Mps1 promotes poleward chromosome movements in meiotic prometaphase

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ABSTRACT In prophase of meiosis I, homologous chromosomes pair and become connected by cross-overs. Chiasmata, the connections formed by cross-overs, enable the chromosome pair, called a bivalent, to attach as a single unit to the spindle. When the meiotic spindle forms in prometaphase, most bivalents are associated with one spindle pole and then go through a series of oscillations on the spindle, attaching to and detaching from microtubules until the partners of the bivalent become bioriented—attached to microtubules from opposite sides of the spindle. The conserved kinase, Mps1, is essential for the bivalents to be pulled by microtubules across the spindle in prometaphase. Here we show that MPS1 is needed for efficient triggering of the migration of microtubule-attached kinetochores toward the poles and promotes microtubule depolymerization. Our data support the model Mps1 acts at the kinetochore to coordinate the successful attachment of a microtubule and the triggering of microtubule depolymerization to then move the chromosome.

INTRODUCTION

In many organisms, cells enter prometaphase of meiosis with improper kinetochore-microtubule attachments that would lead to segregation errors if they were not corrected (Nicklas, 1997; Meyer et al., 2013; Chmátil et al., 2015). In budding yeast each partner chromosome in the homologue pair (called a bivalent) can attach one microtubule to its kinetochore (Winey et al., 2005; Sarangapani et al., 2014). The bivalents begin meiosis mono-oriented (both partners at one pole) and, through a series of steps, become bioriented and prepared to separate away from each other at anaphase I (Figure 1A). The microtubule-organizing centers, called spindle pole bodies (SPBs) in yeast, are duplicated in premeiotic S-phase resulting in an older SPB and a newly formed SPB. In late prophase the homologous chromosome pairs (called bivalents) cluster at the side-by-side SPBs in a microtubule-dependent manner (Figure 1A). The end of prophase and entry into prometaphase is marked by the formation of a spindle between the SPBs, forcing them apart with the bivalents attached mainly to the older SPB. The bivalents are released from this monopolar attachment in an Aurora B–dependent manner (Monje-Casas et al., 2007; Meyer et al., 2013) as was previously demonstrated in mitotic cells (Biggins et al., 1999; Cheeseman et al., 2002; Tanaka et al., 2002). Then, following a series of migrations back and forth across the spindle that include a series of microtubule releases (via Aurora B) and reattachments, the partners of the bivalents become attached to microtubules from opposite SPBs (Meyer et al., 2013). During this process, the spindle assembly checkpoint senses the state of kinetochore-microtubule attachments and delays cell cycle progression into anaphase until all chromosome pairs are bioriented (Shonn et al., 2000; Cheslock et al., 2005). The process of attaching the kinetochores to microtubules appears to be controlled at several levels (reviewed in Tanaka, 2010; Godé et al., 2015; Lampson and Grishchuk, 2017). A series of studies from the Tanaka laboratory defined these steps in yeast mitosis (Figure 1B). They found that, in yeast, as in other organisms, the kinetochores first attach most often to lateral surfaces of microtubules (Hayden et al., 1990; Merdes and De Mey, 1990;
A

Prophase I | Clustering | Pro-Metaphase I | Metaphase I

B

Girling

MT

Lateral attachment

MT shortens

Kinetochoore reaches MT tip

End-on and Pulling

End-on and pause

MT rescue (MT polymerization)

MT release

FIGURE 1: Kinetochore-microtubule interactions in budding yeast meiosis. (A) In prophase I, chromosomes have released their attachments to microtubules. At the exit from prophase I, centromeres cluster at the side-by-side SPBs. When SPBs separate to form a spindle, most centromeres are attached to the older SPB. Following a period of oscillations on the spindle including microtubule releases and reattachments, the homologous partners become bioriented. (B) Studies in mitotic cells suggest that most initial attachments are lateral (adapted from Tanaka, 2010). Microtubules depolymerize until they meet the kinetochore. In some organisms, kinetochores can glide toward the microtubule plus end. When the microtubule plus end meets the kinetochore, the illustrated outcomes have been observed.

Rieder and Alexander, 1990; Tanaka et al., 2005; Franco et al., 2007; Gachet et al., 2008; Magidson et al., 2011). Second, the microtubule depolymerizes to bring the microtubule plus end to the kinetochore (Kitamura et al., 2007; Tanaka et al., 2007). The kinetochore and microtubule plus end can then have any of several fates (Figure 1B). The microtubule can repolymerize, the kinetochore can release the microtubule, or the kinetochore can form an end-on attachment that can move the kinetochore poleward as the microtubule depolymerizes. In this process, the protein composition at the kinetochore-microtubule 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).

Mps1 is a conserved kinase with a central role in the spindle assembly checkpoint (Hardwick et al., 1996; Weiss and Winey, 1996; Abrieu et al., 2001). In budding yeast, Mps1 also has an essential role in meiotic chromosome segregation (Straight et al., 2000). An analysis of the role of the Mps1 in meiosis revealed that it was needed for the efficient poleward migration of centromeres during the biorientation process (Figure 1A) (Meyer et al., 2013). In addition, Mps1 is needed for an efficient spindle checkpoint in meiosis I. In MPS1 mutants, following anaphase I, most chromosomes end up associated with the spindle pole with which they were initially associated when the spindle first formed (Meyer et al., 2013). This is because they cannot move across the spindle to the opposite pole in prometaphase. Because most chromosomes connect to the older SPB just before prometaphase, even in wild-type cells, MPS1 mutants exhibit more than 80% nondisjunction, nearly all to the older SPB at anaphase I. The Ipl1 kinase, but not Mps1, is critical for releasing these monopolar attachments and for controlling the restructuring of kinetochores in meiosis prophase, but is not critical for poleward migration during prometaphase of meiosis I (Miller et al., 2012; Meyer et al., 2013, 2015; Chen et al., 2020).

This role of Mps1 in promoting force-generating kinetochore-microtubule attachments is critical for meiosis but less so in mitosis (Meyer et al., 2013). In budding yeast as in many other organisms, MPS1 is an essential gene, but separation-of-function alleles have been identified that result in severe defects in meiotic biorientation but very mild defects in mitosis (Meyer et al., 2013). This suggests either that meiosis is particularly sensitive to defects in the biorientation machinery, or alternatively, that meiotic sensitivity to MPS1 mutations reflects a meiosis-specific process. Interestingly, similar meiosis-specific mutant alleles of MPS1 have also been isolated in Drosophila and zebrafish (Poss et al., 2004; Gilliand et al., 2005).

The manner in which Mps1 promotes the formation of force-generating attachments between kinetochores and microtubule plus ends is unclear. Does Mps1 promote the movement of kinetochores toward the spindle midzone so they can encounter microtubules from the opposite pole, or convert lateral attachments to end-on attachments, or stabilize end-on kinetochore-microtubule attachments, or trigger microtubule depolymerization to drag kinetochores poleward (Figure 1B)? Because Mps1 kinase is known to have many targets, it could be involved in coordinating multiple steps in the biorientation process. Here we use live cell imaging experiments to explore the meiotic roles of Mps1. The results of these experiments suggest that Mps1 mutants can form end-on kinetochore-microtubule attachments but these mutants are defective in the subsequent microtubule depolymerization that pulls kinetochores poleward.

RESULTS

Mps1 is necessary for chromosome movements across the meiotic spindle

Previous work has shown that Mps1 is needed for the efficient establishment of force-generating attachments of kinetochores to microtubules. This is a multistep process (Figure 1B), and the step, or steps, at which MPS1 mutants are defective is unknown. Therefore, we used live cell imaging to track chromosome movements at various stages of the meiotic biorientation process in order to identify the deficiencies that occur when MPS1 is inactive.

We focused on the mps1-R170S mutation because this separation-of-function allele has only mild mitotic defects and severe meiotic defects, thus providing clues as to the critical roles that Mps1 plays in meiosis. As a control, we used an analogue-sensitive allele that allowed us to inactivate the Mps1 kinase activity with an ATP analogue (mps1-as1) (Jones et al., 2005). Prior studies revealed that both mutations result in high levels of meiosis I nondisjunction (Meyer et al., 2013). To track chromosome movement, one chromosome (chromosome I) was tagged adjacent to its centromere with an array of lac operator repeats, and the cells expressed lacI-GFP, which binds to the repeats, from a meiotic promoter (Straight et al., 1996). The movement of this GFP-tagged centromere was tracked in cells with a deletion of SPO11. In this background, homologous partner chromosomes do not become connected by recombination events to form bivalents (Figure 2A) (Klapholz et al., 1985; Loidl et al., 1994). The resulting partnerless univalents, each with only one kinetochore, can never biorient on the spindle and thus go through repeated cycles of microtubule attachment, migration on the spindle, and microtubule detachment (Figure 2B) (Meyer et al., 2013). Using this assay, both mps1-as1 and mps1-R170S mutants exhibit a considerable loss in the ability of chromosomes to traverse across the spindle, while in wild-type cells the GFP-tagged chromosome...
movements that occur on bipolar spindles. However, in assays with microtubules it is presumably the major driving force for the poleward movement (Figure 2, C and D).

3. The reorientation process in the spo11 background can be evaluated by quantifying the traverses of a GFP-tagged centromere across the spindle. (C, D) spo11 diploid cells, with the indicated genotypes, with one GFP-tagged CEN1 and the SPB marker (SPC42-DsRed) were sporulated and released from a pachytene arrest (Pcgal1-ND780 GAL4-ER) at 6 h after meiotic induction by the addition of 5 μM β-estradiol. The experiment was performed in three biological replicates, and 20 cells were scored in each replicate of the experiment. The pooled data from the three replicates (60 cells for each genotype) are presented. Images were collected at 45 s (one replicate) or 2 min (two replicates) intervals for 75 min. Representative kymographs from wild-type and mps1-R170S cells are shown. Scale bar: 2 μm. (D) For each cell, the number of traverses was recorded for the first 20 min after the spindle formed. The data (as traverses/minute) for each cell are plotted. Error bars are the average and SD for each set of 60 cells; ****p < 0.0001 (ordinary one-way analysis of variance [ANOVA], multiple comparisons).

To examine the directionality of chromosome movements on microtubules in meiosis, we assayed the movements of a univalent chromosome (spo11 background) on the monopolar microtubule array that emanates from the side-by-side SPBs as cells exit pachytene (Figures 1A and 3A). On these monopolar arrays, all poleward movements of chromosomes are minus end directed and all movements away from the pole are toward the microtubule plus ends. In this experiment, cells were released from a prophase arrest and chromosome movements on the monopolar array were monitored (Figure 3, A–C). In cells expressing the wild-type MPS1 gene the univalents migrated toward the side-by-side SPBs (clustering) in consecutive cycles (Figure 3, B and C) and as cells approached the time of spindle assembly, GFP-tagged centromeres were more and more likely to have become positioned against the SPBs (Supplemental Figure S1). The beginning of clustering, about 30 min before spindle assembly, may correspond to the time at which new Ndc80 complexes, capable of interacting with microtubule plus ends, are added to the meiotic kinetochore (Miller et al., 2012; Meyer et al., 2015; Chen et al., 2020). This clustering does not occur in ndc80-ml mutants that cannot produce new outer kinetochore parts after exiting prophase, arguing that the movements depend on kinetochore-microtubule interactions (Supplemental Figure S1). The majority of wild-type cells cluster the GFP-tagged centromere 5–10 min before spindle assembly, while clustering is significantly delayed in the MPS1 mutants (Figure 3D). Further, the length of time centromeres spent at the SPBs during the consecutive cycles of clustering is shorter in MPS1 mutants (Figure 3E). Similar observations were obtained by monitoring bivalent pairs (SPO11) (Supplemental Figure S2). The trend in these experiments is for centromeres to migrate toward the minus ends of microtubules in an MPS1 and NDC80-dependent manner. Although we cannot visualize individual kinetochore-microtubule attachments in these experiments, the data are consistent with the model that Mps1 is needed to promote minus-end-directed locomotion, via Ndc80-mediated attachments to the plus ends of microtubules to get the centromeres to the SPBs. They do not eliminate the possibility that there is also a plus-ended gliding process in budding yeast meiosis. Indeed, this could be one of the forces that moves the centromeres away from the SPBs in the repeated cycles of clustering.

mps1-R170S mutants exhibit pausing defects during the biorientation process

The imaging experiments above (and a prior characterization of Mps1 in meiosis (Meyer et al., 2013), employ relatively long frame intervals (from 45 s to 2 min) to allow acquisition of data for cells proceeding from prometaphase thru anaphase I without photosensitivity or toxicity. At this frame rate, a traverse of a centromere across the entire spindle can occur in the interval between sequential frames and details about pauses, restarts, and reversals of direction that occur as the kinetochore interacts with a microtubule are not detected. Understanding these details might clarify at which

FIGURE 2: Mps1 promotes migration across the meiotic spindle. (A) Cartoon illustrating the process of re-orientation in the absence of links between homologues (spo11.1 background). As the univalent does not have the ability to biorient, it will reorient indefinitely. (B) The reorientation process in the spo11 background can be evaluated by quantifying the traverses of a GFP-tagged centromere across the spindle. (C) spo11 diploid cells, with the indicated genotypes, with one GFP-tagged CEN1 and the SPB marker (SPC42-DsRed) were sporulated and released from a pachytene arrest (Pcgal1-ND780 GAL4-ER) at 6 h after meiotic induction by the addition of 5 μM β-estradiol. The experiment was performed in three biological replicates, and 20 cells were scored in each replicate of the experiment. The pooled data from the three replicates (60 cells for each genotype) are presented. Images were collected at 45 s (one replicate) or 2 min (two replicates) intervals for 75 min. Representative kymographs from wild-type and mps1-R170S cells are shown. Scale bar: 2 μm. (D) For each cell, the number of traverses was recorded for the first 20 min after the spindle formed. The data (as traverses/minute) for each cell are plotted. Error bars are the average and SD for each set of 60 cells; ****p < 0.0001 (ordinary one-way analysis of variance [ANOVA], multiple comparisons).

crosses the spindle, on average, about once every 6 min during prometaphase (Figure 2, C and D).

The coupling of kinetochores to the plus 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. The rapid and processive migrations across the midzone and to the opposite pole are most consistent with the kinetochore being dragged by a depolymerizing plus end–attached microtubule toward the spindle pole where its minus end is attached (Tanaka et al., 2007). However, it is formally possible that these movements could be gliding of the centromere along the side of a microtubule in the opposite direction, away from the SPB and toward the plus end of the microtubule it is tracking (Figure 1B) (Kapoor et al., 2006; Windlecker et al., 2009; Akera et al., 2015).

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steps in the biorientation process Mps1 is playing a critical function. To identify smaller-scale chromosome movements that occur within a single traverse, we imaged chromosome behavior at much faster acquisition rates (2 s intervals) over the course of 5 min, again using a spo11 mutant background so the resulting green fluorescent protein (GFP)-tagged chromosome I univalent could not become bioriented. 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 then 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 spindle arrays. (A) Schematic representation of centromere clustering on a monopolar microtubule array. (B) Images of a representative wild-type cell exhibiting cycles of clustering of GFP-tagged CEN1 (green) at the side-by-side SPBs (red) before spindle formation (the last image). Scale bar: 2 μm. (C) The pulling of the chromosome can be separated in two alternating phases where CEN1 is either moving toward the SPBs (Clustering) or at a relative constant distance from the SPBs. (D) Clustering of GFP-tagged CEN1 was monitored using live cell imaging of spo11 diploid cells with MPS1, mps1-R170S, or mps1-as1 alleles. Experiments were performed in three biological replicates imaged at 45 s (once) or 2 min (twice) frame rates. The graph shows the timing of the final clustering of CEN1 (within 0.5 μm) relative to the time of SPB separation for each individual cell. The red dotted line represents the time at which the SPBs separated. *p < 0.05, ****p < 0.0001 (ordinary one-way ANOVA, multiple comparisons). (E) MPS1 and mps1-R170S cells from the 45 s frame rate replicate were evaluated to determine the amount of time that CEN1 was positioned at the side-by-side SPBs (within 0.5 μm) in individual cells in the 45 min preceding SPB separation to make the prometaphase spindle (duration of clustering). The total time that CEN1 was at the SPBs in each cell is shown (n = 25 cells for the wild-type control and n = 16 cells for mps1-R170S; unpaired t test, *p < 0.05).

**FIGURE 3:** Mps1 promotes minus end-directed migration to the base of monopolar microtubule arrays. (A) Schematic representation of centromere clustering on a monopolar microtubule array. (B) Images of a representative wild-type cell exhibiting cycles of clustering of GFP-tagged CEN1 (green) at the side-by-side SPBs (red) before spindle formation (the last image). Scale bar: 2 μm. (C) The pulling of the chromosome can be separated in two alternating phases where CEN1 is either moving toward the SPBs (Clustering) or at a relative constant distance from the SPBs. (D) Clustering of GFP-tagged CEN1 was monitored using live cell imaging of spo11 diploid cells with MPS1, mps1-R170S, or mps1-as1 alleles. Experiments were performed in three biological replicates imaged at 45 s (once) or 2 min (twice) frame rates. The graph shows the timing of the final clustering of CEN1 (within 0.5 μm) relative to the time of SPB separation for each individual cell. The red dotted line represents the time at which the SPBs separated. *p < 0.05, ****p < 0.0001 (ordinary one-way ANOVA, multiple comparisons). (E) MPS1 and mps1-R170S cells from the 45 s frame rate replicate were evaluated to determine the amount of time that CEN1 was positioned at the side-by-side SPBs (within 0.5 μm) in individual cells in the 45 min preceding SPB separation to make the prometaphase spindle (duration of clustering). The total time that CEN1 was at the SPBs in each cell is shown (n = 25 cells for the wild-type control and n = 16 cells for mps1-R170S; unpaired t test, *p < 0.05).

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FIGURE 4: Mps1 promotes chromosome mobility on the meiotic prometaphase spindle. (A–C) spo11Δ diploid cells, with the indicated genotypes, with one CEN1-GFP-tagged chromosome and a SPB marker (SPC42-GFP) were sporulated and released from a pachytene arrest (PGAL1::NDT80 GAL4-ER) at 6 h after introduction to sporulation medium by the addition of 5 μM β-estradiol. Subsequently, cells were harvested and observed by time-lapse imaging during meiosis at 2 s intervals for 5 min. Chromosomes were scored in cells with 1.5–3.5-μm-long spindles (cells in prometaphase–metaphase [Meyer et al., 2013]). The experiment was performed as three biological replicates per genotype with 40 cells scored per replicate. (A) Cells were placed in one of five categories according to the primary behavior of the GFP-tagged centromere during the 5 min interval: clustered (remaining close to one SPB), nonpolar (positioned away from the poles and not migrating toward a pole), low-mobility half spindle (making small movements within one half spindle), high mobility (moving poleward or toward the midzone, covering a distance of approximately...
targets at the end-on attached kinetochore-microtubule interface to help convert paused kinetochores to moving kinetochores. To investigate this model, we characterized the behavior of centromeres making poleward migrations in wild-type and mps1-R170S cells. We identified centromeres that in the course of our 5 min snapshot of prometaphase moved from a position that was about 1 micron (0.9–1.2 μm) away from a spindle pole toward that pole (Figure 5A). Such cells are rare in the mps1-R170S population due to the preponderance of locked-in-place centromeres. These poleward migrations could come from either pushing or pulling forces, but because the migrations occur within a half spindle (the 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 (Figure 5A). The chart of the movements of each tracked centromere as it moves poleward (Figure 5B) reveals first, that all centromeres exhibit some reversals and pauses during the journey. Some of these might be artifactual as 1) the measurements are taken from two-dimensional projections of three-dimensional spindles so spindle rotations in the Z-dimension could distort the true centromere-SPB distance, and 2) the movements are relatively small compared with the sizes of the centromere GFP and SPB foci—distances measured are from the center of each focus. Measuring protocols were used to minimize these issues (see Materials and Methods). Tracking the individual centromeres showed that poleward migrations took significantly less time in wild-type cells than in mps1-R170S mutants (Figure 5, B and C). To determine whether this was because centromeres reach higher velocities in wild-type cells, we measured the velocities of both poleward and anti-poleward centromere movements over the course of migrations to the pole (Figure 5D). Measurements were obtained as a sliding three-frame window (4 s) in which the centromere moved in the same direction between frames 1 and 2 and between frames 2 and 3. There was no obvious difference in the average speeds of either poleward or anti-poleward movements of the GFP-tagged centromere in wild-type and mps1-R170S strains; the velocities exhibited by the GFP-tagged centromere as it made poleward migrations were indistinguishable (Figure 5D; average forward velocity, WT 76.23 nm/s, n = 13, mps1-R170S 58.80 nm/s, n = 15; p = 0.0758; average reverse velocity, WT 38.58 nm/s, n = 12, mps1-R170S 41.33 nm/s, n = 15; p = 0.65, unpaired t tests). If the centromere movements during poleward migration are driven mainly by microtubule depolymerization, then kinetochore microtubule depolymerization occurs at indistinguishable rates in wild-type cells and mps1-R170S mutants.

Because migration to the pole takes much longer in mps1-R170S mutants than in wild-type cells but the velocities of poleward movements are indistinguishable, this argues that the mps1-R170S mutants must pause or reverse more often. To test this, we measured the frequency with which the GFP-tagged centromere paused or reversed direction in its poleward migration (Figure 5E). The MPS1 mutants exhibited significantly more pauses, or reversals of direction, in their journeys to the pole (Figure 5F), and the distance traveled between pauses or reversals was significantly shorter (Figure 5G). Because the velocities of movement in wild-type cells and mps1-R170S mutants are indistinguishable, the higher numbers of pauses in MPS1 mutants results in longer times for poleward journeys of centromeres in these cells.

If Mps1 acts during prometaphase to promote depolymerization of kinetochore microtubules, and kinetochore microtubules are stabilized in MPS1 mutants, then microtubule turnover should be reduced in prometaphase in MPS1 mutants (Figure 6A). To test this, we measured microtubule turnover in cells expressing a photoconvertible mEos2-tagged α-tubulin subunit (Markus et al., 2015). mEos2-Tub1 has properties of a GFP until it is pulsed with 405 nm light, at which point it switches to a red fluorescent protein (RFP) (Figure 6B). To measure turnover of kinetochore microtubules, we pulsed half of the spindle of cells expressing mEos2-Tub1 with 405 nm light and then measured turnover of the red fluorescent signal (Table 1). Previous measurements of microtubule turnover in budding yeast have been in mitotic cells, but the majority of defects we have examined with MPS1 mutants have been in meiotic cells. Therefore, we first compared microtubule turnover in metaphase spindles of yeast meiotic and mitotic cells and found them to be indistinguishable (Figure 6C). To confirm that our methods could detect variations in microtubule turnover rates in meiosis, we measured turnover in cells expressing an auxin-degradable version of the microtubule plus-end protein Stu2 (Stu2-AID*), which helps to regulate microtubule dynamics in mitotic metaphase (Woyniak et al., 2006; Podolski et al., 2014; Miller et al., 2016; Humphrey et al., 2018; Miller et al., 2019). Cells were induced to enter meiosis, and microtubule turnover was measured in the presence or absence of auxin. As observed previously in mitotic cells, (Kosco et al., 2001; Pearson et al., 2003), inactivating Stu2 in meiotic cells reduced microtubule turnover (Figure 6D). If Mps1 is, like Stu2, promoting microtubule turnover in metaphase cells, then inactivating Mps1 should give a similar outcome. To test this, we compared microtubule turnover in metaphase meiotic wild-type cells and mps1-as1 cells (both in the presence of the Mps1-as1 inhibitor 1-NMPP1). Microtubule turnover rates in metaphase, with or without Mps1 activity, were indistinguishable. This finding is consistent with the reduction in Mps1 levels at kinetochores as they become bioriented and the spindle checkpoint is satisfied (Dou et al., 2003; Howell et al., 2004; Aravamudhan et al., 2015; Koch et al., 2019). Our failure to

one-fourth to three-fourths of a spindle length, and traverse (moving pole-to-pole across the entire spindle). Examples of each classification are shown. Scale bar, 2 μm. *p < 0.05 (unpaired t tests). (B) Traces of the position of CEN1 relative to the SPBs from representative wild-type and mps1-R170S cells that were classified as “non-polar” in panel A. (C) The top left panel is a schematic of the relative positions of the GFP-tagged CEN1 in two sequential imaging frames (SPBs are shown in red). The spindle-centered reference system has three key parameters: The position of SPB1 is constant at x = 0 and y = 0, the position of SPB2 depends on the spindle length (variable over time), and the coordinates x and y (in microns) define the distance of CEN1 from SPB1 at that imaging frame. Shown are traces of the location of CEN1, relative to the SPBs, in 150 sequential time points (every 2 s for 5 min) in five representative cells from the nonpolar category. (D, E) Detailed analysis of centromeres exhibiting nonpolar behavior. (D) We calculated the median position of CEN1 over the course of the 5 min imaging period and then determined the distance of CEN1 from that median position (nanometers) for each frame (150 total) of the acquisition (see cartoon). The graph shows the distribution of distances (in 100 nm bins) from the mean centromere position per cell. The error bars represent the average and SD, n = 8 cells for WT, 17 cells for mps1-R170S, and 13 cells for ndc80-md. (E) The proportion of individual CEN1 movements (in D) that were less than 100 nm from the median position was calculated for each indicated genotype. Mutant genotypes were compared with the wild-type control. **p < 0.01 (ordinary one-way ANOVA).
detect a role for Mps1 in metaphase microtubule dynamics could suggest that it is simply not involved in that function. The meiotic defects we have observed in MPS1 mutants were in prometaphase, before chromosomes are bioriented, raising the question of whether microtubule dynamics are discernibly different in prometaphase and metaphase cells using our microtubule turnover assay. In wild-type yeast meiosis, most of the chromosomes are bioriented within a few minutes after spindle formation (Meyer et al., 2013). Therefore, we used the spo11 mutation to obtain a population of cells in which none of the chromosomes are bioriented. Consistent with the higher rates of turnover for unattached versus stably attached kinetochore microtubules (Gorbsky and Borisy, 1989; Zhai et al., 1995), the spindles in the spo11 cells exhibited higher rates of microtubule turnover than were seen in metaphase cells (Figure 6, B and F). If Mps1 promotes depolymerization of the kinetochore microtubules of non-bioriented chromosomes in prometaphase, then this higher rate of turnover seen in prometaphase should be reduced in MPS1 mutants. For both mps1-as1 and mps1-R170S this proved to be the case (Figure 6, G and H). Both mutations reduce the rate of turnover to levels like those seen in metaphase cells, where inactivating Mps1 has no discernible effect on microtubule turnover.

**DISCUSSION**

Previous work has shown that Mps1 is essential for proper chromosome segregation in meiosis in a variety of organisms (Straight et al., 2000; Poss et al., 2004; Gilliland et al., 2005). We have found that, in budding yeast meiosis, Mps1 impacts at least three steps in the biorientation process (Meyer et al., 2013, 2018). First, Mps1 promotes the migration of bivalents to the side-by-side SPBs at the base of a monopolar microtubule array following the exit from meiotic prophase (clustering). Second, Mps1 promotes the processive poleward movements on the prometaphase meiosis I spindle that occur...
before bivalents become bioriented. Third, through phosphorylation of Dam1, and possibly other targets, Mps1 helps to stabilize end-on attachments of the prometaphase kinetochores to microtubules.

The failure of \(\text{MPS1}\) mutants to phosphorylate Dam1 does not explain the massive defects in meiotic chromosome segregation exhibited by \(\text{MPS1}\) mutants. Despite their defects in kinetochore-
microtubule interactions, dam1-2A mutants that cannot be phosphorylated by Mps1 exhibit rather mild meiotic chromosome segregation defects (Shimogawa et al., 2006; Meyer et al., 2018). Thus, there must be another role (or roles) of Mps1 that explains its essentiality for meiotic chromosome segregation. Our experiments have not revealed a critical meiotic substrate but have refined our understanding of the ways in which Mps1 affects chromosome dynamics in meiosis I.

Our results suggest that the major defect in MPS1 mutants is in regulating microtubule dynamics at the kinetochore interface. A number of observations point to this conclusion. First, when kinetochores are moving poleward in MPS1 mutants, the average velocity is indistinguishable from that of wild-type cells (Figure 5D). This suggests that Mps1 is not essential for kinetochores to track depolymerizing microtubules. In addition, it demonstrates that once a kinetochore microtubule begins depolymerizing, its rate of depolymerization is not affected by Mps1. However, the distances traveled between pauses by poleward-migrating centromeres in MPS1 mutants are shorter than in wild-type cells (Figure 5G) and the pauses are more frequent (Figure 5F). The pauses during poleward migration of the centromeres could represent losses of kinetochore-microtubule plus-end attachment or pauses in microtubule depolymerization, or both. Given that phosphorylation of Dam1 by Mps1 strengthens kinetochore attachments to plus ends (Shimogawa et al., 2006; Meyer et al., 2018), some of the pauses in MPS1 mutants are probably due to failures in maintaining the kinetochore-plus-end connection. However, other results suggest that this is not the major defect. First, MPS1 mutants exhibit low levels of the lagging chromosomes that are an indicator of a defect in attaching kinetochores to microtubules (Meyer et al., 2013, 2018). Second, MPS1 mutants exhibit a stuck-in-the-middle phenotype in which kinetochores maintain a very stable position in midspindle. This is unlike DAM1 mutants, in which kinetochores and plus ends become uncoupled, or NDC80 mutants, in which kinetochores do not attach to microtubules (Meyer et al., 2018); in these two mutants the apparently unconnected centromeres move much more freely than in MPS1 mutants. One explanation for the stuck-in-the-middle phenotype is that MPS1 mutants may be defective in promoting the initiation of depolymerization of kinetochore-coupled MT plus ends. We propose that when a microtubule plus end attaches to a kinetochore, the proximity of the microtubule plus end–associated proteins to Mps1 allows Mps1 to phosphorylate key substrates associated with the plus end, changing their activity or localization in a way that favors microtubule catastrophe over rescue (Figure 6A). The identity of these Mps1 substrates and how their phosphorylation biases microtubule dynamics remains an important unanswered question.

The above model does not solve another unknown. Why is it that meiotic chromosome segregation is more vulnerable to defects in Mps1 activity than is mitosis? We offer three possible explanations. First, when mitosis begins, kinetochores are already attached to microtubules. In contrast, the chromosome paring process of meiotic prophase demands that kinetochores be released from microtubules for an extended time period. When meiotic prometaphase begins, the kinetochores are dispersed across the nucleus and are then gathered into the microtubule-dense region around the SPBs (clustering) just before the SPBs separate to form a spindle. Mps1 is required for this clustering (Meyer et al., 2013). It may be that in the absence of clustering the formation of initial kinetochore-microtubule attachments on the nascent bipolar spindle is highly inefficient, leading to biorientation defects. A phenomenon similar to clustering, referred to as kinetochore retrieval, has been reported in Schizosaccharomyces pombe meiosis (Kakui et al., 2013; Cojoc et al., 2016). Here, mutations that lead to defects in meiotic kinetochore retrieval also result in subsequent biorientation defects, but it is difficult to know whether the segregation defects are purely due to the failure to cluster the dispersed meiotic kinetochores before spindle formation, or to other effects of the mutations.

Second, the vulnerability of meiotic cells to MPS1 defects might lie in differences between meiotic and mitotic spindles. When yeast meiotic spindles form, most chromosomes are mono-oriented, with most chromosomes clustered near the older SPB (Meyer et al., 2013). Mitosis starts in a similar way (Marco et al., 2013). Thus, in both meiosis and mitosis, chromosomes that become bioriented have made their way to the spindle midzone from the pole. But yeast meiotic spindles are longer, possibly making them more dependent on processes that get them from the poles to the midzone (Meyer et al., 2013). Movement from the pole to the midzone could be accomplished by pulling of the kinetochore by a long microtubule extending across the spindle from the opposite pole—a process that our results show is defective in MPS1 mutants (Meyer et al., 2013) both because failure to phosphorylate Dam1 results in defective end-on attachments and because processive poleward movements are defective in MPS1 mutants. An alternate means to get to the midzone from the pole is by movement of chromosomes along microtubules.

### Table 1: Microtubule turnover measurements.

| Type of cells | Stage | # of replicates | # of cells | Half-time (s<sub>1/2</sub>) | Spindle length (μm) | R²  |
|--------------|-------|----------------|-----------|-----------------|-------------------|-----|
| WT           | Mitotic metaphase | 3              | 10 (4, 3, 3) | 119.5           | nd                | 0.938 |
| WT           | Meiotic metaphase | 4              | 25 (7, 6, 6, 6) | 119.5           | 2.69              | 0.961 |
| WT (1-NMPP1) | Meiotic metaphase | 3              | 26 (8, 12, 6) | 126.0           | 2.62              | 0.986 |
| mps1-as1 (1-NMPP1) | Meiotic metaphase | 4              | 29 (8, 5, 7, 9) | 133.3           | 3.55              | 0.986 |
| STU2-AID*    | Meiotic metaphase | 3              | 29 (10, 12, 7) | 135.9           | 2.46              | 0.973 |
| STU2-AID* (+Auxin) | Meiotic metaphase | 3              | 23 (11, 4, 8) | 223.6           | 2.15              | 0.945 |
| spo11        | Meiotic prometaphase | 3             | 35 (9, 13, 13) | 79.7            | 3.34              | 0.979 |
| spo11 mps1-R170S | Meiotic prometaphase | 3             | 25 (8, 10, 7) | 115.5           | 3.11              | 0.932 |
| spo11 (1-NMPP1) | Meiotic prometaphase | 3             | 20 (6, 7, 7) | 80.6            | 3.59              | 0.981 |
| spo11 mps1-as1 (1-NMPP1) | Meiotic prometaphase | 4             | 29 (8, 8, 4, 9) | 119.5           | 3.09              | 0.995 |
from that pole toward their plus ends. This chromosome gliding mechanism has been reported in S. pombe and animal cells but not budding yeast (Kapoor et al., 2006; Windecker et al., 2009; Akera et al., 2015). In S. pombe the process involves proteins (Bub1, Bub3, Mad1, kinesin-5) whose kinetochore localization depends on Mps1 (Windecker et al., 2009; Akera et al., 2015) and is especially critical for chromosome biorientation in cells with long spindles. There is as yet no evidence that this mechanism is important in budding yeast. However, consistent with this model is the recent demonstration that BUB1 and BUB3 mutants, like MPS1 mutants, both exhibit much higher levels of meiotic than mitotic segregation defects and missegregate homologous chromosomes to the older SPB in meiosis I, though not at the high levels seen in MPS1 mutants (Cairo et al., 2020).

Finally, the flexibility of the connections between homologous meiotic centromeres could make them vulnerable to deficiencies in Mps1. This is true of meiotic chromosomes across species and may explain the shared dependence on Mps1 in yeast, Drosophila, and zebrafish meioses. Mitotic sister kinetochore-attached chromosomes are arranged back-to-back, and tightly cohered. Bioriented attachments of sister chromatids are thus probably very quickly under tension and stabilized. In contrast, homologous meiotic kinetochore-attached chromosomes are connected by chiasma and therefore a longer tether. This predicts that greater microtubule depolymerization is required in meiosis to separate the homologous kinetochore sufficiently that they are under tension. It may be that in the time interval between the formation of an initial bipolar attachment and the generation of stabilizing tension, one or both of the kinetochore-microtubule connections is lost, and the process must restart. This more challenging meiotic attachment process may render the cell vulnerable to defects that diminish the efficiency of establishing kinetochore-microtubule attachments. The observation that in budding yeast, meiotic chromosomes are much more sensitive to defects in the spindle checkpoint than are mitotic cells reinforces the idea that biorientation in meiosis faces greater hurdles than in mitosis (Shonn et al., 2000; Cheslock et al., 2005). But work remains to reveal the greatest vulnerabilities of the meiotic biorientation process and how the cell deals with them.

**MATERIALS AND METHODS**

Request a protocol through Bio-protocol.

**Yeast strains and culture conditions**

All strains are derivatives of two strains termed X and Y described previously (Dresser et al., 1994). Strain genotypes are listed in Supplemental Tables S1 and S2. We used standard yeast culture methods (Amberg et al., 2005). To induce meiosis, cells were grown in YP (yeast peptone) acetate to 4–4.5 × 10⁷ cells/ml and then shifted to 1% potassium acetate at 10⁶ cells/ml. Mitotic cells were grown in SD-TRP (complete synthetic defined medium missing tryptophan) media (Sunrise Science).

**Genome modifications**

Heterozygous and homozygous CEN1-GFP dot strains: An array of 256 lac operon operator sites on plasmid pJN2 was integrated near the CENT locus (coordinates 153583–154854). lacI-GFP fusions under the control of P_CYC and P_DMC1 were also expressed in this strain to visualize the location of the lacO operator sites during meiosis as described in Meyer et al. (2013).

PCR-based methods were used to create complete deletions of ORFs and promoter insertions (Longtine et al., 1998; Janke et al., 2004). spo11::kanMX, spo11::his5, spo11::HIS3MX6, lacI-GAL4(848)-ER-UAA3::mCherryNT1, natNT2::P_GAL1-NDT80, KANMX::P_GAL1-NDT80, mps1::KANMX, TRPl::10×myc/mps1-as1 (= mps1-as1), mps1-R170S::his5, KANMX::P_GAL1-NDT80, KANMX::P_GAL1-NDT80, mps1::KANMX, TRPl::10×myc/mps1-as1 (= mps1-as1), mps1-R170S::his5, KANMX::P_GAL1-NDT80, KANMX::P_GAL1-NDT80, mps1::KANMX, TRPl::10×myc/mps1-as1 (= mps1-as1), mps1-R170S::his5, KANMX::P_GAL1-NDT80, KANMX::P_GAL1-NDT80, mps1::KANMX, TRPl::10×myc/mps1-as1 (= mps1-as1), mps1-R170S::his5, KANMX::P_GAL1-NDT80, KANMX::P_GAL1-NDT80.

**Fluorescence microscopy**

Long-term live cell imaging experiments (every 45–120 s for 3–4 h) were performed with cells in liquid media. 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 of CEN1 movement, two different exposure programs were defined, depending on the presence (Spo11) or absence (spo11Δ) of chiasma. In the presence of chiasma, the intervals were every 2 min for 2 h and later every 5 min for 2 h (Supplemental Figure S2). Without chiasma, images were acquired every 45 s or 2 min for 75 min followed by every 10 min for 3 h (Figures 2 and 3).

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 beginning of clustering was defined when CEN1-GFP first reached a position within 0.5 μm of the SPB and remained within this distance for three consecutive frames. Traverses (CEN1 crossing the spindle from one pole to the other) were counted only when the CEN1-GFP signal was overlapping with the SPB signal for at least one frame. Homologues were considered to be bioriented when the homologous CEN1-GFP signals were distinctly separated in two foci.

For high-speed live cell imaging, images were collected every 2 s for 5 min using a Roper CoolSNAP HQ2 camera on a Zeiss Axio Imager 7.1 microscope fitted with a 100×, NA1.4 plan-Apo objective (Carl Zeiss MicroImaging), 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. The coverslip was then inverted over a silicone rubber gasket attached to a glass slide. Thru-focus images were acquired as described previously and then deconvolved to provide a two-dimensional projected image for each acquisition (Conrad et al., 2008). For the analysis of centromere movements on bipolar spindles, the coordinates of the two SPBs (labeled by SPC42-GFP) and the centromeres (marked by CEN1-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 = 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 axis 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 remains the same or increases over time. Therefore, for instances in which the SPB1-SPB2 distances decreased in sequential frames, the value was corrected by replacing the SPB1-SPB2 distance with the prior maximum spindle length (dMax SPB1-SPB2). The magnitude of this correction was also then applied to correct the SPB1-CEN1 distance; the following formula was applied for each interval: Distance SPB1-CEN1 = Observed distance SPB1-CEN1 × dMax 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 the time interval (4 s). The median position for CEN1 was determined in 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 its 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. During this 0.9–1.2 µm migration, the intermediate steps were considered poleward movement when the distance between SPB and CEN1 from one interval to the other 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 ≥ 0), 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).

Measuring microtubule turnover

Microtubule turnover was evaluated in yeast cells expressing mEos2-Tub1, harvested from either log-phase vegetative cultures (in YPAD [yeast peptone adenine dextrose] medium [Majdan et al., 2005]) or meiotic cultures. For meiotic experiments, cells in a synchronized arrest were induced to exit prophase by the addition of estradiol to the medium, using previously published methods (Meyer et al., 2013). Where indicated, auxin (2 mM; Sigma Aldrich I5148-10G), CuSO4 (200 µM; Sigma Aldrich 451657-10G), or 1-NMPP1 (5 µM; Calbiochem; 5 mM stock in dimethyl sulfoxide) were added to the medium at the time of prophase exit. One hour after prophase exit was induced, cells were concentrated, spread across polyethyleneimine-treated coverslips, and then covered with a thin 1% agarose pad to anchor the cells to the coverslip. The coverslip was then inverted over a silicone rubber gasket attached to a glass slide. Cells synchronously entering prometaphase were then subjected to imaging to measure microtubule turnover.

Cells were imaged using a 100×, NA 1.4 objective on a Zeiss Axio Observer inverted microscope equipped with a Yokogawa CSU-22 (Yokogawa) spinning disk, Mosaic (digital mirror device; Photonic Instruments/Andor), a Hamamatsu ORCA-Flash4.0LT (Hamamatsu Photonics), and Slidebook software (Intelligent Imaging Innovations). Photoconversion was achieved by targeting a selected area in half the spindle with filtered light from the HBO 100 via the Mosaic, and confocal GFP and RFP images were acquired at 15 s intervals for ~5 min. At each acquisition, we acquired seven images in the Z-dimension with 0.5 µm spacing. To quantify fluorescence dissipation after photoconversion, we measured pixel intensities within an area surrounding the region of highest fluorescence intensity and background subtracted using an area from the nonconverted half spindle using MetaMorph software.

Fluorescence values were normalized to the first time point after photoconversion for each cell, and the average intensity at each time point was fitted to a single exponential decay curve $F = A \times \exp(-k \times t)$, using SigmaPlot (SYSTAT Software), where $A$ represents the microtubule population with a decay rate of $k$, respectively. $t$ is the time after photoconversion. For each experiment, we performed at least three biological replicates with at least three cells imaged per experiment. Cell numbers for each experiment are given in Table 1. Sample identity for scoring fluorescent signals was blinded. The half-life for the microtubule population was calculated as $\ln(2)/k$. Graphs were prepared using GraphPad Prism. Graphs represent the averages and SEM for combined replicates.

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