Published in "Molecular Biology of the Cell doi:10.1091/mbc.E10-08-0673, 2011" which should be cited to refer to this work.

Bub1, Sgo1 and Mps1 mediate a distinct pathway for chromosome bi-orientation in budding yeast

Zuzana Storchová*,†,‡, Justin Becker*, Nicolas Talarek§, Sandra Kögelsberger†, David Pellman‡

Howard Hughes Medical Institute, Department of Pediatric Oncology, Dana-Farber Cancer Institute, Children's Hospital, Department of Cell Biology, Harvard Medical School, Boston, Massachusetts 02115, USA

* these two authors contributed equally to the work;
† Max-Planck Institute of Biochemistry, Group Maintenance of Genome Stability, 82152 Martinsried, Germany;
§ Current address: Department of Medicine, Division of Biochemistry, University of Fribourg, CH-1700 Fribourg, Switzerland
‡ corresponding authors: storchov@biochem.mpg.de; david_pellman@dfci.harvard.edu;

Running head: Bub1, Sgo1 and Mps1 in bi-orientation

ABSTRACT

The conserved mitotic kinase Bub1 performs multiple functions that are only partially characterized. Besides its role in the spindle assembly checkpoint and chromosome alignment, Bub1 is crucial for the kinetochore recruitment of multiple proteins, among them Sgo1. Both Bub1 and Sgo1 are dispensable for growth of haploid and diploid budding yeast, but they become essential in cells with higher ploidy. We find that over-expression of *SGO1* partially corrects the chromosome segregation defect of *bub1*Δ haploid cells and restores viability to *bub1*Δ tetraploid cells. Using an unbiased high-copy suppressor screen, we identified two members of the chromosome passenger complex (CPC), *BIR1* (survivin) and *SLI15* (INCENP), as suppressors of the growth defect of both *bub1*Δ and *sgo1*Δ tetraploids, suggesting that these mutants die due to defects in chromosome bi-orientation. Over-expression of *BIR1* or *SLI15* also complements the benomyl sensitivity of haploid *bub1*Δ and *sgo1*Δ cells. Mutants lacking *SGO1* fail to bi-orient sister chromatids attached to the same spindle pole (syntelic attachment) after nocodazole treatment. Moreover, the *sgo1*Δ cells accumulate syntelic attachments in unperturbed mitoses, a defect that is partially corrected by *BIR1* or *SLI15* over-expression. We show that in budding yeast neither Bub1 nor Sgo1 is required for CPC localization and does not affect Aurora B activity. Instead we identify Sgo1 as a possible partner of Mps1, a mitotic kinase suggested to have an Aurora B –independent function in establishment of bi-orientation. We found that Sgo1 over-expression rescues defects caused by metaphase inactivation of Mps1; and that Mps1 is required for Sgo1 localization to the kinetochore. We propose that Bub1, Sgo1, and Mps1 facilitate chromosome bi-orientation independently of Aurora B - mediated pathway at the budding yeast kinetochore, and that both these pathways are required for the efficient turnover of syntelic attachments.

INTRODUCTION

Proper chromosome segregation is essential for successful cell division . Eukaryotic cells do not explicitly monitor the fate of individual chromosomes during mitosis; rather they have evolved elaborate surveillance mechanisms to recognize and correct errors in microtubule-kinetochore attachments. These mechanisms are so efficient that under normal conditions, wild-type cells missegregate their chromosomes once in thousands of cell divisions. This is not true, however, for polyploid cells, which contain more than two chromosome sets. In animals and fungi, tetraploids (cells with four copies of each chromosome) missegregate their chromosomes at high rates producing aneuploid cells (Mayer and Aguilera, 1990; Andalis *et al.*, 2004; Fujiwara *et al.*, 2005; Storchova *et al.*, 2006; Ganem *et al.*, 2009). Indeed, it has been suggested that cancer cells, which frequently exhibit abnormal karyotypes, might in some cases be the descendents of unstable tetraploid cells, whose elevated rate of chromosome missegregation might facilitate transformation (Shackney *et al.*, 1989; Andalis *et al.*, 2004; Storchova and Pellman, 2004; Fujiwara *et al.*, 2005; Margolis, 2005; Storchova and Kuffer, 2008).

We have previously reported the genetic phenomenon of "ploidy-specific lethality" in budding yeast, where a deletion of a particular gene kills tetraploid cells even though haploids and diploids bearing the null allele are viable (Lin *et al.*, 2001). Using a genome-wide screening approach, we identified a set of 39 gene deletions that cause ploidy-specific lethality (Storchova *et al.*, 2006). Interestingly, nearly all of these genes have known roles in chromosome segregation or the repair of DNA breaks, further supporting the idea that the maintenance of genetic stability is the principal cellular function compromised by polyploidization.

The proteins that are essential to tetraploids but not diploids likely participate in pathways that are either directly impaired or under increased demand following a whole-genome duplication. Because tetraploidy increases the severity of certain mutant phenotypes, we anticipated that ploidy-specific lethality could be exploited to facilitate the genetic analysis of factors with poorly understood roles in chromosome segregation. One tetraploid-essential gene, *BUB1*, encodes a conserved serine-threonine kinase that is required for the activity of the spindle assembly checkpoint (SAC), which delays anaphase until all kinetochores form stable attachments to microtubules (Hoyt *et al.*, 1991). In yeast and vertebrate cells, Bub1 binds the SAC protein Bub3 (which is also essential in tetraploids (Storchova *et al.*, 2006)), targets other checkpoint proteins to unattached kinetochores, and forms a complex with Mad1 in the presence of a checkpoint signal (Musacchio and Salmon, 2007). These interactions are essential for inactivation of the anaphase promoting complex (APC) in the presence of microtubule-attachment errors (for review see (Musacchio and Salmon, 2007)). The finding that loss of Bub1 is lethal to tetraploid yeast raised the possibility that the SAC is required for the viability of these cells, in contrast to budding yeast with normal ploidy. However, this possibility can be excluded because *mad1*Δ and *mad2*Δ tetraploids, which are similarly defective for the SAC, are viable (Storchova *et al.*, 2006). The requirement for Bub1 in tetraploids is consistent with reports of a SAC-independent function in chromosome segregation that requires its kinase domain (Warren *et al.*, 2002; Fernius and Hardwick, 2007).

A checkpoint-independent requirement for Bub1 in the maintenance of genetic stability may be attributable, at least in part, to its conserved role in targeting the shugoshin proteins (Sgo1 in budding yeast) to the centromere (Kitajima *et al.*, 2005; Fernius and Hardwick, 2007). It was recently discovered that shugoshin centromere recruitment requires the phosphorylation of the histone H2A by Bub1 (Kawashima *et al.*, 2010). Deletion of *SGO1* also results in ploidy-specific lethality (Storchova *et al.*, 2006). In organisms from yeast to man, shugoshin prevents

premature separation of sister chromatids in meiosis (and mitosis in vertebrates) by recruiting protein phosphatase 2A (PP2A), which in turn promotes the retention of centromeric cohesin by antagonizing the function of mitotic kinases (Wang and Dai, 2005; Riedel *et al.*, 2006). However, it appears that budding yeast Sgo1, despite being essential for accurate chromosome segregation, is dispensable for the protection of centromeric cohesin in mitosis (Katis *et al.*, 2004; Indjeian *et al.*, 2005).

Instead, Sgo1 has been shown to play a role in the establishment of sister-chromatid bi-orientation – that is, the tension-generating attachment of sister chromatids to microtubules emanating from opposite spindle poles (also called "bipolar attachment"). Such bi-orientation is a necessary condition for sister chromatids to segregate evenly between daughter cells. In normal cells, improper attachments of both sisters to the same spindle pole - syntelic attachments- are recognized by proteins of chromosome passenger complex (CPC) and disrupted by the activity of its effector kinase Aurora B (Ipl1 in budding yeast). This release of the syntelic attachments activates the SAC and allows bipolar attachments to form in their place (Biggins and Murray, 2001; Tanaka *et al.*, 2002; Pinsky *et al.*, 2006). However, *sgo1*Δ mutants enter anaphase with an elevated frequency of unrepaired syntelic attachments and fail to arrest under experimental conditions that abolish kinetochore tension (Indjeian *et al.*, 2005; Indjeian and Murray, 2007). Cells that lack Bub1 kinase activity do not properly localize Sgo1 to the kinetochore and hence exhibit similar defects in bi-orientation (Fernius and Hardwick, 2007; Indjeian and Murray, 2007). However, the precise mechanism by which Sgo1 (and, in turn, its upstream regulator Bub1) promotes the attachment of sister kinetochores to opposing spindle poles remains unknown. Furthermore, given its role in the centromeric recruitment of protein phosphatase 2A (Wang and Dai, 2005), it is likely that budding yeast Sgo1 functions in multiple mitotic processes. Hence, the reason for Sgo1's importance for tetraploid viability is unclear.

In the experiments reported here, we have characterized the functions of Bub1 and Sgo1 that are essential for the growth of tetraploid budding yeast. By identifying genetic suppressors that can restore viability to *bub1*Δ and *sgo1*Δ tetraploids, we first determined the specific mitotic defects that cause the lethality of these cells and second gained insight into the function of Bub1 and Sgo1 in chromosome segregation. Our findings suggest that the ploidy-specific lethality of the *bub1*Δ mutation is due to a failure to properly localize Sgo1 to the kinetochore. Both *bub1*Δ and *sgo1*Δ tetraploids die due to the persistence of uncorrected syntelic attachments, which results in high rates of sister-chromatid nondisjunction. The role of Sgo1 in sister-chromatid bi-orientation can be bypassed—in cells of all ploidies—by over-expression of factors which activate Ipl1 kinase. We found that budding yeast Bub1 and Sgo1 is dispensable for the localization of the CPC and observed no change in Ipl1-dependent phosphorylation events in cells lacking Bub1 or Sgo1. This suggests that Sgo1 might regulate microtubule attachment without affecting the Ipl1-mediated phosphorylation of kinetochore proteins. In searching for factors that cooperate with Sgo1, we identified a reciprocal genetic interaction between *SGO1* and *MPS1*, which encodes a mitotic checkpoint kinase that promotes the turnover of syntelic attachments (Maure *et al.*, 2007). Notably, we found that Mps1 activity is required to maintain the kinetochore localization of Sgo1, and *SGO1* over-expression allows cells to survive mitosis after Mps1 inactivation. Taken together, our data suggest that Sgo1 is not only a recruiting and activating factor of Aurora B. Instead, we propose that Sgo1 and Msp1 acts in parallel to the CPC and that both pathways are required for the establishment of the chromosome bi-orientation.

RESULTS

The defect of *bub1*Δ and *sgo1*Δ cells is suppressed by the over-expression of *BIR1* or *SLI15*

BUB1, which codes for a mitotic kinase involved in the spindle assembly checkpoint and chromosome segregation, was previously identified as one of the 39 genes whose deletion results in ploidy-specific lethality in budding yeast (Storchova *et al.*, 2006). Tetraploid cells lacking *BUB1* grow poorly at 24°C and are inviable at higher temperatures; this contrasts with haploid *bub1*Δ mutants, which propagate at any temperature, although with a slightly reduced growth rate compared to wild-type cells. We found that over-expression of *SGO1* on a 2-micron plasmid rescues the growth defect of *bub1*Δ tetraploids (Fig. 1A), consistent with the requirement of the kinase domain of Bub1 for the centromeric localization of Sgo1 (Supplementary Fig. 1A; Fernius, 2007#18). We reasoned that the ploidy-specific lethality of *bub1*Δ would facilitate its genetic analysis and thus could be exploited to elucidate the function of Bub1 in chromosome segregation. Using a high-copy number suppressor screen, we identified two suppressor genes, *BIR1* and *SLI15*, whose over-expression bypasses the requirement for Bub1 in tetraploid cells (Fig. 1A). Similar to *bub1*Δ tetraploids, *sgo1*Δ tetraploids are inviable at higher temperatures, (Storchova *et al.*, 2006) and we found that *BIR1* or *SLI15* over-expression also bypass the growth defect of these cells (Fig. 1B). Moreover, the over-expression of *BIR1* or *SLI15* suppresses the sensitivity of haploid *sgo1*Δ (Fig. 1C) and *bub1*Δ cells (Supplementary Fig. 5B, see below) to the microtubule-depolymerizing drug benomyl. This verifies that the observed genetic interaction between CPC components and Bub1/Sgo1, although more easily detected in mutant tetraploid strains, is relevant to cells of any ploidy.

Bir1 and Sli15 facilitate accurate chromosome segregation by activating the kinase Ipl1/Aurora B, which promotes the release of microtubules from kinetochores that are not properly attached (Tanaka *et al.*, 2002; Pinsky *et al.*, 2006). However, we found that *IPL1* over-expression does not restore the viability of the *bub1*Δ and *sgo1*Δ tetraploids (Supplementary Fig. 2A), just as it cannot bypass a deficiency of either Bir1 or Sli15 (Kim *et al.*, 1999). Presumably, this reflects the inactivity of Ipl1 kinase in the absence of binding to other CPC members.

Finally, the genetic interaction between CPC components and Sgo1 is not reciprocal. The temperature-sensitive *sli15-3* mutation results in impaired Ipl1 kinase activation, and the defects of *sli15-3* cells can be suppressed by *IPL1* over-expression (Kim *et al.*, 1999) (Fig. 1D). By contrast, we find that *SGO1* over-expression does not rescue the temperature-sensitivity of the *sli15-3* strain (Fig. 1D). In conclusion, our results suggest that the growth defect of *bub1*Δ or *sgo1*Δ tetraploids can be ameliorated by increased expression of Sli15 or Bir1, consistent with recent evidence that Sgo1 plays a role in establishment of sister-chromatid bi-orientation (Indjeian *et al.*, 2005; Kiburz *et al.*, 2008).

Rescue of ploidy-specific lethality of *bub1*Δ and *sgo1*Δ requires the activation of Ipl1

Over-expression of Ipl1, the third subunit of the CPC, fails to cause any discernible improvement in the viability of either *bub1*Δ or *sgo1*Δ tetraploids at any temperature tested (Supplementary Fig. 2A). Since Bir1 and Sli15 have an Ipl1-independent role in anaphase septin organization (Thomas and Kaplan, 2007), we tested the possibility that the Ipl1 kinase activity is not required to improve the growth of *bub1*Δ and *sgo1*Δ tetraploids. We used recently identified alleles of *BIR1* that selectively abolish its physical interaction with associated kinetochore proteins (Thomas and Kaplan, 2007). In particular, the W901A point mutation in *BIR1* selectively disrupts the Bir1-Ipl1 interaction but leaves the Bir1-Sli15 interaction unperturbed; by contrast, the doubly mutated Bir1^{A931E,I935E} fails to associate with either Ipl1 or Sli15 (Thomas and Kaplan, 2007). High-copy plasmids expressing Bir1^{W901A} and Bir1^{A931E,I935E} failed to restore viability to *bub1*Δ and *sgo1*Δ

tetraploids at the restrictive temperature (Supplementary Fig. 2B). This result suggests that the mechanism by which *BIR1* and *SLI15* rescue the mitotic defects of *bub1*Δ and *sgo1*Δ mutants requires the kinase activity of Ipl1.

The suppressors of *bub1*Δ and *sgo1*Δ promote accurate chromosome segregation without restoring SAC function

The lack of Bub1 in haploid strains causes two different mitotic defects: the cells fail to delay anaphase in the presence of unattached kinetochores (Hoyt *et al.*, 1991), and they exhibit sister-chromatid nondisjunction at a rate higher than that caused by checkpoint dysfunction alone (Warren *et al.*, 2002), possibly due to a failure to localize Sgo1 at the centromere (Fernius and Hardwick, 2007). Loss-of-function mutations in *SGO1* and *BIR1* have been shown to compromise the ability of the SAC to respond to a lack of tension at mitotic kinetochores (Indjeian *et al.*, 2005; Shimogawa *et al.*, 2009) without affecting the ability to arrest mitosis after microtubule depolymerization. This finding is consistent with a role for Bir1 and Sgo1 in regulating microtubule attachment; however, it remains possible that over-expression of these proteins directly delays anaphase. Thus, the over-expression of the suppressors could potentially rescue the defects of *bub1*Δ mutants by restoring the SAC rather than by promoting sister-chromatid bi-orientation.

To test this possibility, we analyzed the ability of diploid *bub1*Δ mutants over-expressing *SGO1* and *SLI15* to arrest in the presence of the microtubule-depolymerizing drug nocadozole. Under these conditions, SAC-deficient cells progress through the cycle and acquire a multi-budded morphology, due to concomitant bud emergence and cytokinesis failure.Over a 6-hour time course, multi-budded cells appeared with the same frequency and timing in *bub1*Δ cells over-expressing *SGO1* and *SLI15* as observed in cells transformed with an empty vector (Fig. 2A). By contrast, cells carrying a *BUB1*-expressing plasmid arrested as large-budded cells for the duration of the experiment. Thus, the high-copy suppression of the ploidy-specific lethality of *bub1*Δ does not involve mechanisms that rescue the SAC defect.

Haploid *bub1*Δ cells missegregate their chromosomes at rates higher than other SAC-deficient mutants (Warren *et al.*, 2002). We reasoned that the high-level chromosome loss of these cells might be suppressed by the Sgo1 and CPC over-expression. To test this hypothesis, we utilized strains carrying a yeast artificial chromosome (YAC) (Huang and Koshland, 2003), which is nonessential but contains selectable markers on both chromosome arms. Haploid *bub1*Δ mutants lost the YAC at a rate about 35-times higher than the same strain expressing a single copy of *BUB1* on a plasmid (Fig. 2B; 2.9×10^{-2} and 8.3×10^{-4} missegregation events per division, respectively). Over-expression of *SGO1* and *SLI15* suppressed YAC loss in *bub1*Δ mutants by approximately 3-fold and 2-fold, respectively (Fig 2B; 9.2×10^{-3} and 1.85×10^{-2} missegregation events per division, respectively). We conclude that the suppressors of *bub1*Δ and *sgo1*Δ tetraploids increase survival by improving the efficiency of chromosome segregation.

The chromosome missegregation defect of *sgo1Δ* cells is due to the inability to establish bipolar attachments

Recent studies have shown that haploid *sgo1* mutants fail to arrest the cell cycle in the presence of chromosomes that cannot form tension-generating attachments. This suggests a defect in tension-sensing in these cells and is consistent with the observed high rates of sister-chromatid nondisjunction (Indjeian *et al.*, 2005; Indjeian and Murray, 2007). We performed a series of experiments to monitor chromosome bi-orientation in *sgo1*Δ and *bub1*Δ tetraploids with and without over-expression of CPC components.

First, we followed the segregation of a single GFP-marked copy of chromosome IV. Whereas wild-type tetraploids missegregate chromosome IV in fewer than 1% of divisions at all temperatures, *sgo1*Δ tetraploids displayed modest chromosome segregation defects at 24°C (8% of the mitotic divisions) and more severe defects at 34°C (21% of divisions) (Fig. 3A). Notably, 70% of nondisjunction events at 34°C were directed toward the bud; this phenotype, which has previously been reported for *ipl1-321* mutants (Tanaka *et al.*, 2002), indicates the persistence of syntelic attachments at the mother SPB. Our finding, moreover, is consistent with the observation of preferential missegregation to the bud in the *sgo1-100* mutant cells with unreplicated DNA (Indjeian and Murray, 2007). Over-expression of *BIR1* reduced the segregation defect of *sgo1*Δ tetraploids more than 3-fold, to 6% (Fig. 3A). Similarly, deletion of *BUB1* in yeast tetraploids caused nondisjunction of chromosome IV in 26% of the cells grown at semi-permissive temperature, and the frequency of the segregation errors dropped more than 3-fold after over-expression of *BIR1* (Fig. 3A).

To directly test the ability of the *sgo1*Δ cells to bi-orient chromosomes during metaphase, we monitored the behavior of an unreplicated minichromosome containing two functional centromeres (Dewar *et al.*, 2004). This assay allows bipolar attachments to be distinguished from monopolar attachments based on whether the minichromosome is positioned between the two SPBs or proximal to one SPB, respectively. Therefore, we can directly evaluate the preference of sister kinetochores for tension-generating versus tensionless attachments, in contrast to previously published experiments in *sgo1*Δ cells that monitored the fate of the obligatory tensionless attachments of unreplicated chromosomes (Indjeian *et al.*, 2005). In our experiment, cells lacking Sgo1 showed a significant accumulation of syntelic attachments, although the effect was not as severe as that observed in cells with defective Ipl1 kinase (Fig. 3B). The increased frequency of monopolar attachments in *sgo1*Δ mutants was partially suppressed by over-expression of *SLI15* (Fig. 3B). Taken together, these experiments show that the primary defect in cells lacking Bub1 or Sgo1 is a high level of chromosome loss due to failed chromosome bi-orientation. In mutant tetraploids, the frequency of chromosome loss reaches a threshold level of chromosomal instability that no longer supports viability, and the frequency of missegregation can be reduced by over-expression of the CPC components Bir1 and Sli15.

The elevated frequency of syntelic attachments in *sgo1*Δ mutants could be due to an increase in the formation of such attachments, a decrease in their turnover by corrective mechanisms, or both. In order to focus specifically on the contribution of corrective mechanisms, we performed live-cell imaging on diploid cells released from nocodazole-induced arrest and followed the movement of GFP-marked sister chromatids as they attached to the spindle. Under these conditions, the initial attachment of sister kinetochores to either the same SPB or opposing SPBs appears to be random (Indjeian and Murray, 2007). Indeed, in wild-type cells released from nocodazole (27 cells analyzed in total), we found that the rate of initial bipolar attachment at chromosome IV was similar to the rate of the monopolar attachment (44% and 56%, respectively). In the *sgo1*Δ mutants (54 cells analyzed in total), the chromosomes initially attached to one pole more often than to both poles (31% bipolar, 69% monopolar). However, this observed difference between *sgo1*Δ and wild type cells might be biased by the fact that a correction from monopolar to bipolar attachment appears to be achieved very quickly in the wild-type strain. Thus, we might have underestimated the frequency of primary monopolar attachment in these cells. Strikingly, *sgo1*Δ mutants that formed initial monopolar attachments failed to convert them to bipolar attachments, even in the moments when the two SPBs moved very close to each other (data not shown). Within 75 minutes of release from nocodazole, 86% of the wild type cells entered anaphase with correct bipolar attachments at chromosome IV,

whereas only 18% of the cells lacking Sgo1 were able to do so (Fig. 3C). Only 8% of wild type cells, but almost 45% of *sgo1*Δ mutants missegregated their chromosomes. The remaining 37% of *sgo1*Δ mutants did not enter anaphase within 75 minutes of release, in comparison to 6% of wild-type cells. The data demonstrate that cells lacking Sgo1 are either unable to recognize the tensionless attachments or cannot release them, which in turn leads to increased levels of chromosome missegregation and subsequent cell death.

The defect in bi-orientation is not due to PP2A-associated functions of Sgo1

Sgo1 was identified as a factor that protects centromeric cohesion in meiosis by recruiting the phosphatase PP2A to the kinetochore (Riedel *et al.*, 2006). The phosphorylation of centromere-associated cohesins triggers their efficient removal but is antagonized by the activity of PP2A. In cells lacking Sgo1, pericentromeric cohesion in meiosis I is prematurely abolished, which results in chromosome missegregation. Thus, the observed defects in chromosome segregation in *bub1*Δ and *sgo1*Δ cells could be due to premature loss of cohesion. However, budding yeast *sgo1*Δ mutants appear to have no discernable cohesion defects in mitosis (Kiburz *et al.*, 2005), a finding which we have replicated here in tetraploids (Supplementary Fig. 3A). Thus, the frequent chromosome missegregation in *sgo1*Δ cells cannot be explained by the precocious separation of sister chromatids. In support of this conclusion, expression of a mutant Sgo1 protein that fails to interact with the phosphatase PP2A (which has the conserved asparagine 51 residue replaced by isoleucine (Kitajima *et al.*, 2006)) can restore viability to *sgo1*Δ tetraploids, although to a lesser extent than the expression of the wild type Sgo1 protein (Supplementary Fig. 3B).

Recently, it was reported that Sgo1 over-expression inhibits the nonproteolytic function of separase and delays anaphase, and that this function of Sgo1 is mediated through its recruitment of the PP2A regulatory subunit Cdc55 (Clift *et al.*, 2009). However, over-expression of *BIR1* and *SLI15* has no effect on the benomyl sensitivity and cold sensitivity of haploid *cdc55*Δ mutants (Supplementary Fig. 4A). Moreover, we found that the defects of *bub1*Δ mutants can be suppressed by over-expression of *SGO1* even in the absence of the PP2A regulatory subunit Cdc55: the benomyl sensitivity of a haploid *bub1*Δ*cdc55*Δ strain can be rescued by *SGO1* over-expression, similar to observations in the *bub1*Δ *strain* (Supplementary Fig. 4B). Taken together, these results demonstrate that the function of Sgo1 in bi-orientation is independent of Cdc55 and Cdc55-mediated PP2A functions.

Bub1 and Sgo1 are dispensable for CPC localization and Ipl1 kinase activity

What is the underlying molecular mechanism that allows the rescue of the chromosome segregation defect in *bub1*Δ and *sgo1*Δ mutants upon Bir1 and Sli15 over-expression? We hypothesized that the absence of Sgo1 at the kinetochore might directly impair the function of the CPC in regulating sister-chromatid bi-orientation. One possibility is that Sgo1, like its homolog Sgo2 in fission yeast (Kawashima *et al.*, 2007), is required to properly localize the CPC to the kinetochore. We studied the effect of the deletion of *SGO1* on the localization of Bir1-GFP, Sli15-GFP and Ipl1-GFP by quantitative fluorescent microscopy. Unlike the observations for fission yeast Sgo2, the loss of *SGO1* in budding yeast has no effect on the accumulation of CPC proteins on pre-anaphase spindles (Fig. 4A). Quantification of fluorescence intensity in mitotic cells expressing Bir1-GFP revealed no difference between wild-type and mutant strains. Values for median fluorescence intensity, averaged across 100 mitotic cells, were 48.5 ± 9.2 and 50.6 ± 12.2 arbitrary units for cells with and without *SGO1*, respectively.

In order to more precisely measure the association of the CPC with centromeric DNA, we performed a chromatin immunoprecipitation (ChIP) of Sli15-TAP in the presence and absence of *BUB1*. Sli15 is enriched at budding yeast centromeres (Kang *et al.*, 2001), albeit to a lesser extent than a constitutive kinetochore protein Ndc10, which we used as a control. Consistent with our epifluorescence data, the enrichment of CENIII DNA in Sli15-TAP immunoprecipitates was not reduced by the deletion of *BUB1* (Fig. 4B), further supporting the idea that the kinetochore localization of the CPC does not require the Bub1/Sgo1 in budding yeast.

We next examined whether the kinase activity of Ipl1 might be impaired in the absence of Sgo1 or Bub1, even if the CPC is properly localized. We measured phosphorylation of Sli15, a direct substrate and one of the cofactors of Ipl1 kinase (Kang *et al.*, 2001), by analyzing the mobility in SDS-PAGE. Indeed, the pattern of migration of the Sli15-TAP protein was not altered in *bub1*Δ and *sgo1*Δ mutant strains in comparison to wild-type. By contrast, the phosphorylation was almost completely abolished in cells expressing *ipl1-321*, a temperature-sensitive mutation that abolishes the kinase activity (Fig. 4C).

Similarly, we analyzed the phosphorylation of another known substrate of Ipl1 kinase, the microtubule-binding protein Dam1 (Cheeseman *et al.*, 2002). Catalytic interaction between Ipl1 and Dam1 is thought to be the primary mechanism by which the CPC destabilizes microtubule attachments that do not produce tension (Cheeseman *et al*., 2002; Gestaut *et al*., 2008). In asynchronous cultures, the relative abundance of the two distinctly migrating populations of Dam1-myc was unaffected by the deletion of *BUB1* at 30°C or 37°C, whereas the *ipl1-321* mutation eliminated the appearance of phosphorylated Dam1-myc at the restrictive temparature (Fig. 4D). These findings suggest that the general activity of the Ipl1 kinase remains intact in cells that lack Bub1 or Sgo1. It should be noted that these experiments are not specific for the subset of phosphorylation events, at particular residues and particular times in the cell cycle, that directly mediate the repair of syntelic attachments. However, they show that the CPC is catalytically active in the absence of Sgo1 and Bub1.

Sgo1 supports chromosome bi-orientation even if Ipl1 is inactive

Our data suggest the possibility that Sgo1 acts in the establishment of chromosome bi-orientation independently of the CPC. To test this model, we used *ipl1-321* strain transformed with either an *IPL1* or *SGO1* carrying 2μ vector, or an empty vector control. The chromosome IV was marked with TetO/TetR-GFP and SPBs were marked with Spc29-RFP. We synchronized the cell by nocodazole treatment, thus effectively abolishing microtubule-kinetochore attachment, and then microscopically analyzed chromosome segregation after release from nocodazole at the restrictive temperature. Bipolar attachments were identified by the positioning of the marked chromosome in the center between the two SPBs. As expected, up to 70 % of $IPL1^{+}$ cells could achieved bi-orientation within 60 min of removal of the microtubule poison, whereas only 20% of cells with inactive Ipl1 could bi-orient chromosome IV (Fig. 5A). Over-expression of Sgo1 improved the bi-orientation rate of the *ipl1-321* strain to approximately 44%, thus suggesting that Sgo1 can alter kinetochore-microtubule attachments even in the absence of functional Ipl1. Consistently, analysis of the cells in anaphase revealed that strains over-expressing Sgo1 missegregated chromosome IV in approximately 46% of anaphases (95% confidence interval 37.5 - 54.6%), whereas the *ipl1-321* strain showed missegregation in 65% (58 – 71%) of anaphases. Wild type strain missegregated chromosome IV at a rate of 8.5% (5.6 – 11.4%) under the identical conditions.

Because Ipl1 functions in the SAC, the $IPL1^{+}$ and *ipl1-321* strains do not respond equivalently after the release from nocodazole treatment. As expected, a higher percentage *ipl1-321*

cells progressed through anaphase and accumulated multibudded cells than *IPL1*$^{+}$ cells over the course of the experiment (Fig. 5B). Importantly, the decrease in the frequency of large-budded cells and increase in the frequency of multi-budded cells were not altered by over-expression of Sgo1 in *ipl1-321* mutants. These results indicate that Sgo1 does not slow progression through mitosis in these cells (Fig. 5B). Taken together, our data suggest that some of the improper attachments created after release from nocodazole can be corrected independently of Ipl1 kinase function by an Sgo1-mediated process.

Mps1 regulates Sgo1 to establish chromosome bi-orientation

Another mitotic protein, the conserved checkpoint kinase Mps1, has recently been shown to promote the turnover of syntelic attachments without any apparent effect on Ipl1 localization or kinase activity (Maure *et al.*, 2007). To test the hypothesis that Sgo1 and Mps1 act in the same pathway, we analyzed the genetic relationship between Mps1 and Sgo1. Indeed, over-expression of *MPS1* (under the control of its own promoter and located on an episomal vector) weakly but reproducibly suppresses the benomyl sensitivity of *sgo1*Δ haploids as well as the growth defect of *sgo1*Δ tetraploids (Supplementary Fig. 5A, B).

However, this effect could be unrelated to Mps1's role in regulating microtubule attachment, since over-expression of Mps1 can also induce a SAC-dependent mitotic arrest. Previous reports suggest that the chromosome segregation defects of *sgo1* mutants can be significantly reduced by simply delaying anaphase onset, either by weak treatment with hydroxyurea or over-expression of Mps1 at levels sufficient to activate the SAC (Indjeian *et al.*, 2005). We therefore investigated whether this genetic interaction, the ability of Mps1 over-expression to partially compensate for the absence of Sgo1, depends upon activation of the SAC. We find that Mps1 over-expression suppresses the benomyl sensitivity of *bub1*Δ*K* cells (mutants lacking the kinase domain of Bub1 but still capable of activing the checkpoint (Fernius and Hardwick, 2007)), whereas no such effect was observed in *bub1*Δ cells, which lack a functional SAC (Supplementary Fig. 5C). Therefore, we conclude that Mps1-dependent activation of the SAC can improve the fitness of cells that lack or mislocalize Sgo1, but that Mps1 cannot bypass Sgo1's role in regulating microtubule attachment.

Next, we analyzed whether *SGO1* over-expression can reduce the mitotic defects of cells lacking Mps1 activity. In addition to its functions in the SAC and microtubule attachment, Mps1 is essential for SPB duplication during the cell cycle (Lauze *et al.*, 1995). In order to be able to inactivate Mps1 only after SPB duplication is complete, we used strains expressing two mutant alleles: *mps1-as* (Jones *et al.*, 2005), which enables chemical inhibition of the kinase by the ATP-analogue 1NM-PP1, and *cdc34-2*, which causes a temperature-sensitive arrest at the G1-S transition (after SPB duplication). The cells were arrested at 37°C, incubated for additional 2 hours at the restrictive temperature in the presence or absence of the inhibitor 1NM-PP1, and then released from the *cdc34* arrest with and without the addition of nocodazole. The cells were then grown at room temperature for two hours, which corresponds approximately to a single cell division, and subsequently plated on YPD plates. The majority of the cells with inactivated Mps1 kinase did not survive the progression through the mitosis, and addition of nocodazole did not affect the survival rates (Fig. 6A). Strikingly, over-expression of Sgo1 allowed for the survival of cells after Mps1 inhibition, such that growth on the YPD plate was indistinguishable between cells treated with 1NM-PP1 and untreated cells (Fig. 6B). Thus, increased abundance of Sgo1 can bypass deficiency of Mps1 kinase activity in mitosis. These observations raise the possibility that Mps1's role in chromosome bi-orientation - a function that appears to be independent of Ipl1 activity (Maure *et al.*, 2007) - involves Sgo1.

We hypothesized that Mps1 might regulate Sgo1 function by ensuring its proper targeting to the kinetochore. To test this possibility, we again utilized the *mps1-as* allele in combination with the *cdc34-2* temperature-sensitive allele. After release from the *cdc34-2* arrest in the absence of the Mps1 inhibitor, these cells properly duplicated SPBs (marked via Spc29-RFP), and Sgo1-GFP localized within the area corresponding to the mitotic spindle (Fig. 6B, D). However, the localization of Sgo1-GFP to the spindle is impaired upon addition of the inhibitor 1NM-PP1 (Fig. 6B,D). In inhibitor-treated cells, accumulation of Sg1 on the spindle is restored to 50 % of normal levels by over-expression of Sgo1-GFP from a multicopy vector (Fig. 6D), consistent with the ability of Sgo1 over-expression to rescue cell survival.The effect of Mps1 on sgo1 localization is specific, since inactivation of its kinase activity does not affect localization of Sli15-GFP (Fig. 6D). Taken together, we propose that Sgo1 and Mps1 act in the same pathway for the establishment of chromosome bi-orientation in mitosis and that contributes to the kinetochore recruitment of the Sgo1.

DISCUSSION

The chromosome segregation apparatus is compromised by increased ploidy, as demonstrated by the fact that budding yeast and mammalian tetraploids exhibit high chromosomal instability and accumulate aneuploid cells (Mayer and Aguilera, 1990; Fujiwara *et al.*, 2005). In fact, several genes involved in the maintenance of genome stability are dispensable in haploid or diploid budding yeast, but become essential in cells with increased ploidy (Storchova *et al.*, 2006). We used this "ploidy-specific lethality" to analyze the function of Bub1, a conserved kinase involved in the spindle assembly checkpoint and chromosome segregation, and Sgo1, a conserved protein of unknown molecular function required for proper chromosome segregation in both mitosis and meiosis. We found that the ploidy-specific requirement for Bub1 can by bypassed by over-expression of Bir1/survivin and Sli15/incenp, conserved proteins that are a part of CPC. The CPC is considered to be the key protein complex involved in the establishment of bi-oriented attachments of sister chromatids. Bir1 and Sli15 are required for the catalytic activity of Ipl1/Aurora B, which phosphorylates several kinetochore proteins, including Dam1 and Ndc80, upon the formation of syntelic or monotelic attachments that do not produce tension. These phosphorylation events help to disrupt the unproductive attachments and enable their replacement by proper bi-oriented attachments (Cheeseman *et al.*, 2002; Tanaka *et al.*, 2002; Pinsky *et al.*, 2006). Our finding that over-expression of CPC components restores viability to *bub1*Δ tetraploids suggests that these cells die due to an excess of unrepaired syntelic attachments.

The role of Bub1 in bi-orientation is independent of its function in the SAC. It has been shown the C-terminal (kinase-containing) part of Bub1is dispensable for mitotic arrest upon spindle depolymerization; instead it promotes the kinetochore recruitment of Sgo1 (Fernius and Hardwick, 2007) by phosphorylating H2A and thus creating a kinetochore mark that directs the localization of Sgo1 (Kawashima *et al.*, 2010). Because Sgo1 over-expression bypasses the requirement for Bub1 in tetraploids, and because over-expression of the same two CPC components reverses the lethality of *bub1*Δ and sgo*1*Δ tetraploids alike, we propose that the ploidy-specific requirement for Bub1 effectively represents a requirement for Sgo1 in establishment of bi-orienation.

We observed that loss of Sgo1 produces a phenotype similar to that previously observed with *ipl1* mutants, in which chromosome missegregation is biased toward the daughter bud and cells fail to reliably bi-orient an unreplicated, dicentric chromosome (Tanaka *et al.*, 2002; Dewar *et al.*, 2004). Sgo1 could play a role in the establishment of bi-oriented attachments by (1) biasing the initial

attachment of kinetochores to the spindle so as to favor bi-orientation, (2) promoting the release of microtubule attachments that are not under tension, or (3) delaying anaphase in a SAC-independent manner and thereby allowing the CPC more time to destabilize syntelic attachments. We observed by live cell imaging that under experimental conditions where cells initially form large numbers of syntelic attachments, *sgo1*Δ mutants were incapable of converting monopolar microtubule attachments to bi-oriented attachments. This observation supports the model that Sgo1 promotes the release of tensionless microtubule attachments. It should be noted that our evidence does not exclude a role for Sgo1 in cell cycle progression (Clift *et al.*, 2009) or in biasing the initial attachment of kinetochores toward bi-orientation (as has been proposed for sister chromatids in meiosis (Kiburz *et al.*, 2008)).

The defects in chromosome segregation that are observed in *sgo1*Δ and *bub1*Δ mutants are similar to those observed in CPC mutants and are suppressed by over-expression of CPC components. This suggests that the localization and/or function of CPC proteins might be impaired by the loss of Sgo1 at the kinetochore. Such a mechanism as was previously observed in fission yeast: Sgo2 interacts with Bir1 and disruption of the interaction impairs the kinetochore localization of Bir1 and Aurorahomolog Ark1 in metaphase (Kawashima *et al.*, 2007). However, by quantitative fluorescence imaging we find that budding yeast lacking Sgo1 and Bub1 show normal localization of Ipl1, Bir1 and Sli15 during mitosis, which was further confirmed by ChIP of Sli15-TAP to the centromeric DNA. The intact CPC localization in Sgo1 deficient cells could have several explanations. First, Sgo1 might act independently of CPC; second, there might be a minor defect in CPC recruitment that our methods failed to detect; or, last, loss of Sgo1 triggers a compensatory response that enhances CPC targeting. However, our data clearly suggest that aberrant CPC localization could not be responsible for the striking phenotype of budding yeast lacking SGO1, which is an inability to repair syntelic attachments once they form (Fig. 3C). Moreover, Western blot analysis of the phosphorylation of Dam1 and Sli15 by Ipl1 did not reveal any difference in cells with and without Bub1and Sgo1. Previous evidence shows that the phosphorylation of Dam1 by Ipl1 is required for disassembly of incorrect chromosome attachments (Cheeseman *et al.*, 2002; Gestaut *et al.*, 2008). Our data shows that this important catalytic interaction is preserved in the absence of Sgo1, though our experiments do not allow for the detection of more subtle changes in phosphorylation at specific residues.

If Sgo1 functions in a pathway independent of Ipl1, then at least some correction of monopolar attachments should be possible in the absence of Aurora B activity. Consistent with this idea, we observe that Sgo1 overexpression in *ipl1-321*mutants increases the frequency of chromosome bi-orientation and successful anaphase by approximately 20%. However, since these cells still missegregate chromosomes at very high rates, they are inviable. In light of this evidence, we favor a model in which multiple modifications at the kinetochore are required to eliminate a syntelic attachment. One crucial set of modifications, the phosphorylation of Dam1 and Ndc80, is catalyzed by Ipl1 and stimulated by CPC proteins Bir1 and Sli15. However, *sgo1*Δ mutants present a situation in which Ipl1 kinase is active and yet syntelic attachments are hardly, if ever, repaired (Fig. 3C). While we cannot rule out the possibility that Ipl1 activity is lost only in a narrow spatiotemporal window, it seems likely instead that Sgo1—or some downstream factor—provides a second signal to release tensionless microtubule attachments. This second set of kinetochore modifications would occur in parallel to Ipl1, in the sense that they are not directly catalyzed by Ipl1 kinase, although the initial "sensing" of kinetochore tension might involve a common mechanism. Analysis of *ipl1* and *sgo1* mutants suggests that both pathways are required for the efficient correction of syntelic attachments, though the contribution of Ipl1 is more significant: nondisjunction of sister chromatids

occurs at a rate of 85% in *ipl1*-321 cells (Biggins *et al.*, 1999) but is rare enough in *sgo1* mutants to permit colony growth. The viability of Sgo1-deficient strains can be explained by the observation that, under normal conditions, these cells achieve bi-orientation on the first try and only rarely need to repair syntelic attachments (Indjeian and Murray, 2007).

Recently, the mitotic kinase Mps1 has been implicated in the establishment sister-chromatid bi-orientation in diverse organisms, in addition to its previously characterized functions in the SAC and spindle pole duplication (Winey and Huneycutt, 2002; Jones *et al.*, 2005; Maure *et al.*, 2007; Jelluma *et al.*, 2008; Hewitt *et al.*, 2010; Maciejowski *et al.*, 2010; Santaguida *et al.*, 2010). Budding yeast with inactivated Mps1 missegregate chromosomes at high levels (Jones *et al.*, 2005) and are unable to release syntelic attachments (Maure *et al.*, 2007). We observed that over-expression of *SGO1* enables cells to survive transient pharmacological inhibition of Mps1 kinase activity. Furthermore, the metaphase localization of Sgo1-GFP is abolished after Mps1 inactivation. This delocalization is not due to a more general defect in kinetochore integrity since microtubule-kinetochore attachments are stable in cells with inactive Mps1 (Maure *et al.*, 2007) . Moreover, the localization of the CPC component Sli15-GFP is not altered upon Mps1 inhibition, confirming the specificity of the effect. Thus, we speculate that Mps1 is an upstream regulator of Sgo1 function and that the bi-orientation defect caused by Mps1 inactivation can be explained, in part, by the loss of Sgo1 kinetochore function. Finally, the fact that Mps1 inhibition does not alter the activity of purified Ipl1 in an *in vitro* kinase assay (Maure *et al.*, 2007), despite a marked loss of Sgo1 localization to the kinetochore, provides additional evidence consistent with the model that Sgo1 does not regulate Ipl1 function.

The proposal that budding yeast Bub1, Sgo1, and Mps1 act in parallel to the CPC implies that these pathways are wired differently in budding yeast than in fission yeast or vertebrates. Bub1 deficiency in *S. pombe*, *X. laevis*, and human cells causes marked abrogation of CPC localization to the inner centromere (Boyarchuk *et al.*, 2007; Kawashima *et al.*, 2010), consistent with the hypothesis that Sgo2 targeting by Bub1 and subsequent Bir1-Sgo2 interaction are required for CPC recruitment (Kawashima *et al.*, 2007; Kawashima *et al.*, 2010). Moreover, emerging evidence suggests that Aurora B and Mps1 regulate one another in human cells: chemical inhibition of Aurora B in mitosis causes mislocalization and hypophosphorylation of Mps1 (Santaguida *et al.*, 2010), and depletion or inhibition of Mps1 prior to mitotic entry diminishes Aurora B kinase activity in metaphase-arrested cells (Jelluma *et al.*, 2008; Kwiatkowski *et al.*, 2010 ; Maciejowski *et al.*, 2010) However, even in higher eukaryotes, the literature is consistent with the existence of second pathway, acting independently or downstream of Ipl1, to repair syntelic attachments. Crucially, three different chemical inhibitors of Mps1, when applied to human cells after mitotic entry, cause errors in attachment error-correction and chromosome alignment without causing detectable changes in Aurora B activation (Hewitt *et al.*, 2010; Maciejowski *et al.*, 2010; Santaguida *et al.*, 2010; Sliedrecht *et al.*, 2010). These studies motivate future work to identify the key downstream Mps1 substrates that are important for bi-orientation. The regulation of microtubule attachment in budding yeast, while distinct from that in other eukaryotes, may very well be an attractive system for manipulating conserved functions of shugoshin or Mps1 without simultaneously affecting CPC localization or activity . Our work illustrates the value of cross-species comparisons for understanding the architecture of signaling networks; different organisms appear to utilize the same players but wire the system somewhat differently. Further experiments are needed to reveal the mechanism by which budding yeast Sgo1 can alter kinetochore-microtubule attachments in the absence of functional Ipl1/Aurora B, and to test whether such a function also exists for shugoshin homologs in other organisms.

MATERIALS AND METHODS

General molecular genetics methods. All strains and plasmids used in this study are listed in Table 1; details on strain construction are available upon request. The strains are derived from the BY series of S288C (*leu2*Δ, *his3*Δ, *ura3*Δ, *lys2*Δ/*met15*Δ), or W303 background (*ade2-101*, *ura3-52*, *trp1-1*, *his3-1,3*, *leu2-1,112*). The tetraploid construction was performed by mating MAT**a**/**a** and MATα/α strains, as previously described (Storchova *et al.*, 2006). Yeast cultivation, α-factor synchronization and nocodazole treatment (30 μg/ml) was performed as described previously. The *SGO1, IPL1, BIR1, SLI15* and *MPS1* were cloned into the plasmid pRS316 (CEN, *URA3*) to obtain plasmids for the shuffling experiments, and into pRS425 (2micron, *LEU2*) for the complementation experiments. Since the strains *sgo1*Δ and *bub1*Δ are genetically unstable, they were transformed with the *URA3*-marked centromeric plasmids containing a functional gene; counter selection using 5-FOA was performed before each experiment to obtain strains of the desired genotypes.

A genome-wide suppressor screen in *bub1*Δ tetraploids. The high-copy suppressor screen was performed using a plasmid-shuffle strategy. In brief, tetraploid deletion strain containing a centromeric vector with a wild-type allele and a *URA3* marker were transformed with a 2 micron genomic library (*LEU2*-marked*)*. Single colonies were replica-plated on media lacking leucine and containing 5-FOA and incubated at 37°C for three days. All robustly growing colonies (<1%) were tested by colony PCR for presence/absence of *BUB1*; approximately 60 % of the colonies grew due to the presence of *BUB1* gene on the suppressing plasmid and were excluded from further analysis. The remaining plasmids were then extracted from the candidate clones, used for secondary verification and sequenced.

Measurement of chromosome loss rates by fluctuation test with yeast artificial chromosome. The fidelity of chromosome segregation in *bub1*Δ mutants was assessed by measuring the rate of loss of a *URA3,TRP1*-marked yeast artificial chromosome (YAC) (Huang and Koshland, 2003). Cells were streaked on media nonselective for the YAC and grown at 30°C for three days. For each strain, seven colonies were cut out of the plate and dispensed in 1 ml sterile water, diluted and plated in duplicate on both non-selective plates (to estimate the total number of cells, N) and 5-FOA plates (to estimate the number of mutants) plates. To ensure specificity for chromosome loss events, as opposed to chromosomal rearrangements, colonies on 5-FOA were replica plated to ensure simultaneous loss of the *TRP1* marker near the centromere. The mutation rate μ (the number of chromosome loss events per cell division) was calculated according to the median method (Lea and Coulson, 1949).

Western Blot Analysis. Protein samples were extracted, run on SDS polyacrylamide gels and transferred to polyvinylidene difluoride membranes. The mouse monoclonal anti-phosphoglycerate kinase antibody (Molecular Probes) was used to detect a loading control, peroxidase-anti-peroxidase rabbit antibody (Sigma-Aldrich) for recognition of the TAP tag and anti-Myc rabbit serum. The secondary antibodies were goat anti-rabbit and goat anti-mouse (Abcam), both used at a dilution of 1:10000.

Chromatin Immunoprecipitation. Chromatin immunoprecipitation was performed using a protocol developed in the laboratory of Stefan Jentsch (Kalocsay *et al.*, 2009). The proteins of interest were tagged with the TAP epitope. 300 ml of fresh culture were fixed at room temperature by

formaldehyde addition to a final concentration 1% and harvested after 16 minutes. Upon lysis (50 mM HEPES-KOH, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% Na Deoxycholate, 0.1% SDS, 1 mM PMSF) in bead beater (6 x 3 min), the chromatin was sheared to an average length of 300-500 bp by water bath sonication (Bioruptor UCD-200, Diagenode) using 30 x 30 s cycles with 30 s breaks at an output of 200 W. Anti- IgG sepharose beads (Roche) were used for immunoprecipiation at 4^{o}C for 120 min. The association of the TAP-tagged protein with centromeric DNA was quantified as the ratio in abundance of a 200-bp centromeric sequence (CEN-III) to a 200-bp telomeric sequence (TEL-V*) in the immunoprecipitated DNA. Quantitative PCR was performed on a Light Cycler LC480 System (Roche) with previously described primers (Keogh *et al.*, 2005).

Fluorescence microscopy. Strains expressing alleles tagged with GFP (Ipl1-GFP, Sli15-GFP, Sgo1-GFP, Bir1-GFP, Bub1-GFP) and RFP (Spc29-RFP) were visualized by a fully automated Zeiss inverted microscope (Carl Zeiss) equipped with a MS-2000 stage (Applied Scientific Instrumentation, Eugene, OR), a CSU-X1 spinning disk confocal head (Yokogawa) and LaserStack Launch with selectable laser lines (Intelligent Imaging Innovations, Denver, CO). Images were captured at intervals of 0.5 μm in the Z focal plane using a CoolSnap HQ camera (Roper Scientific, Tuscon, AZ) under the control of the Slidebook software (Intelligent Imaging Innovations, Denver, CO). For quantification of the fluorescence signal in metaphase cells, we analyzed all cells showing two RFP dots (spindle pole body marker, Spc29-RFP) less than 2 μm apart and positioned orthogonal to the plane of the bud neck. The GFP signal localized between the spindle poles was quantified by extracting both the median and maximum fluorescent intensities over a 36-pixel mask (positioned between the RFP dots), after application of standardized signal renormalization settings.

Acknowledgment: We thank Kevin Hardwick, Kenneth Kaplan, Tomo Tanaka and Mark Winey for plasmids and strains. We thank Karolina Peplowska, Markus Räschle, Satoshi Yoshida and the members of the Storchová group for critical reading of the manuscript and helpful discussions. We are thankful to Susanne Gutmann and Regina Dagher for technical assistance.

References:

Andalis, A.A., Storchova, Z., Styles, C., Galitski, T., Pellman, D., and Fink, G.R. (2004). Defects arising from whole-genome duplications in Saccharomyces cerevisiae. Genetics *167*, 1109-1121.

Biggins, S., and Murray, A.W. (2001). The budding yeast protein kinase Ipl1/Auroraallows the absence of tension to activate the spindle checkpoint. Genes & development *15*, 3118-3129.

Biggins, S., Severin, F.F., Bhalla, N., Sassoon, I., Hyman, A.A., and Murray, A.W. (1999). The conserved protein kinase Ipl1 regulates microtubule binding to kinetochores in budding yeast. Genes & development *13*, 532-544.

Boyarchuk, Y., Salic, A., Dasso, M., and Arnaoutov, A. (2007). Bub1 is essential for assembly of the functional inner centromere, vol. 176, 919-928.

Cheeseman, I.M., Anderson, S., Jwa, M., Green, E.M., Kang, J., Yates, J.R., 3rd, Chan, C.S., Drubin, D.G., and Barnes, G. (2002). Phospho-regulation of kinetochore-microtubule attachments by the Aurorakinase Ipl1p. Cell *111*, 163-172.

Clift, D., Bizzari, F., and Marston, A.L. (2009). Shugoshin prevents cohesin cleavage by PP2A(Cdc55)-dependent inhibition of separase. Genes & development *23*, 766-780.

Dewar, H., Tanaka, K., Nasmyth, K., and Tanaka, T.U. (2004). Tension between two kinetochores suffices for their bi-orientation on the mitotic spindle. Nature *428*, 93-97.

Fernius, J., and Hardwick, K.G. (2007). Bub1 kinase targets Sgo1 to ensure efficient chromosome biorientation in budding yeast mitosis. PLoS genetics *3*, e213.

Fujiwara, T., Bandi, M., Nitta, M., Ivanova, E.V., Bronson, R.T., and Pellman, D. (2005). Cytokinesis failure generating tetraploids promotes tumorigenesis in p53-null cells. Nature *437*, 1043-1047.

Ganem, N.J., Godinho, S.A., and Pellman, D. (2009). A mechanism linking extra centrosomes to chromosomal instability. Nature *460*, 278-282.

Gestaut, D.R., Graczyk, B., Cooper, J., Widlund, P.O., Zelter, A., Wordeman, L., Asbury, C.L., and Davis, T.N. (2008). Phosphoregulation and depolymerization-driven movement of the Dam1 complex do not require ring formation. Nature cell biology *10*, 407-414.

Hewitt, L., Tighe, A., Santaguida, S., White, A.M., Jones, C.D., Musacchio, A., Green, S., and Taylor, S.S. (2010). Sustained Mps1 activity is required in mitosis to recruit O-Mad2 to the Mad1-C-Mad2 core complex. The Journal of cell biology *190*, 25-34.

Hoyt, M.A., Totis, L., and Roberts, B.T. (1991). S. cerevisiae genes required for cell cycle arrest in response to loss of microtubule function. Cell *66*, 507-517.

Huang, D., and Koshland, D. (2003). Chromosome integrity in Saccharomyces cerevisiae: the interplay of DNA replication initiation factors, elongation factors, and origins. Genes & development *17*, 1741-1754.

Indjeian, V.B., and Murray, A.W. (2007). Budding yeast mitotic chromosomes have an intrinsic bias to biorient on the spindle. Curr Biol *17*, 1837-1846.

Indjeian, V.B., Stern, B.M., and Murray, A.W. (2005). The centromeric protein Sgo1 is required to sense lack of tension on mitotic chromosomes. Science (New York, N.Y *307*, 130-133.

Jelluma, N., Brenkman, A.B., van den Broek, N.J., Cruijsen, C.W., van Osch, M.H., Lens, S.M., Medema, R.H., and Kops, G.J. (2008). Mps1 phosphorylates Borealin to control Aurora B activity and chromosome alignment. Cell *132*, 233-246.

Jones, M.H., Huneycutt, B.J., Pearson, C.G., Zhang, C., Morgan, G., Shokat, K., Bloom, K., and Winey, M. (2005). Chemical Genetics Reveals a Role for Mps1 Kinase in Kinetochore Attachment during Mitosis. Curr Biol *15*, 160-165.

Kalocsay, M., Hiller, N.J., and Jentsch, S. (2009). Chromosome-wide Rad51 spreading and SUMO-H2A.Z-dependent chromosome fixation in response to a persistent DNA double-strand break. Molecular cell *33*, 335-343.

Kang, J., Cheeseman, I.M., Kallstrom, G., Velmurugan, S., Barnes, G., and Chan, C.S. (2001). Functional cooperation of Dam1, Ipl1, and the inner centromere protein (INCENP)-related protein Sli15 during chromosome segregation. The Journal of cell biology *155*, 763-774.

Katis, V.L., Galova, M., Rabitsch, K.P., Gregan, J., and Nasmyth, K. (2004). Maintenance of cohesin at centromeres after meiosis I in budding yeast requires a kinetochore-associated protein related to MEI-S332. Curr Biol *14*, 560-572.

Kawashima, S.A., Tsukahara, T., Langegger, M., Hauf, S., Kitajima, T.S., and Watanabe, Y. (2007). Shugoshin enables tension-generating attachment of kinetochores by loading Aurorato centromeres, vol. 21, 420-435.

Kawashima, S.A., Yamagishi, Y., Honda, T., Ishiguro, K.-i., and Watanabe, Y. (2010). Phosphorylation of H2A by Bub1 Prevents Chromosomal Instability Through Localizing Shugoshin. Science (New York, N.Y *327*, 172-177.

Keogh, M.C., Kurdistani, S.K., Morris, S.A., Ahn, S.H., Podolny, V., Collins, S.R., Schuldiner, M., Chin, K., Punna, T., Thompson, N.J., Boone, C., Emili, A., Weissman, J.S., Hughes, T.R., Strahl, B.D., Grunstein, M., Greenblatt, J.F., Buratowski, S., and Krogan, N.J. (2005). Cotranscriptional set2 methylation of histone H3 lysine 36 recruits a repressive Rpd3 complex. Cell *123*, 593-605.

Kiburz, B.M., Amon, A., and Marston, A.L. (2008). Shugoshin Promotes Sister Kinetochore Biorientation in Saccharomyces cerevisiae. Molecular biology of the cell *19*, 1199-1209.

Kiburz, B.M., Reynolds, D.B., Megee, P.C., Marston, A.L., Lee, B.H., Lee, T.I., Levine, S.S., Young, R.A., and Amon, A. (2005). The core centromere and Sgo1 establish a 50-kb cohesin-protected domain around centromeres during meiosis I. Genes & development *19*, 3017-3030.

Kim, J.H., Kang, J.S., and Chan, C.S. (1999). Sli15 associates with the ipl1 protein kinase to promote proper chromosome segregation in Saccharomyces cerevisiae. The Journal of cell biology *145*, 1381-1394.

Kitajima, T.S., Hauf, S., Ohsugi, M., Yamamoto, T., and Watanabe, Y. (2005). Human Bub1 defines the persistent cohesion site along the mitotic chromosome by affecting Shugoshin localization. Curr Biol *15*, 353-359.

Kitajima, T.S., Sakuno, T., Ishiguro, K.-i., Iemura, S.-i., Natsume, T., Kawashima, S.A., and Watanabe, Y. (2006). Shugoshin collaborates with protein phosphatase 2A to protect cohesin. Nature *441*, 46-52.

Kwiatkowski, N., Jelluma, N., Filippakopoulos, P., Soundararajan, M., Manak, M.S., Kwon, M., Choi, H.G., Sim, T., Deveraux, Q.L., Rottmann, S., Pellman, D., Shah, J.V., Kops, G.J., Knapp, S., and Gray, N.S. (2010). Small-molecule kinase inhibitors provide insight into Mps1 cell cycle function. Nature chemical biology *6*, 359-368.

Lauze, E., Stoelcker, B., Luca, F.C., Weiss, E., Schutz, A.R., and Winey, M. (1995). Yeast spindle pole body duplication gene MPS1 encodes an essential dual specificity protein kinase. The EMBO journal *14*, 1655-1663.

Lea, D., and Coulson, C. (1949). The distribution of the numbers of mutants in bacterial populations. Journal of Genetics *49*, 264-285.

Lin, H., de Carvalho, P., Kho, D., Tai, C.Y., Pierre, P., Fink, G.R., and Pellman, D. (2001). Polyploids require Bik1 for kinetochore-microtubule attachment. The Journal of cell biology *155*, 1173-1184.

Maciejowski, J., George, K.A., Terret, M.E., Zhang, C., Shokat, K.M., and Jallepalli, P.V. (2010). Mps1 directs the assembly of Cdc20 inhibitory complexes during interphase and mitosis to control M phase timing and spindle checkpoint signaling. The Journal of cell biology *190*, 89-100.

Margolis, R.L. (2005). Tetraploidy and tumor development. Cancer cell *8*, 353-354.

Maure, J.-F., Kitamura, E., and Tanaka, T.U. (2007). Mps1 Kinase Promotes Sister-Kinetochore Bi-orientation by a Tension-Dependent Mechanism. Curr Biol *17*, 2175-2182.

Mayer, V.W., and Aguilera, A. (1990). High levels of chromosome instability in polyploids of Saccharomyces cerevisiae. Mutation research *231*, 177-186.

Musacchio, A., and Salmon, E.D. (2007). The spindle-assembly checkpoint in space and time. Nature reviews *8*, 379-393.

Pinsky, B.A., Kung, C., Shokat, K.M., and Biggins, S. (2006). The Ipl1-Auroraprotein kinase activates the spindle checkpoint by creating unattached kinetochores. Nature cell biology *8*, 78-U28.

Riedel, C.G., Katis, V.L., Katou, Y., Mori, S., Itoh, T., Helmhart, W., Galova, M., Petronczki, M., Gregan, J., Cetin, B., Mudrak, I., Ogris, E., Mechtler, K., Pelletier, L., Buchholz, F., Shirahige, K., and Nasmyth, K. (2006). Protein phosphatase 2A protects centromeric sister chromatid cohesion during meiosis I. Nature *441*, 53-61.

Santaguida, S., Tighe, A., D'Alise, A.M., Taylor, S.S., and Musacchio, A. (2010). Dissecting the role of MPS1 in chromosome biorientation and the spindle checkpoint through the small molecule inhibitor reversine. The Journal of cell biology *190*, 73-87.

Shackney, S.E., Smith, C.A., Miller, B.W., Burholt, D.R., Murtha, K., Giles, H.R., Ketterer, D.M., and Pollice, A.A. (1989). Model for the genetic evolution of human solid tumors. Cancer research *49*, 3344-3354.

Shimogawa, M.M., Widlund, P.O., Riffle, M., Ess, M., and Davis, T.N. (2009). Bir1 is required for the tension checkpoint. Molecular biology of the cell *20*, 915-923.

Sliedrecht, T., Zhang, C., Shokat, K.M., and Kops, G.J. (2010). Chemical genetic inhibition of Mps1 in stable human cell lines reveals novel aspects of Mps1 function in mitosis. PloS one *5*, e10251.

Storchova, Z., Breneman, A., Cande, J., Dunn, J., Burbank, K., O'Toole, E., and Pellman, D. (2006). Genome-wide genetic analysis of polyploidy in yeast. Nature *443*, 541-547.

Storchova, Z., and Kuffer, C. (2008). The consequences of tetraploidy and aneuploidy. Journal of cell science *121*, 3859-3866.

Storchova, Z., and Pellman, D. (2004). From polyploidy to aneuploidy, genome instability and cancer. Nature reviews *5*, 45-54.

Tanaka, T.U., Rachidi, N., Janke, C., Pereira, G., Galova, M., Schiebel, E., Stark, M.J., and Nasmyth, K. (2002). Evidence that the Ipl1-Sli15 (Aurorakinase-INCENP) complex promotes chromosome bi-orientation by altering kinetochore-spindle pole connections. Cell *108*, 317-329.

Thomas, S., and Kaplan, K.B. (2007). A Bir1p Sli15p kinetochore passenger complex regulates septin organization during anaphase. Molecular biology of the cell *18*, 3820-3834.

Wang, X., and Dai, W. (2005). Shugoshin, a guardian for sister chromatid segregation. Experimental cell research *310*, 1-9.

Warren, C.D., Brady, D.M., Johnston, R.C., Hanna, J.S., Hardwick, K.G., and Spencer, F.A. (2002). Distinct chromosome segregation roles for spindle checkpoint proteins. Molecular biology of the cell *13*, 3029-3041.

Winey, M., and Huneycutt, B.J. (2002). Centrosomes and checkpoints: the MPS1 family of kinases. Oncogene *21*, 6161-6169.

Table 1 Used plasmids and strains

Name	Relevant markers	Type	Backbone	Origin
BZ242	*URA3,SGO1*	cen	pRS316	This work
pBS199	*URA3,BUB1*	cen	pRS316	A. Murray
BZ11	*LEU2, SGO1*	2 µ	pRS425	This work
BZ9	*LEU2, BIR1*	2 µ	pRS425	This work
BZ40	*LEU2, SLI15*	2 µ	pRS425	This work
pTR168	*LEU2,BUB1*	2 µ	pRS315	A. Hoyt
BZ278	*LEU2,IPL1*	2 µ	pRS425	This work
T431	*pGAL1-10-CEN4, TetOx112, RS-ARS-RS, CEN4*	cen	N/A	T. Tanaka
BZ277	*LEU2, MPS1*	2 µ	pRS425	This work
PB2303	*URA3,MPS1*	cen	pRS316	This work
BZ279	*LEU2, BIR1^W901A*	2 µ	pRS425	This work/K.Kaplan
BZ280	*LEU2, BIR1^A931E,I935E*	2 µ	pRS425	This work/K.Kaplan
BJ25	*LEU2,BIR^deltaN*	2 µ	pRS425	This work
BZ299	*LEU2, SGO1-eGFP*	2 µ	pRS425	This work

YZ669	S288C	1N	MATα *CEN4-tet0::HIS3 TetR-GFP-LEU2*	Storchova *et al* , 2006
YZ855	S288C	1N	MATa *sli15-3*	This work
YZ1063	S288C	1N	MATa *ipl1-321, SPC42-GFP-HIS3; pMET-rec; TetR-GFP*	Dewar et al, 2004
YZ1064	S288C	1N	MATa *SPC42-GFP-HIS3; pMET-rec; TetR-GFP*	Dewar et al, 2004
YZ640	S288C	4N	MATa/a/0/α *CEN4 tet0::HIS3;TetRGFP-LEU2/TetRGFP-LEU2/leu2Δ/leu2Δ; sgo1/sgo1/sgo1/sgo1; SPC29-*	This work
YZ997	S288C	4N	MATa/a/α/α *bub1/bub1/bub1/bub1, CEN4-tet0::HIS3 TeTRGFP-LEU2/TeTRGFP-LEU2/leu2Δ0/leu2Δ0, SPC29-mRFP/SPC29-mRFP/SPC29/SPC29*	This work
YZ841	S288C	2N	MAT**a/α** *SLI15-GFP-HIS3/SLI15, SPC29-mRFP/SPC29*	This work
NT745	S288C	2N	MAT**a/α** *SLI15-GFP-HIS3/SLI15; SPC29-mRFP/SPC29; sgo1/sgo1*	This work
NT712	S288C	2N	MAT**a/α** *BIR1-GFP-HIS3/BIR1; SPC29-mRFP/SPC29*	This work
NT713	S288C	2N	MAT**a/α** *BIR1-GFP-HIS3/BIR1; SPC29-mRFP/SPC29; sgo1/sgo1*	This work
NT571	S288C	2N	MAT**a/α** *IPL1-GFP-HIS3/IPL1; SPC29-mRFP/SPC29*	This work
NT608	S288C	2N	MAT**a/α** *IPL1-GFP-HIS3/IPL1; SPC29-mRFP/SPC29; sgo1/sgo1*	This work
YZ728	S288C	1N	MAT**a** *SLI15-TAP-HIS3*	Ghaemmaghami S et al. 2003
YZ741	S288C	1N	MAT**a** *SLI15-TAP-HIS3; bub1::kanMX*	This work
YZ725	S288C	1N	MAT**a** *NDC10-TAP-HIS3*	Ghaemmaghami S et al. 2003
YZ735	S288C	1N	MAT**a** *NDC10-TAP-HIS3; bub1::kanMX*	This work
YZ704	S288C	1N	MAT**a** *YAC(URA3,TRP1,ADE2,CEN)*	Storchova *et al* , 2006
YZ406	S288C	1N	MAT**a** *bub1::kanMX; YAC(URA3,TRP1,ADE2,CEN)*	This work
YZ1013	S288C	1N	MATα *sgo1::kanMX, spc29-mRFP-kanMX; pMET-rec; TetR-GFP*	This work
JF98	W303	1N	MAT**a** *bub1delK::hph*	K. Hardwick
YZ1071	W303	1N	MAT**a** *bub1delK::hph; cdc55::kanR*	This work
YZ1003	W303	1N	MAT**a** *bub1delK::hph SGO1-GFP-HIS3, SPC29-mRFP*	This work
YZ986	W303	1N	MAT**a** *bub1::kanMX; DAM1-9myc-TRP1*	This work
YZ1101	W303	1N	MAT**a** *ipl1-321::natMX; DAM1-9myc-TRP1*	This work
YZ990	W303	1N	MAT**a** *DAM1-9myc-TRP1*	This work
YZ535	S288C	1N	MAT**a** *IPL1-TAP-HIS3*	This work
YZ808	S288C	1N	MAT**a** *bub1::kanMX IPL1-TAP-HIS3*	This work
YZ991	S288C	1N	MAT**a** *sgo1::hph IPL1-TAP-HIS3*	This work
3108	W303	1N	MAT**a** *mps1-as cdc34-2*	Mark Winey
YZ1056	W303	1N	MAT**a** *mps1-as cdc34-2 SGO1-GFP-HIS3, SPC29-mRFP*	This work
YZ1182	W303	1N	MAT**a** *mps1-as BUB1-GFP-HIS3, SPC29-mRFP*	This work
YZ650	S288C	1N	MAT**a** *ipl1-321::kanMX; CEN4-tetO::HIS3, TetR-GFP-LEU2 Spc29-mRFP*	
YZ1174	W303	1N	MAT**a** *mps1-as SLI15-GFP-HIS3, SPC29-mRFP*	This work

FIGURE LEGEND

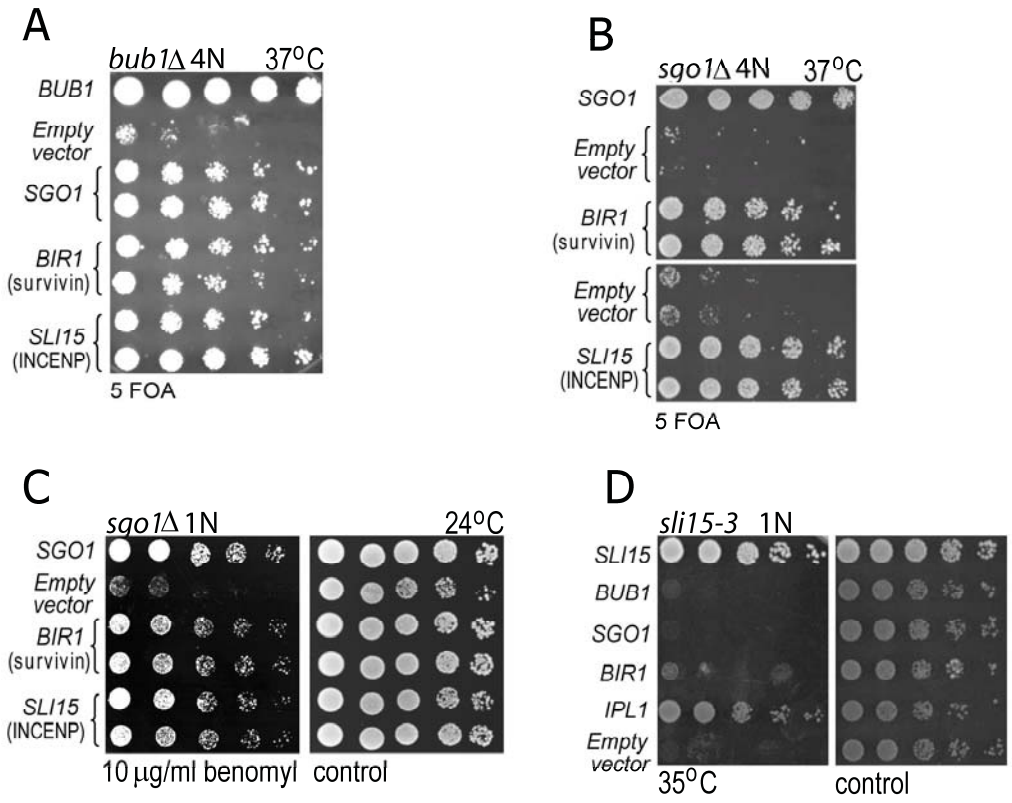

Fig. 1 The defect of *sgo1*Δ and *bub1*Δ strains is suppressed by *BIR1* or *SLI15* over-expression

A. Over-expression of *SGO1*, *BIR1* and *SLI15* under control of their own promoter and carried on 2-micron plasmids suppresses the temperature sensitivity of *bub1*Δ tetraploids. Two independent clones were tested for each genotype, and cells were plated by 5-fold serial dilution on plates lacking leucine (to select for the plasmid presence) and containing 5-FOA (counter selection against plasmid containing the wild type *BUB1*). The 2-micron *LEU2* vector pRS425 ("empty") and a *LEU2*-marked centromeric plasmid containing *BUB1* were used as negative and positive controls, respectively. **B.** Over-expression of *BIR1* and *SLI15* suppresses the lethality of *sgo1*Δ tetraploids at 37°C. The set up is identical as in the previous experiment. **C.** *BIR1* and *SLI15* over-expression suppresses the sensitivity of haploid *sgo1*Δ cells to the microtubule-depolymerizing drug benomyl. **D.** The genetic interaction is not reciprocal, as over-expression of *SGO1* does not improve the growth of *sli15-3* mutants at the minimal restrictive temperature (35° C).

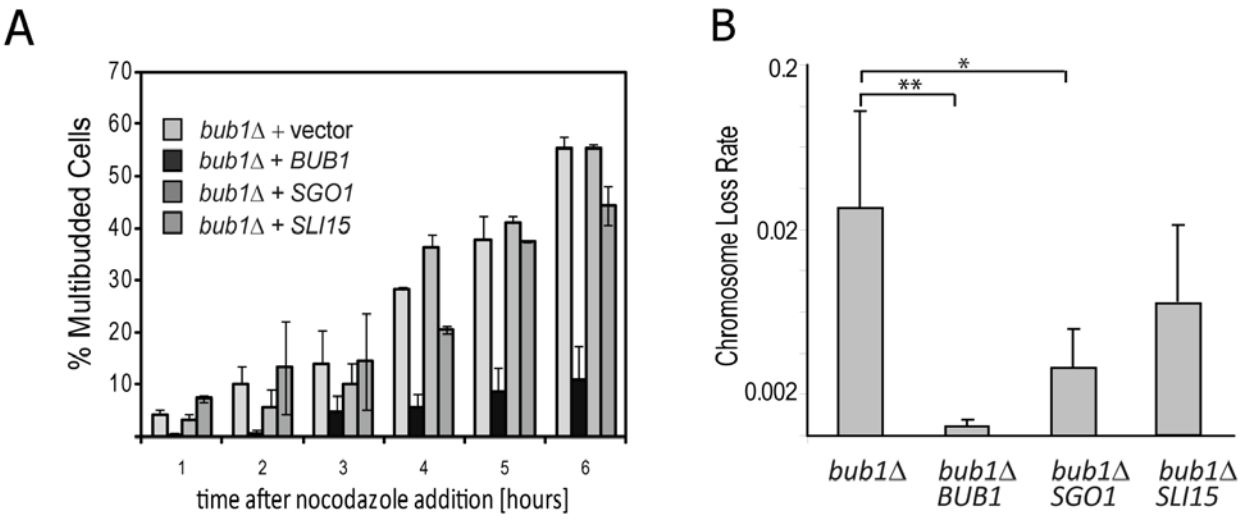

Fig. 2 Over-expression of *SGO1* or *SLI15* reduces the chromosome segregation defect in *bub1*Δ cells without restoring spindle checkpoint function

A. Strains were grown in liquid culture and analyzed for their ability to arrest in the prolonged presence of the microtubule poison nocodazole. Whereas wild-type cells arrest as large-budded cells, mutants lacking a functional spindle assembly checkpoint (SAC) reenter the cycle, which is indicated morphologically by the formation of a second bud. The accumulation of multibudded cells in *bub1*Δ was unaffected by over-expression of *SGO1* or *SLI15*, indicating that the SAC remains defective. **B.** The elevated chromosome loss rate in haploid *bub1*Δ strain bearing a yeast artificial chromosome (YAC), measured by genetic fluctuation test, decreases significantly upon *SGO1* or *SLI15* over-expression. The experiments were performed at 30° C. (*P < 0.05, Student's unpaired T-test, two-tailed).

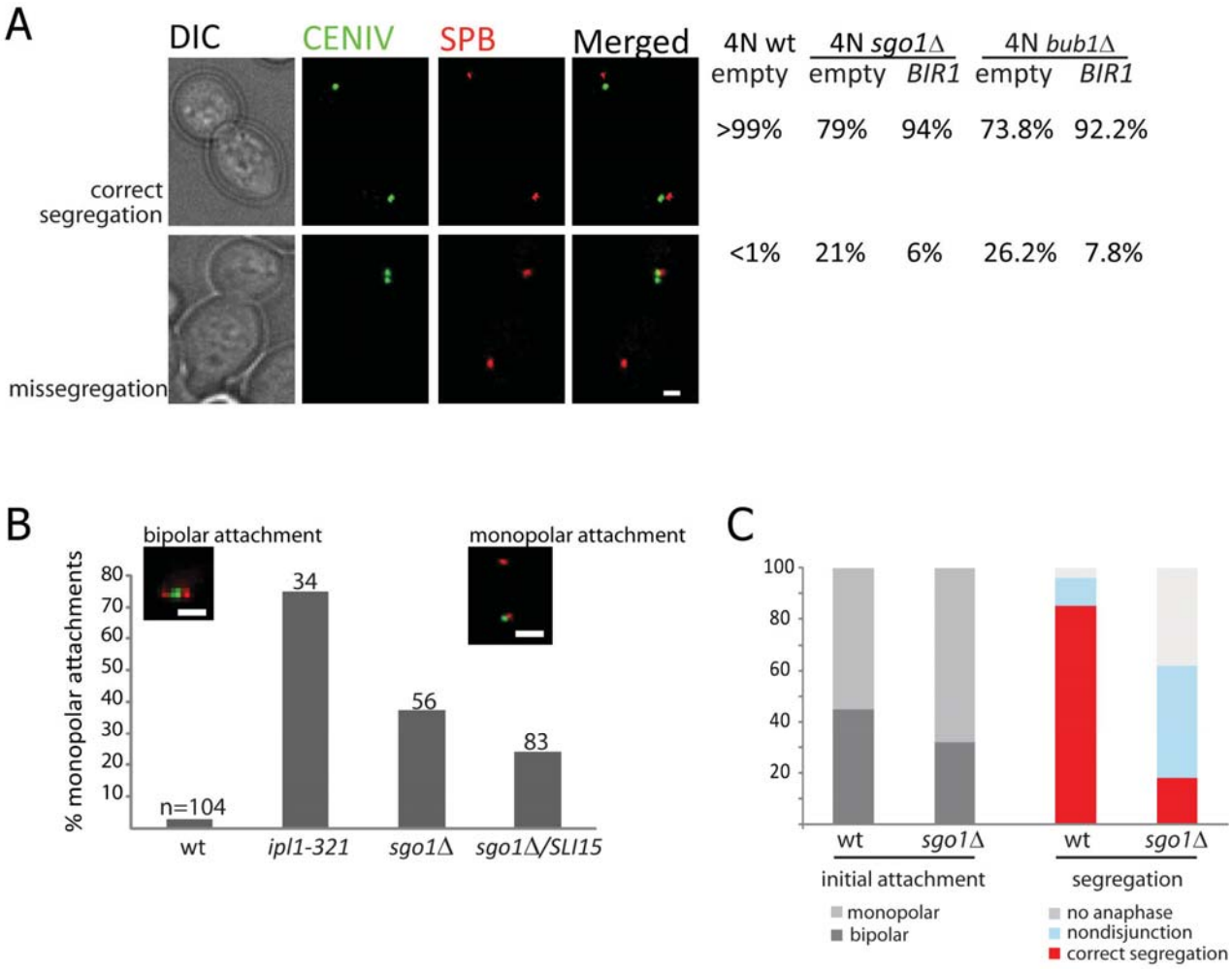

Fig. 3 Frequent chromosome missegregation in *sgo1*Δ mutants is due to defects in bi-orientation and can be bypassed by over-expression of *BIR1* or *SLI15*

A. *sgo1*Δ and *bub1*Δ tetraploid strains exhibit elevated rates of nondisjunction at chromosome IV; this defect can be suppressed by *BIR1* over-expression. Chromosome IV was visualized using a TetO/TetR-GRP array, and spindle pole bodies (SPBs) were marked by expression of Spc29-RFP. At least 150 anaphase cells were scored for each genotype. The experiments were performed at 33° C in order to enhance the phenotype . Bar - 1 μm. **B.** Haploid cells lacking *SGO1* show frequent monopolar attachment of an unreplicated dicentric minichrosome, albeit less so than mutants with reduced Ipl1 kinase activity. The missegregation defect in the *sgo1*Δ strain can be partially suppressed by over-expression of *SLI15*. The experiments were performed at 33° C. The two inset images show examples of attachments: L*eft:* The GFP-labeled is positioned between the two SPBs (marked with RFP), indicating a bipolar attachment; *Right* the minichromosome localizes in

proximity of one of the SPBs, indicating monopolar attachment. **C.** Diploid cells were released from treatment with nocodazole in order to create conditions in which a large number of syntelic attachments would be formed, even in wild-type cells. Live-cell imaging was used to monitor the initial attachment (left) and eventual segregation (right) of a GFP-marked copy of chromosome IV. *sgo1*Δ cells never converted monopolar attachment into bipolar attachments during the time of observation (75 min.) and were consequently much more likely than wild-type cells to undergo non-disjunction in anaphase.

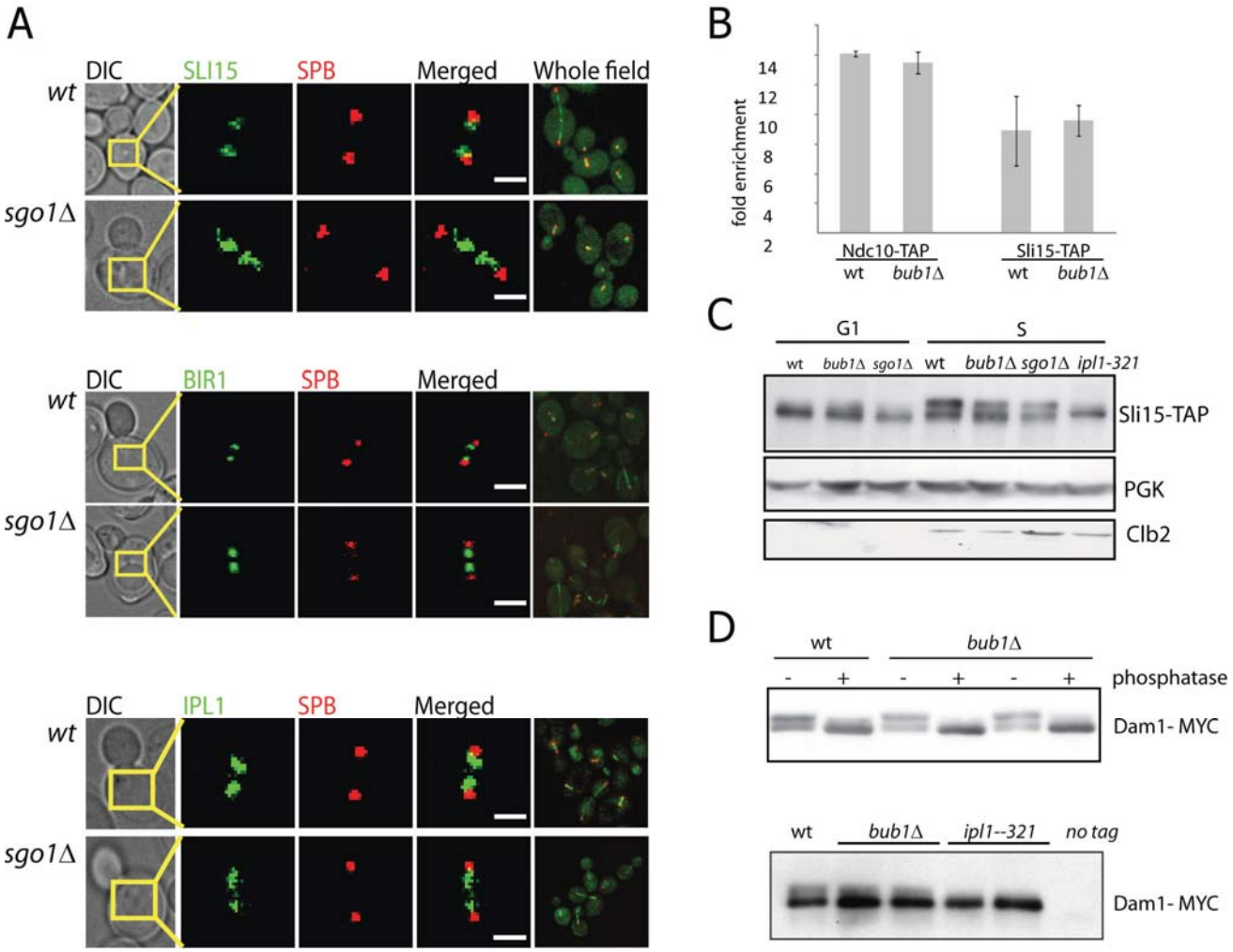

Fig. 4 The localization and activity of CPC proteins is not affected by the absence of Bub1 or Sgo1

A. The localization of Sli15-GFP, Bir1-GFP and Ipl1-GFP to pre-anaphase spindles is not diminished in the diploid *sgo1*Δ cells. SPBs are marked with Spc29-RFP. Bar - 1 μm. **B.** Sli15-directed chromatin immunoprecipation (ChIP) revealed a seven-fold enrichment of centromeric DNA relative to telomeric DNA in wild-type cells. This enrichment is not affected by absence of Bub1, similar to results obtained with Ndc10-directed ChIP. **C.** Phosphorylation of Sli15-TAP is not affected by the presence or absence of *BUB1* and *SGO1*. Cells were grown at 35°C, synchronized in α-factor (G1 phase) or hydroxyurea (S phase), harvested and analyzed by PAGE and western blotting. Slower migrating forms of Sli15-TAP were eliminated by mutation of the Ipl1 kinase domain, but not by deletion of the *SGO1* or *BUB1*. **D.** The phosphorylation of Dam1, the crucial substrate of Ip11 in the

release of microtubule attachments, is unaltered by the deletion of *BUB1* (*upper panel,* 30°C) but is abolished in the *ipl1-321* mutants at 37°C (*lower panel*). Two independent clones of both the *bub1*Δ and *ipl1-321* mutants were tested, and the separation between phosphoforms of Dam1-myc9 was enhanced by adding 10 μM Phos-Tag AAL-107 (Kinoshita *et al.*, 2006) to the polyacrylamide gel mixture. The slower migrating forms of Dam1-myc9 are sensitive to alkaline phosphatase treatment (*upper panel*).

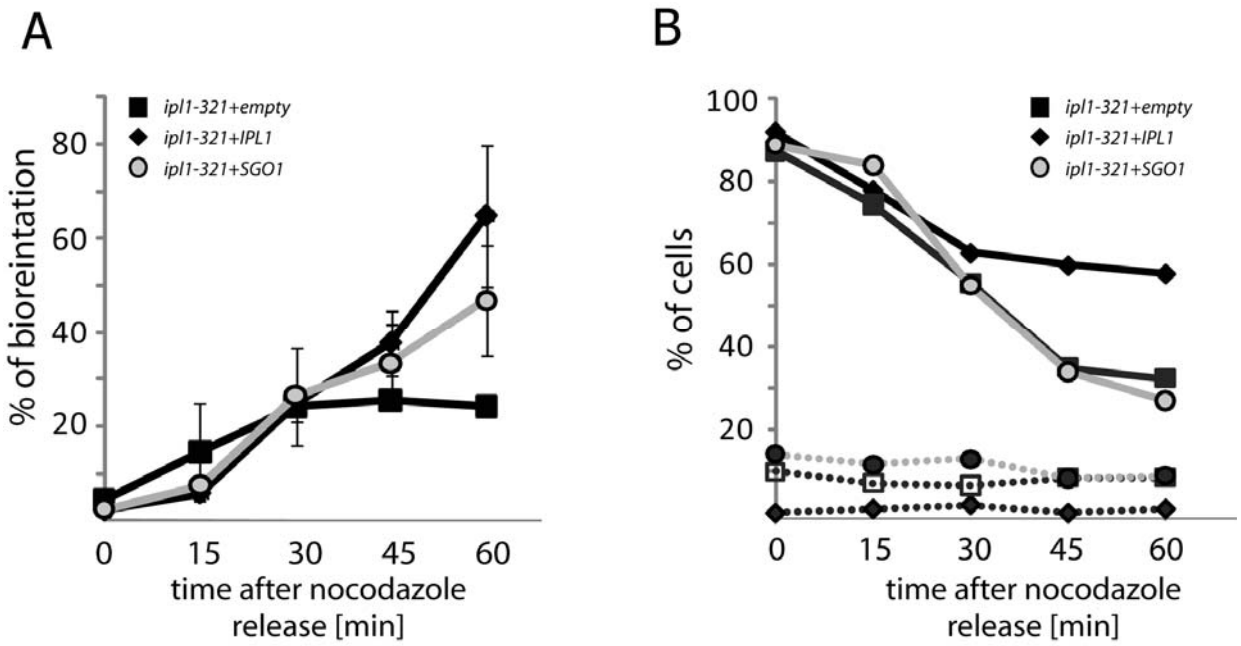

Fig. 5 Some monopolar attachments can be corrected independently of the chromosome passenger complex

A. Chromosome IV was marked with a TetO/TetR-GFP array. The percentage of cells with bi-oriented chromosome IV (as judged by the localization of the GFP dot in relation to the SPB marked) among all large budded cells was assessed after release from nocodazole. All strains carry a genomic *ipl1-321* allele as well as a multicopy plasmid with either functional *IPL1* gene (*black diamonds*), *SGO1* (*light circles*) or marker only (*grey squares*). **B**. Progression through anaphase after release from nocodazole is accelerated in cells lacking functional *IPL1*, as it is evidenced by the decrease of the percentage of large budded cells in population (full lines). These strains also accumulate bi-budded cells at higher level than wild type (dotted lines). Over-expression of *SGO1* did not alter the cell-cycle progression of *ipl1-321* mutants, even though it affected bi-orientation (panel A). The experiments were performed at 35^{o}C.

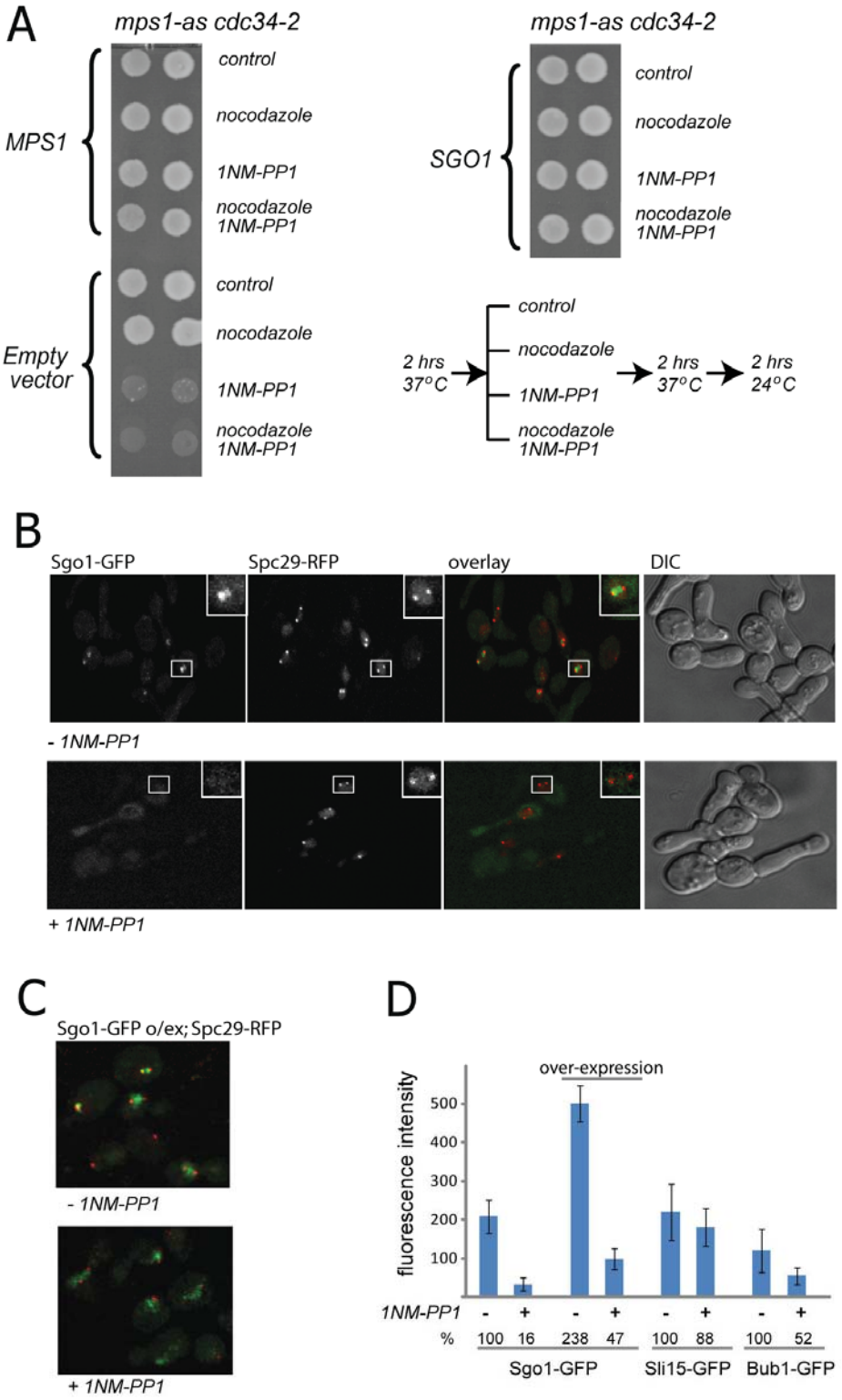

Fig.6 Sgo1 can partially bypass the role of Mps1 in chromosome segregation and appears to act in the same pathway

A. Over-expression of *SGO1* allows cells to survive transient pharmacological inhibition of Mps1 kinase during mitosis. The cells were synchronized in S phase (at the restrictive temperature for the *cdc34-2* allele), released into mitosis with or without the *mps1-as* inhibitor and with or without nocodazole, and then spotted on non-selective plates. **B.** Localization of Sgo1-GFP to pre-anaphase spindles requires active Mps1. The images were taken 10 min after adding DMSO (control samples) or the ATP analog 1NM-PP1 to inhibit Mps1. **C.** Over-expression of Sgo1-GFP on a 2-micron plasmid increases retention of Sgo1 on the mitotic spindle, even in the absence of targeting by Mps1. **D**. Quantification of the fluorescent signal on the spindle for Sgo1-GFP, over-expressed Sgo1-GFP, Sli15-GFP and Bub1-GFP in cells with active or inactive Mps1 kinase.