle poles during mitosis and interacts with the nuclear mitotic apparatus protein (NuMA) and more weakly but specifically with dynein and β-tubulin. Similar to NuMA, cohesin also interacts with the nuclear matrix during interphase. Further analysis showed that cohesin is required for mitotic spindle aster assembly in vitro. Our studies suggest a novel function of cohesin in mitotic spindle organization.

EXPERIMENTAL PROCEDURES

Cell Lines and Cell Culture—HeLa cells were grown in Dulbecco’s modified Eagle’s medium (Sigma) supplemented with 10% fetal bovine serum, 1-glutamate, and penicillin/streptomycin.

Synchronization of HeLa Cells—HeLa cells were synchronized to the S and G2 phases by a double thymidine block, and to G1 phase by the arrest of cells at mitosis as described above. Mitotic cells were collected by shake-off and incubated with 20 μg/ml cytochalasin B. Cells were then washed with phosphate-buffered saline and resuspended in KHM buffer, 78 mM KCl, 50 mM Hepes, pH 7.0, 4 mM MgCl2, 2 mM EGTA, 1 mM dithiothreitol, and 0.2 mM 2(2-aminoethyl)-benzenesulfonyl fluoride (containing cytosol in a concentration of 10–20 μg/ml). Cells were then lysed by a Dounce homogenizer, and the crude extract was subjected to ultracentrifugation at 100,000 × g for 15 min. 

RESULTS

the hSMC1–hSMC3 Heterodimer Is the Cohesin Complex in Both Interphase and Mitosis—Previously, we identified the stable heterodimeric complex of hSMC1 and hSMC3 in human cells (14). Reciprocal coimmunoprecipitation of the endogenous hSMC1 and hSMC3 proteins from HeLa nuclear extracts indicates that the SMC molecules are engaged in a highly stable heterodimeric complex resistant to mild denaturant (2 M guanidine) in an equimolar ratio in the absence of any associated proteins (Fig. 1A). Because microinjection of an-
tibody against hSMC1 blocked the progression of metaphase in the previous study (14), we first wanted to determine whether hSMC1-hSMC3 is still in the cohesin complex in mitotic cells. Under the coimmunoprecipitation condition, including a 1 M salt wash, human cohesin containing hRAD21 and SA1/SA2 is coimmunopurified with antibody specific for hSMC1 (Fig. 1, B and C). The association of hRAD21 and SA1/SA2 with hSMC1-hSMC3, although resistant to the 1 M salt wash, is sensitive to 2 M guanidine unlike the interaction between hSMC1 and hSMC3 (Fig. 1A). Therefore, an hSMC1-hSMC3 heterodimer is a core subunit of cohesin, and hRAD21 and SA1/SA2 associate with it. The presence of hRad21 was also confirmed by peptide sequence analysis of the 115-kDa protein corresponding to hRad21 (KINHL (amino acid 210) and KGEADNLDEFLK (amino acid 410)) (GenBank™ accession number D38551) (21). The amounts of hRAD21 and SA1/SA2 coimmunoprecipitated with anti-hSMC1 antibody are comparable between S- and M-phase extracts, indicating that hSMC1-hSMC3 is part of cohesin during both S phase and mitosis (Fig. 1, B and C). Anti-SA1N antibody (directed against the N terminus of SA1) detected both SA1 and SA2, whereas anti-SA1C (against the C terminus) antibody detected a single protein species (SA1), consistent with the different C-terminal sequences in SA1 and SA2 (Fig. 1, C and D) (5). Because bovine SMC1 and SMC3 was found to be part of the recombination repair complex, RC-1 (22), the presence of DNA ligase III in the hSMC1 coimmunoprecipitation was also examined. DNA ligase III was not detected in our coimmunoprecipitation from HeLa nuclear extracts, indicating that the RC-1 repair complex represents a very minor complex in human cells (data not shown). Taken together, these results indicate that hSMC1-hSMC3 is in the cohesin complex in both S phase and mitotic cells at similar levels. The specificity of the antigen affinity-purified antibodies specific for hSMC1, hSMC3, hRAD21, and SA1N and SA1C was demonstrated by Western blot analysis using crude HeLa extracts from various cell cycle stages (14) as well as mitotic whole cell extracts (Fig. 1D).

Cohesin Localizes to Spindle Poles during Mitosis and Interacts with NuMA, Dynein, and β-Tubulin—Using the above antibodies, we next tested whether cohesin localizes to specific sites in mitotic cells. We performed a partial in situ extraction of soluble proteins for this purpose (CSK extraction, see “Experimental Procedures”). We found that a subpopulation of cohesin localizes to spindle poles in mitotic cells, both in metaphase and anaphase (Fig. 2). Spindle pole staining was detected using antibodies against hSMC1 (produced in two different rabbits), hSMC3, SA1N (which also recognizes SA2), and SA1C (Fig. 2 and data not shown). The corresponding preimmune antisera and antigen-depleted antibodies did not show spindle pole staining, indicating that the staining is antigen-specific (data not shown). The spindle pole localization was verified by co-staining with both β- and γ-tubulin antibodies (data not shown). Thus, cohesin is present at spindle poles during mitosis.

Based on the above results, we next tested for potential interactions of cohesin with proteins that are known to localize to the spindle poles. We found that cohesin specifically interacts with NuMA. NuMA plays an important role in mitotic spindle organization in vivo and in vitro (20, 23). NuMA interacts with dynein/dynactin and β-tubulin and is targeted to the
mitotic spindle poles by the dynein/dynactin-dependent transport mechanism (23, 24). An anti-hSMC3 antibody co-precipitated NuMA from mitotic HeLa extracts, which is resistant to a 1 M salt wash (Fig. 3, lane 4). Neither the protein A beads alone nor the preimmune antibody precipitated NuMA (Fig. 3, lanes 2 and 3). Because NuMA interacts with dynein and β-tubulin, cohesin interaction with these proteins was also tested. Anti-hSMC3 antibody coimmunoprecipitated dynein and β-tubulin from mitotic extracts (Fig. 3, lanes 5–15). No dynein or β-tubulin was precipitated with protein A beads alone or with preimmune IgG (lanes 6, 7, 9, 10, 13, and 14). In contrast to the NuMA interaction, however, these interactions were entirely sensitive to 1 M salt, indicating that the interaction is weaker and may be indirect (Fig. 3, lanes 8 and 15).

The interaction of NuMA and cohesin was also examined in S phase (Fig. 4, A and B). Anti-hSMC1 antibody co-precipitated NuMA from both S and M phase extracts (Fig. 4A, lanes 5 and 6). In contrast, CNAP1 (hCAP-D2), a component of the condensin complex that is present abundantly in the mitotic cytoplasm as well as on chromosomes (17), failed to co-precipitate with hSMC1, demonstrating the specificity of the coimmunoprecipitation (Fig. 4A, lanes 9–12). Consistently, NuMA reciprocally coimmunoprecipitated hSMC1 from both S- and M-phase extracts (Fig. 4B). Furthermore, SA1/SA2, hRAD21, and NuMA co-precipitated each other in a 1 M salt-resistant manner (data not shown). Interestingly, among the multiple species of NuMA, only a subspecies of NuMA appears to tightly interact with cohesin (Fig. 3, compare lanes 1 and 4; Fig. 4A, compare lanes 1, 2, 5, and 6). The one that preferentially interacts with cohesin in a 1 M salt-resistant manner differs between S and M phases, a faster migrating species in S phase and a slower one in M phase (Fig. 4A, lanes 5 and 6). NuMA was shown to be hyperphosphorylated in a mitosis-specific manner by cdk2 kinase, which results in slower migration of the protein in SDS-PAGE (25). This phosphorylation was shown to be critical for the proper targeting of NuMA to spindle microtubules and is thus important for the function of NuMA in spindle organization (26). Therefore, our results strongly suggest that cohesin specifically interacts with NuMA localized at the spindle structure in mitosis (Fig. 3, lane 4; Fig. 4A, lane 6). However, the tight interaction was only seen with a subpopulation of each form of NuMA (Fig. 4A, compare 1 M salt and guanidine eluate), and the peak of NuMA in sucrose density gradient centrifugation did not coincide with that of cohesin, indicating that NuMA is not a component of cohesin (Fig. 4C). Taken together, the identified interaction of cohesin with the known spindle pole-associated protein NuMA, as well as weaker but specific interactions with dynein and β-tubulin, further supports the observed localization of cohesin at the spindle poles.

Human Cohesin Interacts with the Nuclear Matrix—NuMA was shown to associate with the nuclear matrix during interphase (27). Because cohesin interacts with NuMA in interphase, we next tested whether cohesin also associates with the nuclear matrix. We performed serial extractions of cells on coverslips with detergent and DNase I to visualize the proteins present at the mitotic coverslips with detergent and DNase I to visualize the proteins. HeLa mitotic extracts were immunoprecipitated with an anti-hSMC3 antibody and the precipitated material was subjected to multiple low salt washes and eluted with 1 M salt followed by 2 M guanidine-HCl. As indicated at the bottom, proteins eluted by each treatment (1 M salt and 2 M guanidine-HCl) were trichloroacetic acid-precipitated and subjected to Western blot analysis. As indicated at the top, protein A beads without an antibody (lanes 2, 6, 9, and 13) or preimmune IgG on beads (lanes 3, 7, 10, and 14) were incubated with the same extract and were treated in an identical manner. Lanes 1, 5, and 12, an input extract as a positive control for the Western blot.
buffers that remove soluble cytoplasmic proteins (panels 2). Cells were treated with CSK and extraction treatment (panel 1). Cells were then treated with DNease I to remove DNA (panel 4).

is due to the precipitation of cohesin proteins. The nuclear matrix association of cohesin contrasts with the observation noted with condensin, the other SMC-containing complex, the nuclear localization of which was sensitive to DNease I treatment (17).

We next performed the same experiments in a test tube. These extractions with salt and detergent as well as DNease I removed soluble cytoplasmic and nuclear proteins as well as DNA-associated proteins in a stepwise manner, leaving proteins that tightly associate with the nuclear matrix. Eluted material from each step was subjected to Western blot analysis with anti-hSMC1 antibody (Fig. 6A). We found three populations of hSMC1. The first population of hSMC1 eluted in the soluble fractions (Fig. 6A, CSK and Extraction, lanes 2 and 3). A second population of hSMC1 was co-eluted with DNA after DNease I digestion (Fig. 6A, lane 4). This elution was dependent on DNease I (Fig. 6A, lane 9). A third population of hSMC1 remained associated with the nuclear matrix (Fig. 6A, lane 5).

In the same experiments, cytoplasmic dynein was removed in the first extraction while NuMA remained in the last nuclear matrix fraction, consistent with their subcellular localization (Fig. 6B). When extractions from S and G2 phase cells were compared, we found that cohesin dissociation from chromosomes is initiated in the G2 phase. In G2 phase, significantly less hSMC1 and hSMC3 were found in the DNA fraction compared with S phase, indicating that they are dissociating from chromosomes during G2 phase (Fig. 6C, compare lanes 4 and 9). Despite their dissociation from chromosomes, however, both hSMC1 and hSMC3 remain associated with the nuclear matrix (Fig. 6C, lane 10). Similar changes were observed with hRAD21 and SA1/SA2 (data not shown). Proper synchronization of cells to S and G2 phases was confirmed by FACS analysis (Fig. 6D). Importantly, at 7 h after release from the thymidine block (the prospective time point for G2 phase), cells were clearly in interphase and not in prophase (Fig. 6E). Therefore, this is different from the results in Xenopus embryos in which cohesin dissociation was shown to occur in prometaphase (6). This may reflect the differences in cell cycling between somatic and embryonic cells. Similar experiments with G1-synchronized cells revealed that cohesin associates with chromosomes, although at a lower level than in S phase, and with the nuclear matrix (data not shown). These results indicate that the association of cohesin with chromosomes is greatest in S phase and that its dissociation from chromosome begins during G2 phase, whereas a subpopulation remains associated with the nuclear matrix throughout interphase.

Cohesin Is Required for Mitotic Aster Assembly in Vitro—Localization of human cohesin at the spindle poles raises the possibility that cohesin may function in the spindle organization during mitosis. To test this hypothesis, in vitro mitotic aster assembly was performed using mitotic HeLa extracts, and the effect of cohesin depletion was examined. In the control assay, NuMA and the major population of hSMC1 co-sedimented with assembled asters consistent with the localization of cohesin at the spindle poles in mitotic cells (Fig. 7A). The extracts were immunodepleted with comparable amounts of affinity-purified antibodies against hSMC1, hSMC3, SA1N, anti-BRCA1, or purified preimmune IgG prior to the assembly reaction. Depletion of hSMC1 from the extracts was confirmed.
Human cohesin is required for mitotic spindle aster assembly in vitro. A, Western blot analysis of sedimented aster assembly. After the normal aster assembly reaction, assembled asters were sedimented and the pellet (lane 1) and supernatant (lane 2) were subjected to Western blot analysis with anti-NuMA or anti-hSMC1 antibody as indicated. B, Western blot analysis of immunodepleted HeLa mitotic extracts. Lane 1, input mitotic extract. In lanes 2–7 antibodies used to deplete the extracts are indicated at the top. Depleted extracts were subjected to Western blot analysis with anti-hSMC1 as indicated on the left. C, mitotic asters assembled in vitro with immunodepleted extracts stained with anti-β-tubulin antibody. The same depleted extracts as in B were used for the assembly reaction. Panel 1, aster assembly reaction carried out in the absence of ATP. The antibodies used to deplete the extracts are indicated at the top of each panel. A representative aster for each depletion is shown. Scale bars are 4 μm. D, Western blot analysis for the presence of NuMA and β-tubulin. Lane 1, input mitotic extract; lane 2, anti-hSMC1-depleted extract; lane 3, preimmune-IgG-depleted extract. The extracts were probed with anti-NuMA and anti-β-tubulin antibodies as indicated.

Human cohesin at the Spindle Poles

A

B

C

D

FIG. 8. Quantification of aster assembly. A, Western blot analysis of immunodepleted mitotic extracts. Extracts were depleted with preimmune IgG (lane 1), anti-hSMC1 (lane 2), and anti-hRAD21 (lane 3) antibodies as indicated at the top. The degree of cohesin depletion was checked by the presence of hSMC1 as indicated. B, the efficiency of aster assembly. The immunodepleted extracts from Fig. 8A were used to assemble asters in vitro. The same volumes of reaction mixtures were placed on coverslips and co-stained with anti-β-tubulin and anti-NuMA antibodies (see Fig. 9 legend). We examined 200 β-tubulin-positive NuMA spots and counted normal asters among them by randomly choosing areas on the coverslip under the microscope. This was repeated three times on multiple coverslips from two independent experiments. The criteria for normal asters in this experiment are: 1) distinct clustering of β-tubulin at the center; 2) co-localization of β-tubulin with NuMA; and 3) at least four spindles emanating from the aster in at least three different directions (see Fig. 9). Percentages of normal asters were averaged with standard deviation and are shown in the bar graph.
ing the timing of cohesin dissociation from chromosomes and cohesin function during mitosis. Namely, cohesin functions in sister chromatid cohesion until the onset of anaphase in yeast, whereas a majority of it dissociates from chromosomes prior to mitosis in metazoans. However, our previous antibody microinjection studies suggested that hSMC1 is still involved in the progression of mitosis, possibly in metaphase plate organization in human mitotic cells (14). Does cytoplasmic cohesin, which no longer interacts with chromosomes, play any role in mitotic chromosome organization? Our current study demonstrates the mitosis-specific localization of cohesin at the spindle poles and a specific interaction between cohesin and NuMA, a factor critical for spindle organization. Finally, we show that cohesin depletion inhibits mitotic spindle aster assembly in vitro. Based on these results, we propose that cohesin may play an additional role in mitotic chromosome organization by acting on mitotic spindle structures.

**Localization of Cohesin at the Mitotic Spindle Poles and the Nuclear Matrix**—Analysis of the subcellular localization of a protein by using immunofluorescent staining has often proven to be valuable in deducing its potential function in the cell. It is important, however, to utilize multiple antibodies because staining patterns can vary significantly depending upon the antigen epitopes, especially for large and/or highly structured proteins. Thus, our criteria for determining specific protein localization are the following. 1) Western blot analysis shows highly specific reactivity to the target protein. 2) At least two different batches of antibodies exhibit the same staining pattern, preferably with antibodies against different domains of the same protein. 3) If the protein is in a complex, antibodies against other subunits exhibit the same staining pattern; and finally, 4) the protein biochemically interacts with other proteins that localize to the same place.

We first established the specificity of antibodies for the cohesin components by Western analysis against the crude HeLa extracts. The recent studies indicated that the non-SMC components of cohesin, SA1 and RAD21, localize to the interface between the two sister chromatids, particularly the centromeric region, during metaphase (5, 15, 16). However, our antibodies against all four cohesin components, including the anti-hSMC1 antibody previously used for antibody microinjection (14), failed to detect any specific localization of the endogenous cohesin components in this location. Instead, we found that a significant population of cohesin localizes to spindle poles. The spindle pole localization of cohesin was confirmed by staining with multiple antibodies against three of the cohesin subunits and was antigen-specific. Interestingly, a previous report demonstrated that hSMC1 interacts with hsHec1, which was shown to localize to the spindle poles (37). Consistent with the spindle pole localization, we observed in mitosis a strong interaction of cohesin with a subpopulation of NuMA, the largest species that most likely represents the mitosis-specific, hyperphosphorylated form (25, 26). Weaker interactions of cohesin with dynein and β-tubulin, which may be indirectly mediated by NuMA, were also observed. Although our antibody against hRAD21 failed to stain the mitotic spindle poles, NuMA-coimmunoprecipitated hRAD21 from mitotic extracts and cohesin depletion by an anti-hRAD21 antibody had a similar inhibitory effect on mitotic aster assembly. Thus, the lack of staining is most likely due to epitope inaccessibility of our antibody. Consistent with this notion, hRad21 was recently observed at mitotic spindles using a different antibody (16).

In interphase cells, sequential extraction revealed that not all cohesin associates with DNA, but some is soluble while a third population associates with the nuclear matrix. Dissociation of all four cohesin components from chromosomes is initiated during G2 phase earlier than previously observed in a Xenopus system, which may be due to differences in cell cycling between somatic cells and embryos (6). Consistent with the nuclear matrix association, the interaction between cohesin and NuMA persists dur-
antibodies failed to co-deplete significant amounts of NuMA effect of any protein at spindle poles. Furthermore, cohesin spindle poles and play an important role in centrosome replication by immunodepletion with an antibody against hSMC1, hSMC3, SA1/SA2, or hRAD21, but not with preimmune IgG, indicating that the cohesin complex is involved in the assembly of mitotic asters. Although BRCA1 was shown to localize to spindle poles and play an important role in centrosome replication (18–20), 30), immunodepletion using anti-BRCA1 antibody did not deplete cohesin and had no effect on mitotic spindle aster assembly, indicating that the inhibition of aster formation is cohesin-specific and not due to a nonspecific depletion effect of any protein at spindle poles. Furthermore, cohesin antibodies failed to co-deplete significant amounts of NuMA and β-tubulin in our system, consistent with the fact that only a subpopulation of NuMA and β-tubulin interacts with cohesin. This result indicates that the abolishment of aster assembly is not due to the lack of NuMA or β-tubulin. This idea is further supported by the observation that NuMA is still associated with the irregularly shaped asters in the cohesin-depleted assembly reactions, indicating that depletion of cohesin does not affect NuMA localization. Rather, it is possible that cohesin is recruited to the spindle poles by NuMA. Because β-tubulin staining of the irregular asters was much weaker, cohesin may be required for the assembly or maintenance of spindles. This effect is specific to cohesin and/or other factors that interact with and therefore are co-depleted with cohesin. We have preliminary evidence that cohesin may interact in a mitosis-specific manner with other factors that may be important partners for the mitotic function of cohesin. Further study is necessary to investigate the NuMA-cohesin interaction as well as whether cohesin interacts with other proteins important for mitotic spindle organization, such as the component(s) of centrosomes and/or pericentrosomal structures. Interestingly, the kinesin-related proteins KIF3A and 3B and their associated protein MAP4 were previously reported to interact with hSMC3 (HCAP), also suggesting the potential function of cohesin in the context of the microtubule network (38). Although currently it is not clear how cohesin participates in mitotic spindle aster assembly, our results indicate the multifunctionality of cohesin in the cell.

Acknowledgments—We thank Drs. T. Hirano, T. Lindahl, and R. Shiekhattar for kindly providing antibodies for XRAD21, DNA ligase III, and BRCA1, respectively. We thank Dr. C. Cooper for permission to call SBL.8 hSMC1. We extend similar thanks to Dr. Y. Takeda for permission to refer to HCAP as HSMC3. We thank Dr. C. Hughes for use of a FACs machine, and H. Liu, M. Nomura, and S. Sandmeyer for access to the Zeiss microscope. We are grateful for critical reading of the manuscript by Drs. B. Hamkalo, S. Sandmeyer, M. Nomura, R. Steele, M. Waterman, and A. Ball.

REFERENCES

1. Guacci, V., Koshide, D., and Strunnikov, A. (1997) Cell 91, 47–57
2. Michaeil, C., Cioc, R., and Nasmyth, K. (1997) Cell 91, 35–45
3. Toth, A., Cioc, R., Uhmann, F., Galvao, M., Schleiffer, A., and Nasmyth, K. (1999) Genes Dev. 13, 320–333
4. Nasmyth, K., Peters, J.-M., and Uhlmann, F. (2000) Science 288, 1379–1384
5. Nosada, A., Yokochi, T., Kobayashi, R., and Hirano, T. (2000) J. Cell Biol. 150, 415–416
6. Sumara, I., Vorlaufer, E., Gieffers, C., Peters, B. H., and Peters, J.-M. (2000) J. Cell Biol.
7. Tomonaga, T., Nagao, N., Kawasaki, Y., Furuya, K., Murakami, A., Morishita, J., Yuasa, T., Futami, T., Keayse, S. E., Uhmann, F., Nasmyth, K., and Yanagida, M. (2000) Genes Dev. 14, 2757–2770
8. Birkenbihl, R. P., and Subramani, S. (1992) Nucleic Acids Res. 20, 6685–6611
9. Uhmann, F., Wernic, D., Pspuart, M.-A., Komin, E. V., and Nasmyth, K. (1999) Cell 100, 375–386
10. Tanaka, T., Cosma, M. P., Wirth, K., and Nasmyth, K. (1999) Cell 98, 847–858
11. Blat, Y., and Kleckner, N. (1999) Cell 98, 249–259
12. Megee, P. C., Misrot, C., Guacci, V., and Koshland, D. (1999) Mol. Cell 4, 445–450
13. Nosada, A., Hirano, M., and Hirano, T. (1998) Genes Dev. 12, 1986–1997
14. Schmiesing, J. A., Ball, A. R., Gregson, H. C., Alderton, J., Zhou, S., and Yokomori, K. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 12906–12911
15. Waizenegger, I. C., Hauf, S., Meinke, A., and Peters, J.-M. (2000) Cell 103, 399–410
16. Hoque, M. T., and Ishikawa, F. (2001) J. Biol. Chem. 276, 5059–5067
17. Schmiesing, J. A., Gregson, H. C., Zhou, S., and Yokomori, K. (2000) Mol. Cell. Biol. 20, 6996–7006
18. Gaglio, T., Dionne, M. A., and Compton, D. A. (1997) J. Cell Biol. 138, 1055–1066
19. Gaglio, T., Saredi, A., Bingham, J. B., Hashani, M. J., Gill, S. R., Schroer, T. A., and Compton, D. A. (1996) J. Cell Biol. 135, 399–414
20. Gaglio, T., Saredi, A., and Compton, D. A. (1995) J. Cell Biol. 131, 693–708
21. Nomura, N., Nagase, T., Miya, J., Naka, T., Tanaka, A., Sato, S., Seki, N., Kawarabayashi, Y., Ishikawa, K., and Tabata, S. (1994) DNA Res. 1, 283–290
22. Jessberger, R., Riwar, B., Baechtold, H., and Akhmedov, A. T. (1996) EMBO J. 15, 4061–4068
23. Moreo, A., Ramyar, K., Veitch, J. D., and Cleveland, D. W. (1996) Cell 87, 447–458
24. Merdes, A., Heald, R., Samejima, K., Earnshaw, W. C., and Cleveland, D. W. (2000) J. Cell Biol. 149, 851–861
25. Hsu, H.-L., and Yeh, N.-H. (1996) J. Cell Sci. 109, 277–288
26. Compton, D. A., and Luo, C. (1995) J. Cell Sci. 108, 621–633
27. Lydersen, B. K., and Pettijohn, D. E. (1980) J. Cell Biol. 82, 489–498
28. Pfeffer, C., and Penman, S. (1984) J. Cell Biol. 98, 1973–1984
29. Hsu, L. C., and White, R. L. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 12983–12988
30. Xu, X., Weaver, L., Llina, S., Li, C., Gotay, J., Wang, X. W., Harris, C. C., Ried, T., and Deng, C. X. (1999) Mol. Cell 3, 389–395
31. de Bette, L. C., and Kohwi-Shigematsu, T. (1998) J. Cell Biol. 141, 353–368
32. Pederson, T. (2000) Mol. Biol. Cell 11, 79–805
33. Zhao, K., Wang, W., Rando, O. J., Xue, Y., Swiderk, K., Kuo, A., and Crabtree, G. R. (1998) Cell 95, 625–636
34. Donze, D., Adams, R. C., Ene, J., and Kamakaka, R. T. (1999) Genes Dev. 13, 698–708
35. Gerasimova, T. I., and Corces, V. G. (1998) Cell 92, 511–521
36. Uv.davved, A. (1999) EMBO J. 18, 1–9
37. Zheng, L., Chen, Y., and Lee, W.-H. (1999) Mol. Biol. Cell 10, 5417–5428
38. Shimizu, K., Shirakata, H., Honda, T., Minami, S., and Takay, Y. (1998) J. Biol. Chem. 273, 6591–6594

2 J. A. Schmiesing and K. Yokomori, unpublished data.
A Potential Role for Human Cohesin in Mitotic Spindle Aster Assembly
Heather C. Gregson, John A. Schmiesing, Jong-Soo Kim, Toshiki Kobayashi, Sharleen Zhou and Kyoko Yokomori

J. Biol. Chem. 2001, 276:47575-47582.
doi: 10.1074/jbc.M103364200 originally published online October 4, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M103364200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 37 references, 22 of which can be accessed free at http://www.jbc.org/content/276/50/47575.full.html#ref-list-1