Mps1 promotes chromosome meiotic chromosome biorientation through Dam1

Régis E. Meyera, Jamin Brownb, Lindsay Beckc, and Dean S. Dawsona,b,c,*
aProgram in Cell Cycle and Cancer Biology, Oklahoma Medical Research Foundation, and bDepartment of Cell Biology, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104

ABSTRACT In budding yeast meiosis, homologous chromosomes become linked by chiasmata and then move back and forth on the spindle until they are bioriented, with the kinetochores of the partners attached to microtubules from opposite spindle poles. Certain mutations in the conserved kinase, Mps1, result in catastrophic meiotic segregation errors but mild mitotic defects. We tested whether Dam1, a known substrate of Mps1, was necessary for its critical meiotic role. We found that kinetochore–microtubule attachments are established even when Dam1 is not phosphorylated by Mps1, but that Mps1 phosphorylation of Dam1 sustains those connections. But the meiotic defects when Dam1 is not phosphorylated are not nearly as catastrophic as when Mps1 is inactivated. The results demonstrate that one meiotic role of Mps1 is to stabilize connections that have been established between kinetochores and microtubules by phosphorylating Dam1.

INTRODUCTION
During meiosis, cells face a critical transition from a stage where chromosomes are dispersed, and unattached to microtubules (prophase), to a stage where paired homologous partners are aligned on the middle of the spindle (metaphase). This transition starts with the capture of chromosomes by the spindle microtubules resulting in mostly incorrect initial attachments. If uncorrected, these kinetochore–microtubule (kMT) attachments would pull the homologous partners to the same pole rather than to opposite poles of the spindle at anaphase (Meyer et al., 2013). These aberrant attachments are corrected to avoid mis-segregation and aneuploidy (reviewed in Duro and Marston, 2015). In yeast meiosis, the correction is done via consecutive cycles of detachment/reattachment of microtubules to the kinetochores, accompanied by movements of the chromosomes back and forth on the spindle as they orient (Meyer et al., 2013). During this process, the spindle assembly checkpoint senses the state of kMT attachments and delays cell cycle progression into anaphase until all chromosome pairs are bioriented (Hoyt et al., 1991; Li and Murray, 1991; Cheslock et al., 2005; Shonn et al., 2000; reviewed in Joglekar, 2016). When the spindle assembly checkpoint is satisfied, anaphase ensues.

A key aspect of the chromosome biorientation process involves modulating the type and the stability of kMT attachments depending on chromosome position. The kMT attachment process appears to be controlled at several levels (reviewed in Tanaka, 2010; Godek et al., 2015; and Lampson and Grishchuk, 2017). In yeast, this has been studied primarily in mitotic cells. First, new attachments must be formed. In yeast, as in other organisms, kinetochores initially attach most often to lateral surfaces of microtubules (Hayden et al., 1990; Merdes and De Mey, 1990; Rieder, 1990; Tanaka et al., 2005; Franco et al., 2007; Gachet et al., 2008; Magidson et al., 2011). Second, when depolymerization of the microtubule brings the end of the microtubule to a laterally attached kinetochore, the connection can be converted to an end-on attachment (Kitamura et al., 2007; Tanaka et al., 2007). Third, the protein composition at the kMT interface, and modifications of those proteins, change, which promotes the ability of the kinetochore to track the shortening microtubule (Asbury et al., 2006; Westermann et al., 2006; Grishchuk et al., 2008; Daum et al., 2009; Gaitanos et al., 2009; Powers et al., 2009; Welburn et al., 2009; Lampert et al., 2010; Schmidt et al., 2012; Volkov et al., 2013; Umbreit et al., 2014). Finally, incorrect connections that do not promote biorientation are released (Biggins et al., 1999; Cheeseman et al., 2002; Tanaka et al., 2002).

Mps1 is a conserved kinase with a central role in the spindle assembly checkpoint (Hardwick et al., 1996; Weiss and Winey, 1996;
In budding yeast meiosis, Mps1 performs an essential function in promoting the formation, maintenance, or regulation of force-generating attachments to the plus ends of microtubules (Meyer et al., 2013). This seems to contradict the emerging picture of Mps1 function in mammalian mitotic chromosome biorientation, where Mps1 acts in part to destabilize kMT attachments (Jelluma et al., 2010; Hiruma et al., 2015; Ji et al., 2015; Maciejowski et al., 2017). In both yeast and mammals, Mps1 binds to the Calponin homology domain of the outer kinetochore protein, Ndc80 (Kemmeler et al., 2009; Nijenhuis et al., 2013). In mammalian cells, Mps1 is displaced from the kinetochores chromosomes once they have become bioriented. Evacuation of Mps1 from the attached kinetochores contributes to turning off the spindle checkpoint “wait” signal (Etemad and Kops, 2016). The binding of microtubules to Ndc80 may displace or prevent Mps1 binding to Ndc80 (Hiruma et al., 2015; Ji et al., 2015). In budding yeast mitosis, tethering Mps1 to kinetochores does not result in a dramatic loss of kMT attachments as it does in mammalian cells (Aravamudhan et al., 2015), consistent with the finding that Mps1 promotes, rather than destabilizes, kMT attachments in budding yeast meiosis.

The manner in which Mps1 promotes force generating attachments between kinetochores and microtubule plus-ends in meiosis is unclear. MPS1 mutations have been described that result in catastrophic errors in meiotic chromosome segregation, but only mild mitotic defects, demonstrating there is a greater need for Mps1 in meiosis than in mitosis, but what that need might be is unknown. One way that Mps1 is known to impact the kMT interface in mitosis is through phosphorylation of Dam1 (Figure 1A). Dam1 is a member of the Dam1 complex, which interacts with the Ndc80 complex and increases its ability to hold on to microtubule plus ends in vitro (Franck et al., 2007; Tien et al., 2010; Lampert et al., 2013; Sarangapani et al., 2013). Two proteins of the Dam1 complex, Dam1 and Duo1, interact directly with microtubules (Asbury et al., 2006; Westermann et al., 2006; Zelter et al., 2015). The Dam1 protein contains six residues that are phosphorylated by Mps1 (Figure 1B) (Shimogawa et al., 2006). Prior work has shown that mutation of two of these residues (serine 218 and 221) to nonphosphorylatable amino acids results in diminished levels of end-on attachments in meiotic yeast cells (Shimogawa et al., 2006; Shimogawa et al., 2010), suggesting that Mps1 might promote the formation or maintenance of end-on attachments thru Dam1. Despite the fact that dam1-2A (S218A S221A) mutants have reduced end-on kMT attachments, they only exhibit minor mitotic chromosome segregation defects, suggesting that normal end-on kMT attachments might not be essential for effective mitotic chromosome segregation in budding yeast. The severe defects of some MPS1 mutants in meiosis, but not mitosis, raises the question of the meiotic consequences of Dam1 phosphorylation by Mps1.

RESULTS

Mps1 works partially through Dam1 to promote meiotic chromosome segregation

To better understand how Mps1 controls meiotic poleward chromosome movement, and how Dam1 might be involved, we analyzed meiotic chromosome movements in dam1 mutants. We assayed both the dam1-2A allele, described above, and a dam1-6A allele in which two serines of Dam1 that are phosphorylated by Mps1 (S218 and S221) have been switched to aspartic acid. *p < 0.05, **p < 0.01, ***p < 0.001 (Fisher’s exact test) (n ≥ 59).

FIGURE 1: Dam1 phosphorylation promotes meiotic segregation.

(A) Cartoon highlighting the distribution of proteins known to be phosphorylated by Mps1 kinase at the kMT interface (Cnn1 is the target for the inner kinetochore), Ndc80, Dam1, and Spc105 represent sub-complexes composed of two or more proteins (reviewed in Biggins, 2013). +TIPs indicates microtubule plus-end tracking proteins such as Stu1, Stu2, and Bim1 (not necessarily in the same location or exactly at the frayed end of the microtubule). The proteins shown are not necessarily at the kMT interface at the same time. Yellow circles indicate known Mps1 phosphorylation sites. (B) Domains of Dam1 protein. Known residues phosphorylated by Mps1 in budding yeast are represented by black lines. (C, D) All strains evaluated were diploids with GFP-tagged centromeres of chromosome 1 (CEN1-GFP) and expressing SPC42-DsRed to mark the SPBs. Cells were sporulated and released from a pachytene arrest (PGAL1-NDT80 GAL4-ER) at 6 h after meiotic induction by the addition of 5 μM β-estradiol. In the diploid cells with long spindles (length ≥ 5 μm), the proportion of cells exhibiting proper CEN1 segregation (white), defective segregation of CEN1 (gray), or numerous lagging chromosomes (black) was monitored 3-4 h postrelease (n ≥ 100). An example of each category is shown. Scale bar: 5 μm. (C) Relevant genotypes of tested strains: WT is wild-type for Dam1, NDC80, and Mps1, dam1-2A is dam1-2A/ dam1-2A, and DAM1-me is Dam1-md/DAM1-me, so meiotic production of Dam1 protein comes from the meiotic IME2 promoter. dam1-6A-me is Dam1-md/dam1-6A-me, so expression of Dam1-6A protein comes from the meiotic IME2 promoter. ndc80-md is PCLB2-NDC80/ PCLB2-NDC80. dam1-md is PCLB2-DAM1/PCLB2-DAM1. mps1-as1 is mps1-as1/ PCLB2-MPS1 so meiotic expression of Mps1 is from the mps1-as1 allele. For mps1-as1 diploid mutants, 1 h after this release (t = 7 h), an inhibitor of the analog-sensitive allele mps1-as1 (1NM-PP1, 10 μM) was added to the medium. (D) The dam1-2D allele expresses a protein in which two serines of Dam1 that are phosphorylated by Mps1 (S218 and S221) have been switched to aspartic acid. *p < 0.05, **p < 0.01, ***p < 0.001 (Fisher’s exact test) (n ≥ 59).
which all the Mps1 phosphorylation sites were converted to alanines. To prevent the accumulation of potential mitotic errors, the dam1-6A allele was placed under control of a promoter (P_{IME2}) that is turned off in mitotic growth but is meiotically expressed (me) (dam1-6A-me). The other DAM1 allele was wild type, but under the control of the CLB2 promoter that is expressed only in mitotic cells, resulting in meiotic depletion (md) (DAM1-md) (Lee and Amon, 2003). Because the Dam1 protein is degraded on meiotic entry and Dam1 complexes are reassembled prior to metaphase I (Miller et al., 2012; Meyer et al., 2015), the DAM1-md/dam1-6A-me combination allows us to assay the effect of preventing Mps1 phosphorylation of Dam1 in meiosis. The segregation of a green fluorescent protein (GFP)-tagged version of chromosome I was monitored in cells harvested from meiotic time courses to assay the effects of the dam1 mutants. The dam1-2A and -6A alleles exhibited similar increases in meiosis I nondisjunction and lagging chromosomes suggesting that the critical residues are the serines mutated in dam1-2A mutants (S218 and S221) (Figure 1C). The lagging chromosomes were mainly associated with metaphase spindles (Supplemental Figure S1A) rather than anaphase spindles (as seen in mps1 null mutants) (Meyer et al., 2013). Depletion of Dam1 (dam1-md) had catastrophic defects (Figure 1C) similarly to what was observed when cells were depleted for the essential outer kinetochore protein Ndc80 (ndc80-md). The dam1-md allele also resulted in failures of meiotic spindle integrity (Supplemental Figure S1B) similarly to what was reported for dam1 null mutants in mitosis (Jones et al., 1999).

These defects of the nonphosphorylatable dam1 mutants were much less severe than the defects of mps1-f-as1 mutants (Figure 1C), demonstrating that Dam1 is not the most critical target of Mps1 in meiotic biorientation. But to test whether the meiotic segregation defects exhibited by mps1 mutants might be partially attributable to a failure to phosphorylate Dam1, we introduced a version of DAM1 with phosphomimetic mutations (S218D, S221D; dam1-2D) into mps1-as1 mutants (Figure 1D). The dam1-2D allele improved segregation fidelity in these strains, suggesting that the severe failure of meiotic chromosome segregation in mps1 mutants is partially attributable to a failure to phosphorylate Dam1. Less clear are the specific contributions that phosphorylating Dam1 makes to the biorientation process.

**Phosphorylation of Dam1 by Mps1 stabilizes associations of centromeres with the microtubules**

Live cell imaging was used to determine whether the dam1-2A mutation affected chromosome movements during the biorientation process. The experiment was performed in a spo11 mutant background in which chromosomes do not become tethered to their homologous partners. In this situation, the resulting individual (univalent) chromosomes, each with one kinetochore, can only attach to a single microtubule (Figure 2A). Thus, they can never biorient, resulting in repeated cycles of forming kMT attachments, chromosome movement toward the poles, and release of the kMT attachment (Figure 2B) (Meyer et al., 2013). The spo11 mutation also results in a longer spindle, making it easier to track long processive movements of chromosomes (Shonn et al., 2000; Meyer et al., 2013). Using this assay, dam1-md mutants exhibit a nearly complete loss in the ability of chromosomes to traverse the spindle, while dam1-2A mutants are significantly compromised in the process (Figure 2, C and D, and Supplemental Figure S2). A similar, but less severe, deficiency was observed for dam1-2A mutants when the behavior of bivalent pairs (SPO11 background) was evaluated in this type of assay (Supplemental Figure S3, A–G). The shorter spindles and the availability of two connected kinetochores to which microtubules can attach may explain why the dam1-2A defect in across-the-spindle traverses is less severe in the SPO11 strain.

The coupling of kinetochores to the ends of depolymerizing microtubules is presumably the major driving force for the poleward movements that occur on bipolar spindles. However, in assays with bipolar spindles (as in Figure 2C), it is difficult to know exactly how the kinetochore of a particular chromosome is attached to a microtubule. To measure more specifically minus-end directed movements, we assayed the clustering of an univalent chromosome (spo11 background) toward the side-by-side spindle pole body (SPBs) before the bipolar spindle is formed (Figure 3A). Because the microtubules form a monopolar array, all poleward movements are...
minus-end directed. Following the release from a prophase arrest, the univalents migrate toward the side-by-side SPBs (cluster) in consecutive cycles (Figure 3, B and C). In wild-type cells, the clustering events were more and more frequent approaching the time of spindle formation (Figure 3D), leading to a final clustering several minutes before the SPBs separated to form a spindle (Figure 3E). The dam1 mutants show significant delays in reaching the final clustering (Figure 3E). Similar observations were obtained by monitoring bivalent pairs (Supplemental Figure S3, H and I).

The delay in clustering in dam1-2A mutants could reflect a deficiency in minus-end-directed movement or a failure in remaining at the SPBs once they have arrived. Indeed, in the dam1-2A mutants, centromeres spent less time at the SPBs before migrating away again (Figure 3F), suggesting the phosphorylation of Dam1 by Mps1 stabilizes the association of the centromeres with the SPBs, perhaps by stabilizing the kMT attachment.

dam1 mutants exhibit pausing defects during the biorientation process

The imaging experiments above employ long acquisition interval times (2 min/frame) to allow acquisition of data for cells proceeding from prometaphase to anaphase I, without photobleaching or toxicity. At this frame rate, a traverse across the entire spindle can occur in the interval between sequential frames. To reveal the pauses and restarts to chromosome movement that might occur within a single traverse, we imaged chromosome behavior at faster acquisition rates (2 s intervals) over the course of 5 min. Images were acquired using a thru-focus method in which a single image is collected as the objective lens focuses through the cell (Conrad et al., 2008). Deconvolution of the acquired data produces a two-dimensional projection of the image. To reduce acquisition times, the SPBs and the centromere of chromosome I were both tagged with GFP.

Chromosome behavior was quantified in cells with bipolar spindles. In the control wild-type (WT) cells, chromosomes were found to exhibit any one of several different behaviors during the 5-min filming. We assigned the behaviors to five categories (Figure 4A). These included high-mobility traverses across all or part of the spindle, pausing at the pole, pausing in a nonpolar area—either midspindle, or to the side of the line between the two spindle pole bodies—and limited movements in a small area (low mobility) (Figure 4A). Whereas visualizing the chromosomes undergoing high-mobility movements across part of the spindle (traverses) or the whole spindle (traverses) was common in the control strain, this was less common in dam1-2A and dam1-md mutants. In contrast, whereas it was uncommon in the control strain for chromosomes to exhibit centromeres that lingered in a nonpolar position, this was a predominant behavior in the dam1-md strain (Figure 4, B–D). In the dam1 mutants, the nonpolar centromeres moved around much like centromeres in nonpolar positions in wild-type cells (Figure 4, B–D). In fact, centromeres that remained in a nonpolar position in the WT, dam1-2A, and dam1-md strains behaved in an indistinguishable manner, the main difference being that this phenotype was much more common in the dam1-md strain. One possible explanation is
that in the dam1 mutants, the kinetochores are less able to efficiently secure microtubule attachments, so they spend more time uncoupled from microtubules and less time undergoing high-mobility, microtubule-mediated movements. But in ndc80 mutants, in which kinetochores are presumably unattached to microtubules, the centromeres in a nonpolar position showed somewhat more movement than was seen in dam1-md, dam1-2A, and wild-type cells. It may be that without connections to microtubules, the centromeres in ndc80 mutants are mobilized by the much faster meiotic telomere-led movements (Conrad et al., 2008). Thus, we speculate that the midspindle centromeres in WT, dam1-md, and dam1-2A are undergoing some microtubule interactions and these interactions impact their movement.

**dam1 exhibit reduced processivity during poleward centromere migrations**

The dam1-2A results suggest the model that phosphorylation of Dam1 by Mps1 in meiosis promotes either the establishment or maintenance of kMT attachments, consistent with observations in mitotic cells (Shimogawa et al., 2006). To attempt to distinguish between these models, we characterized the behavior of centromeres making poleward migrations in wild-type and dam1-2A cells. We identified centromeres that moved from a position of 1 micron away from a spindle pole to the pole (Figure 5A). These movements toward a pole could come from either pushing or pulling forces, but since the migrations occur within one half spindle (average spindle length was more than 2 microns), they are presumably mediated most often by minus-end-directed movements along a microtubule that emanates from the destination pole. These poleward migrations took significantly less time in wild-type cells than in dam1-2A mutants (Figure 5, B and C). To determine whether this was because centromeres reach higher velocities in wild-type cells, we measured the distance that each centromere moved over each frame (2 s) of acquisition (Figure 5D) for both poleward and antipoleward centromere movements that occurred over the course of migration to the pole. There was no significant difference in the velocities of the centromeres in the two strains. The peak movement at ~45 nm/s translates to 2.7 µm/min, which is close to the previously published rate at which plus-end-attached mitotic kinetochores move on depolymerizing microtubules (1.5–2.0 µm/min; Gandhi et al., 2011). The slightly faster rate, observed here, is likely due to the fact that we are reporting movement over 2-s intervals while previous reports represent the average speed over longer time intervals that probably included short pauses (as in the migrations plotted in Figure 5B). The fact that centromeres dam1-2A mutants are able to move at the same maximal velocity as in wild-type cells is consistent with the model that force-generating, end-on microtubule attachments can be formed without Mps1 phosphorylation of Dam1.

What did differ between the WT and dam1 strains is the processivity of movement toward the pole (Figure 5, E–G). The dam1 mutants exhibited more pauses, or reversals of direction, in their journeys to the pole (Figure 5, F and G).

**dam1-2A mutants cannot maintain midspindle biorientation**

A possible explanation for the frequent pauses and reversals of poleward migrating univalents in dam1-2A mutants is that the force exerted on the kinetochores of poleward migrating
univalents is sufficient to sever the kMT connection. In wild-type control cells, the kinetochores of bioriented chromosomes are positioned midspindle, with the centromeres alternating between being visibly separated or together (fused) (Figure 6A). Sometimes the centromere pair will migrate to one pole, generally with the centromeres in the fused configuration. Presumably, this reflects the loss of a connection between one kinetochore and its microtubule. As a first assessment of the ability of dam1-2A cells to maintain their kMT connections, we monitored the persistence of bioriented bivalents in the midspindle position (over long time periods with images collected at 2-min intervals; example traces for two cells are shown in Figure 6B). In dam1-2A mutants, the bivalents spent significantly less time in a midspindle position (Figure 6C). Shorter acquisitions, with rapid frame rates (every 2 s for 10 min) were used to evaluate breathing of the centromeres of bioriented bivalents and the frequency with which they collapsed to a single pole with nonseparated centromeres. The breathing rate—the rate at which the centromeres of bioriented bivalents resepared after coming together—was not dramatically different in dam1-2A and control cells (Figure 6D). Thus, kMT connections in control and dam1-2A mutants seem similarly able to generate tension across the bioriented kinetochores. However, in the dam1-2A mutants, the bioriented pair often lost their midspindle position and collapsed to one pole with unseparated centromeres (Figure 6E). If the collapse to one pole is because kMT attachments are unstable, then this should activate the spindle checkpoint. Indeed, when the spindle checkpoint is inactivated by deletion of the MAD2 gene in the dam1-2A mutants they exhibit elevated rates on meiosis I nondisjunction (Supplemental Figure S4).
to a failure in phosphorylating Dam1. In mutants in which Dam1 cannot be phosphorylated by Mps1 on serines 218 and 221, mitotic kinetochores show reduced co-localization with microtubule plus-ends, leading to the model that phosphorylation of Dam1 by Mps1 is required for the conversion of low affinity plus-end microtubule attachments to high-affinity attachments (Shimogawa et al., 2006). This work provides three new observations that support the notion that phosphorylation of Dam1 by Mps1 is not necessary for the formation of kinetochore attachments to microtubule plus-ends but rather to sustain those attachments. First, in dam1-2A mutants, the maximal velocity of minus-end directed movements is indistinguishable from that seen in wild-type controls. Second, in dam1-2A mutants, the inefficiency in poleward movement is not due to reduced velocity but instead is due to frequent pausing during the poleward migration. Because we measured the movement of kinetochores on intact spindles, we were not able resolve the individual microtubules to which our marked kinetochores were attached. Thus, we could not distinguish whether the pauses in dam1-2A cells were due to a failure to retain plus-end attachments or to pauses or reversals of microtubule depolymerization (Figure 7A). This second possibility is consistent with the finding that in mitotic dam1-2A mutants the microtubule plus-ends are often not colocalized with kinetochores and the microtubules are longer than in wild-type cells, leading to the suggestion that Dam1 phosphorylation affects the dynamics of kinetochore microtubules (Shimogawa et al., 2006). Third, in dam1-2A mutants, the stretched-apart centromeres of bioriented chromosomes frequently snap together, coupled with a collapse of the bivalent to one pole. This behavior is consistent with a failure to maintain kMT connections under tension. Together these observations suggest that dam1-2A mutants can form plus-end kMT attachments that support maximal rapid poleward movement but cannot maintain those attachments.

The failure of mps1 mutants to phosphorylate Dam1 does not explain the massive defects in meiotic chromosome segregation exhibited by mps1 mutants. Despite their defects in kMT interactions, dam1-2A mutants exhibit rather mild meiotic chromosome segregation defects, just as has been reported for mitotic cells (Shimogawa et al., 2006). Consistent with this, expression of DAM1 phosphomimetic alleles could only modestly improve the very high meiotic error-rate of mps1 mutants. Thus, there must be another role (or roles) of Mps1 that explains its essentiality for mitotic chromosome segregation. Further work is required to identify these Mps1 substrates that are essential for meiotic chromosome biorientation.

DISCUSSION

Previous work has shown that Mps1 is essential for chromosome segregation in meiosis (Straight et al., 2000; Poss et al., 2004; Gilliland et al., 2005). In budding yeast, Mps1 is critical for promoting the force-generating kMT attachments that support poleward migration of the chromosomes in meiosis I (Meyer et al., 2013). But it is unclear what steps in forming, maintaining, or regulating these attachments require Mps1.

Because Dam1 is a known substrate of Mps1, we tested whether the catastrophic meiotic defects displayed by mps1 mutants are due

FIGURE 6: Dam1 phosphorylation stabilizes bioriented attachment. (A) Cartoon illustrating events occurring after establishment of a bioriented attachment until anaphase I onset. A bioriented bivalent alternates between phases in which the GFP-tagged centromeres appear as separate dots (“split”) and phases in which the signals overlap (“fused”). The time between losing the split configuration and the next split configuration is defined as “breathing time.” The bioriented bivalent sometimes moves off a midspindle position with a single centromere losing the split configuration and the next split configuration is defined as “breathing time.” (B) The duration of each breathing event was determined and graphed for 4 h. (B) Traces of the chromosomes in a single cell alternating between a midspindle breathing position and off-center fused position. Representative cells for the indicated genotypes are shown. (C) For each cell, the time (total consecutive frames) spent with the configuration “Mid-spindle breathing” was determined. The graph represents their distributions for wild-type and dam1-2A mutant cells (n ≥ 32). * p < 0.05 (Student’s t test). (D, E) Diploid cells of the indicated genotype expressing homozygous CEN1-GFP and the SPB marker (SPC42-DsRed) were sporulated and released from a pachytene arrest (pGAL1-NDT80 GAL4-ER) at 6 h after meiotic induction by the addition of 5 μM β-estradiol. Cells were imaged at 2-min intervals for 4 h. (B) Traces of the chromosomes in a single cell alternating between a midspindle breathing position and off-center fused position. Representative cells for the indicated genotypes are shown. (C) For each cell, the time (total consecutive frames) spent with the configuration “Mid-spindle breathing” was determined. The graph represents their distributions for wild-type and dam1-2A mutant cells (n ≥ 32). * p < 0.05 (Student’s t test). (D, E) Diploid cells of the indicated genotype expressing homozygous CEN1-GFP and the SPB marker (SPC42-DsRed) were sporulated and released from a pachytene arrest (pGAL1-NDT80 GAL4-ER) at 6 h after meiotic induction by the addition of 5 μM β-estradiol. A short-term time-lapse movie (2-s intervals for 10 min) was done. (D) The duration of each breathing event was determined and graphed (n ≥ 63). ns = nonsignificant, p > 0.05 (Student’s t test). (E) Time spent in each of the following configurations was scored: “midspindle breathing,” “off-centered/fused,” and “stretched or split sister chromatids.” This last category was rare and appears as a less than full GFP signal pulled away from the main bright GFP signal. The graph represents the proportion of time each cell spent with its CEN1’s either off-center/fused or with split sister chromatids (n ≥ 15). Representative pictures of the different configurations are shown on the right. The blue arrows represent the SPBs. The yellow asterisks represent split CEN1s, the pink asterisk represents a fused CEN1, and the green plus sign represents the split of CEN1 sister chromatids. ** p < 0.01 (Student’s t test).
PCR-based methods were used to substitute the endogenous DAM1 by dam1-6A-md/DAM1 in the diploid O619 (dam1-md/+) by replacing the endogenous DAM1 by P_{MEZ}-dam1-6A in the diploid O619 (dam1-md/+) diploid strains were generated by replacing the endogenous DAM1 by P_{MEZ}-dam1-md in the diploid O619 (dam1-md/DAM1). The transformation was done with the digested plasmid OPL284 (pTRP-P_{MEZ}-dam1-6A by Sall and Hpal). P_{MEZ}-DAM1/dam1-md (dam1-me) diploid strains were generated by replacing the endogenous DAM1 by P_{MEZ}-DAM1 in the diploid O619 (dam1-md/DAM1). The transformation was done with the PCR product (OPR167-1161 with OPL284 as a template). Both transformations were done in diploid strains as the expression of Dam1 is restricted to meiosis with the IME2 promoter and will not allow cell survival of haploid strains.

**Fluorescence microscopy**

When analyzing fixed cells, images were collected using a Roper CoolSNAP HQ camera on a Zeiss Axio Imager 7.1 microscope. Images were processed and analyzed using Axiosvision software. For analyzing chromosome behavior (lagging, nondisjunction), cells were fixed with 2% formaldehyde and stained with DAPI (4,6-diamino-2-phenylindole, dihydrochloride) to visualize DNA. For homologue segregation assays (Figure 1, C and D, and Supplemental Figure S1A), cells were sporulated 9 h 30 min (3 h 30 min after release from pachytene arrest), and samples were taken for in situ immunofluorescence microscopy. Metaphase I cells were defined as cells with one DNA mass spanned by a meiotic spindle measuring 1–3.5 ßm in length. Cells with long spindle were defined as cells with spindles measuring at least 3.5 ßm. Mononucleate cells were defined as cells with a single DNA mass. Binucleate cells were defined as cells with two distinct separated DNA masses.

**Long-term time-lapse microscopy**

Time-lapse imaging (every 45–120 s for 3–4 h) were performed with CellASIC microfluidic flow chambers (www.cellasic.com) using Y04D plates with a flow rate of 5 psi. Images were collected with a Nikon Eclipse TE2000-E equipped with the Perfect Focus system, a Roper CoolSNAP HQ2 camera automated stage, an X-cite series 120 illuminator (EXFO) and NIS software. Images were processed and analyzed using NIS software. For time-lapse imaging following the spindle integrity using markers for microtubules (TUB1-GFP) and SPBs (SPC42-DsRed), the intervals were every 5 min for 4 h (Supplemental Figure S1B). For the time-lapse imaging of CEN1 movement, two different exposure programs were defined depending of the presence (SPO1I) or absence (spo11A) of chiasmata. In the presence of chiasmata, the intervals were either every 2 min for 2 h and later every 5 min for 2 h (Figure 6 and Supplemental Figure S3). Without chiasmata, images were acquired every 45 s for 75 min followed by every 10 min for 3 h (Figures 2 and 3 and Supplemental Figure S2).

For monitoring movements of CEN1-GFP on monopolar spindles (side-by-side SPBs), following the release from prophase, centromeres were considered as unattached if they did not remain at a constant distance from the SPBs for at least four consecutive frames. Centromeres were considered to be attached if they stayed
at a constant distance from the SPBs for at least three consecutive frames or moved incrementally in one direction. The first event of clustering was defined after observing three consecutive frames when $CEN1\text{-GFP}$ reaches a position within 0.75 µm of the SPB. Later events of clustering were defined when centromeres returned to their previous clustered position for at least one frame. Traverses ($CEN1$ crossing the spindle from one pole to the other one) were counted only when the $CEN1\text{-GFP}$ signal was overlapping with the SPB signal for at least one frame. Homologues were considered to be bioriented when the signals for $CEN1\text{-GFP}$ were distinctly separated. For monitoring the configuration of bivalents after the first bipolar attachment (Figure 6), centromeres were considered to be “split or fused” when the signals for $CEN1\text{-GFP}$ were for at least one frame clearly separated in two masses in the middle of the spindle and/or fused for the remaining frames. The centromeres were considered to be “Off-centered/fused” when the signals for $CEN1\text{-GFP}$ were overlapping with the SPBs for at least one frame and without being split.

**High-speed time-lapse microscopy.** Time-lapse imaging (every 2 s for 5–10 min) were collected using a Roper CoolSNAP HQ2 camera on a Zeiss Axios Imager 7.1 microscope fitted with a 100 x NA1.4, plan-apo objective (Carl Zeiss Microlmaging), an X-cite series 120 illuminator (EXFO), and a BNC555 pulse generator (Berkeley Nucleonics) to synchronize camera exposure with focusing movements and illumination. Cells from sporulating cultures were concentrated, spread across polyethyleneimine-treated coverslips, and then covered with a thin 1% agarose pad to anchor the cells to the coverslip (Yumura et al., 1984). The coverslip was then inverted over a silicone rubber gasket attached to a glass slide. Thru-focus images were acquired as described and then deconvolved to provide a two-dimensional projected image for each acquisition (Conchello and Dresser, 2007; Conrad et al., 2008). For the analysis of centromere movements on bipolar spindles, the coordinates of the two SPBs (labeled by $SPC42\text{-GFP}$) and the centromeres (marked by $CEN1\text{-GFP}$) were defined for each interval. To separate the movement inherent to spindle rotation inside the cells and the movement of $CEN1$ on the spindle, a relative position for $CEN1$ and the two SPBs was assigned for each interval. For one SPB ($SPB1$) this position was defined as being constant as $x = 0$ and $y = 0$. For the other SPB ($SPB2$), the position was defined as $x = \text{distance between the SPBs in each frame and } y = 0$. Finally, the relative position of $CEN1$ was determined by the distance between $CEN1$ and $SPB1$ and the angle formed between the axes $SPB1$-$SPB2$ and $SPB1$-$CEN1$. As the acquisitions were done in two dimensions, the impact of the spindle rotating in three dimensions was corrected by assuming that the spindle length remained the same or increased over time. So for instances in which the $SPB1$-$SPB2$ distance decreased in sequential frames, the value was corrected by replacing by the $SPB1$-$SPB2$ distance with the prior maximum spindle length ($d\text{Max}$ $SPB1$-$SPB2$). The magnitude of this correction was also then applied to correct the $SPB1$-$CEN1$ distance, and the following formula was applied for each interval: \( \text{Distance } SPB1\text{-CEN1} = \text{Observed distance } SPB1\text{-CEN1} \times d\text{Max} \) $SPB1$-$SPB2$/observed distance $SPB1$-$SPB2$. The velocity of $CEN1$ movement on the spindle was calculated for each interval by adding the distance between interval $n$–1 to $n$+1 and dividing by time the interval (4 s). The median position for $CEN1$ was determined in sliding 5-min intervals for each cell by calculating the average position. The dispersion distance was determined for each interval by calculating the distance between $CEN1$ and this average position. Cells with the following characteristics were selected to monitor poleward migration (Figure 5): The $CEN1$ exhibited a migration of 0.9–1.2 µm to a final destination within 0.25 µm of one SPB. The angle of approach had to be within 15°C on the pole to pole spindle axis. The migrations started within the same half-spindle of the destination SPB. Inside this 0.9- to 1.2-µm-distance movement, the intermediate steps were considered poleward movement when the distance between SPB and $CEN1$ from one interval to the other one was decreasing and anti-poleward movement when increasing. The pauses and reversals of direction were determined as follows. First, the distance ($D$) between the final SPB destination and $CEN1$ was calculated for each interval (frame). Second, the average distance for each sequential pair of steps was determined. Third, sequential positions in this sliding average were compared. If the distance between the SPB and $CEN1$ was increasing ($D \geq 0$), then the movement was considered to be paused/reversed. The number of consecutive poleward steps was determined as the number of consecutive steps showing continued decreasing distance ($D < 0$).

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