Current Biology, Vol. 15, 2263–2270, December 20, 2005, ©2005 Elsevier Ltd All rights reserved. DOI 10.1016/j.cub.2005.11.034

Report

Control of Shugoshin Function during Fission-Yeast Meiosis

Sabine Vaur,[1,2] Fabien Cubizolles,[1,2] Guillaume Plane,[1,2] Sylvie Genier,[1,2] Peter K. Rabitsch,[3] Juraj Gregan,[3] Kim Nasmyth,[3] Vincent Vanoosthuyse,[4] Kevin G. Hardwick,[4] and Jean-Paul Javerzat[1,2,*]

[1] Centre National de la Recherche Scientifique
Institut de Biochimie et Génétique Cellulaires
Unité mixte de recherche 5095
Bordeaux, F-33077
France

[2] Université Victor Segalen Bordeaux 2
Bordeaux F-33077
France

[3] Research Institute of Molecular Pathology
Dr. Bohr-Gasse 7
A-1030 Vienna
Austria

[4] Wellcome Trust Centre for Cell Biology
University of Edinburgh
Edinburgh EH9 3JR
United Kingdom

Summary

Meiosis consists of a single round of DNA replication followed by two consecutive nuclear divisions. During the first division (MI), sister kinetochores must orient toward the same pole to favor reductional segregation. Correct chromosome segregation during the second division (MII) requires the retention of centromeric cohesion until anaphase II. The spindle checkpoint protein Bub1 is essential for both processes in fission yeast [1, 2]. When *bub1* is deleted, the Shugoshin protein Sgo1 is not recruited to centromeres, cohesin Rec8 does not persist at centromeres, and sister-chromatid cohesion is lost by the end of MI. Deletion of *bub1* also affects kinetochore orientation because sister centromeres can move to opposite spindle poles in approximately 30% of MI divisions. We show here that these two functions are separable within the Bub1 protein. The N terminus of Bub1 is necessary and sufficient for Sgo1 targeting to centromeres and the protection of cohesion, whereas the C-terminal kinase domain acts together with Sgo2, the second fission-yeast Shugoshin protein, to promote sister-kinetochore co-orientation during MI. Additional analyses suggest that the protection of centromeric cohesion does not operate when sister kinetochores attach to opposite spindle poles during MI. Sgo1-mediated protection of centromere cohesion might therefore be regulated by the mode of kinetochore attachment.

*Correspondence: jpaul.javerzat@ibgc.u-bordeaux2.fr

Results and Discussion

Bub1 is an essential component of the spindle checkpoint, a surveillance mechanism that delays the onset of anaphase in the presence of incorrectly attached kinetochores. The control of Sgo1 function by Bub1 suggested that other spindle-checkpoint components might be involved in this process. To test this idea, we looked at chromosome segregation patterns during meiosis in all known mutants of the fission-yeast spindle checkpoint. These mutants are *mad1Δ* [3], *mad2 Δ* [4], *mad3 Δ* [5], *bub3 Δ* [6], and *mph1 Δ* [7]. We monitored chromosome segregation in tetrads by crossing a strain (*cen1-GFP* [8]) harboring a GFP mark close to *cen1* with an unmarked mating partner. As previously reported [1], deletion of *bub1* affects both kinetochore orientation and the protection of centromeric cohesion. In approximately 40% of MI divisions (Figure 1A), GFP-marked sister centromeres moved to opposite spindle poles (equational segregation), indicative of a defect in kinetochore mono-orientation. In the approximately 60% remaining MI divisions, sister centromeres segregated normally (reductionally) during MI but randomly during MII, consistent with a complete loss of sister-chromatid cohesion past the first metaphase-to-anaphase transition. As shown in Figure 1A, only the *bub1* mutant caused such a marked defect in meiotic chromosome segregation. These data strongly suggest that Bub1 functions during meiosis are likely to be distinct from Bub1's role in the spindle checkpoint.

Bub1 controls two key features of the first meiotic division: the monopolar attachment of sister kinetochores and the protection of centromeric cohesion through the recruitment of Sgo1. Deletion of *sgo1* alone, however, does not affect chromosome segregation during MI [2, 9], implying that equational segregation in *bub1Δ* cells must be due to a defect distinct from the mislocalization of Sgo1. These observations suggested that kinetochore mono-orientation and protection of centromeric cohesion are separable functions within the Bub1 protein. To test this idea, we made serial deletions, starting from the kinase domain at the C terminus (Figure 1B). All deletions were made by homologous recombination within the *bub1* locus, leaving the truncated ORFs under the control of the endogenous *bub1* promoter. Chromosome segregation was monitored in tetrads from a cross in which only one mating partner harboured *cen1-GFP*. As shown in Figure 1C, all deletions gave a similar phenotype, that is, approximately 20% of meiosis I divisions showing equational segregation during MI and approximately 80% showing reductional segregation. However, when sister *cen1-GFP* dots segregated reductionally during MI, the segregation was mostly equational during MII. This is in sharp contrast with the random segregation of sister *cen1-GFP* observed during MII when the entire *bub1* gene is deleted. This observation suggests that cohesion was not lost between sister centromeres at the end of MI in the kinase-deleted mutants. Consistent with

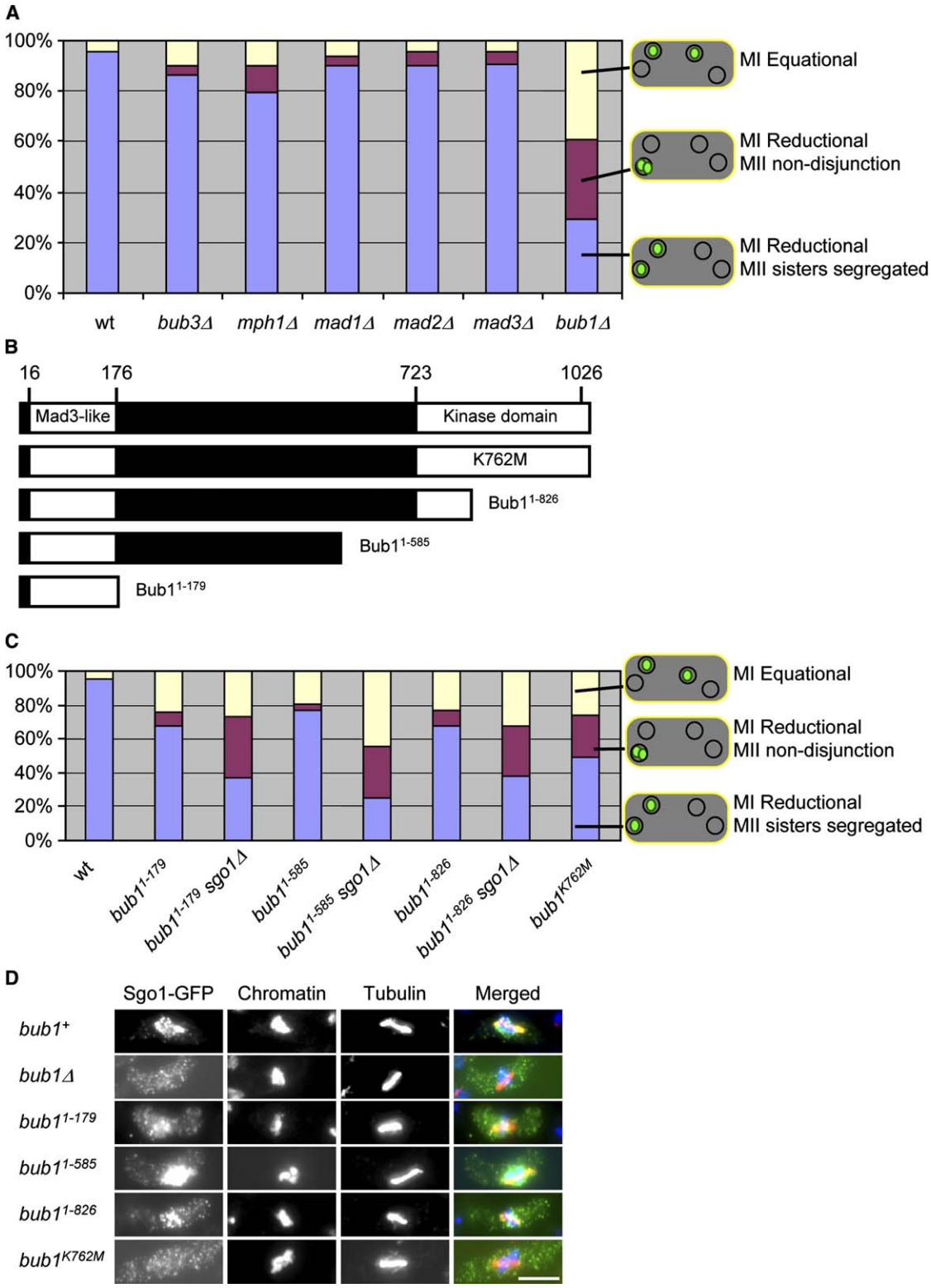

Figure 1. The N-Terminal Domain of Bub1 Is Sufficient for Sgo1 Localization and Function

(A) Meiotic chromosome segregation in spindle-checkpoint mutants. Crosses were made with only one mating partner bearing *cen1-GFP*. At least 100 tetrads were examined for each cross.

(B) Schematic representation of the *bub1* alleles used in this study. The *bub1*^{K762M} allele bears a point mutation in the ATP binding pocket. *bub1*$^{1–826}$ removes most of the kinase domain. The kinase domain is entirely deleted in *bub1*$^{1–585}$ and *bub1*$^{1–179}$.

(C) *cen1-GFP* segregation in the *bub1* mutants. At least 100 tetrads were examined for each cross heterozygous for *cen1-GFP*.

(D) Sgo1-GFP was examined by indirect immunofluorescence on fixed cells with antibodies against GFP. Anti-tubulin antibodies were used to visualize the spindle and identify metaphase I cells. Chromatin was counterstained with DAPI. The scale bar represents 5 μm.

this idea, equational segregation during MII in the kinase-truncated mutants relied on a functional *sgo1* gene (Figure 1C). This analysis indicates that the protection of centromeric cohesion is functional in the *bub1*-truncated mutants. To confirm this assumption, we looked at Sgo1 localization in cells by using immunofluorescence. In wild-type cells, Sgo1 is recruited at centromeres shortly before MI, and by metaphase I, Sgo1 is seen as nuclear staining with punctate dots of fluorescence along the spindle (Figure 1D and [2, 9]). This localization is abolished in a *bub1Δ* background but preserved in the truncated mutants (Figure 1D), although the signal intensity was variable; in $bub1^{1-585}$, Sgo1 staining was as strong as in the wild-type, but it was weaker in $bub1^{1-179}$ and $bub1^{1-826}$ backgrounds. We conclude that the N terminus of Bub1 is sufficient to promote correct Sgo1 localization and function during MI. The corollary is that the kinase domain is dispensable for Sgo1 localization and function. This finding appears to contradict a previous report [2] showing that Sgo1 was mislocalized in a kinase-dead allele of Bub1 (K762R). Similarly, we found that Sgo1 was mislocalized in a different allele (Figure 1D, *K762M* [6]), and analysis of *cen1-GFP* segregation revealed a high frequency of sister-chromatid nondisjunction during MII (Figure 1C), consistent with Sgo1's being largely nonfunctional in this mutant background. Therefore, we originally thought that the kinase activity of Bub1 was involved. However, our subsequent deletion analysis of Bub1 clearly showed that the kinase domain was dispensable for correct Sgo1 localization and function. Because the catalytic mutant is nearly as affected as the null allele, one trivial explanation would be that $Bub1^{K762M}$ is unstable, mislocalized, or both. However, we found that $Bub1^{K762M}$ was properly localized in metaphase I cells and that the amount of protein at centromeres was close to wild-type levels (Figure S1). Others made similar observations for the $bub1^{K762R}$ allele [10]. Altered Sgo1 function in the *K762M* and *K762R* mutants might result from a dominant-negative effect of the catalytically inactive kinase on the N-terminal domain. For instance, one intriguing possibility would be that the kinase activity is required for an autophosphorylation event that triggers a Bub1 conformational change allowing the unmasking of the N-terminal domain and its interaction with downstream effectors. Deletion of the whole kinase domain would result in the loss of this regulatory module and constitutive unmasking of the N-terminal domain, whereas the catalytically inactive mutant would tether the N terminus in an inactive state. Further experiments are required to evaluate this possibility.

Bub1's N-terminal 179 amino acids sufficient for correct Sgo1 localization and function are part of the Mad3-like domain involved in the nuclear accumulation and kinetochore targeting of Bub1 [11]. In a *bub1Δ* background, the Sgo1-GFP fluorescence is dispersed within the whole cell. This raised the possibility that Bub1 might be solely required for promotion of the nuclear retention of Sgo1, in which case the targeting of Sgo1 to the nucleus by an exogenous nuclear localization signal should bypass the requirement of Bub1 for correct Sgo1 localization and function. To test this idea, we fused an exogenous SV40 nuclear localization signal (NLS), which functions efficiently in *S. pombe* [11], to the Sgo1 protein and expressed the resulting construct (*sgo1-NLS-GFP*) from the genomic *sgo1* promoter at the endogenous locus.

Analysis of *cen1-GFP* segregation in tetrads showed that Sgo1-NLS-GFP was functional; *cen1-GFP* segregated mostly as wild-type (Figure 2A), and Sgo1-NLS-GFP was found in the nucleus in a dotted pattern very similar to that of Sgo1-GFP without exogenous NLS (Figure 2B). In a *bub1Δ* background, Sgo1-GFP was dispersed throughout the cell. By contrast, Sgo1-NLS-GFP was still enriched in the nucleus but appeared as a diffuse nuclear signal without dots, indicating a defect in centromere localization (Figure 2B). Accordingly, *cen1-GFP* segregated randomly during the second division (Figure 2A). By contrast, the dotty pattern and chromosome segregation were restored in a $bub1^{1-585}$ background (Figures 2A and 2B). Therefore the addition of a NLS to Sgo1 allowed its nuclear enrichment in the absence of Bub1, but Sgo1 was not properly localized and was nonfunctional. This analysis rules out the possibility that Bub1's sole role would be to target Sgo1 to the nucleus and further confirms that the N terminus of Bub1 is necessary and sufficient for Sgo1 localization and function.

To gain more insight into the mechanism by which Bub1 controls Sgo1 localization, we looked at Bub1 localization by chromatin immunoprecipitation on metaphase I-arrested cells. Strikingly, although Bub1 is required for Sgo1 localization, the two proteins are bound to distinct domains within the meiosis I centromere. Sgo1 localizes within the pericentromeric regions *dg* and *dh* [2], whereas Bub1 is enriched within the central domain (*cnt1*, Figure 2C). Although we did not compare Bub1 and Sgo1 localization within the same experiment (for technical reasons related to the use of the TAP tag), we are pretty confident that Bub1 does not bind to the outer repeats of the centromere because we repeated this experiment several times. Accordingly, we could not detect complex formation between the two proteins by coimmunoprecipitation experiments made from a cell population enriched in metaphase I cells (data not shown). Therefore, the mechanism by which Bub1 promotes Sgo1 localization to the centromere is not straightforward and might involve multiple steps early in meiosis I (end of prophase), when Sgo1 and Bub1 first associate with centromeric chromatin.

Deletion analyses showed that the kinase domain of Bub1 is dispensable for Sgo1 function but required for correct segregation during the first meiotic division (Figure 1C). For all kinase-truncated mutants, equational segregation occurred in approximately 20% of meiosis I divisions, indicating a defect in monopolar attachment of sister kinetochores. This phenotype is reminiscent of that of *sgo2Δ* (approximately 20% equational in MI; Figure 3A and [9]). Interestingly, no additive effect was seen in the $bub1^{1-179}$ *sgo2Δ* double mutant, indicating that Sgo2 and the kinase domain of Bub1 act in the same pathway (Figure 3A). Sgo2 localization, function, or both might be under the control of Bub1 kinase activity. However, Sgo2 localization was indistinguishable from that of the wild-type in cells lacking Bub1 (Figure 3C), and reciprocally, Bub1 appeared properly localized in *sgo2Δ* cells (Figure 3B). We conclude that the kinase activity of Bub1 does not regulate Sgo2 localization. It remains

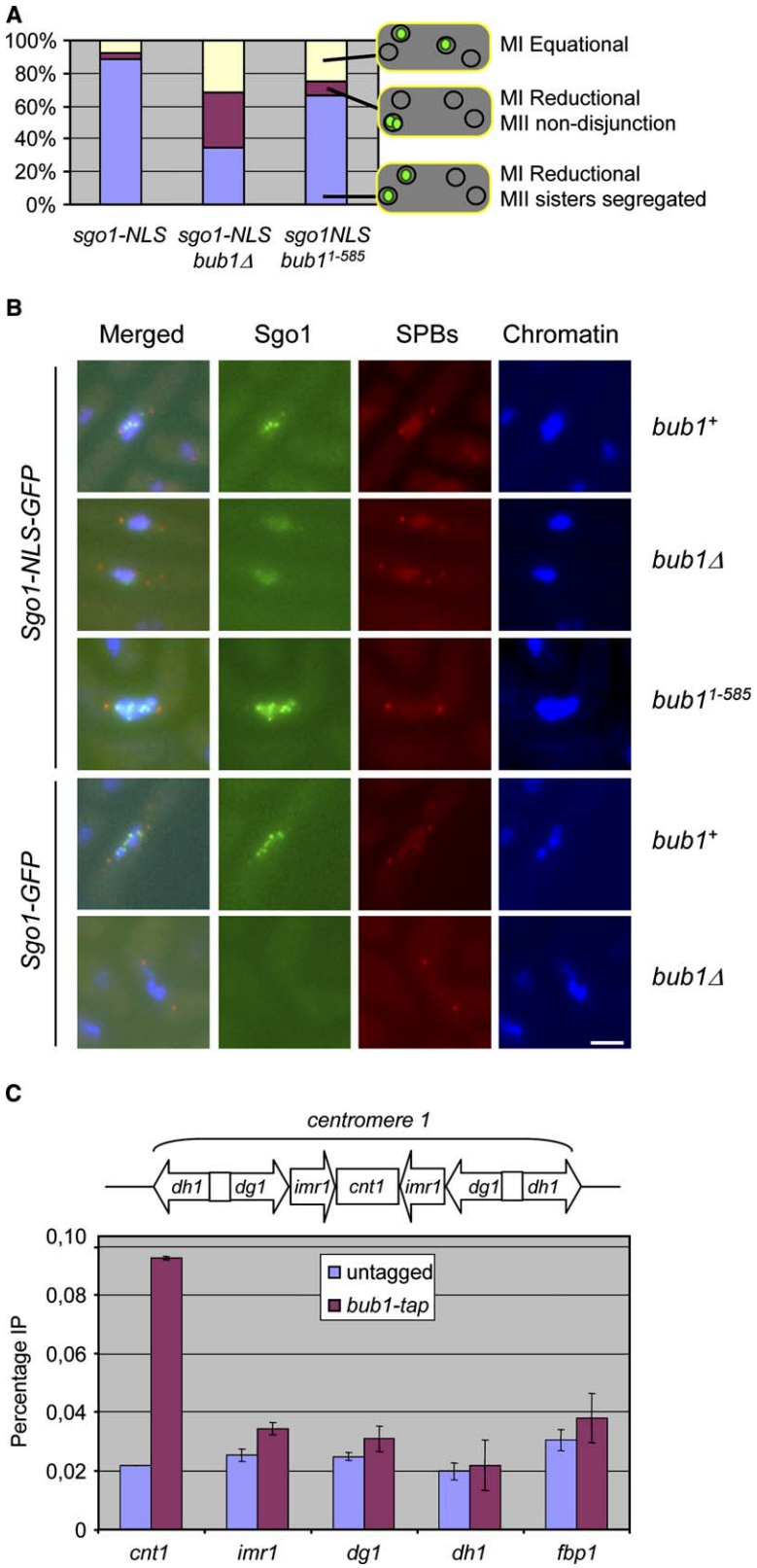

Figure 2. Tethering Sgo1 to the Nucleus Does Not Bypass the Requirement of Bub1 for Correct Sgo1 Localization and Function

(A) Cen1-GFP segregation from crosses heterozygous for *cen1-GFP*. At least 100 tetrads were examined for each cross.

(B) GFP fluorescence was examined in unfixed cells arrested at metaphase I by depletion of the APC activator Slp1 (a strain in which *slp1* is under the control of the *rad21* promoter, not induced during meiosis [2], was used). The spindle pole bodies (SPBs) were visualized with Cut12-CFP [16], and chromatin was stained with Hoescht 33342. The scale bar represents 2.5 μm.

(C) A chromatin immunoprecipitation assay was used to measure Bub1 levels throughout centromere 1 and within the *fbp1* gene in metaphase I-arrested diploid *pat1-114* cells. The upper panel shows a schematic representation of *S. pombe* centromere 1. The experiment was repeated twice, and the standard deviation was calculated (error bars).

possible that Sgo2 function is regulated through Bub1-mediated phosphorylation, although this does not seem to be the case during the vegetative cycle (V.V. and K.G.H., unpublished data).

The segregation pattern observed in *sgo2Δ* and the kinase-deleted alleles of *bub1* raises an important question. When sister *cen1-GFP* segregate reductionally during MI, they segregate normally during MII in a

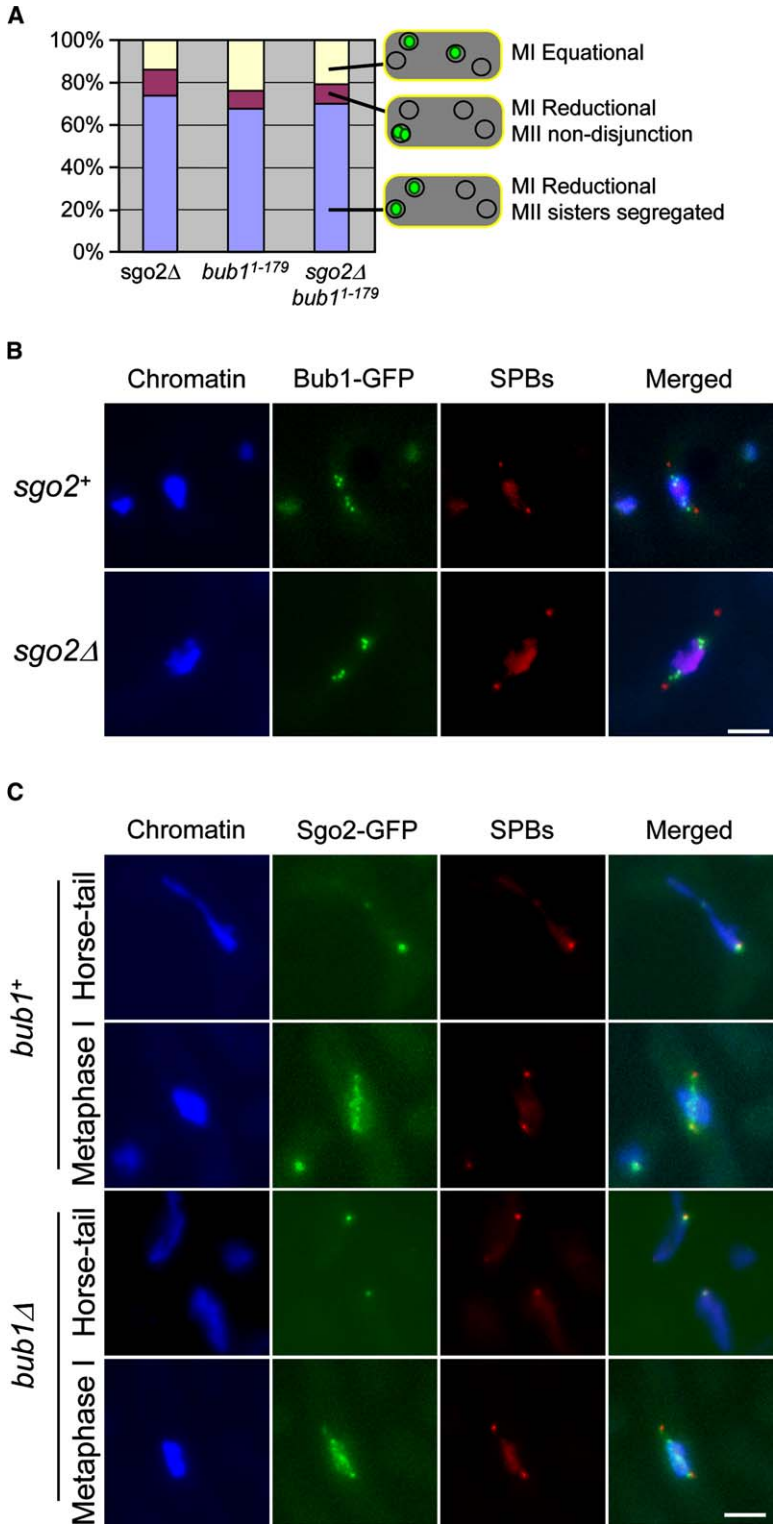

Figure 3. Sgo2 and the Kinase Domain of Bub1 Act in the Same Pathway

(A) *cen1-GFP* segregation from crosses heterozygous for *cen1-GFP*. At least 100 tetrads were examined for each cross.

(B) The lack of Sgo2 does not affect Bub1 localization. Bub1-GFP was observed in unfixed metaphase I-arrested cells.

(C) Reciprocally, Sgo2-GFP appeared properly localized in *bub1Δ* cells during the horse-tail stage (prophase I) and in metaphase I-arrested cells. The SPBs were visualized with Cut12-CFP, and chromatin was stained with Hoescht 33342. The scale bar represents 2.5 μm.

sgo1-dependent manner (Figure 1C and [9]). This indicates that Sgo1 is fully functional and protects centromeric Rec8 from cleavage during the first division. However, sister *cen1-GFP* can segregate equationally during the first division in these mutant backgrounds (approximately 20% of equational segregation; see Figure 1C and Figure 3A). For equational segregation to occur, cohesion must be released along the entire length of the chromosome, including the centromere. How can this be achieved? The fact that *sgo2* mutants have co-orientation defects and premature loss of cohesion suggests that Sgo2 might have independent roles in both processes. If this is true, one would expect cohesion defects to occur in the *sgo2* mutant even when

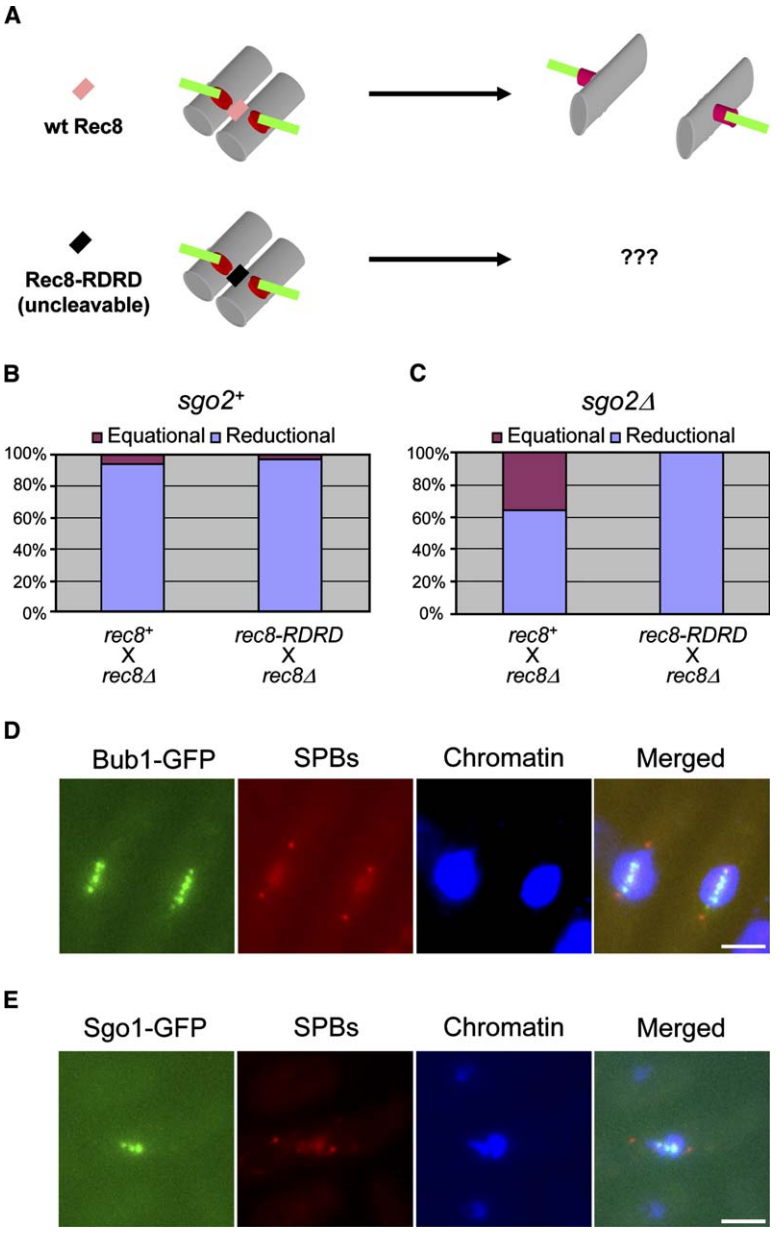

Figure 4. Control of Sgo1 Function by the Mode of Kinetochore Attachment

(A–C) Is Sgo1-mediated protection still operating upon bilateral attachment of sister-kinetochores? (A) Schematic drawing of the experimental design. See text for details. In a *rec11Δ* background, cohesion is restricted to the centromere region. In *rec11Δ sgo2Δ* cells, equational segregation can occur, either by Rec8 cleavage (if Sgo1 protection is OFF) or breakage (if Sgo1 function is still operating). In the former, expression of uncleavable Rec8 should inhibit equational segregation, but it should not do so in the latter. (B and C) Expression of non-cleavable Rec8 inhibits MI equational segregation in *sgo2Δ* meiosis. Binucleated cells were identified by chromatin staining with Hoescht 33342. For each cross, at least 100 binucleated cells were scored for *cen1-GFP* fluorescence.

(D) Bub1-GFP and (E) Sgo1-GFP localization in *rec8Δ* cells arrested at metaphase I. The SPBs were visualized with Cut12-CFP, and chromatin was stained with Hoescht 33342. The scale bar represents 2.5 μm.

kinetochores were properly oriented during MI. However, no cohesion defect was detectable in that situation (sister chromatids segregated faithfully during MII when MI segregation was reductional). We therefore came to the idea that the lack of Sgo2 only affects kinetochore orientation but that the protection of centromeric Rec8 is not effective when sister kinetochores are connected to opposite spindle poles (bilateral attachment). In this scenario, Rec8 would be normally protected upon monopolar attachment, but it would be cleaved along the entire length of the chromosome upon bilateral attachment. Alternatively, equational segregation might occur without Rec8 cleavage if centromeric cohesion cannot resist spindle forces acting on bilaterally attached kinetochores. To distinguish these two possibilities, we expressed a noncleavable form of Rec8 in *sgo2* mutant cells and asked whether equational segregation was suppressed.

There are two distinct cohesin complexes in fission-yeast meiotic cells. Rec8 complexes at centromeres contain Psc3, whereas Psc3 is replaced by Rec11 along chromosome arms [12]. Expression of noncleavable Rec8 (Rec8-RDRD) prevents homolog segregation during MI. Deleting *rec11* disrupts arm cohesion and restores homolog segregation, but MII segregation is prevented by the presence of noncleavable Rec8 at the centromere [13].

To see whether equational segregation in *sgo2Δ* MI cells required Rec8 cleavage at the centromere, we expressed Rec8-RDRD (or, as a control, wild-type Rec8) in cells lacking Sgo2 and the cohesin arm subunit Rec11 (Figures 4A–4C). Sister-chromatid segregation was monitored with *cen1-GFP* in cells that had completed anaphase I (binucleated cells). The experiment was carried out in a *mes1* background to arrest cells at the end of MI (in a *mes1*$^{+}$ background, cells attempt

a second division, which often results in deformed nuclei and sometimes in spurious GFP signals).

As reported previously [13], expression of Rec8RDRD in wild-type cells prevented homolog segregation (no binucleate cells), but homolog segregation was restored to a certain extent by the deletion of *rec11* (approximately 40% binucleated cells). In those binucleated cells, segregation of *cen1-GFP* was mainly reductional (Figure 4B). In *sgo2Δ rec11Δ* cells expressing wild-type Rec8, equational segregation occurred in approximately 36% of binucleated cells (Figure 4C), which is nearly twice the rate observed in a *rec11*$^{+}$ background, suggesting that disrupting arm cohesion might increase equational segregation in this mutant. Crucially, equational segregation was abolished in *sgo2Δ rec11Δ* binucleate cells expressing noncleavable Rec8 (Figure 4C). Spindle forces are therefore not sufficient to pull apart sister-chromatids when centromeric cohesion cannot be eliminated. This observation strongly suggests that equational segregation in *sgo2Δ* requires Rec8 cleavage at the centromere.

These data are consistent with the idea that the status of centromere cohesion in *sgo2Δ* meiosis relies on the mode of sister-kinetochore attachment to spindle microtubules. When sister kinetochores are properly attached (monopolar attachment), cohesion is preserved at the centromere, and faithful equational segregation occurs during MII. By contrast, when sister kinetochores are bilaterally attached during MI, centromeric Rec8 is not protected and is cleaved, and MI equational segregation occurs.

One possible mechanism would be that Bub1 and Sgo1 are associated with centromeres upon monopolar attachment of sister kinetochores but are absent from bilaterally attached kinetochores. This was technically difficult to assess in *sgo2Δ* meiosis, however, because a single metaphase I cell is likely to contain a mixture of monopolarly and bilaterally attached kinetochore pairs.

To circumvent this caveat, we looked at Sgo1-GFP and Bub1-GFP localization in *rec8Δ* metaphase I cells. Rec8 is required for reductional segregation in fission yeast [14]. In *rec8Δ* cells, all six sister centromeres in the cell segregate equationally during MI. Hence, in a metaphase I cell, all kinetochore pairs are expected to exhibit a bilateral attachment to the spindle.

As shown in Figures 4D and 4E, Bub1 and Sgo1 were readily detected in those cells, in a pattern very similar to that observed in wild-type metaphase I. Hence, the bilateral attachment of sister kinetochores does not seem to prevent Bub1 and Sgo1 localization to centromeres in metaphase I.

Taken together, our data suggest that the mode of kinetochore attachment does govern the status of centromeric cohesion. The experiment using noncleavable Rec8 strongly suggests that equational segregation requires Rec8 cleavage and hence that centromeric Rec8 is not protected upon bilateral attachment. The mechanism remains enigmatic, however, because the absence of protection would not be achieved through the loss of Bub1/Sgo1 from centromeres, as suggested by the persistence of Bub1 and Sgo1 foci in *rec8Δ* metaphase cells.

In contradiction with the above data, ectopic expression of both Rec8 and Sgo1 during the vegetative cycle appeared to be sufficient to prevent anaphase [2], suggesting that the bilateral attachment does not prevent Sgo1 from protecting Rec8 from Separase. However, it must be noted that both proteins were overexpressed to high levels and that the experiment was conducted in mitotic cells. It remains formally possible that the control of centromere cohesion by the mode of kinetochore attachment only occurs in meiotic cells. In favor of this idea, overexpression of Sgo1 during MI did not suppress equational segregation in *sgo2Δ* cells (our unpublished observation), and the ectopic expression of Sgo1 during MII did not prevent sister-chromatid separation [9]. Similarly, data from *Drosophila* male meiosis indicate that Sgo1 homolog protein MEI-S332 function and localization are separable because cohesion between sister chromatids can be released at the metaphase II/anaphase II transition even if MEI-S332 remains localized at centromeres [15]. The mere presence of Sgo1 at the centromere might not be sufficient to protect Rec8 from cleavage, leaving the possibility that other factors might be required for restricting Sgo1 activity to monopolarly attached kinetochores.

In summary, our data indicate that fission-yeast Shugoshin proteins are subjected to multiple levels of regulation: (1) The N-terminal domain of Bub1 controls Sgo1 localisation and function; (2) Sgo2 and the kinase domain of Bub1 act in the same pathway to promote monopolar attachment during MI; and (3) Sgo1-mediated protection of centromeric cohesion might be suppressed by the bilateral attachment of sister-kinetochores.

Supplemental Data

Supplemental Experimental Procedures as well as a supplemental table and figures are available with this article online at http://www.current-biology.com/cgi/content/full/15/24/2263/DC1/.

Acknowledgments

We thank Keith Gull, Tomohiro Matsumoto, Shelley Sazer, Takashi Toda, Yoshinori Watanabe, and Mitsuhiro Yanagida for strains and reagents. S.V. was supported by a grant from the Fondation pour la Recherche Médicale, F.C. by a Centre National de la Recherche Scientifique post-doctoral position, and J.G. by a European Molecular Biology Organization long-term fellowship. This work was supported by the Centre National de la Recherche Scientifique, the Association pour la Recherche sur le Cancer, and grant BCM0131 from the Ministère de la Recherche.

Received: September 2, 2005
Revised: October 20, 2005
Accepted: November 1, 2005
Published: December 19, 2005

References

1. Bernard, P., Maure, J.F., and Javerzat, J.P. (2001). Fission yeast Bub1 is essential in setting up the meiotic pattern of chromosome segregation. Nat. Cell Biol. *3*, 522–526.
2. Kitajima, T.S., Kawashima, S.A., and Watanabe, Y. (2004). The conserved kinetochore protein shugoshin protects centromeric cohesion during meiosis. Nature *427*, 510–517.
3. Ikui, A.E., Furuya, K., Yanagida, M., and Matsumoto, T. (2002). Control of localization of a spindle checkpoint protein, Mad2, in fission yeast. J. Cell Sci. *115*, 1603–1610.
4. Kim, S.H., Lin, D.P., Matsumoto, S., Kitazono, A., and Matsumoto, T. (1998). Fission yeast Slp1: An effector of the Mad2-dependent spindle checkpoint. Science *279*, 1045–1047.
5. Millband, D.N., and Hardwick, K.G. (2002). Fission yeast Mad3p is required for Mad2p to inhibit the anaphase-promoting

complex and localizes to kinetochores in a Bub1p-, Bub3p-, and Mph1p-dependent manner. Mol. Cell. Biol. *22*, 2728–2742.

6. Vanoosthuyse, V., Valsdottir, R., Javerzat, J.P., and Hardwick, K.G. (2004). Kinetochore targeting of fission yeast Mad and Bub proteins is essential for spindle checkpoint function but not for all chromosome segregation roles of Bub1p. Mol. Cell. Biol. *24*, 9786–9801.
7. He, X., Jones, M.H., Winey, M., and Sazer, S. (1998). Mph1, a member of the Mps1-like family of dual specificity protein kinases, is required for the spindle checkpoint in S. pombe. J. Cell Sci. *111*, 1635–1647.
8. Nabeshima, K., Nakagawa, T., Straight, A.F., Murray, A., Chikashige, Y., Yamashita, Y.M., Hiraoka, Y., and Yanagida, M. (1998). Dynamics of centromeres during metaphase-anaphase transition in fission yeast: Dis1 is implicated in force balance in metaphase bipolar spindle. Mol. Biol. Cell *9*, 3211–3225.
9. Rabitsch, K.P., Gregan, J., Schleiffer, A., Javerzat, J.P., Eisenhaber, F., and Nasmyth, K. (2004). Two fission yeast homologs of Drosophila Mei-S332 are required for chromosome segregation during meiosis I and II. Curr. Biol. *14*, 287–301.
10. Yamaguchi, S., Decottignies, A., and Nurse, P. (2003). Function of Cdc2p-dependent Bub1p phosphorylation and Bub1p kinase activity in the mitotic and meiotic spindle checkpoint. EMBO J. *22*, 1075–1087.
11. Kadura, S., He, X., Vanoosthuyse, V., Hardwick, K.G., and Sazer, S. (2005). The A78V mutation in the Mad3-like domain of Schizosaccharomyces pombe Bub1p perturbs nuclear accumulation and kinetochore targeting of Bub1p, Bub3p, and Mad3p and spindle assembly checkpoint function. Mol. Biol. Cell *16*, 385–395.
12. Kitajima, T.S., Yokobayashi, S., Yamamoto, M., and Watanabe, Y. (2003). Distinct cohesin complexes organize meiotic chromosome domains. Science *300*, 1152–1155.
13. Kitajima, T.S., Miyazaki, Y., Yamamoto, M., and Watanabe, Y. (2003). Rec8 cleavage by separase is required for meiotic nuclear divisions in fission yeast. EMBO J. *22*, 5643–5653.
14. Watanabe, Y., and Nurse, P. (1999). Cohesin Rec8 is required for reductional chromosome segregation at meiosis. Nature *400*, 461–464.
15. Clarke, A.S., Tang, T.T., Ooi, D.L., and Orr-Weaver, T.L. (2005). POLO kinase regulates the Drosophila centromere cohesion protein MEI-S332. Dev. Cell *8*, 53–64.
16. Garcia, M.A., Koonrugsa, N., and Toda, T. (2002). Spindle-kinetochore attachment requires the combined action of Kin I-like Klp5/6 and Alp14/Dis1-MAPs in fission yeast. EMBO J. *21*, 6015–6024.