csi2p modulates microtubule dynamics and organizes the bipolar spindle for chromosome segregation

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ABSTRACT Proper chromosome segregation is of paramount importance for proper genetic inheritance. Defects in chromosome segregation can lead to aneuploidy, which is a hallmark of cancer cells. Eukaryotic chromosome segregation is accomplished by the bipolar spindle. Additional mechanisms, such as the spindle assembly checkpoint and centromere positioning, further help to ensure complete segregation fidelity. Here we present the fission yeast csi2\textsuperscript{+}. csi2p localizes to the spindle poles, where it regulates mitotic microtubule dynamics, bipolar spindle formation, and subsequent chromosome segregation. csi2 deletion (csi2\textsuperscript{Δ}) results in abnormally long mitotic microtubules, high rate of transient monopolar spindles, and subsequent high rate of chromosome segregation defects. Because csi2\textsuperscript{Δ} has multiple phenotypes, it enables estimates of the relative contribution of the different mechanisms to the overall chromosome segregation process. Centromere positioning, microtubule dynamics, and bipolar spindle formation can all contribute to chromosome segregation. However, the major determinant of chromosome segregation defects in fission yeast may be microtubule dynamic defects.

INTRODUCTION Absolute fidelity of chromosome segregation is essential for proper cellular development (Siegel and Amon, 2012). Eukaryotic chromosome segregation is achieved by the bipolar spindle, a dynamic structure composed of microtubules, microtubule-associated proteins (MAPs), motors, and other regulatory proteins (Walczak and Heald, 2008; Tanenbaum and Medema, 2010; Meunier and Vernos, 2012). Of key importance is the correct attachment of the chromosomes, via its kinetochore, to the microtubules responsible for subsequent chromosome separation to the opposite spindle poles (Verdaasdonk and Bloom, 2011; Foley and Kapoor, 2013). Indeed, cells have evolved the spindle assembly checkpoint (SAC), active at the kinetochore-microtubule interface, to ensure correct chromosome-to-microtubule attachment (Musacchio and Salmon, 2007; Lara-Gonzalez et al., 2012; Vleugel et al., 2012).

The fission yeast Schizosaccharomyces pombe serves as a good model system with which to dissect different aspects of the chromosome segregation pathway, from spindle formation (Hagan and Yanagida, 1992, 1995), to kinetochore structure (Goshima et al., 1999), to checkpoint regulators (He et al., 1997). It is reported that the fission yeast csi1\textsuperscript{+} regulates chromosome segregation by positioning the centromeres at the spindle pole body (SPB) during interphase, so that when mitosis starts, the chromosomes are efficiently captured by the spindle microtubules (Hou et al., 2012). Alternatively, csi1\textsuperscript{−} also organizes the bipolar spindle, which is required for proper chromosome segregation (Zheng et al., 2014). Of interest, it was reported in mammalian cells that the precise timing of bipolar spindle formation at mitosis onset is required for proper chromosome segregation, as induced transient monopolar spindles and/or delays in bipolar spindle formation lead to kinetochore-microtubule misattachment and chromosome segregation defects (McHedlishvili et al., 2012; Silkworth et al., 2012). In addition, regulation of microtubule lengths and dynamics also affects chromosome segregation, as
depletion of the mitotic kinesin-8 MCAK, which depolymerizes microtubules, and depletion of the microtubule-associated proteins EB1, which stabilizes microtubules, both lead to chromosome segregation defects (Kline-Smith et al., 2004; Lan et al., 2004; Green et al., 2005). Thus proper centromere positioning, timing of bipolar spindle formation, and regulation of microtubule dynamics are all important for subsequent chromosome segregation. However, what are the relative contributions of these different mechanisms?

We report here the fission yeast csi2+. csi2p localizes to the SPB, similar to the reported localization of csi1p, and their localization is interdependent. csi2 deletion (csi2Δ) has similar chromosome segregation defects as reported for csi1Δ. However, csi2Δ does not have the centromere-positioning defects of csi1Δ (Hou et al., 2012), although it does have microtubule and transient monopolar spindle defects (Zheng et al., 2014). Thus csi2Δ uncouples the relative contribution of centromere positioning from microtubule dynamics and bipolar spindle formation, enabling establishment of their respective and relative contributions to chromosome segregation.

RESULTS

We used the S. pombe genome-wide yellow fluorescent protein (YFP)–tagged collection (Matsuyama et al., 2006) and the haploid deletion collection (Kim et al., 2010) to visually identify novel genes whose products localize to the SPB and whose deletions lead to spindle defects. We identified SPBC2G2.14 and SPAC4D7.07C. Consistent with the logic of our screen, we confirmed that the protein products of these genes localize to the SPB, and their deletions lead to transient monopolar spindles and chromosome missegregation (see later description). We thus named SPBC2G2.14 and SPAC4D7.07C as csi1+ and csi2+ (chromosome segregation impaired 1 and 2), respectively. The role of csi1+ in centromere–SPB anchoring has been described (Hou et al., 2012), as has its role in bipolar spindle formation (Zheng et al., 2014). Here we focus on the function of csi2+.

csi2Δ has spindle assembly defects

csi2Δ yielded viable cells. However, csi2Δ cells were sensitive to the microtubule-depolymerizing drug benzimidazol-2-yl carbamate (MBC; Supplemental Figure S1A), suggesting that csi2Δ cells have the centromere assembly defects in the microtubule cytoskeleton. We thus examined microtubule organization and dynamics in csi2Δ and wild-type cells expressing mCherry-αtub2p (tubulin). No striking differences in interphase microtubules were observed in csi2Δ and wild-type cells. However, spindle microtubule organization and dynamics were markedly different in csi2Δ cells compared with wild type. In wild type, entry into mitosis is concurrent with the disassembly of cytoplasmic interphase microtubules (Hagan, 1998). To standardize measurements of mitosis time, we define the start of mitosis, time 0 min, as the complete disassembly of cytoplasmic interphase microtubules (Figure 1A). In wild type, time 0 min coincides with the assembly of a microtubule “bar” (~83% of cells) or a “dot” (~17% of cells) that quickly transitions into a bar (n = 18), representing the bipolar spindle (Figure 1, A and B). In contrast, only 18% of csi2Δ cells exhibited bars at time 0 min. The rest exhibited delayed bipolar spindle formation (Figure 1A), where the spindle dot occurred more frequently (60% of cells) and took longer to form bars (n = 51; Figure 1, A and B). Of interest, 22% of csi2Δ cells formed transient microtubule protrusions defined as monopolar spindle (mono; Figure 1, A and B). These microtubule protrusions emanated from both mother and daughter SPB (Figure 1D). Whereas wild-type microtubule dots quickly transitioned into bars (<1 min), the csi2Δ dots took significantly longer (2.8 ± 2.1 min; n = 43; p < 10−14); and the csi2Δ mono spindles persisted 5.3 ± 4.2 min (n = 11) before becoming the bipolar bar (Figure 1C). No wild-type cells exhibited monopolar spindles.

We note that csi1 deletion (csi1Δ) cells also yielded delay in bipolar spindle formation similar to csi2Δ (Supplemental Figure S1B), with 95% of cells exhibiting the transient monopolar microtubule protrusion phenotype and 5% exhibiting the transient dot phenotype. Monopolar spindle defects were recently observed in csi1Δ (Zheng et al., 2014). Here we highlight only similar roles of csi1p and csi2p in bipolar spindle formation.

It is known that the kinesin-5 cut7p localizes to the spindle and is essential for bipolar spindle formation (Hagan and Yanagida, 1992; Fu et al., 2009). We compared the recruitment of cut7–green fluorescent protein (GFP) to the spindle in wild-type and csi2Δ cells. Both wild-type and csi2Δ cells exhibited similar recruitment time of cut7p to the spindle, approximately 4 min before mitosis onset or t = 0 min (Figure 1E and Supplemental Figure S1C). Nevertheless, wild type took 5.1 ± 1.4 min (n = 32) after cut7p arrival to form a bipolar spindle bar, in contrast to csi2Δ, which took 7.4 ± 2.0 min (n = 15; p < 10−3; Supplemental Figure S1D). Taking the results together, we conclude that csi2p (and csi1p) functions in bipolar spindle formation. The observed defects in the bipolar spindle are not due to lack or delay of kinesin-5 recruitment to the spindle at the onset of mitosis.

csi2Δ has chromosome segregation defects

In wild-type cells, once spindle bipolarity has been achieved, the spindle elongates to its steady-state metaphase spindle length (Syrovtakina et al., 2013). Using cdc13-GFP (cyclin) signal degradation as a marker for the metaphase-to-anaphase transition (Tatebe et al., 2001), we compared the final metaphase spindle lengths of wild-type and csi2Δ cells (Figure 2, A and C). Whereas the total duration of mitosis was relatively similar between wild type (35.5 ± 4.2 min, n = 12) and csi2Δ (36.5 ± 5.8 min, n = 12, p = 0.65; Figure 2C), metaphase spindle lengths were different. Wild type had metaphase spindle length of 2.93 ± 0.37 μm (n = 16), significantly shorter than csi2Δ length of 4.30 ± 0.52 μm (n = 14, p < 10−4; Figure 2, B and C). We also observed that the csi2Δ metaphase spindles were not stable in length, but continued to slowly elongate (Figure 2C).

Spindle defects are known to correlate with chromosome segregation defects (Goshima and Scholey, 2010). We thus probed for chromosome segregation defects in wild-type and csi2Δ cells. First, using the artificial minichromosome loss color assay (Niwa et al., 1989), we observed that wild type had zero minichromosome loss (<0.003%; n = 300), represented by the white colonies, compared with 5% (n = 300, p < 0.02) of csi2Δ cells that had minichromosome loss, represented by the pink colonies (Figure 2D). Second, using either the kinetochore marker mis12-GFP (Goshima et al., 1999) or the centromere marker CEN1-GFP (Nabeshima et al., 1998), we observed kinetochore “lagging” in 70% of csi2Δ cells during anaphase B when the spindle length increased dramatically, compared with zero lagging in wild-type cells (Figure 2, E and F, and Supplemental Figure S2A).

Lagging kinetochores are indicative of microtubule–kinetochore attachment defects, which suggests that the spindle assembly checkpoint would have been activated (May and Hardwick, 2006). We thus monitored for checkpoint time delay before anaphase. In wild type, the duration from mitotic onset to the end of metaphase was 16.7 ± 3.0 min (n = 19), which has no statistical significance compared with 19.1 ± 4.6 min (n = 20; p = 0.06) for csi2Δ (Supplemental Figure S2B). This is consistent with total mitosis duration being similar between wild type and csi2Δ (Figure 2C). Nevertheless, in the
FIGURE 1: csi2p organizes the prophase bipolar spindle.

(A) Time-lapse images of wild-type and csi2Δ mitotic cells expressing mCherry-atb2p (tubulin). Wild-type cells typically show a stable bipolar spindle (bar) within 1 min after the start of mitosis, when interphase microtubules have completely depolymerized (time = 0 min). In contrast, some csi2Δ cells exhibit a transient prophase monopolar spindle (mono) or a faint dot (dot) instead of the bar. Other csi2Δ cells show no microtubule signal at mitotic entry (yellow box). Scale bar, 5 μm. (B) Comparison of different spindle structures (bar, mono, dot). (C) Images of wild-type and csi2Δ cells showing the bipolar spindle (bar) compared with 18% of csi2Δ cells (n = 51). Wild-type cells have zero monopolar spindles (mono), compared with 22% of csi2Δ cells. Wild-type cells have 17% of dot spindles (dot) that persisted <1 min, compared with csi2Δ cells having 60% of dot spindles that persisted up to 10 min (p < 10^{-6}; see C). (D) Box-and-dot plot comparison of duration of mono and dot spindle persistence in wild-type and csi2Δ cells. Wild-type cells exhibit zero mono spindles (n = 18), and the dot spindles are transient and persist for <1 min (n = 3). In contrast, csi2Δ cells exhibit zero mono spindles (n = 18), and the dot spindles are transient and persist for <1 min (n = 3). (E) Images of wild-type and csi2Δ prophase mitotic spindle (mono) or a faint dot (dot) instead of the bar. Other csi2Δ cells show no microtubule signal at mitotic entry (yellow box). Scale bar, 5 μm.

sad1p and csi1p are required for csi2p localization to the spindle pole body

We next examined csi2p localization throughout the cell cycle. Fluorescent tagging of csi2p at its native locus revealed that csi2p localizes to the SPB during interphase and mitosis (Figure 3A and Supplemental Figure S3A), consistent with the previous genome-wide YFP-tagged overexpression study (Matsuyama et al., 2006). csi2p localization at the SPB was further confirmed by its colocalization with other known SPB-localized proteins, such as sad1p (Hagan and Yanagida, 1995) and sid4p (Chang and Gould, 2000; Figure 3B and Supplemental Figure S3A). The conserved essential SUN-domain inner nuclear membrane protein sad1p has been proposed to act as a scaffold for the recruitment of other proteins to the SPB (Hiraoka and Dernburg, 2009). Using the temperature-sensitive mutant sad1Δts, in which sad1p is inactivated at the nonpermissive temperature of 37°C, we observed no csi2p-mCherry signal at the SPB (Figure 3C). However, sad1-YFP signal was still present at the SPB in csi2Δ cells (Figure 3D). The result suggests that sad1p directly or indirectly recruits csi2p to the SPB.

It was shown that csi1p localizes to the SPB throughout the cell cycle, and its deletion leads to chromosome segregation defects (Hou et al., 2012; Zheng et al., 2014), very similar to the localization and function of csi2p. We thus checked SPB-localization dependence between cs1p and csi2p. In the absence of csi1Δ, no csi2-GFP signal was observed at the SPB (Figure 3E). Of interest, in the absence of csi2Δ, csi1-GFP failed to localize to the spindle SPB precisely at mitosis onset until anaphase, after which time, csi1-GFP signal gradually returned to the SPB (Figure 3F). Thus csi2p requires csi1p to be recruited to the SPB throughout the cell cycle. However, csi1p is only dependent on csi2p for recruitment to the SPB specifically during mitosis. Accordingly, we observed that the monopolar spindle defect in csi1Δ is dominant over csi2Δ cells. Whereas csi2Δ cells showed 21% transient monopolar spindles, the double-deletion csi1Δcsi2Δ cells showed 88%, which is similar to csi1Δ cells, at 95% (Supplemental Figure S3B).

In fission yeast, the three chromosomes are clustered at the interphase SPB via direct coupling between the centromere and the SPB (Kniola et al., 2001). csi1p was shown to be a coupler of centromere to SPB, as csi1Δ cells exhibited declustered centromeres (Hou et al., 2012). Probable direct or indirect interaction between csi1p and csi2p prompted us to test for centromere declustering in csi2Δ cells using the centromere (kinetochore) marker mis12-GFP (Goshima et al., 1999). Surprisingly, whereas 38% of csi1Δ interphase cells showed declustered centromeres, represented by >1 dot of cells expressing mCherry-atb2p and alp4p-GFP (α-tubulin complex protein, marking the SPB). The wild-type cell shows a well-organized microtubule bar spindle between the two SPBs. In contrast, the csi2Δ cell, in addition to having the bar spindle, has microtubules protruding from both SPBs, suggesting that both mother and daughter SPBs are competent microtubule nucleators. Scale bar, 2 μm. (E) Time-lapse images of wild-type and csi2Δ mitotic cells expressing mCherry-atb2p and cut7p-GFP (kinesin-5; Hagan and Yanagida, 1992). For both wild type and csi2Δ, cut7p is recruited to the spindle approximately at the same time before the onset of mitosis (time = 0 min). Scale bar, 5 μm.
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 CSI2p regulates metaphase spindle length and chromosome segregation. (A) Time-lapse images of wild-type and csi2Δ mitotic cells expressing mCherry-atb2p and cdc13p-GFP (cyclin B; Tatebe et al., 2001). cdc13p is degraded at the metaphase–anaphase transition (yellow arrow), marking the final length of the metaphase spindle. In the wild-type cell the final metaphase spindle length was 3.3 ± 0.4 μm (n = 16), compared with 4.7 ± 0.6 μm (n = 14) in csi2Δ (p < 10⁻⁴). A longer metaphase spindle suggests spindle checkpoint delay and/or misregulation of microtubule dynamics. (C) Spindle length vs. time for wild-type and csi2Δ cells. The proceeding table summarizes mitosis duration, spindle final length at end of mitosis breakdown, and metaphase spindle length. The duration of mitosis was the same for wild-type and csi2Δ cells (p = 0.65), suggesting that the spindle checkpoint is satisfied relatively quickly in csi2Δ cells. (D) Minichromosome loss assay (Niwa et al., 1989) for wild-type and csi2Δ cells. Wild-type cells exhibited all-white colonies on selection plates (n > 300), suggesting none or very low (<0.3%) minichromosome loss. In contrast, 5% (n > 300) of csi2Δ colonies were pink, indicating chromosome segregation defects (p < 0.05). (E) Kymograph comparison of wild-type and csi2Δ cells expressing mCherry-atb2p and mis12p-GFP (kinetochore marker; Goshima et al., 1999). By the start of anaphase B, when the spindle exhibits fast elongation, all kinetochores are properly segregated to the spindle poles in the wild-type cell. In contrast, the csi2Δ cell exhibits lagging kinetochores (yellow arrow) during anaphase B, suggesting improper kinetochore–microtubule attachment during mitosis. (F) Bar plot comparison of lagging kinetochores in wild-type and csi2Δ cells. Lagging kinetochores were not observed in wild-type cells (n = 19). In contrast, 70% (n = 32) of csi2Δ cells exhibited lagging kinetochores (p < 10⁻⁴).
from defects of kinetochore–microtubule attachment and leading to chromosome segregation defects (Goshima and Scholey, 2010; Syrovatkina et al., 2013). We thus tracked wild-type and csi2Δ metaphase spindle lengths using cdc13-GFP, whose degradation marks the start of anaphase (Tatebe et al., 2001), and centromere lagging using the kinetochore marker mis6-RFP (Goshima et al., 1999). We found a strong correlation between abnormally long metaphase spindle and kinetochore lagging at anaphase (Figure 4, C and D). Wild-type metaphase spindle lengths ranged from 2 to 3 μm, with a median length of 2.8 μm (Figure 4D). None of the 13 observed wild-type spindles showed lagging kinetochores (Figure 4D). In contrast, csi2Δ final metaphase spindle lengths

interphase centromere declustering (Hou et al., 2012) nor the monopolar spindle (McHedlishvili et al., 2012; Silkworth et al., 2012; Zheng et al., 2014) hypothesis can completely account for the chromosome segregation defects seen in csi2Δ cells. There have to be additional mechanisms.

Abnormally long metaphase spindles correlate with chromosome segregation defect

We noted that csi2Δ cells have significantly longer metaphase spindles compared with wild type (Figure 2, A–C). Abnormally long metaphase spindles are often a consequence of a defective force-balance mechanism for maintaining spindle length, resulting from defects of kinetochore–microtubule attachment and leading to chromosome segregation defects (Goshima and Scholey, 2010; Syrovatkina et al., 2013). We thus tracked wild-type and csi2Δ metaphase spindle lengths using cdc13-GFP, whose degradation marks the start of anaphase (Tatebe et al., 2001), and centromere lagging using the kinetochore marker mis6-RFP (Goshima et al., 1999). We found a strong correlation between abnormally long metaphase spindle and kinetochore lagging at anaphase (Figure 4, C and D). Wild-type metaphase spindle lengths ranged from 2 to 3 μm, with a median length of 2.8 μm (Figure 4D). None of the 13 observed wild-type spindles showed lagging kinetochores (Figure 4D). In contrast, csi2Δ final metaphase spindle lengths
Abnormally long metaphase spindle lengths observed in csi2Δ indicate that spindle microtubules are also longer than wild type, which suggests that microtubule dynamics are perturbed in csi2Δ cells. Because the fission yeast spindle contains many individual microtubules (Ding et al., 1993), it is currently not possible to monitor individual microtubule dynamics within the bipolar spindle structure using optical microscopy. However, we reasoned that the monopolar spindle resulting from kinesin-5 cut7.24Δ inactivation, which is not expected to alter mitotubule dynamics (Hagan and Yanagida, 1992; Fu et al., 2009), would enable measurements of individual microtubules emanating from the spindle poles (Costa et al., 2013). We thus compared individual mitotubule dynamics between cut7.24Δ (control) and cut7.24Δ csi2Δ cells expressing mCherry-atb2 (tubulin). At the nonpermissive temperature 37°C, control and csi2Δ cells exhibited the expected monopolar spindles, with mitotubule protrusions likely composed of multiple mitotubules (p = 0.7; Figure 5A and Supplemental Figure S4A). The number of mitotubule protrusions was similar between control and csi2Δ monopolar spindles (Figure 5B), with control cells having 3 ± 1 (n = 18) microtubule bundles and csi2Δ cells also having 3 ± 1 (n = 19) microtubule bundles. However, the mitotubule protrusions are longer in the csi2Δ cells compared with control (Figure 5C). Whereas the control cells have mitotic microtubule length of 0.7 ± 0.3 μm (n = 53), csi2Δ cells have mitotic microtubule length of 1.2 ± 0.3 μm (n = 59), -40% longer than control (p < 10⁻⁵).

When we could unambiguously determine a single mitotubule, based on homogeneous fluorescence intensity along the entire length of the microtubule, we measured its length over time (Figure 5D). Although mitotubule growth and shrinkage velocities were similar for control and csi2Δ cells (Figure 5E), spindle microtubules of csi2Δ cells consistently grew to longer lengths (2 μm) compared with control (1 μm; Figure 5, D and E). Of importance, the time until microtubule catastrophe was twice as long for csi2Δ cells (21 ± 1 s, n = 3) as for control (11 ± 3 s, n = 3; p < 0.05; Figure 5E), and the frequency of microtubule catastrophe was reduced by half in csi2Δ cells (2.9 ± 0.2 min⁻¹, n = 3) compared with control (5.6 ± 1.7 min⁻¹, n = 3; p < 0.05; Figure 5E). Of note, we observed that csi2Δ monopolar spindles had less microtubule fluorescence signal and less signal area than control cells (Supplemental Figure S4A). Whereas control spindle fluorescence signal was 2951 ± 615 a.u. (n = 24), for csi2Δ it was 2009 ± 526 a.u. (n = 16), or 32% less than control (p < 10⁻⁵; Supplemental Figure S4B). Similarly, whereas control spindle area was 158 ± 22 pixels squared (n = 24), for csi2Δ it was 114 ± 39 pixels squared (n = 16), or 28% less than control (p < 10⁻⁴).

We conclude that csi2Δ cells have defects in spindle microtubule dynamics, leading to the abnormally long metaphase spindle and resulting, in large part, in the observed chromosome segregation defects.

**DISCUSSION**

csi2Δ is a new gene involved in microtubule dynamics, bipolar spindle formation, and chromosome segregation. Our results suggest that sad1Δ recruits both csi2Δ and csi1Δ to the SPB, and csi2Δ and csi1Δ localization at the SPB are partially interdependent. We attempted to show probable physical interaction between csi1Δ and csi2Δ by coimmunoprecipitation but without success (unpublished data), likely due to the fact that csi2Δ is a nuclear protein with a predicted transmembrane domain.

Although both csi2Δ and csi1Δ have transient monopolar spindle defects (Zheng et al., 2014) and chromosome segregation defects (Hou et al., 2012), csi2Δ does not have centromere-positioning defects exhibited by csi1Δ. Thus csi2Δ uncouples the two different

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**FIGURE 4:** Abnormally long metaphase spindle length in csi2Δ correlates positively with chromosome segregation defects. (A) Initial monopolar spindle defects do not correlate with subsequent chromosome segregation defects. Shown are csi2Δ cells expressing mCherry-atb2p and mis12-GFP. Images show the initial state of the spindle, and kymographs show spindle and kinetochore dynamics. Top, an initial monopolar spindle, which subsequently exhibits no kinetochore lag at anaphase B. Bottom, in contrast, a seemingly “normal” initial bipolar spindle, which subsequently exhibits kinetochore lagging (yellow arrowhead). Scale bar, 2 μm. (B) Bar plot quantification of the initial spindle structure (mono, dot, and bar) and subsequent anaphase B kinetochore dynamics (NO Lag, Lag) in csi2Δ cells. No correlation exists between the initial state of the spindle and subsequent kinetochore lagging (n = 40). (C) Time-lapse images of csi2Δ cells expressing cdc13-GFP and mis6-mRFP (kinetochore marker), cdc13p (cyclin B) is degraded at the metaphase/anaphase transition (yellow arrowhead). In the left csi2Δ cell, the final metaphase pole-to-pole distance is 3.1 μm, and the cell has no kinetochore lag. In contrast, the right csi2Δ cell has pole-to-pole distance of 3.6 μm, and it has kinetochore lag. Scale bar, 5 μm. (D) Box-and-dot plot comparison between final metaphase spindle length and kinetochore lagging at anaphase B for wild-type and csi2Δ cells. Wild-type final metaphase spindle length is 2.8 ± 0.4 μm (n = 13), with no cell exhibiting kinetochore lagging. In contrast, csi2Δ final metaphase spindle length is 3.3 ± 0.5 μm (n = 15). Of these, the shorter spindles tend to have no kinetochore lag, and the longer spindles tend to have kinetochore lag.

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3.1 μm > No Lag 3.6 μm > Lag

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range from 3 to 4.5 μm, with a median length of 3.5 μm (Figure 4D). In the csi2Δ spindles, eight of 15 showed lagging kinetochores, and all of these spindles are equal to or above the median length (Figure 4D). Only one spindle above the median length did not exhibit centromere lagging (Figure 4D). Thus, abnormally long metaphase spindle length positively correlates with lagging kinetochores.
control cut7.24 before undergoing catastrophe after 20 s of pole. In contrast, the cut7.24 emanating from the monopolar spindles of cut7.24 lengths. (D) Time-lapse images of single microtubule dynamics plot comparison of the lengths of the microtubule protrusions from have 3 microtubule bundles protruding from the monopolar spindles of cut7.24 and cut7.24Δ cells at 37°C. Control cut7.24Δ cells also have 3 ± 1 (n = 19) microtubule protrusions, and cut7.24Δcsi2Δ cells also possess 0.7 ± 0.3 μm (n = 53). In contrast, cut7.24Δcsi2Δ microtubule protrusion length is 1.2 ± 0.3 μm (n = 59; p < 10^-10), suggesting that csi2p regulates mitotic microtubule lengths. (D) Time-lapse images of single microtubule dynamics emanating from the monopolar spindles of cut7.24Δ and cut7.24Δcsi2Δ cells at 37°C. In the control cut7.24Δ cell, the mitotic microtubule elongates up to 1 μm in length before undergoing catastrophe after 10 s of growth and shrinks back to the spindle pole. In contrast, the cut7.24Δcsi2Δ mitotic microtubule elongates up to 2 μm in length before undergoing catastrophe after 20 s of growth. Scale bar, 2 μm. (E) Length vs. time of individual microtubule dynamics of control cut7.24 (green) and cut7.24Δcsi2Δ cells (red). In control cut7.24Δ cells, the mitotic microtubules grow for 11.3 ± 3.1 s (n = 3) before undergoing catastrophe. In contrast, cut7.24Δcsi2Δ mitotic microtubules grow for 20.7 ± 1.2 s (n = 3), approximately two times as long (p < 0.05). The proceeding table summarizes the growth rate, shrinkage rate, catastrophe frequency, and time before catastrophe of control and csi2ΔΔ cells.

FIGURE 5: csi2p regulates mitotic microtubule length. (A) Images of cut7.24Δ (control) and cut7.24Δcsi2Δ cells expressing mCherry-atb2p. At the nonpermissive temperature (37°C), cut7.24Δ cells fail to form bipolar spindles, but instead make monopolar spindles, which can be evaluated by measuring the individual mitotic microtubule dynamics (Costa et al., 2013). Control cut7.24Δ monopolar spindles exhibit relatively shorter microtubules than cut7.24Δcsi2Δ. Scale bar, 2 μm. Box-and-dot plot comparison of the number of mitotic microtubule bundles protruding from the monopolar spindles of cut7.24Δ and cut7.24Δcsi2ΔΔ cells at 37°C. Control cut7.24Δ cells have 3 ± 1 (n = 18) microtubule protrusions, and cut7.24Δcsi2ΔΔ cells also possess 3 ± 1 (n = 19) microtubule protrusions (p = 0.7). Box-and-dot plot comparison of the lengths of the microtubule protrusions from cut7.24Δ and cut7.24Δcsi2ΔΔ cells at 37°C. Control cut7.24Δ microtubule protrusion length is 0.7 ± 0.3 μm (n = 53). In contrast, cut7.24Δcsi2Δ microtubule protrusion length is 1.2 ± 0.3 μm (n = 59; p < 10^-10), suggesting that csi2p regulates mitotic microtubule lengths.
analyses revealed that 40% of csi1Δ cells have declustered centromeres at interphase, yet 95% of cells have lagging chromosomes at anaphase B (Zheng et al., 2014). This implies that centromere declustering only partially contributes to chromosome segregation defects. Of interest, csi2Δ cells have no declustered centromeres but still have 70% of cells with lagging chromosomes. This implies that the declustered centromeres seen in csi1Δ cells contribute 25% of the chromosome segregation defects and that transient monopolar spindle and/or longer metaphase microtubules seen in both csi1Δ and csi2Δ cells account for the remaining 70% chromosome segregation defects. We do not see a strong correlation between monopolar spindles and subsequent chromosome lagging. Instead, we see a very strong correlation between abnormal spindle lengths, a consequence of defective mitotic microtubule dynamics, and subsequent chromosome lagging. Thus we favor a model in which defective mitotic microtubule dynamics seen in csi1Δ and csi2Δ accounts for 70% of chromosome segregation defects. Sequence homology revealed that csi1+ and csi2+ are both unique to fission yeast. However, given its important and diverse roles during mitosis, functional homology seems likely in higher eukaryotes.

MATERIALS AND METHODS
S. pombe strains and plasmid construction
Standard yeast media and genetic methods were used to create yeast strains, as previously described (Moreno et al., 1991; Forsburg and Rhind, 2006). Strains of csi2 deletion and GFP/mCherry tagging were carried out by a previously described PCR-based method (Bahler et al., 1998).

Screen for csi2+
We used the S. pombe genome-wide YFP-tagged overexpression collection (Matsuyma et al., 2006) and the haploid deletion collection (Kim et al., 2010) to identify novel genes whose products localize to the SPB and whose deletions lead to spindle defects. The novel gene SPAC4D7.07C was found to have spindle defects and chromosome segregation defects. We thus named this gene par2+ (poles separation regulator 2), but subsequently renamed it csi2+ (chromosome segregation impaired 2) to be consistent with published convention.

Minichromosome loss assay
A strain containing the artificial minichromosome Ch16 was introduced into wild-type and csi2Δ cells by mating and random spore analysis, and selection was carried out on minimum media EMM plates lacking the selection marker adenine, according to the published protocol (Niwa et al., 1989). Equal amount of wild-type and csi2Δ cells carrying the minichromosome were plated on selective EMM plates lacking adenine. The plates were incubated at 30°C for 4 d, and colonies were examined for the color red, which indicated minichromosome loss.

Microtubule drug sensitivity assay
Wild-type and csi2Δ cells were grown in YE5S (yeast extract + 5 amino acid supplements) medium to mid log phase (OD600 nm = 0.5), and then a series of fivefold cell dilutions was spotted onto agar plates containing YE5S plus 4 μg/ml MBC (Sigma-Aldrich, St. Louis, MO). These plates were incubated at 30°C for 3 d and then assayed for colony growth.

Microscopy
Live-cell imaging was carried out at either room temperature (21°C) or 37°C, when temperature-sensitive mutants were used. We used a spinning-disk confocal microscope equipped with a PlanApo 100×/1.40 numerical aperture objective (Nikon, Melville, NY) and an ORCA charge-coupled device (CCD) camera (Hamamatsu, Hamamatsu, Japan) or IMage EM electron-multiplying CCD camera (Hamamatsu) as previously described (Tran et al., 2004). MetaMorph 7.5 (Molecular Devices, Sunnyvale, CA) was used to acquire and process all images.

For high temporal resolution, images were acquired at 200-ms exposures for mCherry at 2-s intervals, with each stack comprising four optical sections of 0.5-μm spacing. For longer time scale, images were acquired at 500- or 1000-ms exposures for GFP and mCherry at 1-min intervals, with each stack comprising 11 optical sections of 0.5-μm spacing.

The temperature-sensitive strain sad1-1ts (Hagan and Yanagida, 1995) was incubated in YE5S medium for 3 h at 37°C before imaging. The temperature-sensitive strain cut7.24ts (Hagan and Yanagida, 1992) was incubated in YE5S medium for 20 min at 37°C before imaging.

Data analysis
Data are presented as mean ± SD or as frequency. Statistical analyses on means were performed using the Student’s t test. Statistical analyses on frequencies were performed using the χ² test. All analyses were performed using Excel 2010 (Microsoft, Redmond, WA). All plots were created using KaleidaGraph 4.0 (Synergy Software, Reading, PA). Dot-and-box plots show all individual data points, and the plots enclose 50% of the data in the box, with the median value displayed as a line. The lines extending from the top and bottom of each box mark the minimum and maximum values within the data set that fall within an acceptable range. Outliers are displayed as individual points.

Temperature sensitivity assay
Wild-type, csi2Δ, mad2Δ, bub3Δ, mph1Δ, csi2Δ.mad2Δ, csi2Δ, bub3Δ, and csi2Δ.mph1Δ cells were grown in YE5S medium to mid log phase (OD600 nm = 0.5), and then a series of fivefold cell dilutions was spotted onto YE5S agar plates. These plates were incubated at 25°C for 5 d, or at 30 or 37°C for 3 d, and then assayed for colony growth.

ACKNOWLEDGMENTS
We thank A. Stout of the University of Pennsylvania CDB Microscopy Core for technical assistance; the laboratories of I. Hagan (University of Manchester, Manchester, United Kingdom), J. R. McIntosh (University of Colorado, Boulder, CO), T. Toda (Cancer Research UK, London, United Kingdom), and M. Sato (Waseda University, Tokyo, Japan) and the Japan National Bio Resource Project for generously providing reagents. We thank E. Gomes, M. Piel, A. Paoletti, and E. Bi for helpful discussions throughout this work. J.C. was supported by a predoctoral fellowship from the Fundação para a Ciência e a Tecnologia, Portugal. This work was supported by grants from the National Institutes of Health and the Agence Nationale de la Recherche.

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