Human Bub1 protects centromeric sister-chromatid cohesion through Shugoshin during mitosis

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Sister chromatids in mammalian cells remain attached mostly at their centromeres at metaphase because of the loss of cohesion along chromosome arms in prophase. Here, we report that Bub1 retains centromeric cohesion in mitosis of human cells. Depletion of Bub1 or Shugoshin (Sgo1) in HeLa cells by RNA interference causes massive missegregation of sister chromatids that originates at centromeres. Surprisingly, loss of chromatid cohesion in Bub1 and Sgo1 RNA-interference cells does not appear to require the full activation of separase but, instead, triggers a mitotic arrest that depends on Mad2 and Aurora B. Bub1 maintains the steady-state levels and centromeric localization of Sgo1. Therefore, Bub1 protects centromeric cohesion through Shugoshin in mitosis.

spindle checkpoint | kinetochore | cohesin

To segregate sister chromatids evenly into daughter cells during mitosis, it is vital to establish physical association between sister chromatids in S phase and maintain this cohesion until their separation (1). Sister-chromatid cohesion is mediated by the cohesin protein complex, consisting of Smc1, Smc3, Scc1 (also known as Mcd1 and Rad21), and Scc3 (known as SA1 and SA2 in vertebrates) (1–3). At the metaphase–anaphase transition, cohesin is removed from chromosomes to allow sister-chromatid separation (1). Two distinct pathways for cohesin removal exist in vertebrate cells (1). In prophase, most of the cohesin residing along the chromosome arms is removed, although a small pool of cohesin persists at the centromeres (4, 5). Removal of arm cohesin requires the mitotic kinases Plk1 and Aurora B (6, 7). At metaphase, the anaphase-promoting complex or cyclosome (APC/C) ubiquitinates securin, which is an inhibitor of a protease called separase, and causes its degradation (8, 9). This process leads to the activation of separase, which then removes the residual centromeric pool of cohesin by means of the cleavage of Scc1 (10). Separase-mediated removal of centromeric cohesin is required for sister-chromatid separation (11). The existence of the prophase pathway creates an interesting puzzle: how do vertebrate cells remove most cohesin from chromosome arms in prophase but spare the cohesin at or near centromeres? Also, mechanisms must exist during meiosis I to protect centromeric cohesin from cleavage by separase (12).

Genetic and cytological studies have established a role for MEI-S332 in centromeric cohesion in *Drosophila* (13–17). Recently, homologs of MEI-S332 were identified in both fission and budding yeasts, named Shugoshin (Japanese for "guardian spirit") (18–22). These fungi Shugoshin proteins protect centromeric cohesion during meiosis and mitosis (19, 20, 22). Very recently, the *Xenopus* and mammalian homologs of MEI-S332 and Shugoshin have been identified (23). The vertebrate Sgo localizes to kinetochores and is required for centromeric cohesion during mitosis (23). Therefore, the MEI-S332 family of proteins is evolutionarily conserved and maintains centromeric cohesion in a wide range of organisms.

The spindle checkpoint senses the existence of misaligned sister chromatids during mitosis and meiosis and uses multiple mechanisms to inhibit the ubiquitin ligase activity of APC/C^{Cdc20}, thus stabilizing securin and delaying the onset of anaphase (24–28). Mad2 and BubR1/Mad3 bind directly to Cdc20, thus blocking the activity of APC/C^{Cdc20} in a stoichiometric manner (28). Bub1 phosphorylates Cdc20 and inhibits APC/C^{Cdc20} catalytically (29). Thus, by inhibiting APC/C, the spindle checkpoint indirectly preserves chromosome cohesion and delays the onset of sister-chromatid separation. However, studies in fission yeast have implicated Bub1 in the protection of centromeric cohesion during meiosis and mitosis (19, 30). Therefore, in addition to its function in APC/C inhibition, Bub1 might have a direct role in the retention of centromeric cohesion.

In this article, we study the kinetochore function of Bub1 in mitosis of mammalian cells. We show that depletion of human Bub1 (hBub1) or human Sgo1 (hSgo1) from HeLa cells by using RNA interference (RNAi) causes massive chromosome missegregation during mitosis. We frequently observe sister chromatids that are separated at their centromeres but remain attached at one or both arms in hBub1 or hSgo1 RNAi cells. We further show that hBub1 maintains the hSgo1 protein levels and is required for the centromeric localization of hSgo1. Although hBub1 and hSgo1 RNAi cells have undergone sister-chromatid separation, they contain high levels of securin and cyclin B1, and experience a mitotic arrest that depends on Mad2 and Aurora B. Therefore, our results establish a role for hBub1 in centromeric cohesion that is distinct from its function in the Mad2-dependent checkpoint pathway.

Materials and Methods

Antibodies. To generate antibodies against hSgo1, two overlapping fragments of hSgo1 (residues 1–264 or 177–351) were produced in bacteria as GST-fusion proteins and purified by using glutathione–agarose beads. The two fusion proteins were combined and used to immunize rabbits at Zymed). The antisera were purified by using Affi-Gel beads (Bio-Rad) coupled to the full-length His_6-hSgo1 protein expressed and purified from Sf9 cells. The production of Bub1, Mad2, APC2, securin, and separase antibodies has been described (31–33). The following antibodies were obtained from commercial sources: Aurora B (BD Transduction, San Jose, CA), CREST (ImmunoVision, Springdale, AZ), and cyclins A2 and B1 (Santa Cruz Biotechnology). For immunoblotting, the antibodies were used at a 1:1,000 dilution of crude serum or at 1 μg/ml affinity-purified antibodies.

Cell Culture and RNAi. HeLa tet-on (Clontech) cells were grown in DMEM (Invitrogen) supplemented with 10% FBS. To arrest cells at G_1/S or mitosis, cells were grown in the presence of 2 mM thymidine or 300 nM nocodazole for 18 h (Sigma), respectively. To release from the thymidine- or nocodazole-mediated arrest, cells were washed with and replated in fresh medium. Samples were taken at various time points and processed for immunoblotting. All small interfering RNA

Abbreviations: APC/C, anaphase-promoting complex or cyclosome; RNAi, RNA interference; siRNA, small interfering RNA; hBub1, human Bub1; hSgo1, human Sgo1.

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(siRNA) oligonucleotides were chemically synthesized. The sequences of the sense strands of siRNAs were as follows: hBub1 siRNA1, 5′-CCAGGCUGAACCCAGAGAGTT-3′; hBub1 siRNA2, 5′-CCAUGGGAUUGGAACCCUGTT-3′; hSgo1 siRNA1, 5′-CCUGCUCAGAACCAGGAAATT-3′; hSgo1 siRNA2, 5′-GAGGGGACCCUUUUACAGATT-3′; Mad2 siRNA, 5′-UACGGACUCACCUUGCUUGTT-3′; Aurora B siRNA, 5′-CGCGGCACUUCACAAUUGATT-3′; and separase siRNA, 5′-GCUUGUGAUGCCAUCCUGATT-3′. The annealing and transfection of siRNAs were performed exactly as described (34).

Immunofluorescence and Mitotic Chromosome Spread. HeLa tet-on cells or various RNAi cells were fixed with 4% paraformaldehyde, permeablized with 0.1% Triton X-100 in PBS, and incubated with 1 μg/ml affinity-purified anti-hSgo1 or anti-hBub1. The cells were also stained with a 1:2,000 dilution of a human CREST autoimmune serum (ImmunoVision) or anti-tubulin antibody. After washing, fluorescent secondary antibodies (Molecular Probes) were added at a 1:500 dilution. The cells were washed again with PBS, counterstained with DAPI, and viewed by using a $\times$63 objective on an Axiovert 200M microscope (Zeiss). Images were acquired at 0.2- or 0.3-μm intervals, deconvolved and stacked by using Intelligent Imaging software, and pseudocolored in PHOTOSHOP (Adobe Systems, San Jose, CA). For live-cell imaging, HeLa-H2B-GFP cells were cultured on chambered cover slips (Labtech, Andover, MA) in a CO_2-independent medium (Invitrogen), transfected with the desired siRNAs, treated with nocodazole, mounted on a heated stage, and imaged with an Axiovert 200M microscope ($\times$63 objective, Zeiss). Mitotic-chromosome spread of normal mitotic cells or various RNAi cells was performed exactly as described (35).

Results

Loss of hBub1 or hSgo1 Causes Massive Chromosome Missegregation and Mitotic Arrest. We depleted hBub1 or hSgo1 from HeLa cells by using RNAi. Two different siRNA oligonucleotides were used

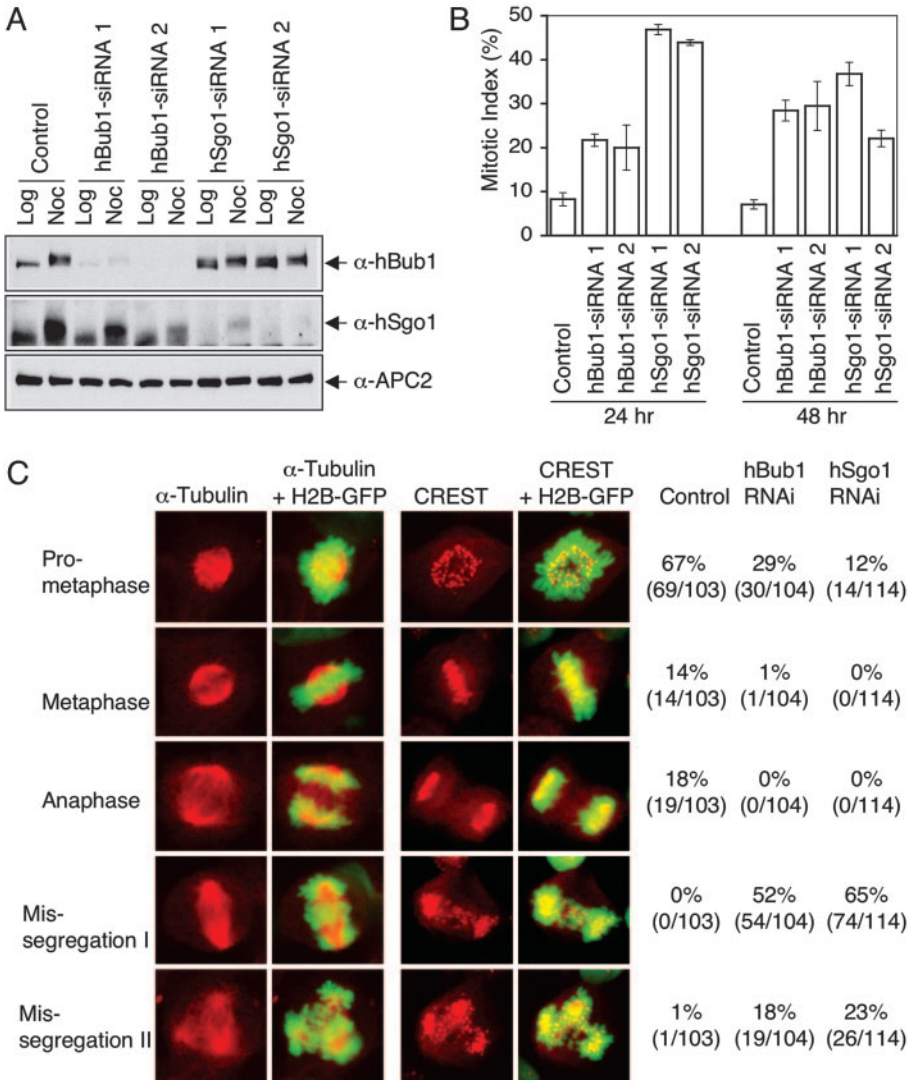

Fig. 1. Loss of hBub1 or hSgo1 causes chromosome missegregation and mitotic arrest. (*A*) Log phase or nocodazole-treated control, hBub1, and hSgo1 RNAi HeLa tet-on cells were lysed with SDS sample buffer and blotted with the indicated antibodies. APC2 was used as the loading control. (*B*) Mitotic indices of control, hBub1 and hSgo1 RNAi cells 24 or 48 h after transfection. Live cells were stained with Hoechst 33342 and observed with an inverted fluorescence microscope. Mitotic cells were scored based on their cell shape and DNA morphology. Three separate fields with >100 cells each were counted. The average values and standard deviation are shown. (*C*) HeLa tet-on cells stably expressing H2B-GFP (green) were transfected with control, hBub1, or hSgo1 siRNA; fixed with paraformaldehyde; and stained with either anti-tubulin or CREST (red). The percentages of cells with a given phenotype and the number of examined cells are shown on the right.

for each gene to rule out potential off-target effects of RNAi. The protein levels of hBub1 and hSgo1 were reduced significantly in cells treated with siRNA against these two genes (Fig. 1*A*). Consistent with hSgo1 being an APC/C substrate (23), the protein levels of hSgo1 were substantially higher in nocodazole-arrested mitotic cells. Interestingly, the protein levels of hSgo1 were reduced in hBub1 RNAi cells, indicating that hBub1 is required for the maintenance of the steady-state levels of hSgo1 (Fig. 1*A*).

Cells treated with hBub1-siRNA accumulated in mitosis (Fig. 1*B*), suggesting that hBub1 is required for normal cell-cycle progression. In contrast, targeted suppression of Mad2 by RNAi in HeLa cells did not cause a significant mitotic delay (36, 37). Depletion of hSgo1 by RNAi caused an even greater percentage of cells to undergo mitotic arrest (Fig. 1*B*). Of mitotic hBub1 and hSgo1 RNAi cells, >50% possessed a bipolar spindle and contained two clusters of mitotic chromosomes with many lagging chromosomes in the spindle midzone (Missegregation I; Fig. 1*C*). An additional 20% of these mitotic cells contained three masses of DNA (Missegregation II), each clustering around an apparent spindle pole. Thus, depletion of hBub1 or hSgo1 by siRNA caused massive chromosome missegregation. Cells with either type of massive chromosome missegregation were rare in control cells (Fig. 1*C*). These results were obtained with two different siRNAs for each protein and were highly reproducible.

To ascertain that the sister chromatids were indeed separated in mitotic hBub1 and hSgo1 RNAi cells, we prepared a chromosome spread of these cells. Because hBub1 and hSgo1 RNAi cells exhibited similar chromosome morphology, only the mitotic chromosome spread for hBub1 RNAi cells was shown in Fig. 2*A*. The statistics of different phenotypes were shown for both types of cells. In early prophase, sister-chromatid cohesion was maintained along the entire length of the chromosomes in both control and hBub1 RNAi cells (Fig. 2*A*). During late prometaphase/metaphase, the sister chromatids remained attached at or near centromeres, whereas the chromosome arms had visibly separated (Fig. 2*A*). Two evenly distributed sets of separated chromatids were observed in normal anaphase cells (Fig. 2*A*). Most hBub1 and hSgo1 RNAi cells in mitosis contained either two clusters of separated chromatids with many chromatids lagging in between or three clusters of separated chromatids (Fig. 2*A*), presumably corresponding to the chromosome missegregation I and II cells described above. Because it was not always easy to distinguish between these two categories of morphology by chromosome spread, the combined percentage of both categories of cells was reported (Fig. 2*A*). Therefore, similar to depletion of hSgo1, depletion of hBub1 causes massive chromosome missegregation during mitosis.

hBub1 and hSgo1 Are Required for Centromeric Cohesion During Mitosis. We next synchronized the hBub1 and hSgo1 RNAi cells at G_1/S with thymidine; harvested mitotic cells at 9, 10, 11, or 12 h after release from the G_1/S arrest; and analyzed the morphology of their chromosome spread (Fig. 2 *B* and *C*). We observed the following four major categories of cells, based on their chromosome morphology: I, most sister chromatids remained attached along their entire length; II, some sister chromatids had fully separated, whereas other sister chromatids were separated at centromeres but remained attached at one or both arms; III, all sister chromatids were fully separated; and IV, all sister chromatids were fully separated and hypercondensed. The

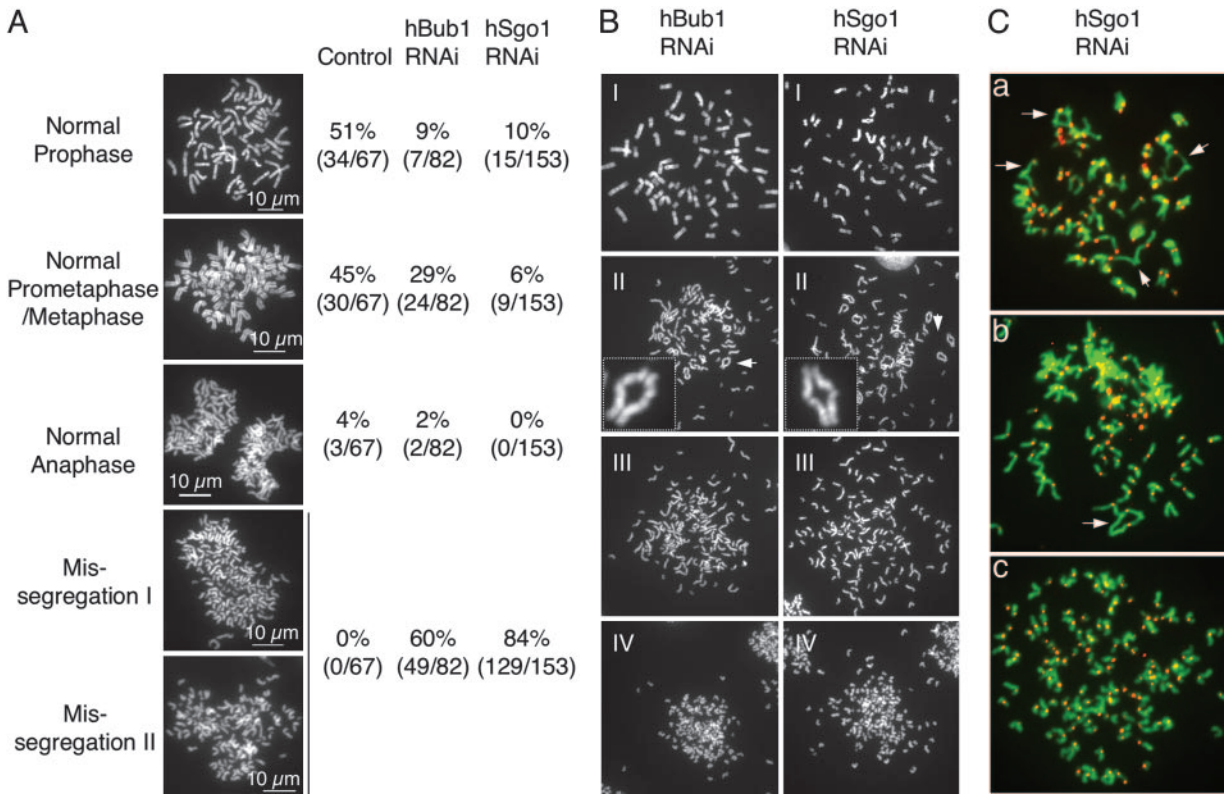

Fig. 2. Loss of centromeric cohesion in hBub1 and hSgo1 RNAi cells. (*A*) Mitotic chromosome spread of control, hBub1, and hSgo1 RNAi cells. The percentages of cells with a given phenotype and the number of cells examined are shown on the right. (Scale bars, 10 μm.) (*B*) Four major categories (I–IV) of phenotypes observed in mitotic chromosome spread of hBub1 and hSgo1RNAi cells. Some of the sister chromatids that are separated only at centromeres are indicated by arrows and magnified in the *Insets*. (*C*) Mitotic chromosome spread of hSgo1 RNAi cells. Chromosomes are shown in green, and the kinetochores stained with CREST are shown in red. Chromosomes that are separated at the centromeres but remain attached at one or both arms are indicated by arrows.

existence of sister chromatids that were separated at centromeres but remained attached at the arms (category II) in hBub1 and hSgo1 RNAi cells was very suggestive. This category of sister-chromatid morphology was not observed in normal mitotic cells. In addition, the percentage of cells in category II decreased at later time points, whereas the percentages of cells belonging to categories III and IV increased (data not shown). Although we did not follow the same cells in a time-lapsed fashion, these data strongly suggest that improper separation of sister chromatids in hBub1 and hSgo1 RNAi cells occurs first at centromeres. Our mitotic chromosome-spread studies reveal the status of sister-chromatid cohesion at a greater detail and complement the live-cell-imaging analysis by Salic *et al.* (23). Therefore, Bub1 and Sgo1 are required for centromeric cohesion in mitotic cells.

The Centromeric Localization of hSgo1 Depends on hBub1. Consistent with earlier reports (23), hSgo1 localized to centromeres in mitosis (Fig. 3*A*). In contrast to earlier reports (23), the centromeric localization of hSgo1 persisted until anaphase and disappeared at telophase (Fig. 3*A*). This result suggests that the mere presence of hSgo1 at centromeres might not be sufficient to protect centromeric cohesion. hBub1 localized to the outer plate of kinetochores, because the two hBub1-staining dots on a given pair of sister chromatids were clearly outside the two CREST dots, which marked the inner kinetochores (Fig. 3*B*). In contrast, hSgo1 localized as one dot between the paired dots of CREST staining (Fig. 3*B*). The localization pattern of hSgo1 is similar to that of centromeric Scc1 and is consistent with it playing a direct role in protecting centromeric cohesion (5). Note that the inner kinetochore localization of the endogenous hSgo1 is different from that described for Myc-tagged *Xenopus* Sgo, which localizes outside the CREST staining (23).

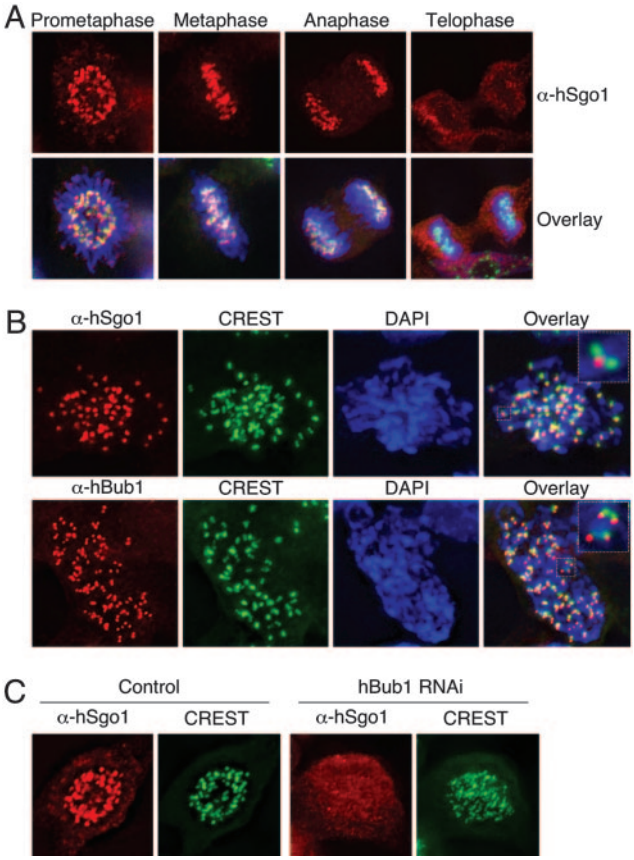

Fig. 3. hBub1 maintains hSgo1 at centromeres. (*A*) HeLa tet-on cells were stained with anti-hSgo1 (red), CREST (green), and DAPI (blue). (*B*) HeLa tet-on cells were stained with anti-hSgo1 or anti-hBub1 (red), CREST (green), and DAPI (blue). The boxed areas are magnified and shown in *Insets*. (*C*) Control and hBub1 RNAi cells in mitosis were stained with anti-hSgo1 (red), CREST (green), and DAPI (blue).

Although the hSgo1 protein level was substantially lower in hBub1 RNAi cells (Fig. 1*A*), there was still significant amount of hSgo1 in these cells. Thus, we examined the localization of hSgo1 in hBub1 RNAi cells. Consistent with earlier findings in fission yeast (19), the centromeric localization of hSgo1 was largely absent in hBub1 RNAi cells (Fig. 3*C*). Thus, in addition to maintaining the steady-state levels of hSgo1, hBub1 is also required for the proper centromeric localization of hSgo1. However, depletion of hSgo1 had no effect on the kinetochore localization of hBub1 (Fig. 6, which is published as supporting information on the PNAS web site).

Loss of hBub1 or hSgo1 Activates the Mad2-Dependent Spindle Checkpoint. Similar to nocodazole-arrested cells (38), mitotic hBub1 and hSgo1 RNAi cells contained high levels of securin and cyclin B1, but low levels of cyclin A2 (Fig. 4*A*; see also Fig. 7*A*, which is published as supporting information on the PNAS web site). Depletion of either Mad2 or Aurora B by RNAi rescued the mitotic-arrest phenotype of hBub1 and hSgo1 RNAi cells (Fig. 4 *B–D*). In contrast, depletion of separase did not affect the mitotic index of hBub1 and hSgo1 RNAi cells (Fig. 4 *C* and *D*). The levels of all of the aforementioned proteins were efficiently reduced by RNAi (Fig. 7 *B–E*). Therefore, the mitotic arrest of hBub1 and hSgo1 RNAi cells depends on the spindle checkpoint.

Despite having undergone sister-chromatid separation, hBub1 and hSgo1 RNAi cells remain arrested in mitosis by the spindle checkpoint and contain high levels of securin. This finding implies that the improper separation of sister chromatids in these cells might not require the full activation of separase. To test this possibility further, we codepleted hSgo1 and separase from HeLa cells by RNAi (Fig. 5). Mitotic cells with both hSgo1 and separase depleted still separated their sister chromatids improperly. Similar results were obtained with hBub1/separase RNAi cells (data not shown). Our results suggest that separase is not required for the aberrant sister-chromatid separation caused by the loss of hBub1 or hSgo1 function.

Discussion

Dual Functions of Bub1. Genetic and biochemical data from several organisms have clearly established Bub1 as a critical component of the spindle checkpoint. This study demonstrates that the extent and penetrance of the chromosome-missegregation phenotype of hBub1 RNAi cells are much greater than that of human Mad2 RNAi cells, only a small percentage of which contain a few lagging chromosomes during anaphase (data not shown). Because depletion of Mad2 by RNAi rescues the mitotic arrest caused by hBub1 RNAi, the Mad2-dependent checkpoint appears to be somewhat active in hBub1 RNAi cells. Therefore, the massive chromosome missegregation observed in hBub1 RNAi cells cannot be attributed solely to its function in the Mad2-dependent spindle checkpoint.

Our data further establish a role for hBub1 in centromeric cohesion during mitosis of mammalian cells. Several lines of evidence indicate that hSgo1 is a target of hBub1 at the kinetochores. First, RNAi-mediated suppression of hBub1 or hSgo1 causes similar phenotypes, including massive chromosome missegregation and spindle-checkpoint-dependent mitotic arrest. Loss of hSgo1 consistently causes more severe phenotypes, as compared with the depletion of hBub1. Depletion of both hBub1 and hSgo1 does not further exacerbate the effect of losing hSgo1 alone (data not shown). These results are consistent with hSgo1 being a downstream target of hBub1.

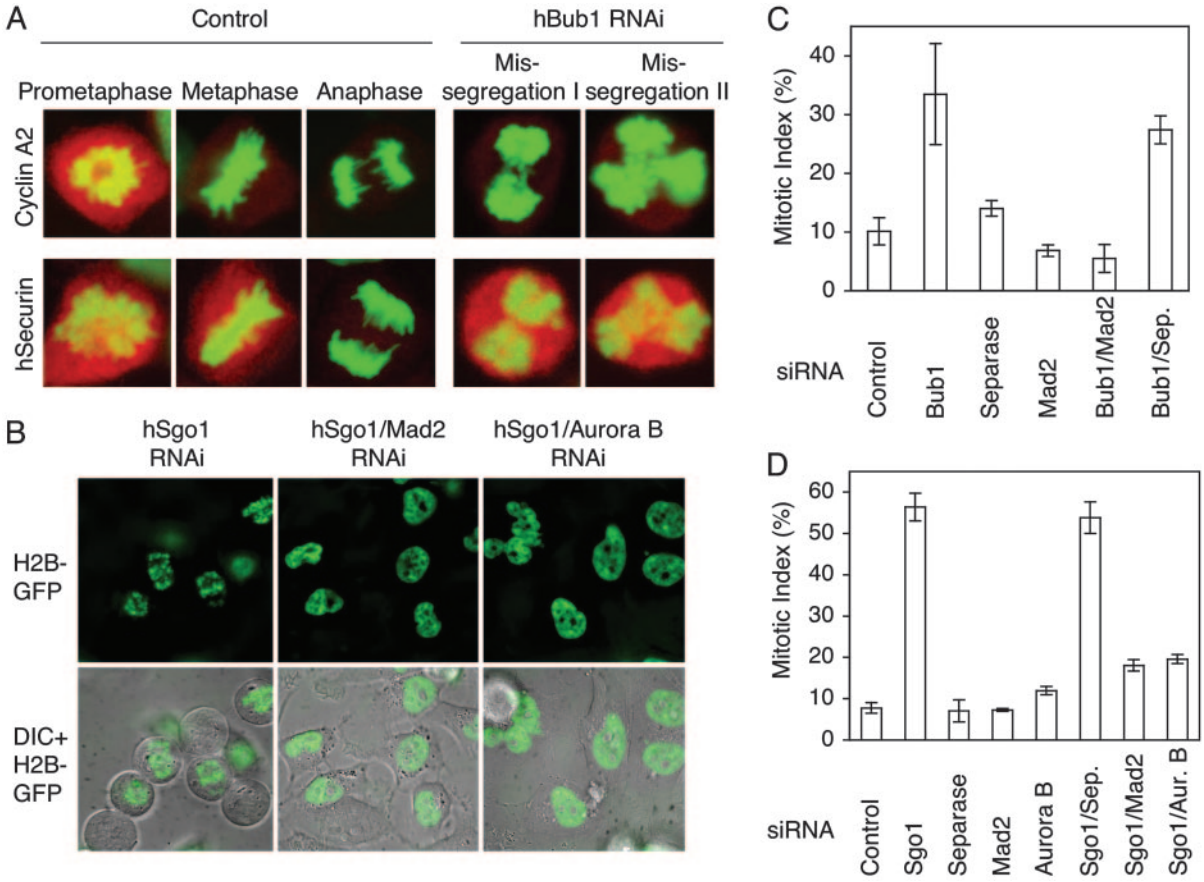

Fig. 4. Loss of hBub1 or hSgo1 triggers the Mad2- and Aurora B-dependent spindle checkpoint. (*A*) HeLa tet-on cells stably expressing H2B-GFP (green) were transfected with control or hBub1 siRNA and stained with anti-cyclin A2 or anti-securin (red). (*B*) Live-cell imaging of hSgo1 RNAi, hSgo1/Mad2 RNAi, and hSgo1/Aurora B RNAi HeLa tet-on cells stably expressing H2B-GFP (green). Note that some cells do not express H2B-GFP. DIC, differential-interference contrast. (*C* and *D*) Mitotic indices of H2B-GFP expressing HeLa tet-on cells transfected with the indicated siRNAs. Three separate fields with >100 cells each were counted. The average values and standard deviation are shown.

Second, hBub1 is required for the maintenance of the steady-state levels and the centromeric localization of hSgo1. Therefore, in addition to its well established role in the spindle checkpoint, Bub1 has an additional role in preserving centromeric cohesion.

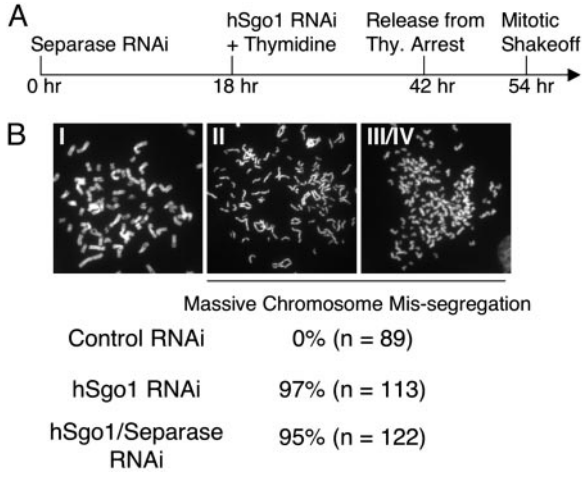

Fig. 5. Depletion of separase by RNAi does not prevent sister-chromatid separation in hSgo1 RNAi cells. (*A*) Time course of the RNAi experiment shown in *B*. (*B*) Percentage of mitotic cells treated as described in *A* that exhibit the indicated phenotype. The number of analyzed cells analyzed is given in parentheses.

Activation of the Mad2-Dependent Checkpoint in the Absence of Bub1 or Shugoshin. What are the defects that activate the spindle checkpoint in hBub1 or hSgo1 RNAi cells? Salic *et al.* (23) have shown also that depletion of hSgo1 in HeLa cells by RNAi causes a mitotic arrest that depends on the spindle checkpoint. Because hSgo1 directly regulates microtubule dynamics, activation of the spindle checkpoint in hSgo1 RNAi cells has been attributed to the unstable kinetochore-microtubule attachment in these cells. Chromosome missegregation is thought to occur as a consequence of a prolonged mitotic arrest. In an alternative and equally plausible model, we propose that loss of hBub1 or hSgo1 weakens centromeric cohesion, leading to sister-chromatid separation before the full activation of APC/C and separase. The separated chromatids can no longer achieve biorientation (24). The lack of tension at their kinetochores and/or the consequent destabilization of kinetochore-microtubule attachment then activate the spindle checkpoint, causing the mitotic arrest. Consistent with this model, we show that Aurora B, a key component of the tension-sensing pathway of the checkpoint (24), is required for the mitotic arrest of hBub1 and hSgo1 RNAi cells.

Paradoxically, loss of Bub1, a key spindle checkpoint protein, triggers a Mad2 and Aurora B-dependent mitotic arrest. One trivial explanation is that the residual amount of hBub1 in RNAi cells, although insufficient for its kinetochore function, does not

abolish its checkpoint function. However, we have observed that fewer hBub1 RNAi cells undergo mitotic arrest than do hSgo1 RNAi cells. Furthermore, as compared with hSgo1 RNAi cells, more hBub1 RNAi cells escape from mitotic arrest in the presence of spindle damaging agents, such as nocodazole (29). Therefore, a partial loss of Bub1 function might have significantly weakened the Mad2 spindle checkpoint pathway. However, it is equally plausible that the function of Bub1 in maintaining Sgo1 at kinetochores is fundamentally separate from its role in the spindle checkpoint. Additional studies are needed to address these issues.

Role of Shugoshin in Centromeric Sister-Chromatid Cohesion. The Shugoshin family of proteins protects centromeric cohesin from cleavage by separase in meiosis I of budding and fission yeasts (19–22). Our results do not allow us to distinguish between a role of hSgo1 in the establishment or the maintenance/protection of centromeric cohesion in mammalian cells. However, we show that, despite having separated their sister chromatids, the hSgo1 RNAi cells contain high levels of securin and undergo a mitotic arrest that requires Mad2 and Aurora B. Removal of the bulk of separase by RNAi does not prevent sister-chromatid separation in hSgo1 RNAi cells. These results strongly suggest that a full, concerted activation of APC/C and separase is not required for the aberrant separation of sister chromatids in hSgo1 RNAi cells. It is possible that chromosome segregation in these cells is a secondary consequence of prolonged mitotic arrest. However, it is equally possible that hSgo1 plays a direct role in maintaining sister-chromatid cohesion.

Conclusion

hBub1 regulates centromeric cohesion through two distinct pathways. It participates in the spindle checkpoint and helps to inhibit APC/C, thus preventing the activation of separase indirectly. hBub1 also maintains hSgo1 at centromeres, thus preserving centromeric cohesion directly.

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1. Nasmyth, K. (2002) *Science* **297,** 559–565.
2. Koshland, D. E. & Guacci, V. (2000) *Curr. Opin. Cell Biol.* **12,** 297–301.
3. Hirano, T. (2002) *Genes Dev.* **16,** 399–414.
4. Losada, A., Hirano, M. & Hirano, T. (1998) *Genes Dev.* **12,** 1986–1997.
5. Waizenegger, I. C., Hauf, S., Meinke, A. & Peters, J. M. (2000) *Cell* **103,** 399–410.
6. Sumara, I., Vorlaufer, E., Stukenberg, P. T., Kelm, O., Redemann, N., Nigg, E. A. & Peters, J. M. (2002) *Mol. Cell* **9,** 515–525.
7. Losada, A., Hirano, M. & Hirano, T. (2002) *Genes Dev.* **16,** 3004–3016.
8. Peters, J. M. (2002) *Mol. Cell* **9,** 931–943.
9. Harper, J. W., Burton, J. L. & Solomon, M. J. (2002) *Genes Dev.* **16,** 2179–2206.
10. Uhlmann, F., Wernic, D., Poupart, M. A., Koonin, E. V. & Nasmyth, K. (2000) *Cell* **103,** 375–386.
11. Hauf, S., Waizenegger, I. C. & Peters, J. M. (2001) *Science* **293,** 1320–1323.
12. Petronczki, M., Siomos, M. F. & Nasmyth, K. (2003) *Cell* **112,** 423–440.
13. Goldstein, L. S. (1980) *Chromosoma* **78,** 79–111.
14. Kerrebrock, A. W., Miyazaki, W. Y., Birnby, D. & Orr-Weaver, T. L. (1992) *Genetics* **130,** 827–841.
15. Kerrebrock, A. W., Moore, D. P., Wu, J. S. & Orr-Weaver, T. L. (1995) *Cell* **83,** 247–256.
16. Tang, T. T., Bickel, S. E., Young, L. M. & Orr-Weaver, T. L. (1998) *Genes Dev.* **12,** 3843–3856.
17. LeBlanc, H. N., Tang, T. T., Wu, J. S. & Orr-Weaver, T. L. (1999) *Chromosoma* **108,** 401–411.
18. Watanabe, Y. (2004) *J. Cell Sci.* **117,** 4017–4023.
19. Kitajima, T. S., Kawashima, S. A. & Watanabe, Y. (2004) *Nature* **427,** 510–517.
20. Katis, V. L., Galova, M., Rabitsch, K. P., Gregan, J. & Nasmyth, K. (2004) *Curr. Biol.* **14,** 560–572.
21. Rabitsch, K. P., Gregan, J., Schleiffer, A., Javerzat, J. P., Eisenhaber, F. & Nasmyth, K. (2004) *Curr. Biol.* **14,** 287–301.
22. Marston, A. L., Tham, W. H., Shah, H. & Amon, A. (2004) *Science* **303,** 1367–1370.
23. Salic, A., Waters, J. C. & Mitchison, T. J. (2004) *Cell* **118,** 567–578.
24. Tanaka, T. U. (2002) *Curr. Opin. Cell Biol.* **14,** 365–371.
25. Cleveland, D. W., Mao, Y. & Sullivan, K. F. (2003) *Cell* **112,** 407–421.
26. Musacchio, A. & Hardwick, K. G. (2002) *Nat. Rev. Mol. Cell Biol.* **3,** 731–741.
27. Bharadwaj, R. & Yu, H. (2004) *Oncogene* **23,** 2016–2027.
28. Yu, H. (2002) *Curr. Opin. Cell Biol.* **14,** 706–714.
29. Tang, Z., Shu, H., Oncel, D., Chen, S. & Yu, H. (2004) *Mol. Cell* **16,** 387–397.
30. Bernard, P., Maure, J. F. & Javerzat, J. P. (2001) *Nat. Cell Biol.* **3,** 522–526.
31. Fang, G., Yu, H. & Kirschner, M. W. (1998) *Mol. Cell* **2,** 163–171.
32. Tang, Z., Bharadwaj, R., Li, B. & Yu, H. (2001) *Dev. Cell* **1,** 227–237.
33. Zou, H., McGarry, T. J., Bernal, T. & Kirschner, M. W. (1999) *Science* **285,** 418–422.
34. Elbashir, S. M., Harborth, J., Lendeckel, W., Yalcin, A., Weber, K. & Tuschl, T. (2001) *Nature* **411,** 494–498.
35. Henegariu, O., Heerema, N. A., Lowe Wright, L., Bray-Ward, P., Ward, D. C. & Vance, G. H. (2001) *Cytometry* **43,** 101–109.
36. Meraldi, P., Draviam, V. M. & Sorger, P. K. (2004) *Dev. Cell* **7,** 45–60.
37. Luo, X., Tang, Z., Xia, G., Wassmann, K., Matsumoto, T., Rizo, J. & Yu, H. (2004) *Nat. Struct. Mol. Biol.* **11,** 338–345.
38. Geley, S., Kramer, E., Gieffers, C., Gannon, J., Peters, J. M. & Hunt, T. (2001) *J. Cell Biol.* **153,** 137–148.