Current Biology *17*, 2175–2182, December 18, 2007 ©2007 Elsevier Ltd All rights reserved DOI 10.1016/j.cub.2007.11.032

Report

Mps1 Kinase Promotes Sister-Kinetochore Bi-orientation by a Tension-Dependent Mechanism

Jean-François Maure,[1] Etsushi Kitamura,[1]
and Tomoyuki U. Tanaka[1],*
[1]Wellcome Trust Centre for Gene Regulation & Expression
College of Life Sciences
University of Dundee
MSI/WTB/JBC Complex
Dundee DD1 5EH
UK

Summary

Segregation of sister chromatids to opposite spindle poles during anaphase is dependent on the prior capture of sister kinetochores by microtubules extending from opposite spindle poles (bi-orientation). If sister kinetochores attach to microtubules from the same pole (syntelic attachment), the kinetochore-spindle pole connections must be re-oriented to be converted to proper bi-orientation [1, 2]. This re-orientation is facilitated by Aurora B kinase (Ipl1 in budding yeast), which eliminates kinetochore-spindle pole connections that do not generate tension [3–6]. Mps1 is another evolutionarily conserved protein kinase, required for spindle-assembly checkpoint and, in some organisms, for duplication of microtubule-organizing centers [7]. Separately from these functions, however, Mps1 has an important role in chromosome segregation [8]. Here we show that, in budding yeast, Mps1 has a crucial role in establishing sister-kinetochore bi-orientation on the mitotic spindle. Failure in bi-orientation with inactive Mps1 is not due to a lack of kinetochore-spindle pole connections by microtubules, but due to a defect in properly orienting the connections. Mps1 promotes re-orientation of kinetochore-spindle pole connections and eliminates those that do not generate tension between sister kinetochores. We did not find evidence that Ipl1 regulates Mps1 or vice versa; therefore, they play similar, but possibly independent, roles in facilitating bi-orientation.

Results and Discussion

Mps1 Kinase Is Required for Sister-Kinetochore Bi-orientation on the Metaphase Spindle

Winey and colleagues developed an *mps1-as1* mutant in budding yeast [8], which can be specifically inactivated by an ATP-analog inhibitor 1NM-PP1 [9]. They arrested this *mps1* mutant after duplication of spindle pole bodies (SPBs; microtubule-organizing centers in yeast) but before DNA replication and SPB separation, by using a *cdc34-2* temperature-sensitive (*ts*) mutant. Upon release from *cdc34-2* arrest by lowering the temperature, they inactivated *mps1-as1* by means of its inhibitor [8]; in this way, the Mps1 requirement for SPB duplication was bypassed in order to study its function in a later phase of the cell cycle. The majority of chromosomes showed missegregation when Mps1 was inactivated, and relevant defects were already detected prior to anaphase entry [8], suggesting that Mps1 plays very crucial roles in chromosome segregation, independently of SPB duplication and the spindle-assembly checkpoint.

To address how Mps1 regulates chromosome segregation, we also used *cdc34-2* and *mps1-as1* and analyzed behavior of centromeres when Mps1 was inactivated. *CEN3* was visualized by adjacent insertion of a *tet* operator array that was bound by Tet repressors fused with green fluorescent protein (GFP) [10], and therefore *CEN3* was visualized as a small GFP dot (Figure 1A, top). Microtubules were also visualized by expression of α-tubulin (*TUB1*) fused with yellow fluorescent protein (YFP). After release from *cdc34-2* arrest and inactivation of Mps1, we depleted Cdc20, an activator of anaphase promoting complex, to arrest cells in metaphase [11] (*CDC20* was under control of the *MET3* promoter, which can be turned off in the presence of methionine). As a control, a wild-type *MPS1*$^+$ strain was treated in the same way as the *mps1-as1* strain.

MPS1$^+$ and *mps1-as1* strains formed a bipolar spindle with similar timing (Figures S1A and S1B in the Supplemental Data available online). Upon formation of bipolar spindle, *MPS1*$^+$ cells frequently showed separated *CEN3* dots on the metaphase spindle, indicative of sister-kinetochore bi-orientation [12–15] (Figure 1A). In *mps1-as1* cells, *CEN3* was also located on the spindle; but in contrast to *MPS1*$^+$ cells, they rarely showed separation of *CEN3* dots on the bipolar spindle. Most of nonseparated *CEN3* signals stayed in the vicinity of the same spindle pole during time-lapse observation (data not shown); they were therefore mono-oriented on the spindle (Supplemental Results and Discussion, note 1). The mono-orientation of *CEN3* signals in *mps1-as1* was not due to failure of DNA replication (Figure S2).

We concluded that Mps1 kinase is required for establishing sister-kinetochore bi-orientation on the metaphase spindle. This is consistent with a previous report that bilobular kinetochore distribution in metaphase is disturbed by Mps1 inactivation [8]. Next we addressed whether a similar defect in *mps1-as1* could also be found without using *cdc34-2* (Supplemental Results and Discussion, note 2; Figures S3 and S4). The bi-orientation defect in *mps1-as1* mutant was not an artifact due to use of *cdc34-2*, as shown by the fact that it was also found in other assays.

The Bi-orientation Defect with Inactive Mps1 Is Not Due to Impaired Spindle-Assembly Checkpoint or Abnormal Spindle Elongation/Discontinuity

Considering that Mps1 is important for a spindle-assembly checkpoint [7], we studied the behavior of GFP-marked *CEN3* in *mad2*-deleted cells, where the

---

*Correspondence: t.tanaka@lifesci.dundee.ac.uk

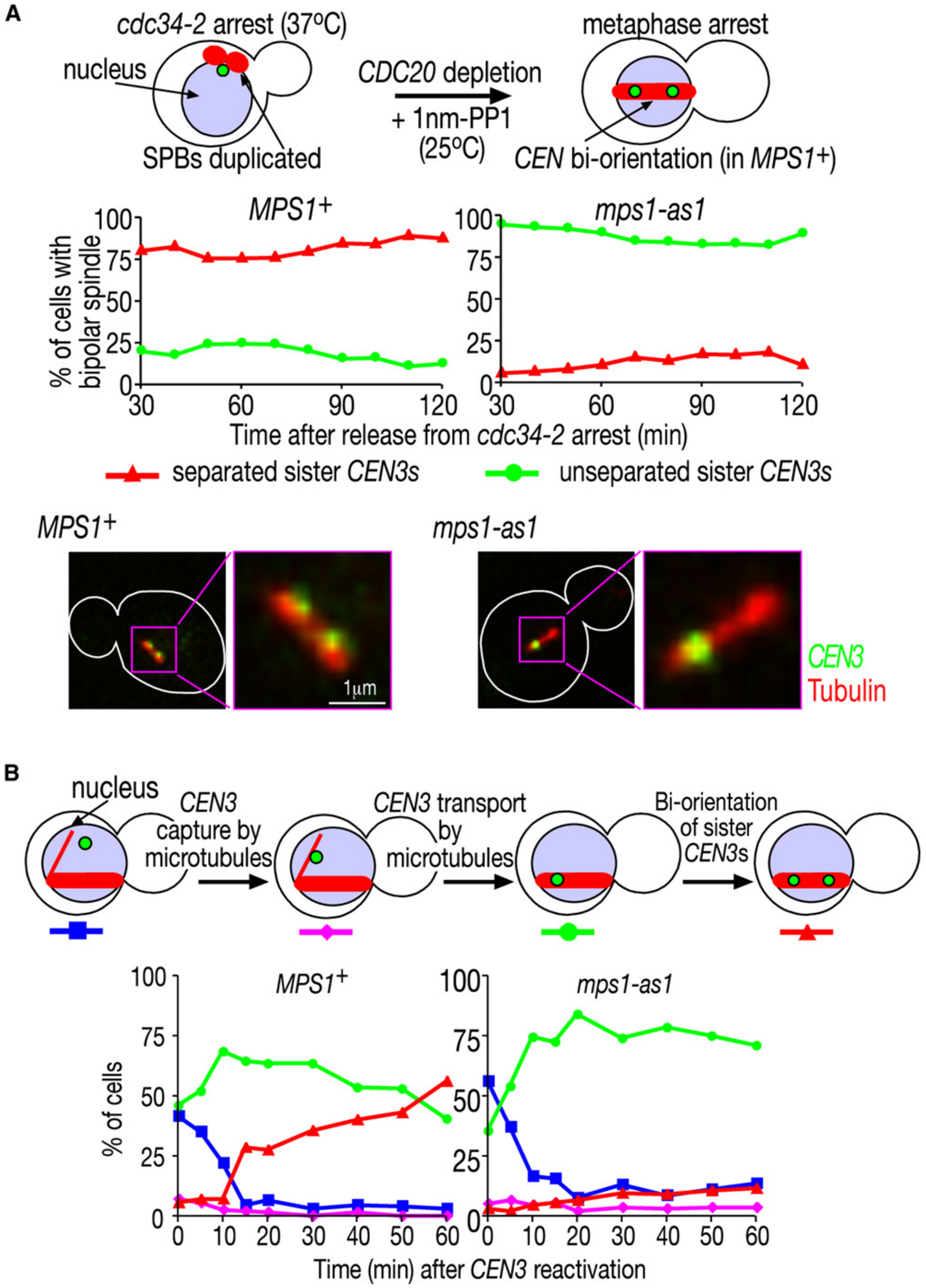

Figure 1. When Mps1 Is Inactivated, an Extensive Defect in Sister-Kinetochore Bi-orientation Is Found on the Metaphase Spindle

(A) *$MPS1^+$* (T4195) and *mps1-as1* (T4174) cells with *PGAL-CEN3-tetOs TetR-GFP YFP-TUB1 cdc34-2 PMET3-CDC20* (*PGAL* and *PMET3* stand for the *GAL1-10* promoter and the *MET3* promoter, respectively) were incubated in methionine dropout medium at 37°C for 2.5 hr, leading to arrest because of *cdc34-2*. Subsequently, they were incubated at 25°C (to release cells from *cdc34-2* arrest) in synthetic complete medium, containing 10 μM 1NM-PP1 (to inactivate *mps1-as1*) and 2 mM methionine (to deplete Cdc20). After 30 min, GFP and YFP images were acquired from live cells every 1 min for 90 min. All media contained glucose, which kept *CEN3* active during the course of the experiment. Top: schematic showing how cells were treated. Middle: graphs show the percentage of cells with bipolar spindle, which has separated GFP signals (red) and a nonseparated signal (green). Bottom: a representative *$MPS1^+$* cell with separated GFP signals (green) on the spindle (red) and an *mps1-as1* cell with a nonseparated GFP signal. Scale bar represents 1 μm. See Figure S1 for data about the spindle of these cells.

(B) When Mps1 is inactivated, kinetochores are captured and transported pole-ward normally by microtubules. T4195 and T4174 cells (see [A]) were released from *cdc34-2* arrest as in (A) and incubated in YP medium, containing raffinose plus galactose (to inactivate *CEN3*), 10 μM 1NM-PP1, and additional 2 mM methionine. Then cells were washed and suspended in the same medium (at time 0) but containing glucose, substituting raffinose/galactose, to reactivate *CEN3*. Subsequently, cells were sampled every 5 min and fixed with paraformaldehyde, and GFP and YFP images were acquired. Each step of kinetochore-microtubule interaction is schematically depicted (top). Each colored line corresponds to the line in the same color in the graphs (bottom) showing the percentage of cells at each step of kinetochore-microtubule interaction.

spindle-assembly checkpoint is defective [16], with the same protocol as in Figure 1A. In contrast to *mps1-as1*, *mad2*-deleted cells showed sister *CEN3* separation as frequently as in *MPS1*$^+$ *MAD2*$^+$ cells on the metaphase spindle (data not shown). The bi-orientation defect with inactive Mps1 was therefore not due to an impaired spindle-assembly checkpoint.

When Mps1 was inactive, the majority of centromeres showed mono-orientation on the bipolar spindle, and in addition, the spindle became longer than normal and was sometimes discontinuous in the middle [8] (Figures S1B and S1C). We next addressed whether such elongated and discontinuous spindles played any causative roles in centromere mono-orientation upon Mps1 inactivation (Supplemental Results and Discussion, note 3; Figure S5). We concluded that *mps1-as1* cells showed defects in bi-orientation before appearance of elongated and discontinuous spindles, which therefore cannot be the sole reason for centromere mono-orientation. Rather, we suspect that the abnormal spindle is the outcome of extensive centromere mono-orientation in *mps1-as1* cells.

Kinetochores Are Captured and Transported Pole-ward Normally by Microtubules when Mps1 Is Inactive

Kinetochores are initially captured by the lateral side of a microtubule and subsequently transported by that microtubule toward a spindle pole, followed by bi-orientation on the spindle [17, 18]. Frequent mono-orientation in *mps1-as1* suggests that one or some of these steps are defective. To identify a defective step, we used an assay system in which we can visualize the initial kinetochore-microtubule interaction in a stepwise manner [17] (Figure 1B, top). In this assay, we regulated the activity of a particular centromere (*CEN3*); *CEN3* was displaced from the spindle and other centromeres by conditional inactivation via transcription from the adjacently inserted *GAL1-10* promoter [19]. Then, during metaphase arrest by Cdc20 depletion, we reactivated *CEN3* by turning off the *GAL1-10* promoter [17]. 1NM-PP1 was added to inactivate *mps1-as1* prior to reactivation of *CEN3*.

After reactivation, the GFP-marked *CEN3* was captured within 10–15 min (Figure 1B, blue line) by the lateral surface of YFP-labeled microtubules and was immediately transported toward a spindle pole (Figure 1B, pink line: time-lapse microscopy, not shown) in the majority of both *MPS1*$^+$ and *mps1-as1* cells. Subsequently, sister *CEN3* dots were separated on the spindle in *MPS1*$^+$, but such separation was rare in *mps1-as1* (Figure 1B, red line). Thus, when Mps1 is inactive, kinetochores are captured and transported pole-ward normally by microtubules, but it is the subsequent establishment of bi-orientation that is extensively defective.

Subsequently we addressed, once bi-orientation is established, whether Mps1 is still required for its maintenance (Supplemental Results and Discussion, note 4; Figure S6). Sister *CEN3* separation was maintained after Mps1 was inactivated, making a sharp contrast with the extensive defect in establishing sister *CEN3* bi-orientation, shown in Figure 1. Thus, Mps1 might not be required for maintenance of bi-orientation once it is established.

Mono-orientation with Inactive Mps1 Is Not Due to Absence or Loss of Kinetochore-Spindle Pole Connections

What causes mono-orientation of sister centromeres when Mps1 is inactive? One explanation is that one of two SPBs is defective in organizing microtubules that extend to capture kinetochores. In particular, because Mps1 is required for SPB duplication [20], the new SPB might be partly defective even after this requirement is bypassed with *cdc34-2*. To address this, we analyzed the behavior of two pairs of sister centromeres on different chromosomes in the same cells (Figure 2A); if mono-orientation is caused by a defect of one SPB, both pairs of sister centromeres will mono-orient always at the same pole, i.e., the more functional one. We marked *CEN5* by the adjacent insertion of a *tet* operator array bound by TetR-3CFP (cyan fluorescent protein) and *CEN15* by a *lac* operator array bound by GFP-lacI [21]. SPBs were also marked by expression of *SPC42* fused with red fluorescent protein (RFP).

After release from *cdc34-2* arrest and addition of 1NM-PP1, *mps1-as1* cells showed extensive mono-orientation of both pairs of sister *CEN5*s and *CEN15*s (Figure 2A), as expected. Importantly, when both *CEN5* and *CEN15* mono-oriented in the same *mps1-as1* cells, they mono-oriented at the same pole or at opposite poles with similar frequency. Therefore mono-orientation cannot be explained by a defect of one SPB in organizing microtubules. The two SPBs are probably equally functional in *mps1-as1* cells, at least under these experimental conditions.

Another explanation for sister centromere mono-orientation with inactive Mps1 is that only a single kinetochore from each pair of sister centromeres is functional. For example, after centromere DNA replication, a kinetochore may be formed on one sister chromatid but not on the other. If this explanation is the case, abolition of sister-chromatid cohesion should permit the sister centromere with an active kinetochore to be drawn to a spindle pole, but should leave the other sister centromere lacking an active kinetochore within the middle of the nucleus.

When we depleted a cohesin subunit Scc1 [11] (also called Mcd1; by means of a strain whose sole *SCC1* gene is under control of the galactose-inducible promoter) to abolish cohesion in *mps1-as1* cells treated with 1NM-PP1, both sister centromeres moved to the vicinity of SPBs in more than 90% of cells, sometimes to the same pole but often to the opposite poles (Figure 2B). Very rarely centromeres stayed between two SPBs and distant from them. A similar result was obtained when we depleted Scc1 in *MPS1*$^+$ cells (Figure 2B); frequent mono-orientation under this condition confirmed that Scc1 was successfully depleted with this procedure [14]. In contrast to the result in *mps1-as1* cells, when Scc1 was depleted in *ndc10-1* cells that lack functional kinetochores, centromeres often did not stay in the vicinity of either SPB [3].

This implies that both sister kinetochores can interact with microtubules in *mps1-as1* cells. Thus, mono-orientation with inactive Mps1 is not caused by a failure to establish functional kinetochores after DNA replication [22]. Taken together, failure in bi-orientation upon Mps1 inactivation is not due to absence or loss

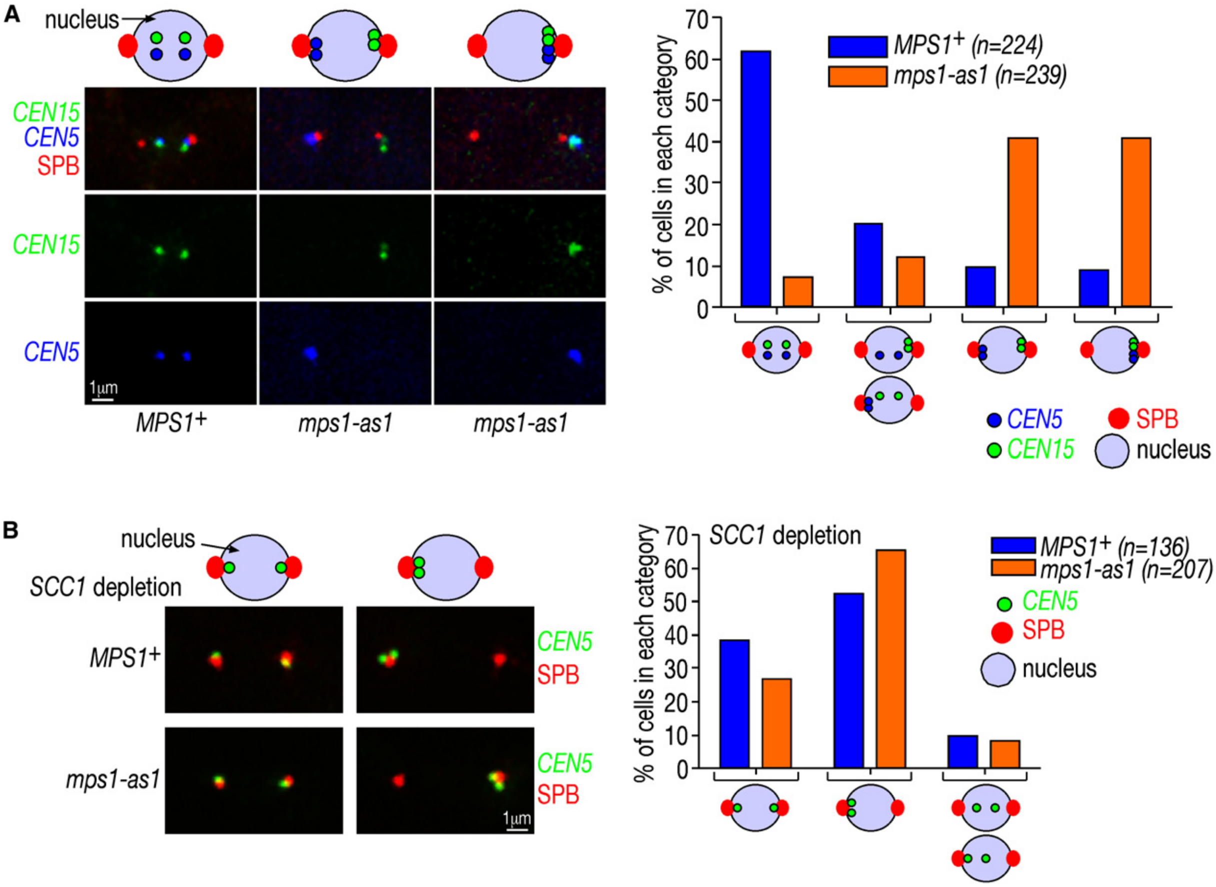

Figure 2. Mono-orientation with Inactive Mps1 Is Not Due to Absence or Loss of Kinetochore-Spindle Pole Connections

(A) When Mps1 is inactivated, mono-orientation of two sister centromere pairs can be simultaneously formed at different spindle poles. *MPS1*$^+$ (T4384) and *mps1-as1* (T4356) cells with *CEN5-tetOs TetR-3CFP CEN15-lacOs GFP-lacI SPC42-RFP cdc34-2 PMET3-CDC20* were released from *cdc34-2* arrest as in Figure 1A and incubated in YP medium, containing 10 µM 1NM-PP1 (to inactivate *mps1-as1*) and additional 2 mM methionine (to deplete Cdc20). After 2.5 hr, cells were fixed with paraformaldehyde, and CFP (*CEN5*, blue), GFP (*CEN15*, green), and RFP (SPB, red) images were collected. Representative *MPS1*$^+$ and *mps1-as1* cells (left) show the three behavior patterns of sister *CEN5*s and *CEN15*s. Scale bar represents 1 µm. Graph (right) shows the percentage of *MPS1*$^+$ (blue) and *mps1-as1* (orange) cells with each behavior pattern of sister *CEN5*s and *CEN15*s.

(B) When Mps1 is inactivated and cohesin is depleted, both sister centromeres can interact with microtubules. *MPS1*$^+$ (T4617) and *mps1-as1* (T5195) cells with *CEN5-tetOs TetR-GFP SPC42-YFP cdc34-2 PGAL-SCC1 PMET3-CDC20* were incubated in methionine dropout medium containing raffinose/galactose and α factor at 25°C. They were washed and cultured in YP medium containing glucose (to deplete Scc1) at 37°C for 2.5 hr, leading to arrest due to *cdc34-2*. Subsequently, they were incubated at 25°C (to release cells from *cdc34-2* arrest) in YP medium, containing glucose, 10 µM 1NM-PP1, and additional 2 mM methionine. After 2.5 hr, cells were fixed with paraformaldehyde, and GFP (*CEN5*, green) and YFP (SPB, red) images were acquired. In representative *MPS1*$^+$ (top) and *mps1-as1* (bottom) cells (left), sister *CEN5*s were pulled to the opposite poles (left) or to the same pole (right). Scale bar represents 1 µm. Graph (right) shows the percentage of each behavior pattern of sister *CEN5*s in *MPS1*$^+$ (blue) and *mps1-as1* (orange) cells.

of kinetochore-spindle pole connections but is due to defects in achieving proper orientation of these connections.

Mps1 Facilitates Kinetochore Bi-orientation in a Tension-Dependent Manner

To facilitate sister-kinetochore bi-orientation on the metaphase spindle, syntelic attachments must be corrected to proper bi-orientation [2]. This error correction stems from stabilization of kinetochore-spindle pole connections by tension, arising from bi-orientation but not syntelic attachment [6, 23]. To investigate error-correction mechanism, we previously developed an unreplicated circular minichromosome harboring two centromeres [6] (Supplemental Results and Discussion, note 5; Figure 3A). In wild-type cells, the two centromeres on this unreplicated dicentric minichromosome always bi-oriented efficiently [6], suggesting that a tension-dependent mechanism suffices for their bi-orientation.

To address whether bi-orientation of the unreplicated dicentric minichromosome, like that of authentic replicated sister centromeres, is dependent on the Mps1 kinase, we compared the behavior of this GFP-marked minichromosome in *MPS1*$^+$ and *mps1-as1* cells after release from *cdc34-2* arrest and addition of 1NM-PP1 (Figures 3B and 3C). In 89% (32/36) of *MPS1*$^+$ cells, GFP signals were found halfway between two YFP-labeled SPBs and often stretched, indicative of bi-orientation. On the other hand, in 92% (33/36) of *mps1-as1* cells, GFP signals remained in the vicinity of one SPB, having

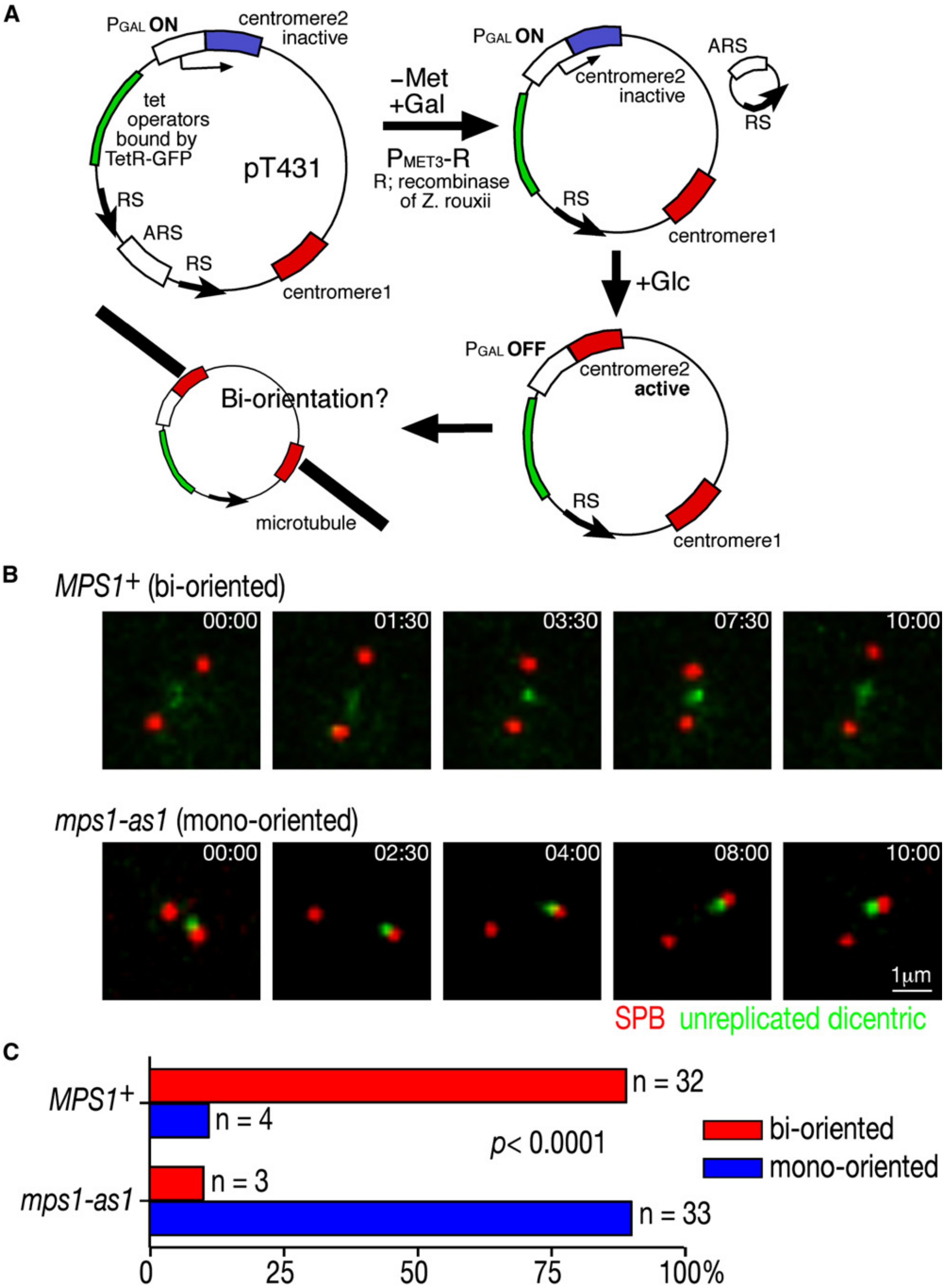

Figure 3. When Mps1 Is Inactivated, the Majority of Unreplicated Dicentric Minichromosomes Show Mono-orientation on the Spindle

MPS1$^+$ (T5292) and *mps1-as1* (T5288) cells with *TetR-GFP SPC42-YFP cdc34-2 PMET3-R* (*R*; recombinase of *Zygosaccharomyces rouxii*), carrying pT431, were incubated in methionine dropout medium (to express the recombinase) containing raffinose/galactose at 25°C. After 1.5 hr, they were incubated in the same medium at 37°C for 2.5 hr, leading to arrest because of *cdc34-2*. Subsequently, they were incubated at 25°C (to release cells from *cdc34-2* arrest) in synthetic complete medium containing glucose (to activate *CEN* under the *PGAL* promoter) and 10 μM 1NM-PP1 (to inactivate *mps1-as1*). After 1 hr and 1.5 hr, GFP and YFP images were acquired every 30 s for 20 min, from live cells.

(A) Schematic showing how an unreplicated dicentric minichromosome was generated from pT431. ARS, DNA replication origin; RS, recombination site; *PMET3*, *MET3* promoter; *PGAL*, *GAL1-10* promoter; Met, methionine; Gal, galactose; Glc, glucose.

(B) In a representative *MPS1*$^+$ (top) and *mps1-as1* (bottom) cell, an unreplicated dicentric minichromosome (green) shows bi-orientation and mono-orientation, respectively. For scoring of these two behaviors see Supplemental Experimental Procedures. Time is shown in min:s and time 0 is defined as the start of image acquisition.

(C) Graph shows the percentage of cells with a bipolar spindle, which showed bi-orientation (red) and mono-orientation (blue) of the unreplicated dicentric minichromosome.

made connections to a single pole, i.e., mono-oriented. Thus, inactivation of Mps1 greatly reduced the incidence of bi-orientation of this minichromosome. A corollary is that Mps1 is able to facilitate bi-orientation of sister kinetochores through a tension-dependent error-correction mechanism.

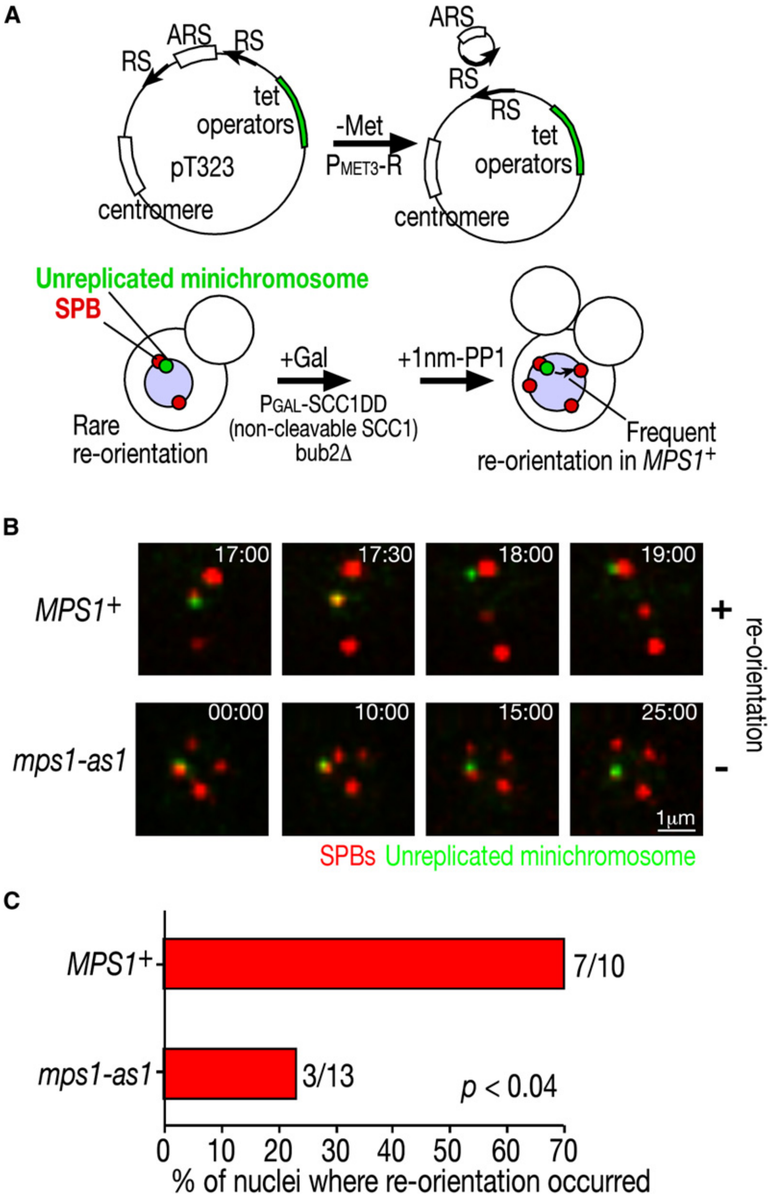

Figure 4. When Mps1 Is Inactivated, Unreplicated Monocentric Minichromosomes Show Less Frequent Re-orientation between SPBs

MPS1$^+$ (T5715) and *mps1-as1* (T5714) cells with *TetR-GFP SPC42-YFP bub2*Δ *PMET3-R PGAL-SCC1DD* (mutant *SCC1* that is resistant to cleavage by separase), carrying pT323, were incubated in methionine dropout medium (to express the recombinase) containing raffinose. After 1 hr, galactose was added to medium to express *SCC1DD*. After 8 hr, 1NM-PP1 was added to inactivate *mps1-as1*. After 4 hr, GFP (pT323 or its derivative minichromosome, green) and YFP (SPB, red) images were acquired every 30 s for 30 min.

(A) Schematic showing is how an unreplicated monocentric minichromosome was generated from pT323. Abbreviations are as in Figure 3A.

(B) Representative *MPS1*$^+$ (top) and *mps1-as1* (bottom) cells showing re-orientation or no re-orientation, respectively, of the unreplicated monocentric minichromosome. For scoring of re-orientation see Supplemental Experimental Procedures. Time is shown in min:s and time 0 is defined as the start of image acquisition.

(C) Graph shows the percentage of cells with 3 or 4 SPB signals, which showed re-orientation of the unreplicated monocentric minichromosome.

Mps1 Promotes Turnover of Kinetochore-Spindle Pole Connections that Do Not Generate Tension

If Mps1 is indeed involved in the tension-dependent error correction, we may see the role of Mps1 in detaching a centromere from one SPB and attaching it to another SPB when tension is not applied on this centromere-SPB connection by microtubules. To increase the chance of detecting this re-orientation between different SPBs, we created cells containing four SPBs, instead of two in normal metaphase, as we did previously [6]. In these cells, we observed the movement of an unreplicated minichromosome with one centromere, on which no tension is applied (Supplemental Results and Discussion, note 6; Figure 4A).

By using time-lapse microscopy, we compared frequency of re-orientation of GFP-marked unreplicated monocentrics between different YFP-labeled SPBs in *MPS1*$^+$ and *mps1-as1* cells (Figures 4B and 4C). During observation, 70% (7/10) of *MPS1*$^+$ cells showed re-orientation whereas only 23% (3/13) of *mps1-as1* cells showed it ($p < 0.04$). These data suggest that the Mps1 kinase does indeed have an important role in eliminating kinetochore-spindle pole connections when they cannot come under tension normally generated by bi-orientation.

Mps1 and Ipl1 Kinase Do Not Appear to Regulate Each Other's Localization or Kinase Activity

Given that Mps1 and Ipl1 kinases play similar roles in promoting sister-kinetochore bi-orientation [3, 6, 24], we addressed whether one may regulate function of the other. First we studied whether Mps1 and Ipl1 localization is altered in *ipl1* and *mps1* mutants, respectively (Supplemental Results and Discussion, note 7; Figures S7 and S8). However, we did not find any significant change in their localization in the mutants.

Next we investigated a possible change in Mps1 kinase activity in an *ipl1* mutant and vice versa. Mps1 was immunoprecipitated from *IPL1*$^+$ and *ipl1-321* cells (*ipl1-321* shows no detectable kinase activity in vitro [25]), whereas Ipl1 was immunoprecipitated from *MPS1*$^+$ and *mps1-as1* cells. The kinase activity was measured in vitro with GST-fused Dam1 as a substrate [26, 27] (Supplemental Results and Discussion, note 8; Figure S9). We did not detect any significant change in either kinase activity in cells in which the other kinase was mutated.

These results are consistent with Mps1 and Ipl1 facilitating bi-orientation through similar mechanisms but in parallel pathways. However, it is still possible that one critically regulates the other but we cannot detect this regulation. For example, one kinase may change the kinase activity of a small population of the other at kinetochores, in such a way that this small change is sufficient to promote bi-orientation.

Conclusions

We have shown that inactivation of Mps1 kinase leads to extensive defects in sister kinetochore bi-orientation on metaphase spindle in budding yeast. The role of Mps1 in bi-orientation is not secondary to its function in SPB duplication or in the spindle-assembly checkpoint (Supplemental Results and Discussion, notes 9 and 10). Mps1 facilitates bi-orientation in a tension-dependent mechanism and eliminates kinetochore-spindle pole connections that do not generate tension; this function is similar to that of Ipl1 (Supplemental Results and Discussion, note 11; Figure S10). This similarity would be explained if one kinase regulates the other; however, we did not find such evidence. Given that both kinases are required for bi-orientation, they may target different substrates at kinetochores (or Mps1 may do so at spindle poles; Supplemental Results and Discussion, note 12) or different sites of the same substrates, to promote re-orientation of kinetochore-spindle pole connections. Intriguingly, both Mps1 and Ipl1 phosphorylate the same Dam1 protein (a kinetochore component in metaphase) at different sites [26, 27]; at least Ipl1-dependent phosphorylation is important for bi-orientation (Supplemental Results and Discussion, note 13). It is also interesting whether the role of Mps1 in promoting bi-orientation is evolutionarily conserved from yeast to humans (Supplemental Results and Discussion, note 14).

Supplemental Data

Ten figures, Results and Discussion, and Experimental Procedures are available at http://www.current-biology.com/cgi/content/full/17/24/2175/DC1/.

Acknowledgments

We thank L. Clayton and M.J.R. Stark for discussions and reading the manuscript; M. Winey for *mps1-as1* strain and discussions; P. Keating for purified GST-Dam1 protein; J.R. Swedlow for discussions; Y. Kitamura and N. Kobayashi for technical help; K. Nasmyth, R. Ciosk, S. Biggins, S.J. Elledge, E. Schiebel, R. Tsien, K. Bloom, H. Araki, J.E. Haber, M. Yanagida, A.W. Murray, A.F. Straight, EUROSCARF, and the Yeast Resource Centre for yeast strains and plasmids. This work was supported by Cancer Research UK, Human Frontier Science Program, Lister Research Institute Prize, and Association for International Cancer Research. J.-F.M. was supported by the EMBO long-term fellowship. T.U.T. is a Senior Research Fellow of Cancer Research UK.

Received: August 2, 2007
Revised: November 13, 2007
Accepted: November 13, 2007
Published online: November 29, 2007

References

1. Nicklas, R.B. (1997). How cells get the right chromosomes. Science *275*, 632–637.
2. Tanaka, T.U., Stark, M.J., and Tanaka, K. (2005). Kinetochore capture and bi-orientation on the mitotic spindle. Nat. Rev. Mol. Cell Biol. *6*, 929–942.
3. Tanaka, T.U., Rachidi, N., Janke, C., Pereira, G., Galova, M., Schiebel, E., Stark, M.J., and Nasmyth, K. (2002). Evidence that the Ipl1-Sli15 (Aurora kinase-INCENP) complex promotes chromosome bi-orientation by altering kinetochore-spindle pole connections. Cell *108*, 317–329.
4. Hauf, S., Cole, R.W., LaTerra, S., Zimmer, C., Schnapp, G., Walter, R., Heckel, A., Van Meel, J., Rieder, C.L., and Peters, J.M. (2003). The small molecule Hesperadin reveals a role for Aurora B in correcting kinetochore-microtubule attachment and in maintaining the spindle assembly checkpoint. J. Cell Biol. *161*, 281–294.
5. Lampson, M.A., Renduchitala, K., Khodjakov, A., and Kapoor, T.M. (2004). Correcting improper chromosome-spindle attachments during cell division. Nat. Cell Biol. *6*, 232–237.
6. Dewar, H., Tanaka, K., Nasmyth, K., and Tanaka, T.U. (2004). Tension between two kinetochores suffices for their bi-orientation on the mitotic spindle. Nature *428*, 93–97.
7. Winey, M., and Huneycutt, B.J. (2002). Centrosomes and checkpoints: the MPS1 family of kinases. Oncogene *21*, 6161–6169.
8. Jones, M.H., Huneycutt, B.J., Pearson, C.G., Zhang, C., Morgan, G., Shokat, K., Bloom, K., and Winey, M. (2005). Chemical genetics reveals a role for Mps1 kinase in kinetochore attachment during mitosis. Curr. Biol. *15*, 160–165.
9. Bishop, A.C., Ubersax, J.A., Petsch, D.T., Matheos, D.P., Gray, N.S., Blethrow, J., Shimizu, E., Tsien, J.Z., Schultz, P.G., Rose, M.D., et al. (2000). A chemical switch for inhibitor-sensitive alleles of any protein kinase. Nature *407*, 395–401.
10. Michaelis, C., Ciosk, R., and Nasmyth, K. (1997). Cohesins: chromosomal proteins that prevent premature separation of sister chromatids. Cell *91*, 35–45.
11. Nasmyth, K., Peters, J.M., and Uhlmann, F. (2000). Splitting the chromosome: cutting the ties that bind sister chromatids. Science *288*, 1379–1385.
12. Goshima, G., and Yanagida, M. (2000). Establishing biorientation occurs with precocious separation of the sister kinetochores, but not the arms, in the early spindle of budding yeast. Cell *100*, 619–633.
13. He, X., Asthana, S., and Sorger, P.K. (2000). Transient sister chromatid separation and elastic deformation of chromosomes during mitosis in budding yeast. Cell *101*, 763–775.
14. Tanaka, T., Fuchs, J., Loidl, J., and Nasmyth, K. (2000). Cohesin ensures bipolar attachment of microtubules to sister centromeres and resists their precocious separation. Nat. Cell Biol. *2*, 492–499.
15. Pearson, C.G., Maddox, P.S., Salmon, E.D., and Bloom, K. (2001). Budding yeast chromosome structure and dynamics during mitosis. J. Cell Biol. *152*, 1255–1266.
16. Musacchio, A., and Salmon, E.D. (2007). The spindle-assembly checkpoint in space and time. Nat. Rev. Mol. Cell Biol. *8*, 379–393.
17. Tanaka, K., Mukae, N., Dewar, H., van Breugel, M., James, E.K., Prescott, A.R., Antony, C., and Tanaka, T.U. (2005). Molecular mechanisms of kinetochore capture by spindle microtubules. Nature *434*, 987–994.
18. Tanaka, K., Kitamura, E., Kitamura, Y., and Tanaka, T.U. (2007). Molecular mechanisms of microtubule-dependent kinetochore transport toward spindle poles. J. Cell Biol. *178*, 269–281.
19. Hill, A., and Bloom, K. (1987). Genetic manipulation of centromere function. Mol. Cell. Biol. *7*, 2397–2405.
20. Winey, M., Goetsch, L., Baum, P., and Byers, B. (1991). MPS1 and MPS2: novel yeast genes defining distinct steps of spindle pole body duplication. J. Cell Biol. *114*, 745–754.

21. Straight, A.F., Belmont, A.S., Robinett, C.C., and Murray, A.W. (1996). GFP tagging of budding yeast chromosomes reveals that protein-protein interactions can mediate sister chromatid cohesion. Curr. Biol. *6*, 1599–1608.
22. Kitamura, E., Tanaka, K., Kitamura, Y., and Tanaka, T.U. (2007). Kinetochore-microtubule interaction during S phase in *Saccharomyces cerevisiae*. Genes Dev., in press.
23. Nicklas, R.B., and Koch, C.A. (1969). Chromosome micromanipulation. 3. Spindle fiber tension and the reorientation of mal-oriented chromosomes. J. Cell Biol. *43*, 40–50.
24. Biggins, S., Severin, F.F., Bhalla, N., Sassoon, I., Hyman, A.A., and Murray, A.W. (1999). The conserved protein kinase Ipl1 regulates microtubule binding to kinetochores in budding yeast. Genes Dev. *13*, 532–544.
25. Kotwaliwale, C.V., Frei, S.B., Stern, B.M., and Biggins, S. (2007). A pathway containing the Ipl1/Aurora protein kinase and the spindle midzone protein Ase1 regulates yeast spindle assembly. Dev. Cell *13*, 433–445.
26. Cheeseman, I.M., Anderson, S., Jwa, M., Green, E.M., Kang, J., Yates, J.R., 3rd, Chan, C.S., Drubin, D.G., and Barnes, G. (2002). Phospho-regulation of kinetochore-microtubule attachments by the Aurora kinase Ipl1p. Cell *111*, 163–172.
27. Shimogawa, M.M., Graczyk, B., Gardner, M.K., Francis, S.E., White, E.A., Ess, M., Molk, J.N., Ruse, C., Niessen, S., Yates, J.R., 3rd, et al. (2006). Mps1 phosphorylation of Dam1 couples kinetochores to microtubule plus ends at metaphase. Curr. Biol. *16*, 1489–1501.