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Kinetochore Orientation during Meiosis Is Controlled by Aurora B and the Monopolin Complex

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SUMMARY

Kinetochores of sister chromatids attach to microtubules emanating from the same pole (coorientation) during meiosis I and microtubules emanating from opposite poles (biorientation) during meiosis II. We find that the Aurora B kinase Ipl1 regulates kinetochore-microtubule attachment during both meiotic divisions and that a complex known as the monopolin complex ensures that the protein kinase coorients sister chromatids during meiosis I. Furthermore, the defining of conditions sufficient to induce sister kinetochore coorientation during mitosis provides insight into monopolin complex function. The monopolin complex joins sister kinetochores independently of cohesins, the proteins that hold sister chromatids together. We propose that this function of the monopolin complex helps Aurora B coorient sister chromatids during meiosis I.

INTRODUCTION

The mitotic cell-division cycle is an alternation of chromosome duplication and segregation. During meiotic cell division, which generates gametes, DNA replication is followed by two rounds of chromosome segregation. During the first division, meiosis I, homologous chromosomes segregate away from each other. During the second division, meiosis II, sister chromatids separate. Central to accurate chromosome segregation is the correct attachment of chromosomes to the spindle apparatus. During mitosis and meiosis II, sister kinetochores attach to microtubules emanating from opposite spindle poles (biorientation). In meiosis I, when homologs segregate away from each other and hence are bioriented, sister chromatids segregate to the same spindle pole. Thus, sister kinetochores must attach to microtubules emanating from the same spindle pole, a phenomenon known as monopolar attachment or sister kinetochore coorientation.

In budding yeast, sister kinetochore coorientation during meiosis I is brought about by the monopolin complex (reviewed in Marston and Amon, 2004). Cells lacking components of this complex biorient sister kinetochores during meiosis I and attempt to separate sister chromatids during the first meiotic division (Rabitsch et al., 2003; Toth et al., 2000; Petronczki et al., 2006). To date, four components of the monopolin complex have been identified. Mam1 is a meiosis-specific protein present at kinetochores from pachytene to metaphase I (Toth et al., 2000). The monopolin complex components Csm1 and Lrs4 are expressed during both mitosis and meiosis. They reside in the nucleolus until G2, when they are released by the Polo kinase Cdc5 (Clyne et al., 2003; Rabitsch et al., 2003). After their release, Csm1 and Lrs4 form a complex with Mam1 and bind to kinetochores (Rabitsch et al., 2003). In addition, Mam1 recruits the ubiquitously expressed casein kinase 1δ/ε Hrr25, which is also required for sister kinetochore coorientation, to kinetochores during meiosis I (Petronczki et al., 2006). The meiosis-specific protein Spo13 is also necessary for kinetochore coorientation. In its absence, the monopolin complex initially associates with kinetochores but cannot be maintained there (Katis et al., 2004b; Lee et al., 2004). How the monopolin complex and proteins that regulate its association with kinetochores bring about sister kinetochore coorientation is poorly understood.

The protein kinase Aurora B is a key regulator of kinetochore-microtubule attachment. Aurora B (Ipl1 in yeast) forms a complex with INCENP (Sli15 in yeast), and this complex controls many aspects of chromosome segregation, including histone H3 phosphorylation (Hsu et al., 2000), cohesin removal (Resnick et al., 2006; Yu and Koshland, 2005), mitotic and meiotic spindle formation and stability (reviewed in Ducat and Zheng, 2004), chiasma resolution (Kaitna et al., 2002), and linking of

cytokinesis to chromosome segregation (Norden et al., 2006). In budding-yeast mitosis, the Ipl1-Sli15 complex was shown to sever kinetochore-microtubule attachments that are not under tension by phosphorylating kinetochore components such as Dam1 (Cheeseman et al., 2002; Dewar et al., 2004; Pinsky et al., 2006; Tanaka et al., 2002). Thereby, Ipl1 generates unattached kinetochores, which activates the spindle checkpoint. The spindle checkpoint inhibits an ubiquitin ligase known as the anaphase-promoting complex (APC) or cyclosome (C; reviewed in Lew and Burke, 2003), whose activity is essential for entry into anaphase through its role in promoting the degradation of securin (Pds1 in yeast). This degradation leads to activation of a protease known as separase (Esp1 in yeast). Once active, separase cleaves a component of cohesin complexes, which hold sister chromatids together. A role for Aurora B in regulating kinetochore-microtubule attachment during meiosis has not been demonstrated.

Here we investigate how Ipl1 and the monopolin complex regulate sister kinetochore orientation during meiosis. We find that Ipl1 is required for homolog biorientation during meiosis I as well as sister chromatid biorientation during meiosis II. Our data further show that Ipl1 is epistatic to the monopolin complex in the regulation of this process. Importantly, we find that an active monopolin complex is sufficient to promote sister kinetochore coorientation during mitosis. The ability to induce sister kinetochore coorientation during mitosis furthermore provides insight into one of the functions of the monopolin complex: it links sister kinetochores in a cohesin-independent manner.

RESULTS

Aurora B Localizes to Kinetochores and the Spindle during Meiosis

To examine the role of Ipl1 in yeast meiosis, we analyzed its protein levels and localization. Ipl1 was expressed throughout meiosis, but levels appeared lower as cells entered the meiotic cell cycle (see Figure S1A in the Supplemental Data available with this article online; 0 time point). Ipl1 activity, as judged by histone H3 phosphorylation, mirrored Ipl1 protein levels (Figure S1A). The localization of Ipl1 in meiosis resembled that in mitosis (Figure S1B; Tanaka et al., 2002; Pereira and Schiebel, 2003). Ipl1 localized to the nucleus in metaphase I and metaphase II. During anaphase I and anaphase II, the protein was also found on the meiotic spindle. Analysis of Ipl1 on chromosome spreads revealed that, early in meiosis, Ipl1 is found on chromosomes but does not localize to kinetochores (data not shown). However, at metaphase I, Ipl1 associates with kinetochores as judged by the colocalization with the kinetochore component Ndc10 (Figure S1C).

IPL1 Is Required for the Biorientation of Homologs during Meiosis I

To determine Ipl1's function during meiosis, we placed the *IPL1* open reading frame under the control of the *SCC1/MCD1* promoter, which is largely repressed during meiosis (Michaelis et al., 1997). This *pSCC1-IPL1* fusion was expressed during the mitotic cell cycle (Figure 1A), but, because Ipl1 is unstable during G1 (Biggins et al., 1999), the protein was rapidly depleted from cells entering the meiotic cell cycle (Figure 1A). Cells carrying the *pSCC1-IPL1* fusion as the sole source of Ipl1 did not exhibit proliferation defects during vegetative growth (data not shown), but progression through the meiotic cell cycle was affected. Cells exhibited a slight delay in entry into S phase (Figure 1B) and a moderate metaphase I and anaphase I delay, with spindles appearing thin and fragile (Figure 1C and data not shown). Despite these delays, 80% of cells eventually progressed through at least one meiotic division (Figure 1D). Similar results were obtained when Ipl1 was depleted by placing the *IPL1* ORF under the control of the mitosis-specific *CLB2* promoter (Figure S2 and data not shown).

To follow the fate of chromosomes during the meiotic divisions in the absence of Ipl1, we integrated a tandem array of tetO sequences near the centromere of chromosome V on both homologs (homozygous CENV GFP dots). These cells also expressed a tetR-GFP fusion, which binds to tetO, to visualize the repeats (Michaelis et al., 1997). The analysis of homozygous GFP dots revealed that 80% of Ipl1-depleted cells segregated homologs to the same spindle pole rather than, as in wild-type cells, to opposite poles (Figures 1E and 1F). Similar results were obtained when we analyzed the chromosome segregation behavior of chromosome III or both chromosomes III and V (data not shown). This highly asymmetric chromosome segregation resulted in the two anaphase I DNA masses being of unequal size (data not shown).

During mitosis, cells defective in *IPL1* function preferentially segregate both sister chromatids with the old spindle pole body (SPB) into the bud (Pereira et al., 2001; Tanaka et al., 2002). This is likely due to the fact that the duplication of kinetochore structures and subsequent microtubule capture occur prior to maturation of the newly synthesized SPB. Consequently, both sister chromatids attach to microtubules emanating from the same spindle pole. Owing to the failure of cells lacking *IPL1* to detach incorrect microtubule attachments, sister chromatids preferentially cosegregate with the old SPB into the bud. Consistent with this idea is the observation that the preferential cosegregation of sister chromatids with the old SPB can be partially rescued by transient microtubule depolymerization (Pereira et al., 2001; Tanaka et al., 2002). Transient treatment with the microtubule-depolymerizing drug benomyl during prophase I (4 hr after induction of meiosis) also partially rescued the cosegregation of homologs in Ipl1-depleted meiotic cells. Whereas 80% of homologs cosegregated to the same pole in mock-treated Ipl1-depleted cells, homolog segregation was nearly random (60% cosegregation and 40% separation) when cells were treated with benomyl (Figure 1F; note that the expected ratio for random

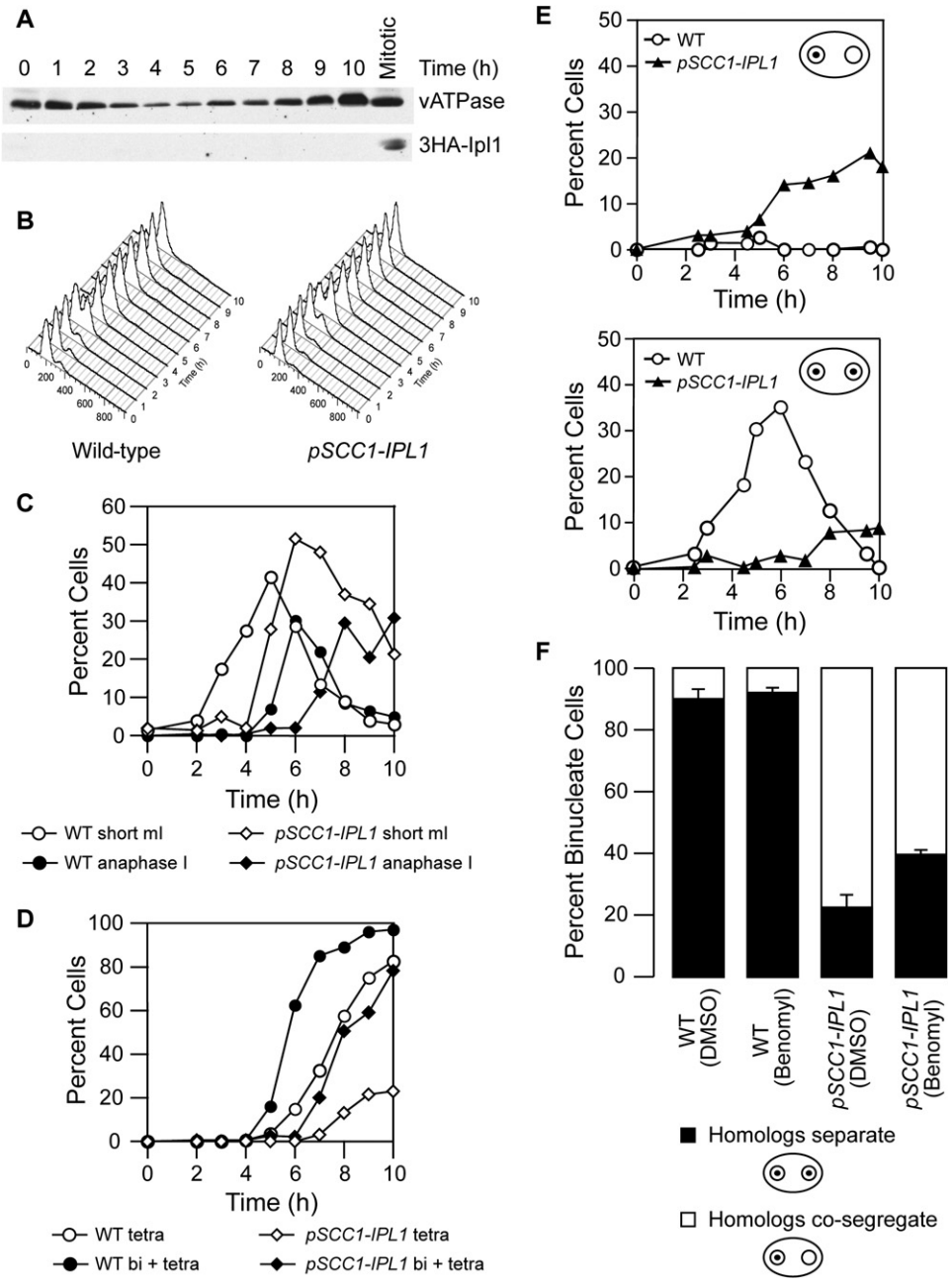

Figure 1. Ipl1 Regulates Meiosis I Chromosome Segregation

(A) *pSCC1-3HA-IPL1* cells (A10423) were induced to sporulate to examine 3HA-Ipl1 levels at the indicated times. vATPase was used as a loading control.

(B) Wild-type (A5811) and *pSCC1-3HA-IPL1* (A10423) cells were induced to sporulate to determine DNA content by FACS.

(C–E) Wild-type (A5715) and *pSCC1-3HA-IPL1* (A14502) cells, both carrying homozygous CENV GFP dots, were induced to sporulate to determine spindle morphology (C), nuclear morphology (D), and CENV GFP dot segregation (E).

(F) Strains described in (C–E) were resuspended in sporulation (SPO) medium containing 120 μg/ml benomyl (benomyl) or DMSO (1%; mock) 4 hr after induction of sporulation. After 30 min, cells were washed and resuspended in SPO medium. Samples were taken between 3 and 6 hr thereafter, and CENV GFP dot segregation was determined. In this and all other figures, error bars represent ± standard deviation.

Note that we only determined the presence of GFP dots but not the number of GFP dots per nucleus.

segregation would be 50%:50%). Our results indicate that *IPL1* is required for accurate homolog segregation during meiosis I. We propose that, as during mitosis, Ipl1 does so by promoting microtubule attachment turnover until all homologs are correctly oriented on the meiosis I spindle.

Aurora B Regulates Meiosis II Chromosome Segregation

To determine the role of Ipl1 in meiosis II chromosome segregation, we examined cells carrying the tetO array on only one of the two homologs (heterozygous CENV GFP dots). Ipl1-depleted cells showed normal segregation of heterozygous CENV GFP dots during the first meiotic division (i.e., the GFP signal was present in one of the two nuclei after the first meiotic division; Figure 2A, upper panels), indicating that sister chromatids did not separate prematurely during meiosis I. However, 60% of the cells that underwent a second meiotic division missegregated chromosomes, resulting in the generation of four nuclei of unequal size (Figure 2A, lower panels and data not shown). Because Ipl1-depleted cells undergo the second meiotic division with poor efficiency, we also examined Ipl1-depleted cells deleted for *SPO11*. *SPO11* encodes the topoisomerase-like enzyme responsible for generating recombination-initiating double-strand breaks (Bergerat et al., 1997; Keeney et al., 1997), and deletion of *SPO11* allowed Ipl1-depleted cells (as is the case in many other meiotic mutants) to progress through the second meiotic division more efficiently (compare Figure 1D and Figure 2B). Missegregation of sister chromatids was even more pronounced in Ipl1-depleted cells lacking *SPO11*: eighty percent of sister chromatids segregated to the same pole during the second meiotic division (Figure 2B). Owing to the resemblance of the meiosis II phenotype of *pSCC1-IPL1 spo11*Δ cells to that of *IPL1*-deficient mitotic cells, we conclude that *IPL1* is required for sister kinetochore biorientation during meiosis II.

Aurora B Affects the Stepwise Loss of Sister Chromatid Cohesion during Meiosis

During mitosis, cohesins are lost along the entire length of chromosomes at the onset of anaphase, whereas during meiosis, cohesins are lost in a stepwise manner (reviewed in Marston and Amon, 2004). Loss of cohesins from chromosome arms is essential for homologs to segregate during meiosis I, and retention of cohesins around centromeres is necessary for sister chromatids to segregate accurately during meiosis II. To determine whether Ipl1 in addition to kinetochore orientation also regulates the loss of sister chromatid cohesion, we examined the localization of the cohesin subunit Rec8 on chromosome spreads. Cells also carried a tagged version of the kinetochore component Ndc10 to identify centromeric regions of chromosomes. In wild-type binucleate cells, Rec8 was found around centromeres (Figure 2C). In contrast, nearly 50% of Ipl1-depleted binucleate cells lacked centromeric Rec8 (Figure 2C). As a control, we also examined the localization of Rec8 in cells lacking *SGO1*, a gene essential to protect Rec8 from removal around centromeres during meiosis I (Katis et al., 2004a; Kitajima et al., 2004; Marston et al., 2004). In such cells, Rec8 was absent in binucleate cells (Figure 2C). Our results indicate that *IPL1* is required to retain Rec8 at centromeres beyond the first meiotic division, though the gene appears to be less important than *SGO1*.

Ipl1-depleted cells also exhibited defects in the localization of the cohesin protector Sgo1, which itself associates with centromeric regions from prophase I until metaphase II (Katis et al., 2004a; Marston et al., 2004). Only 50% of mononucleate and binucleate Ipl1-depleted cells exhibited Sgo1 localization (Figure 2D and data not shown). Deletion of *SPO13*, a gene required for the maintenance of Sgo1 at centromeres (Figure 2D; Lee et al., 2004), did not affect Sgo1 localization in mononucleate cells but had more severe effects on Sgo1 localization than Ipl1 depletion in binucleate cells (Figure 2D and data not shown; Katis et al., 2004b; Lee et al., 2004). How Ipl1 affects cohesin loss and why Ipl1 depletion only partially affects Rec8 and Sgo1 localization are at present unclear. The severity of the homolog-cosegregation phenotype of Ipl1-depleted cells (80% cosegregation of homologs during meiosis I) argues against incomplete inactivation of Ipl1 being responsible for the partial effects on Rec8 and Sgo1 localization. Parallel pathways could account for the incomplete penetrance of the phenotype. We note that our findings are consistent with observations in *Drosophila*, where the Sgo1 homolog MEI-S332 requires Aurora B and INCENP for its association with pericentric regions (Resnick et al., 2006). Our results indicate that *IPL1* is required for two key aspects of the second meiotic division, sister kinetochore biorientation and the correct timing of loss of cohesins from chromosomes.

Depletion of Ipl1 Suppresses the Coorientation Defect of *mam1*Δ and *spo13*Δ Mutants

Having established that Ipl1 regulates kinetochore orientation during meiosis, we next examined the relationship between Ipl1 and coorientation factors. The majority of cells lacking *MAM1* and *SPO11* carrying heterozygous CENV GFP dots segregate sister chromatids during the first observable chromosome segregation phase, leading to the formation of binucleate cells with a GFP dot in each of the two nuclei (Toth et al., 2000; Figure 3A). Remarkably, depletion of Ipl1 in such cells led to the cosegregation of sister chromatids to one spindle pole (Figure 3A). Similar results were obtained when Ipl1 was depleted in cells lacking *SPO11* and *SPO13*. *spo13*Δ *spo11*Δ mutants undergo a single meiotic division during which sister chromatids segregate to opposite poles (Klapholz et al., 1985; Figure 3B). Depletion of Ipl1 in these cells led to the cosegregation of sister chromatids (Figure 3B). Our results indicate that biorientation of sister kinetochores in *mam1*Δ or *spo13*Δ mutants requires *IPL1* function. Inactivation of *SPO13* or *MAM1* changed neither Ipl1 localization (Figures S3A and S3B) nor its ability to phosphorylate histone H3 (Figure S3C), indicating that the two proteins did not affect Ipl1 function. The simplest interpretation of our findings is that Ipl1 performs the same function during meiosis I as it does during mitosis and meiosis II—that is, severing microtubule-kinetochore attachments that are not under tension. The monopolin complex's function is to change sister kinetochores in such a way that they are only under tension when homologs are bioriented.

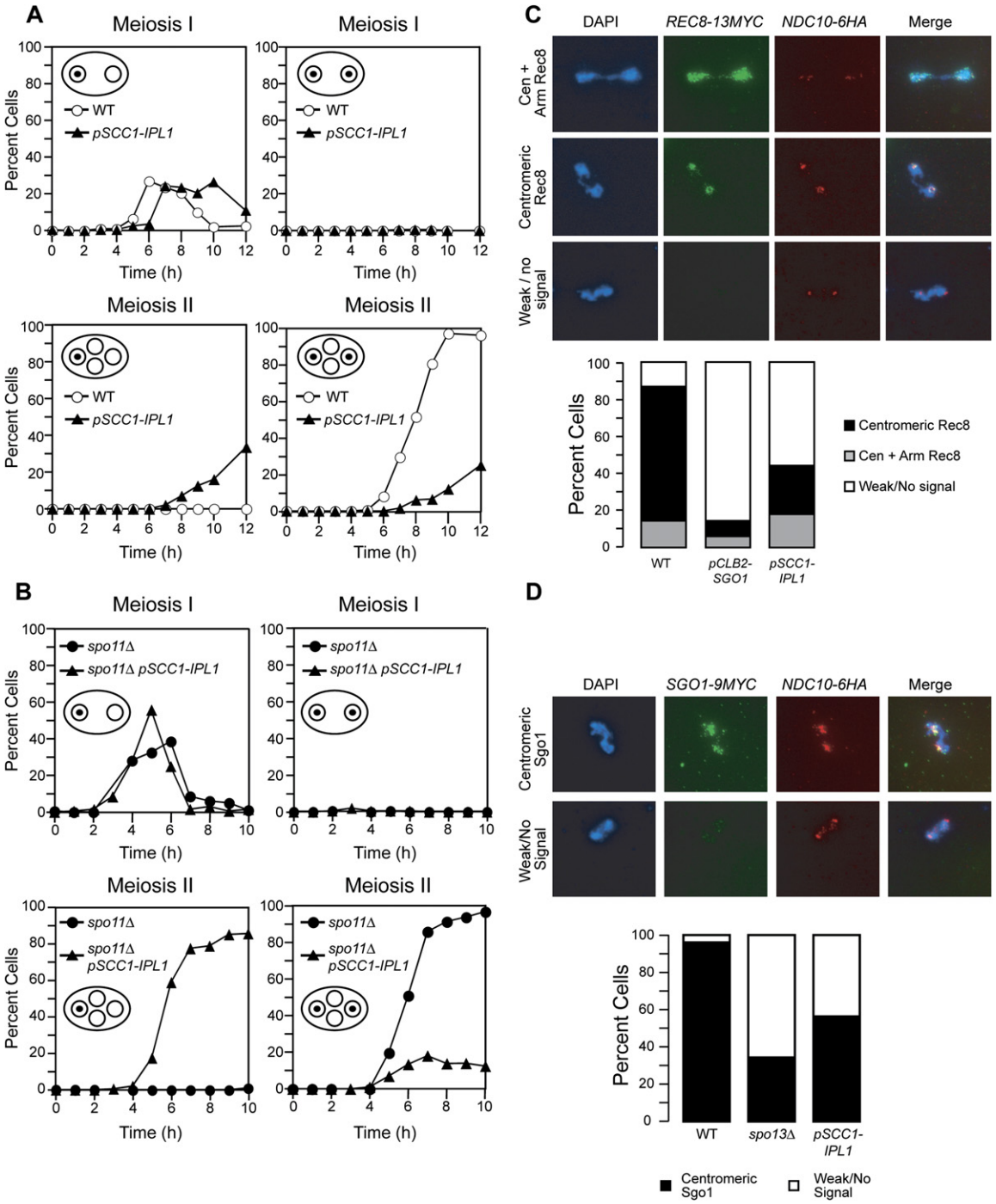

Figure 2. *IPL1* Controls Multiple Meiosis II Events

(A) Wild-type (A5811) and *pSCC1-3HA-IPL1* (A10423) cells carrying heterozygous CENV GFP dots were induced to sporulate to determine GFP dot segregation at the indicated times.

(B) *spo11*Δ (A9498) and *spo11*Δ *pSSC1-3HA-IPL1* (A10425) cells carrying heterozygous CENV GFP dots were induced to sporulate to determine GFP dot segregation at the indicated times.

(C) Wild-type (A10483), Ipl1-depleted (A15201), and Sgo1-depleted (A15056) cells carrying a *NDC10-6HA* and a *REC8-13MYC* fusion were induced to sporulate. Chromosome spreads were prepared at 5, 6, and 8 hr after sporulation induction, and Rec8 localization was analyzed in binucleate cells (n = 50).

(D) Wild-type (A10461), *spo13*Δ (A10755), and *pSCC1-3HA-IPL1* (A15169) cells carrying a *NDC10-6HA* and a *SGO1-9MYC* fusion were induced to sporulate to examine Sgo1 localization as described in (C).

Note that we only determined the presence of GFP dots but not the number of GFP dots per nucleus.

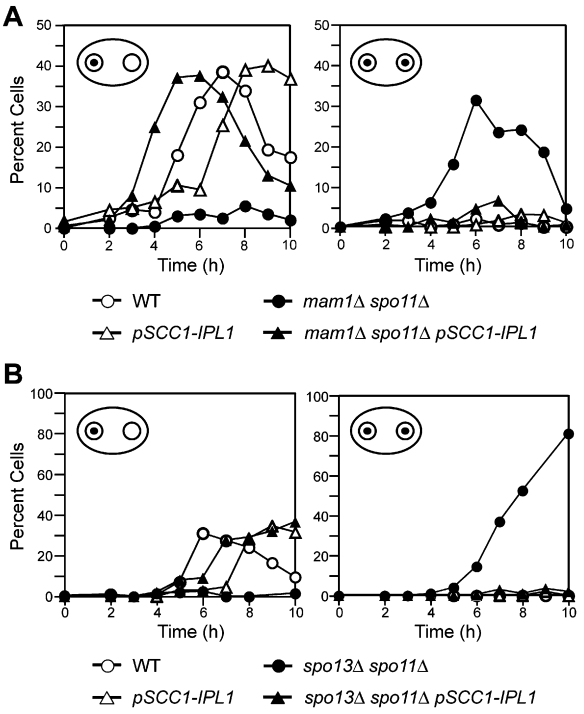

Figure 3. The Ipl1-Depletion Phenotype Is Epistatic to that Caused by the Inactivation of *MAM1* or *SPO13*

Wild-type (A5811), *pSCC1-3HA-IPL1* (A10423), *mam1*Δ *spo11*Δ (A8128), and *mam1*Δ *spo11*Δ *pSCC1-3HA-IPL1* (A15164) cells (A) and wild-type (A5811), *pSCC1-3HA-IPL1* (A10423), *spo13*Δ *spo11*Δ (A7170), and *spo13*Δ *spo11*Δ *pSCC1-3HA-IPL1* (A11432) cells (B), all carrying heterozygous CENV GFP dots, were induced to sporulate to determine GFP dot segregation at the indicated times.

An Active Monopolin Complex Is Sufficient to Promote Sister Kinetochore Coorientation during Mitosis

To gain further insights into how the monopolin complex brings about sister kinetochore coorientation, we wished to define the minimal number of genes necessary for this process to occur during mitosis. The monopolin complex component Mam1 is not expressed during mitosis. Overexpression of *MAM1* alone is, however, not sufficient for sister kinetochore coorientation to occur during mitosis (Figure 4A; Toth et al., 2000). As Mam1 requires Lrs4 and Csm1 to associate with kinetochores (Rabitsch et al., 2003), the fact that Lrs4 and Csm1 are not released from the nucleolus during mitotic G2 (Toth et al., 2000; Figure S4) could be responsible for Mam1's inability to promote sister kinetochore coorientation during mitosis.

To release Lrs4 and Csm1 from the nucleolus, we overexpressed *CDC5* from the galactose-inducible *GAL1* promoter. The presence of a single copy of *CDC5* expressed from the *GAL1* promoter did not interfere with cell-cycle progression (data not shown) but led to the release of Lrs4 from the nucleolus (Figure S4). As Csm1 localization and Lrs4 localization are interdependent (Rabitsch et al., 2003), Csm1 release is also likely to occur. Lrs4, however, failed to associate with kinetochores in *GAL-CDC5* cells (Figure 4B). Co-overexpression of *MAM1* and *CDC5* from the *GAL1* promoter led to Lrs4 association with kinetochores (Figure 4B), indicating that *CDC5* is required to release the Lrs4-Csm1 complex from the nucleolus and that only when Mam1 is present are the two proteins efficiently recruited to kinetochores.

Cells overproducing Cdc5 and Mam1 progressed through mitosis with kinetics similar to that of wild-type cells (Figure 4C). Degradation of Pds1, however, was delayed by 15 min (Figure 4D), indicating that the spindle checkpoint was transiently activated. The analysis of CENIV GFP or CENV GFP dot segregation revealed that 35% of *GAL-CDC5 GAL-MAM1* cells segregated both sister chromatids to the same spindle pole (Figure 4A and data not shown). The cosegregation of sister chromatids depended on the monopolin complex components Lrs4 and Csm1. Deletion of *LRS4* reduced sister chromatid cosegregation to 13%. Inactivation of both *LRS4* and *CSM1* reduced it further to 4% (Figure 4A). Overexpression of *SPO13* did not lead to an increase in *LRS4*/*CSM1*-dependent sister chromatid cosegregation in *GAL-CDC5 GAL-MAM1* cells (see Supplemental Results and Figure S5), suggesting that high levels of Spo13 do not enhance sister kinetochore coorientation when Cdc5 and Mam1 are overproduced. We conclude that overexpression of *CDC5* and *MAM1* is sufficient to promote coorientation of sister kinetochores. This cosegregation of sister chromatids is accompanied by a slight delay in Pds1 degradation, suggesting that the lack of tension caused by the cosegregation of sister chromatids leads to Ipl1-dependent microtubule severing, which results in a transient activation of the spindle checkpoint.

Establishing Sister Kinetochore Coorientation during Mitosis Does Not Interfere with *IPL1* Function

Our *mam1*Δ *pSCC1-3HA-IPL1* and *spo13*Δ *pSCC1-3HA-IPL1* double-mutant analysis indicated that coorientation factors either functioned as inhibitors of Ipl1 or were modifying sister kinetochores in such a way that Ipl1 was not able to biorient them. Several observations argue against Spo13 and Mam1 inhibiting Ipl1 function. First, overexpression of *CDC5* and *MAM1* during mitosis promotes sister kinetochore cosegregation, which is accompanied by a modest delay in Pds1 degradation (Figure 4D). Second, Ipl1 levels, localization, and overall kinase activity (as judged by histone H3 phosphorylation) were not affected in *GAL-CDC5 GAL-MAM1* strains (data not shown). Third, we did not detect any genetic interactions between coorientation factors and *IPL1* gain- and loss-of-function alleles. Overexpression of *CDC5* and *MAM1* did not enhance the chromosome segregation defect of temperature-sensitive *ipl1-321* mutants (Biggins et al., 1999) at intermediate growth temperatures. At 34°C, *ipl1-321 GAL-CDC5 GAL-MAM1* mutants exhibited the same phenotype as *ipl1-321* mutants (Figure 5A). At 25°C and 30°C, the strain showed the same phenotype as the *GAL-CDC5 GAL-MAM1* strain (Figure 5A). Fourth, overexpression of

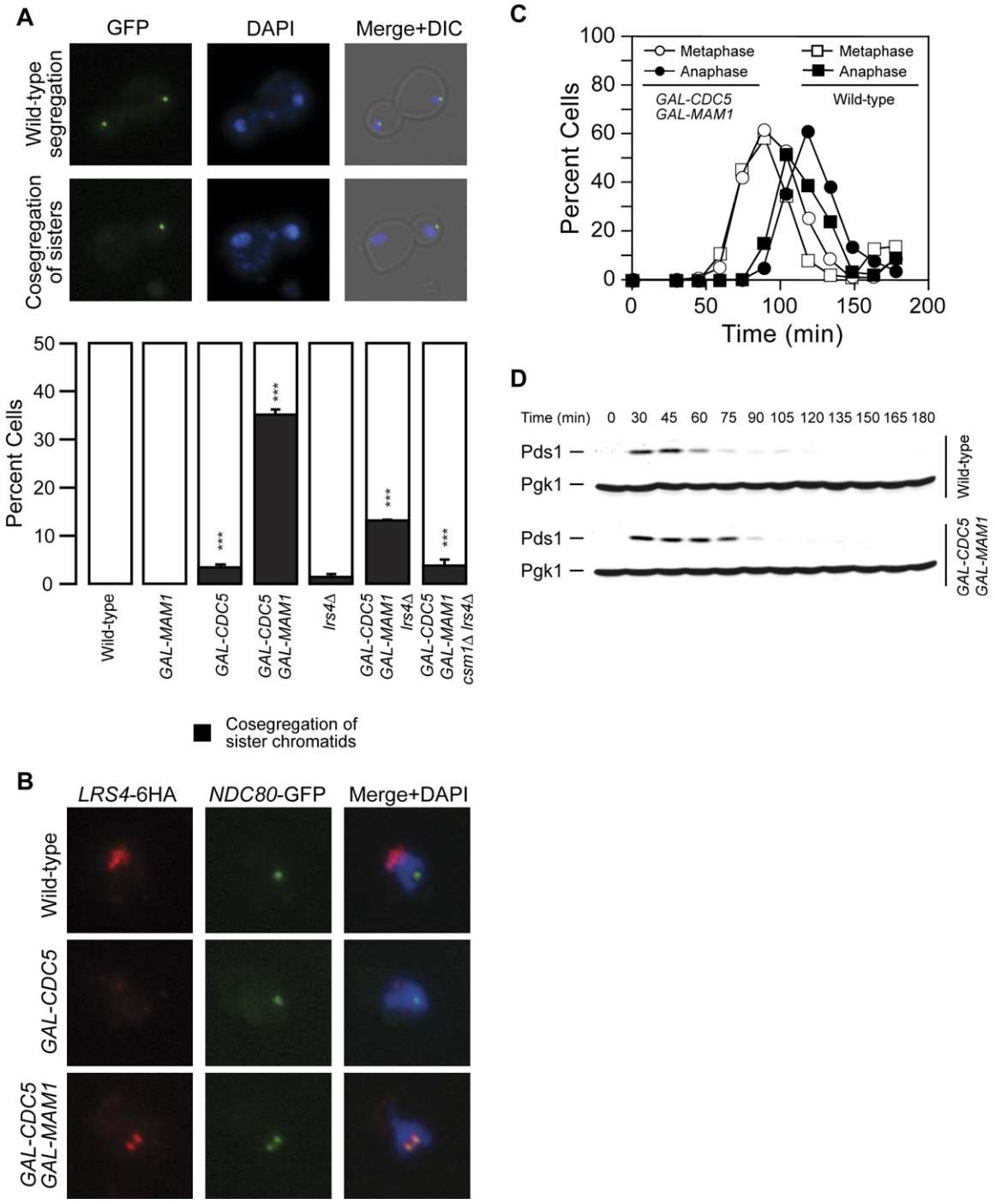

Figure 4. An Active Monopolin Complex Is Sufficient to Promote Sister Kinetochore Coorientation

(A) Wild-type (A5244), *GAL-MAM1* (A12315), *GAL-CDC5* (A12325), *GAL-CDC5 GAL-MAM1* (A12312), *Irs4*Δ (A15911), *GAL-CDC5 GAL-MAM1 Irs4*Δ (A15910), and *GAL-CDC5 GAL-MAM1 Irs4*Δ *csm1*Δ (A16882) cells, all carrying CENIV GFP dots, were arrested in G1 using 5 μg/ml α factor and treated with galactose for 1 hr prior to release. When arrest was complete, cells were released into medium lacking pheromone and containing 2% galactose. Samples were taken to determine GFP dot segregation. Data represent the average of three experiments; ***p ≤ 0.001 relative to wild-type.

(B) Wild-type (A15127), *GAL-CDC5* (A15926), and *GAL-CDC5 GAL-MAM1* (A15925) cells carrying *LRS4-6HA* and *NDC80-GFP* were grown as in (A) to determine the localization of Lrs4-HA on chromosome spreads. Lrs4-6HA is shown in red, Ndc80-GFP in green, and DNA in blue.

(C and D) Wild-type (A15912, squares) and *GAL-CDC5 GAL-MAM1* (A15915, circles) cells, all carrying *PDS1-3HA* fusions, were grown as in (A) except that α factor was added again (5 μg/ml) 90 min after release from G1 arrest. Samples were taken to determine the percentage of metaphase (open symbols; C) and anaphase (closed symbols; C) spindles and Pds1-3HA protein levels (D). Pgk1 was used as a loading control.

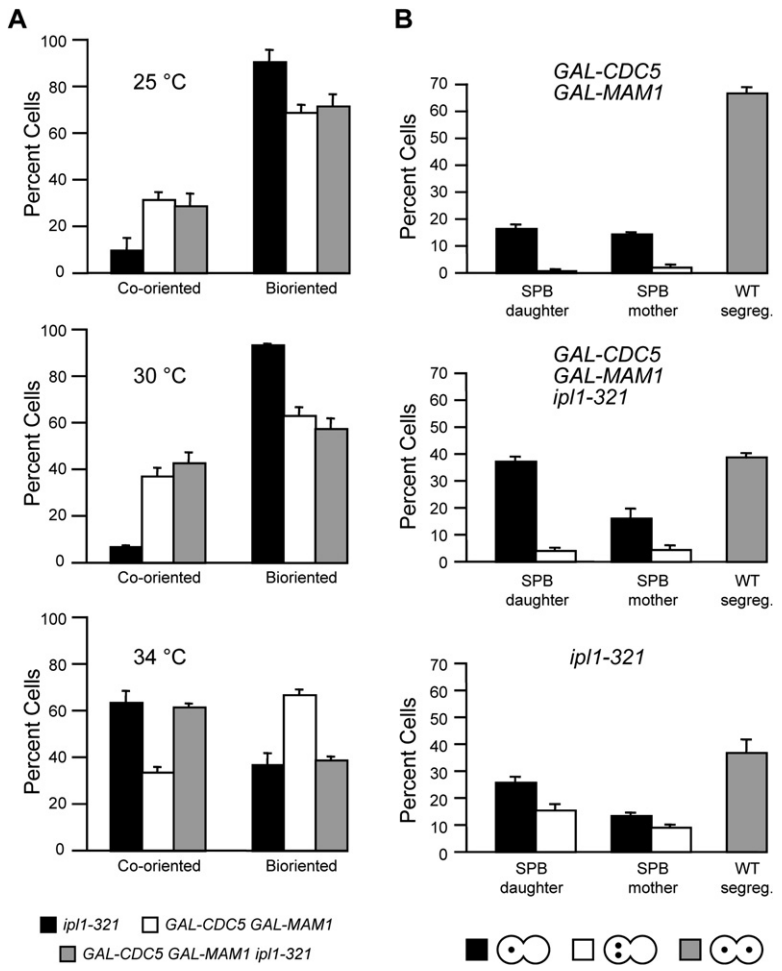

Figure 5. Effects of Overproducing Cdc5 and Mam1 on *ipl1-321* Mutants

GAL-CDC5 GAL-MAM1 (A12312), *ipl1-321* (A16485), and *GAL-CDC5 GAL-MAM1 ipl1-321* (A15931) cells, all carrying CENIV GFP dots, were arrested in G1 as described in Figure 4A, followed by release into medium lacking pheromone and containing 2% galactose at 25°C, 30°C, or 34°C.

(A) The percentage of cosegregating and correctly segregating (bioriented) sister chromatids was determined in anaphase cells. Data represent the average of three experiments.

(B) The percentage of the following three classes of anaphase cells was determined at 34°C: cosegregating sister chromatids that segregated into the bud (SPB daughter), cosegregating sister chromatids that segregated into the mother (SPB mother), and correctly segregating sister chromatids (WT segreg.). Within the first two classes, distinction was made between cosegregating sister chromatids tightly paired (black bars) and cosegregating sister chromatids not paired (white bars).

IPL1 did not affect sister chromatid cosegregation in *GAL-CDC5 GAL-MAM1* cells (data not shown). Finally, the cosegregation of sister chromatids in *GAL-CDC5 GAL-MAM1* cells differed from that observed in *ipl1-321* mutants. Whereas sister chromatids preferentially segregate together with the old SPB into the bud during mitosis in *ipl1-321* mutants (65%–70%; Tanaka et al., 2002; Figure 5B), cosegregation of sister chromatids did not show a SPB preference in *GAL-CDC5 GAL-MAM1* cells (47% in the mother, 53% in the daughter; Figure 5B). These observations, together with the finding that inactivation of the monopolin complex does not affect Ipl1 localization and kinase activity during meiosis, indicate that the monopolin complex does not inhibit Ipl1 but rather acts on the kinetochore to facilitate cosegregation of sister chromatids.

The Monopolin Complex Joins Sister Kinetochores Independently of Cohesins during Mitosis

Insights into monopolin complex function came from the analysis of GFP dots in mitotic cells induced to cosegregate sister chromatids. We observed that cosegregating CENIV GFP dots were always tightly paired in *GAL-CDC5 GAL-MAM1* cells (Figure 4A). In contrast, cosegregating telomeric (TELV) GFP dots were paired only half of the time (Figure 6C). The tight association of sister chromatids at centromeres is specific to cosegregation brought about by overproduction of Cdc5 and Mam1 and is not a phenomenon that generally occurs when sister chromatids cosegregate to the same spindle pole. We observed two distinct GFP signals during anaphase in wild-type cells carrying GFP dots 1.4 and 2 kb away from the centromere of chromosomes IV and V, respectively (Figure 6A). More importantly, in two other mutants that cosegregate sister chromatids, two individual GFP dots were seen in a significant fraction of anaphase cells. In cells lacking cohesins due to the depletion of the cohesin subunit Scc1/Mcd1, approximately 50% of cosegregating sister chromatids were pulled to the spindle pole individually, as judged by the fact that two distinct GFP dots were visible in one of the two nuclear lobes when sister chromatids segregated to the same pole (Figure 6B). Overexpression of *CDC5* and *MAM1* led to an increase in sister chromatid cosegregation from 29% to 44% in such cells, and, importantly, sister centromeres remained tightly associated during anaphase under these

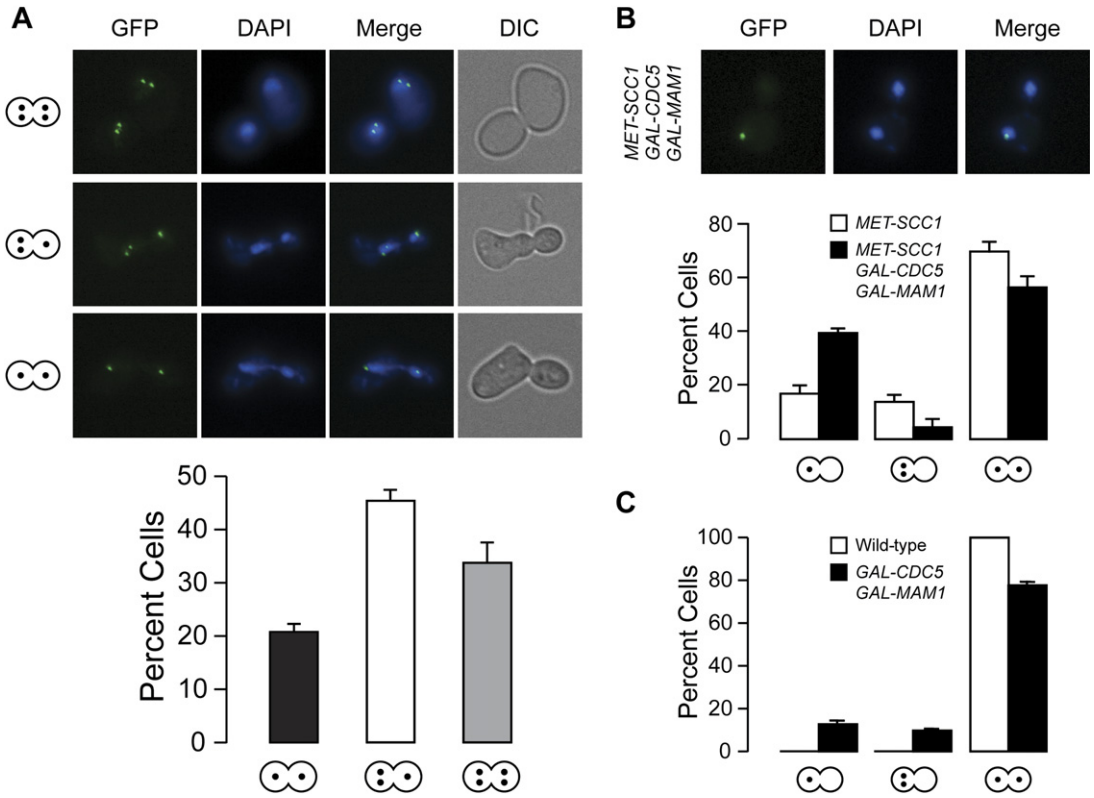

Figure 6. The Monopolin Complex Fuses Sister Kinetochores Together

(A) Haploid wild-type cells carrying GFP dots on both chromosomes IV and V (A15978) were arrested in G1 using 5 μg/ml α factor. Galactose was added 1 hr prior to release. When arrest was complete, cells were released into medium containing galactose but lacking pheromone. The presence of one or two GFP dots in each nuclear lobe was determined in anaphase cells.

(B) Haploid *MET-SCC1* (A16486) and *GAL-CDC5 GAL-MAM1 MET-SCC1* (A16023) cells carrying CENV GFP dots were arrested in G1 in media lacking methionine using 5 μg/ml α factor, preinduced with 2% galactose and 8 mM methionine for 1 hr, and released into YEP medium lacking pheromone and containing 2% galactose and 8 mM methionine at 25°C. Cells were analyzed as in (A).

(C) Wild-type (A5237) and *GAL-CDC5 GAL-MAM1* (A16883) cells carrying TELV GFP dots were grown as described in Figure 4A to determine GFP dot segregation. In this set of strains, only 25% of cells cosegregated sister chromatids. The reasons for the lower levels of cosegregation in this strain are unclear.

conditions (Figure 6B). In another mutant that cosegregates sister chromatids, the *ipl1-321* mutant, two distinct GFP signals were observed in approximately 40% of cells with cosegregating sister chromatids, but GFP dots appeared as one again in most cells when Cdc5 and Mam1 were overproduced in the mutant (Figure 5B).

Could the cosegregation of sister chromatids in *GAL-CDC5 GAL-MAM1* mutants depleted of cohesins be due to only one of the sister kinetochores attaching to a microtubule and the second sister chromatid being dragged along due to cohesin-independent linkages? We can exclude this possibility because in cells lacking cohesins and functional kinetochores (by inactivating *NDC10*), single chromatids are left behind at the metaphase plate during chromosome segregation (data not shown; Tanaka et al., 2002). Together, our data indicate that sister chromatids normally segregate independently even under conditions when they cosegregate to the same spindle pole, but overexpression of *CDC5* and *MAM1* induces a tight association between the cosegregating sister chromatids at centromeres that is independent of cohesins.

A *MAM1*-Dependent Linkage Joins Sister Chromatids in the Absence of *REC8*

Next we investigated whether sister kinetochores are also joined by the monopolin complex during meiosis I. If sister kinetochores were linked during meiosis I in a cohesin-independent manner, sister chromatids should cosegregate to the same spindle pole even in the absence of sister chromatid cohesion. Previous studies indicated that, in cells lacking *REC8*, 65% of sister chromatids segregate to the same pole during anaphase I. However, the percentage of cells progressing past prophase I in the absence of *REC8* is exceedingly small (10%–15%) because of defects in recombination leading to the activation of the recombination checkpoint (Klein et al., 1999). We therefore investigated the segregation behavior of sister chromatids in *rec8*Δ cells in the absence of recombination

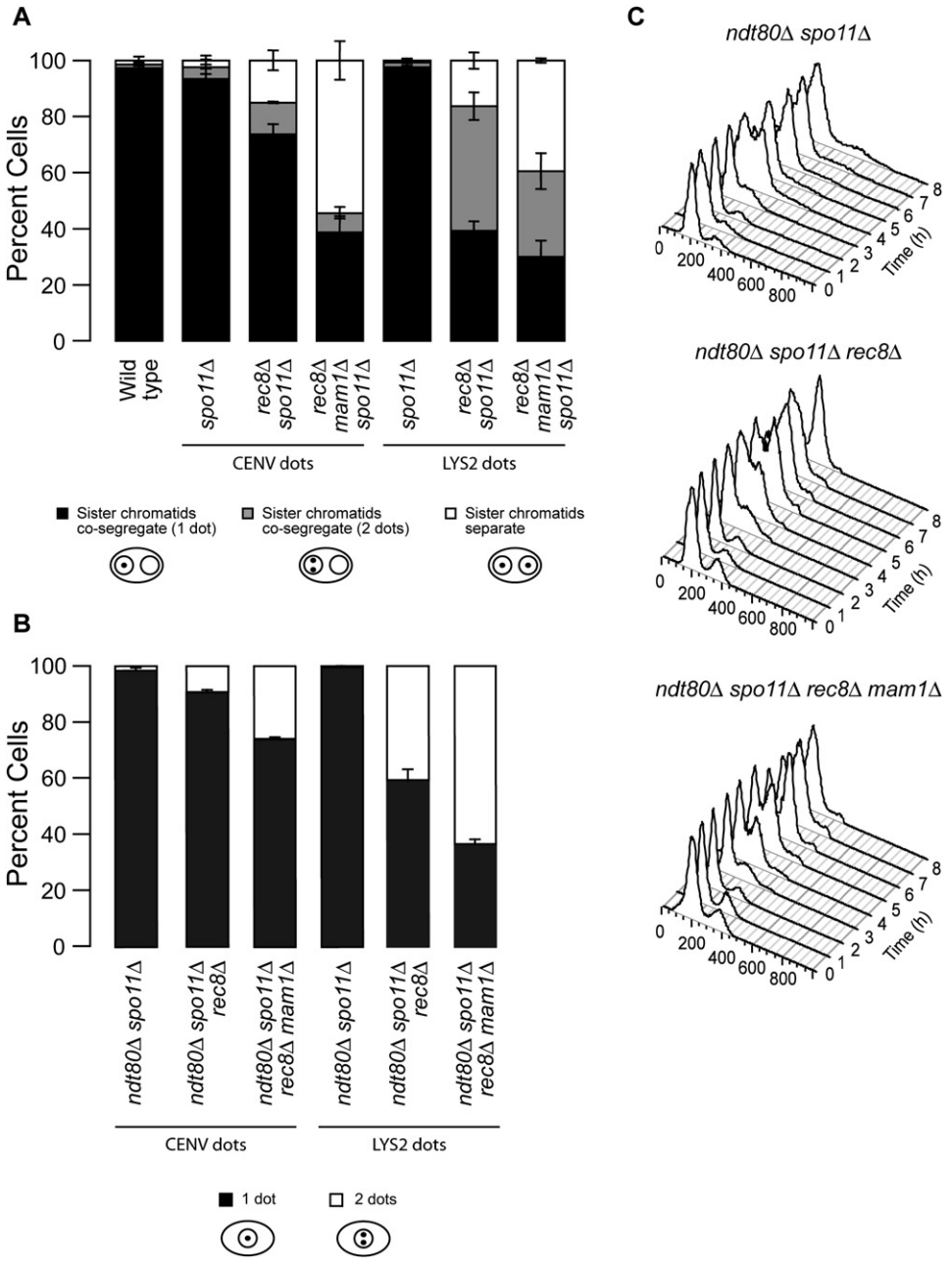

Figure 7. The Cosegregation of Sister Chromatids Observed in *rec8*Δ Cells Depends in Part on *MAM1*

(A) Wild-type (A5811), *spo11* (A9498), *rec8*Δ *spo11*Δ (A16020), and *rec8*Δ *mam1*Δ *spo11*Δ (A16342) cells carrying heterozygous CENV GFP dots and *spo11*Δ (A16725), *rec8*Δ *spo11*Δ (A16838), and *rec8*Δ *mam1*Δ *spo11*Δ (A16839) cells carrying heterozygous LYS2 GFP dots were induced to sporulate to examine the distribution of GFP dots in binucleate cells 6 hr after induction. Strains A16838 and A16839 were analyzed after 8 hr to allow for a more complete segregation of chromosome arms away from the midzone. (n = 100 cells for A16838 and n = 150 cells for A16839; n = 200 cells for all other strains.)

(B and C) *spo11*Δ *ndt80*Δ (A16840), *rec8*Δ *spo11*Δ *ndt80*Δ (A16841), and *rec8*Δ *mam1*Δ *spo11*Δ *ndt80*Δ (A16842) cells carrying heterozygous CENV GFP dots and *spo11*Δ *ndt80*Δ (A16835), *rec8*Δ *spo11*Δ *ndt80*Δ (A16836), and *rec8*Δ *mam1*Δ *spo11*Δ *ndt80*Δ (A16837) cells carrying heterozygous LYS2 GFP dots were induced to sporulate to examine the association of GFP dots (B; 6 hr time point was analyzed) and DNA content (C; CENV GFP dot strains are shown).

brought about by the deletion of *SPO11*. Remarkably, more than 80% of sister chromatids segregated to the same spindle pole in *rec8*Δ *spo11*Δ mutants carrying GFP dots either near the centromere (CENV dots) or at chromosome arms (LYS2 dots, Figure 7A). Furthermore, the majority of CENV GFP dots appeared as one, while

chromosome arms (LYS2 dots) were paired only half of the time (Figure 7A), indicating that the tight association of sister chromatids is restricted to the centromeric region. Importantly, the cosegregation of sister chromatids was in part dependent on a functional monopolin complex, because it was reduced in *rec8*Δ *spo11*Δ *mam1*Δ triple mutants (Figure 7A).

To examine whether the monopolin complex also affects the association of sister chromatids prior to meiosis I chromosome segregation, we examined the effects of deleting *MAM1* in *rec8*Δ *spo11*Δ cells arrested in prophase I by the deletion of the transcription factor *NDT80* (Xu et al., 1998). Six hours after the induction of meiosis, CENV GFP dots were paired in 91% of *rec8*Δ *spo11*Δ *ndt80*Δ cells (Figure 7B). In contrast, GFP dots (LYS2 dots) at chromosome arms appeared less frequently paired (60% of cells; Figure 7B). The appearance of only one dot was not due to the lack of DNA replication, because most cells had replicated their DNA at the time that GFP dots were examined (Figure 7C). Deletion of *MAM1* reduced the pairing of GFP dots in cells carrying CENV GFP dots to 74%. It also reduced pairing of arm sequences from 59% to 37% (Figure 7B), which probably reflects the fact that arm sequences are more likely to interact when centromeres are linked. We conclude that, although it is clearly not the only factor linking sister chromatids at centromeres in the absence of cohesins, the monopolin complex joins sister kinetochores in a cohesin-independent manner during meiosis I.

DISCUSSION

Aurora B kinases affect diverse mitotic events; most prominent among these are chromosome morphogenesis and segregation. We have investigated the protein kinase's role in kinetochore-microtubule attachment during the two meiotic divisions and found that Aurora B is required for homolog biorientation during meiosis I as well as sister chromatid biorientation during meiosis II. Our data further implicate the meiosis I-specific monopolin complex in allowing Aurora B to biorient homologs rather than sister chromatids during meiosis I. Consistent with this central role in determining kinetochore orientation is the observation that the monopolin complex is sufficient to induce coorientation of sister kinetochores. The ability to establish sister kinetochore coorientation during mitosis furthermore provides insights into one of the complex's functions: providing a link between sister kinetochores.

The Roles of Aurora B during Meiosis

Aurora B has been shown to regulate chromosome alignment and segregation, cytokinesis, and microtubule dynamics during meiosis in several organisms (Bishop et al., 2005; Kaitna et al., 2002; Ohi et al., 2004; Rogers et al., 2002; Schumacher et al., 1998). Depletion of Aurora B in budding yeast reveals that the protein kinase is required for several aspects of meiotic cell division in this organism as well. First, Ipl1-depleted cells are somewhat delayed in entry into premeiotic S phase, the basis of which is unclear at present. Second, Ipl1 is required for the coordinated stepwise loss of cohesion in a fraction of cells, which is consistent with recent results in *Drosophila* (Resnick et al., 2006). The third function of Aurora B during meiosis that we uncovered is in promoting homolog and sister chromatid biorientation during meiosis I and meiosis II, respectively. The mechanisms whereby Ipl1 accomplishes this appear to be the same as during mitosis: the protein kinase severs microtubule-kinetochore attachments that are not under tension. The crucial factor that allows the protein kinase to biorient homologs rather than sister chromatids during meiosis I is the monopolin complex.

Establishing Sister Kinetochore Coorientation during Mitosis

By co-overexpressing Cdc5 and Mam1, we were able to induce cosegregation of sister chromatids during mitosis. Does this cosegregation reflect genuine coorientation of sister kinetochores as it exists during meiosis I, or does this regimen lead to nonspecific interference with kinetochore function? Abolishing kinetochore function through the inactivation of core kinetochore components such as *NDC10* leads to spindle elongation in the absence of chromosome segregation, with many chromosomes remaining at the metaphase plate (Goh and Kilmartin, 1993). Interference with kinetochore-microtubule attachment delays and/or prevents entry into anaphase. These phenotypes are not observed in *GAL-CDC5 GAL-MAM1* cells, arguing against a general kinetochore defect in these cells.

Several lines of evidence indicate that the cosegregation of sister chromatids observed in *GAL-CDC5 GAL-MAM1* mutants is also not due to a loss of *IPL1* function. Overproduction of Cdc5 and Mam1 did not enhance the *ipl1-321* phenotype at the semipermissive temperature, nor did overexpression of *IPL1* affect sister chromatid cosegregation in *GAL-CDC5 GAL-MAM1* cells. Furthermore, the cosegregation phenotype of *GAL-CDC5 GAL-MAM1* mutants differs from that of *ipl1-321* mutants. Finally, the fact that Pds1 degradation was delayed in cells overproducing Cdc5 and Mam1 indicates that Ipl1 is active in these cells. Together, our studies indicate that general kinetochore defects and effects on Ipl1 function are not the reason for the cosegregation of sister chromatids in *GAL-CDC5 GAL-MAM1* cells. The finding that the cosegregation of sister chromatids in cells overproducing Cdc5 and Mam1 depends on the monopolin complex components Csm1 and Lrs4 furthermore leads us to conclude that the cosegregation observed during mitosis reflects genuine coorientation of sister kinetochores during meiosis I.

Mechanisms of Sister Kinetochore Coorientation

Aurora B kinases play an essential role in biorienting sister kinetochores during mitosis. It was therefore possible that factors promoting the coorientation of sister kinetochores

during meiosis I would be inhibitors of Aurora B function. However, our studies indicate that this is not the case. Rather, they point toward Ipl1 performing the same function during meiosis I and II as it does during mitosis—that is, severing microtubule-kinetochore attachments that are not under tension. The monopolin complex modifies sister kinetochores so that they are only under tension when homologs are bioriented. How does the monopolin complex accomplish this? Several lines of evidence indicate that the complex functions as a link between sister kinetochores that is distinct from cohesins. When overproduced during mitosis, Cdc5 and Mam1 induce the cosegregation of sister chromatids, with the two sisters being tightly associated near centromeres but not at arm regions. The tight association of sister centromeres is not observed in other mutants that cosegregate sister chromatids to the same pole during anaphase, such as *ipl1-321* mutants or cells depleted for cohesins. Importantly, high levels of Cdc5 and Mam1 are capable of linking cosegregating sister chromatids in cells lacking *IPL1* or cohesin. Even in the absence of the cohesin subunit *REC8*, we observed that 91% of sister chromatids are associated at centromeres during prophase I (*ndt80*Δ block) and preferentially (85%) cosegregate to the same pole during anaphase I. During this cosegregation, centromeric sequences appear tightly paired, whereas arm sequences do not. Importantly, this association of sister chromatids in *spo11*Δ *rec8*Δ cells is in part dependent on *MAM1*, indicating that the protein has sister centromere-connecting abilities not only when overproduced during mitosis but also during meiosis I.

How could the joining of sister kinetochores force them to attach to microtubules emanating from the same pole? The fusion of sister kinetochores could put steric constraints on the kinetochores, hence favoring attachment of both kinetochores to microtubules emanating from the same spindle pole. Ultrastructural analyses of meiosis I spindles in the salamander *Amphiuma tridactylum* and several grasshopper species support this hypothesis (reviewed in Moore and Orr-Weaver, 1998). We favor the idea that, at least in yeast, the monopolin complex, in addition to joining sister kinetochores, prevents attachment of microtubules to one of the two sister kinetochores because this model is more consistent with ultrastructural analyses of meiosis I spindles in budding yeast. In *S. cerevisiae*, in which kinetochores bind to only one microtubule, the number of microtubules in the meiosis I spindle is more consistent with one microtubule attaching to one homolog (Winey et al., 2005). We note that in other organisms such as *Drosophila* and mouse, sister kinetochores also appear to form a single microtubule-binding surface during metaphase I (Goldstein, 1981; Parra et al., 2004). The second observation leading us to favor the model in which the monopolin complex links sister centromeres and prevents one kinetochore from attaching to microtubules is that overexpression of a functional monopolin complex allows 35% of cells treated with the microtubule-depolymerizing drug nocodazole, which causes activation of the spindle checkpoint, to escape the checkpoint arrest (Figure S6).

The mechanisms whereby the monopolin complex links sister kinetochores remain to be determined. We propose that, after DNA replication, sister chromatids are initially topologically linked due to catenation even in the absence of cohesins. Mam1 assembles onto the kinetochores of these sisters, joining them at centromeres. Whether this link is able to withstand the pulling forces exerted by microtubules is unclear, but we envision that the monopolin complex bridges the sister kinetochores in a way that ensures their concerted movement and conceals one of the two microtubule attachment sites. The monopolin complex could itself bridge sister chromatids or induce changes in kinetochore substructures to induce their interaction with each other. In this regard, it is interesting to note that a component of the monopolin complex, Hrr25, forms multimers only during meiosis I (Petronczki et al., 2006), potentially providing a bridging function. In *S. pombe*, coorientation factors appear to bring about sister kinetochore coorientation through cohesin complexes (Yokobayashi and Watanabe, 2005). Our results suggest that, in *S. cerevisiae*, coorientation factors themselves have the ability to join sister chromatids. We propose that this function is important to promote sister kinetochore coorientation. Whether these linkages simply impose steric constraints or additionally control the attachment of microtubules to kinetochores will be an important question to examine in the future.

EXPERIMENTAL PROCEDURES

Strains and Plasmids

Derivatives of SK1 are described in Table S1; derivatives of W303 strains are described in Table S2. To deplete Ipl1 during meiosis, the *IPL1* ORF was placed under the control of the *SCC1* or *CLB2* promoter by the PCR-based method described in Longtine et al. (1998). The *GAL1-10* promoter fusions as well as tagged alleles of various genes are described in the Supplemental Experimental Procedures.

Sporulation Conditions

Cells were grown to saturation in YPD (YEP + 2% glucose) for 24 hr, diluted into YPA (YEP + 2% KAc) at OD_{600} = 0.3, grown overnight, and then washed with water and resuspended in SPO medium (0.3% KAc [pH 7.0]) at OD_{600} = 1.9 at 30°C to induce sporulation.

Western Blot Analysis

Cells were harvested, incubated in 5% trichloroacetic acid (TCA), and lysed as described in Moll et al. (1991). Immunoblots were performed as described in Cohen-Fix et al. (1996). Antibody concentrations are listed in the Supplemental Experimental Procedures.

Localization Techniques

Indirect in situ immunofluorescence was carried out as described in Visintin et al. (1999). Chromosomes were spread as described in Nairz and Klein (1997). Antibody concentrations are listed in the Supplemental Experimental Procedures. GFP dots were analyzed in cells fixed in 2.5% formaldehyde for 10 min, washed twice, and stored in potassium phosphate buffer (pH 7.4). Before microscopic analysis, samples were fixed with 80% EtOH for 10 min and resuspended in 1 mg/ml 4′,6-diamidino-2-phenylindole (DAPI) solution. Two hundred cells were counted per time point unless otherwise noted.

Statistical Analysis

Results are the mean ± standard deviation for n = 3 experiments. Statistical significance was evaluated using ANOVA followed by post hoc multiple comparison according to the Student-Newman-Keuls method. $p \leq 0.01$ was considered significant.

Supplemental Data

Supplemental Data include Supplemental Results, Supplemental Experimental Procedures, Supplemental References, six figures, and two tables and can be found with this article online at http://www.cell.com/cgi/content/full/128/3/477/DC1/.

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